NOVEL INSIGHTS INTO STRAND CLEAVAGE AT AN ABASIC SITE IN DNA

A dissertation

presented to

the Faculty of the Graduate School

at the University of Missouri-Columbia

In Partial Fulfillment

of the Requirements for the Degree

Doctor of Philosophy

By

Tuhin Subhra Haldar

Dr. Kent S. Gates, Dissertation Supervisor

May 2022

The undersigned, appointed by the dean of the Graduate School, have examined the dissertation entitled

NOVEL INSIGHTS INTO STRAND CLEAVAGE AT AN ABASIC SITE IN DNA

Presented by Tuhin Haldar

A candidate for the degree of Doctor of Philosophy

and hereby certify that, in their opinion, it is worthy of acceptance

_____

Dr. Kent S. Gates, Chair

_____

Dr. Timothy E. Glass

_____

Dr. Gary A. Baker

_____

Dr. Li-Qun (Andrew) Gu

Acknowledgement

Herein, I take the opportunity to express my deep sense of gratitude and profound feeling of admiration for my research advisor Dr. Kent S. Gates for accepting me as a PhD student and teaching me everything in science and life. I would like to thank him for introducing and teaching me various aspects of DNA chemistry. While I may not have always been the easiest graduate student to mentor, I am sincerely grateful for all his time and effort to mold me into being a better scientist and a better person. Our lengthy conversations are invaluable to me. I consider myself fortunate to have worked under the best advisor ever.

I would like to thank Dr. Timothy E. Glass, Dr. Gary A. Baker and Dr. Li-Qun (Andrew) Gu for being part of my research and dissertation committee. Your guidance has shaped me to be a better individual professionally.

To that, I am thankful for being a part of this research group, and thankful to all Gates' group members past and present for helping me through some of my research challenges and for their input regarding this work.

I would like to personally thank Dr. Chris Nel and Dr. Kurt Housh. In these two, I sought to make friends, and ending up finding a family. I would like to thank Marjan Heidari for helping me correct this thesis. A very special thanks goes to Jerry D. Brightwell and Holly Oswald for all their support and help.

This thesis is dedicated to my late father who believed in me and encouraged me to go on every adventure, especially this one; and to my doting mother who always loved me unconditionally. Their support through this process is beyond words, and my love for them knows no depths.

Table of Contents

Contents ***Page No.***

List of Figures

Chapter 1

Chapter 2

Chapter 3

List of Schemes

Chapter 1

Chapter 2

Chapter 3

List of Table

Chapter 2

Novel Insights into Strand Cleavage at an Abasic Site in DNA

Tuhin Haldar

Dr. Kent S. Gates, Dissertation Supervisor

ABSTRACT

Loss of a nucleobases from the deoxyribose backbone of DNA generates Abasic sites in DNA. Abasic sites are the most common unavoidable DNA damage lesion. It is important to understand the properties of Abasic site. The acidic nature of the α-protons of the ring-opened abasic aldehyde residue facilitates the β-elimination of the 3'-phosphoryl group. This reaction is expected to generate a DNA strand break with a phosphoryl group on the 5'-terminus and a trans-α,β-unsaturated aldehyde residue on the 3'-terminus; however, a handful of studies have identified noncanonical sugar remnants on the 3'-terminus, suggesting that the products arising from strand cleavage at apurinic/apyrimidinic sites in DNA may be more complex than commonly thought. The strand cleavage in DNA at an abasic site induced by the treatment of heat, NaOH, piperidine, spermine, and the base excision repair glycosylases Fpg and Endo III has been characterized. DNA oligomer generated noncanonical sugar remnants including cis-α,β-unsaturated aldehyde, 2-deoxyribose, and 3-thio-2,3-dideoxyribose products on the 3'-terminus of the strand break. Cells contain millimolar concentration of spermine, which can induce strand cleavage at an abasic site in DNA generate very reactive α,β-unsaturated aldehyde iminium ion. Deoxyguanosine Triphosphate (dGTP) can react with the α,β-unsaturated aldehyde iminium ion of the on the 3’-end of the Ap-derive strand cleavage and generates a previously uncharacterized 3’dGTP-ddR adduct.

1 Chapter 1: Generation and Consequence of Abasic (AP) Sites in DNA

1.1 Introduction

DNA is the central molecule of the cell which regulates the efflux of genetic information within biological systems. The iconic double helix consists of two intertwined strands of DNA held together by Watson-Crick base pairs[1] (Figure 1.1). These DNA strands are composed of a deoxyribose phosphate backbone, in which each dR sugar is decorated with one of four heterocyclic nucleobases: adenine (A), thymine (T), guanine (G) or cytosine (C) (Figure 1.1.1). The hydrogen bond between nitrogenous heterocyclic base pairs, A-T and G-C holds the two strands of DNA together[2]. The sequence of nucleobases carries the genetic code of an organism. Every function of DNA requires separation of the two strands so that the genetic sequence can be read. The two strands must be separated and accurately transcribed to make mRNAs that ultimately produce proteins that serve as the structures and machinery of cells and organisms[3]. During cell division the DNA double helix must be unwound and faithfully copied to generate daughter cells with identical copies of the genetic code[4]. Therefore, any covalent modification of cellular DNA has significant biological ramification. Damage to cellular DNA can lead to inhibition of cell division, mutation, or cell death[5–7]. Generation of abasic (Ap) sites are the most common form of endogenous DNA damage[8]. The N-glycosidic bond which holds the nucleobases to the deoxyribose sugar can be spontaneously hydrolyzed and leads to loss of nucleobases and forms abasic site[8,9]. Covalent modification to nucleobases can destabilize the N-glycosidic bond and lead to abasic site[10–12]. Abasic sites can also generate

as a base excision repair intermediate by removal of a nucleobase by DNA Glycosylases[13,14]. Unrepaired Abasic sites have serious biological consequences. Abasic sites can stall DNA replication and transcription and bypass of these lesions leads to mutation[15,16]. Abasic sites can generate DNA interstrand crosslinks[17] and DNA protein crosslinks[18] which are cytotoxic. Strand breakage at the abasic site in DNA is deleterious towards genomic stability[19]. Given these factors, understanding the formation and consequences of abasic site in cellular DNA is essential.

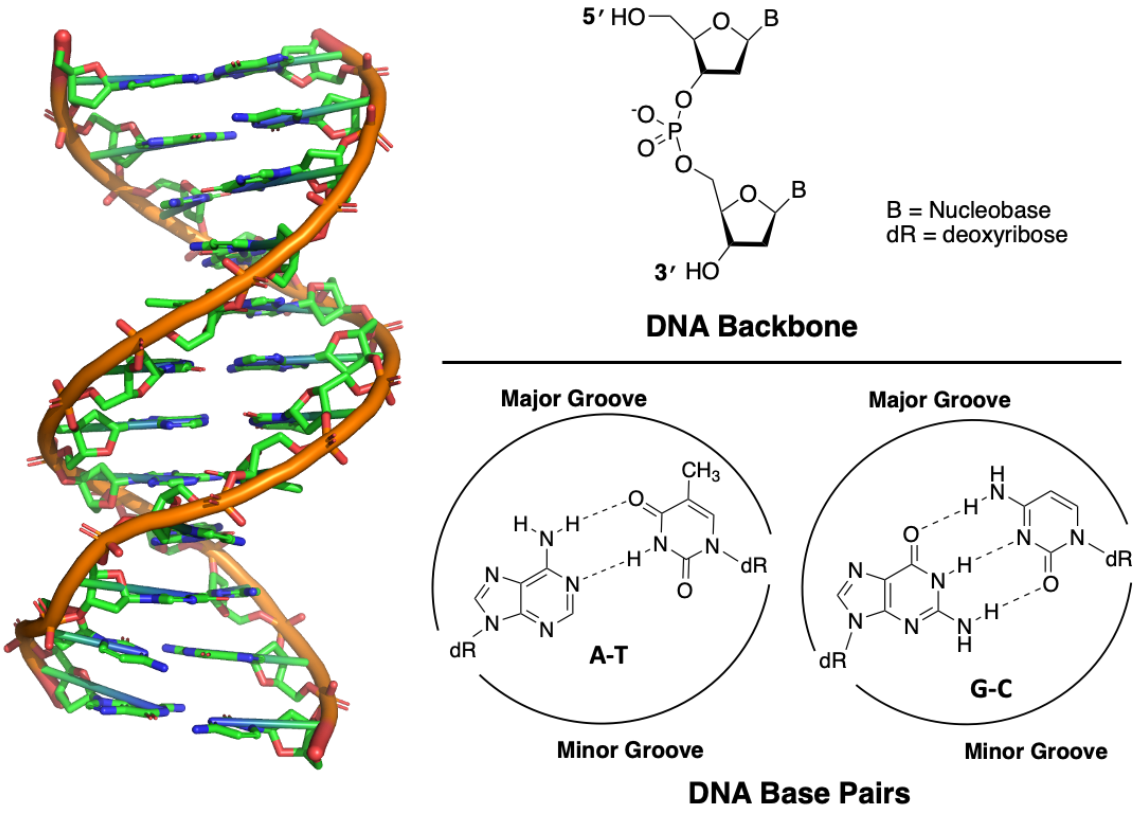


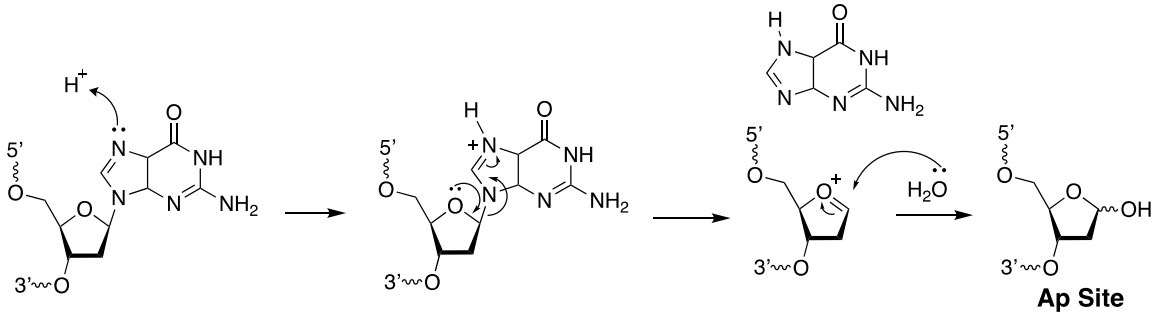
Figure 1.1.1 Structure of DNA. (PBD code: 1BNA)

1.2 Formation of Abasic Sites

Loss of a nucleobase generates an abasic site in DNA. Abasic sites can form via various pathways in cellular DNA by endogenous and exogenous factors. They can generate spontaneously by hydrolysis of glycosidic bonds. Also, enzymatic removal of bases can yield abasic site as an intermediate of DNA repair pathways. Damage to nucleobases can lead to formation of abasic site.

1.2.1 Spontaneous Hydrolysis of the N-Glycosidic Bonds

The N-glycosidic bond between the bases and deoxyribose sugar are susceptible towards hydrolytic attack. The reaction mechanism of the hydrolysis of N-glycosidic bonds is an acid catalyzed SN1.[20] Protonation of nucleobases at specific sites destabilizes the N-glycosidic bond via making the protonated base a better leaving group. Cleavage of the glycosidic bond releases the free base and forms an oxocarbenium ion which further gets hydrolyzed and generates an abasic site (Scheme 1.2.1).[21]



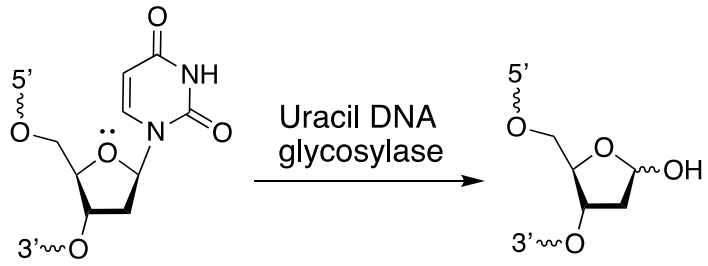
Scheme 1.2.1. Generation of AP sites via spontaneous hydrolysis of guanine.

Spontaneous loss of purine bases (guanine, adenine) is faster than the pyrimidine bases (cytosine, thymine). Under physiological conditions, the rate of depuration is 3.0 × 10−11 nucleotides per second ($t_{1/2}$ = 730 y), whereas the rate of depyrimidination is 1.5 × 10−12 nucleotides per second ($t_{1/2}$ = 14,700 y).[8,9] AP sites generated from spontaneous hydrolysis are most likely caused by depurations, because evidently the N-glycosidic bonds of pyrimidines are more stable than purines. Between purine bases, loss of guanine bases are 1.5 times more favorable than loss of adenine. As a result of spontaneous depurination in a mammalian cell around 10,000 AP sites gets generates per day.[8] The rate of depurination is four times faster in a single-stranded DNA compared to double-stranded DNA.[8] So, during replication the single strands of DNA are more prone towards spontaneous hydrolysis and generating abasic sites.

1.2.2 Enzymatic formation of Abasic Sites

Abasic sites can be generated as an intermediate during the Base Excision Repair (BER) pathway. Damaged bases get removed via hydrolysis of N-glycosidic bonds, catalyzed by DNA glycosylases.[13] Removal of misincorporated uracil in DNA strand via Uracil DNA glycosylase is a common pathway for abasic site formation in cellular DNA.[14] Uracil can be misincorporated in DNA via several pathways. Hydroxide attack on C4 of neutral or N3-protonated cytosine causes deamination and yields uracil.[22–24] Spontaneous hydrolytic deamination of cytosine generates ~100-500 uracils in human cells. In physiological conditions, cytosine deamination is faster ($t_{1/2}$ ~ 200 years) in single-stranded DNA compared to double stranded DNA ($t_{1/2}$ ~30,000–85,000 y).[9,25–27] Uracil can also be misincorporated in place of Thymine by DNA polymerases.[28] Removal of damaged bases

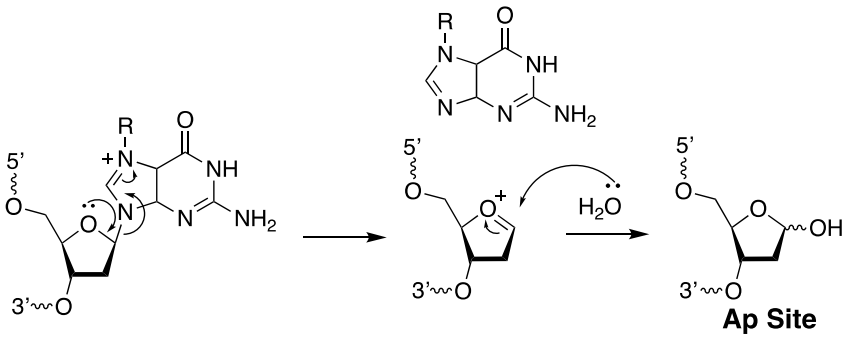
8-oxoG and FapyG by 8-oxoguanine glycosylase (OGG1) generates abasic site.[13] Repair of damaged lesions like 3meA, 7meG and 3meG by N-methyl-purine DNA glycosylase (MPG) introduces abasic site in cellular DNA.[13]



Scheme 1.2.2. Uracil DNA glycosylase catalyzed formation of AP site.

1.2.3 Hydrolysis of labile nucleobases

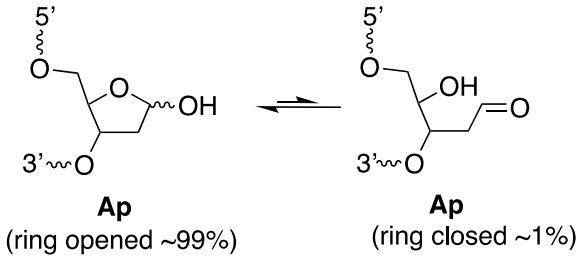
Various endogenous and exogenous factors like alkylating agents, oxidizing agents and UV light and therapeutics like anticancer drugs can damage nucleobases and form abasic sites.[12,29,30] Alkylation at specific endocyclic nitrogens (N7G, N7A, N3G, N3A, N1A, and N3C) of nucleobases destabilizes the N-glycosidic bond by generating a positive charge on the nucleobase (Scheme 1.2.3).[31] These labile lesions lead to deglycosylation and formation of abasic site. The most preferred site for alkylation is N7G in DNA because it is the most nucleophilic site.[32] Endogenous metabolites like reactive oxygen species (ROS) can damage nucleobases. Oxidative damage lesions of guanine, such as 7,8-dihydro-8-oxoguanine (8-oxoG), and formamidopyrimidine can form abasic site.[29] Hydroxyl radicals generated from ionization radiation (IR) can destabilize glycosidic bond and lead to AP site formation.[30]



Scheme 1.2.3. Generation of AP sites via hydrolysis of alkylated guanine.

1.3 Consequences of Abasic site in Cellular DNA

Abasic sites in cellular DNA are cytotoxic and mutagenic. AP sites in cells exist as equilibrium mixture of ring closed hemiacetal (~ 99%) and extremely reactive ring open aldehyde (~ 1%) form (Scheme 1.3.1).[31] The ring opened aldehyde form drives most of the chemistry of AP sites. Unrepaired abasic sites can cause strand breaks in DNA. Absence of a nucleobase in a DNA strand can block transcription and replication and can lead to mutation if not repaired correctly. Abasic site in DNA can also form DNA interstrand and DNA-Protein crosslinks.



Scheme 1.3.1 AP site exists as an equilibrium mixture of hemiacetal and aldehyde.

1.3.1 Replication Block and Mutation

Abasic sites can block DNA polymerases (Pol α, δ, and ε) and cause replication fork stalling.[16,33] If an Ap site is bypassed it can cause mutation by inserting a wrong nucleobase opposite to the abasic site by DNA polymerases.[12] In E. Coli it is observed that preferentially adenine gets inserted opposing AP sites. This is known as "A-rule".[34] A different study shows that in Saccharomyces cerevisiae (budding yeast), dCMP gets preferentially inserted over dAMP.[35] In higher eukaryotes preference towards particular nucleotides gets more complicated. Evidently, the preference of inserted base across abasic site depends on the organism and cells.[36–39]

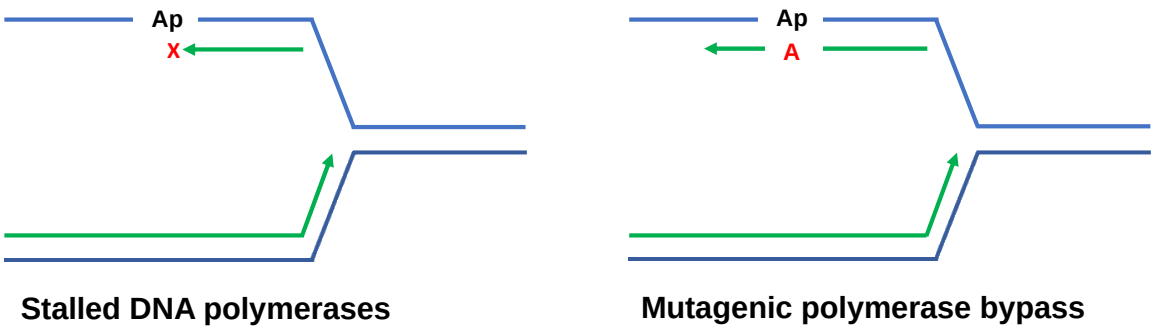


Figure 1.3.1.1 Stalled replication fork at an abasic site and bypass of an abasic site can cause mutation.

1.3.2 Transcription Block and Mutation

Abasic sites present in transcribed strands can block RNA polymerases like T7 RNAP and RNAPII and stall transcription.[40] Stalled RNA polymerases at an abasic site signals for recruitment of Nucleotide excision repair machinery to repair AP site. Bypass of this lesion can lead to mutation by insertion of the wrong base opposite to abasic site.[41]

RNAPII preferentially inserts AMP across AP site.[42] In this scenario, transcription-coupled Nucleotide excision repair (TC-NER) is the main repair pathway for abasic site.[43] Studies show that deficiencies in NER cause higher transcriptional mutagenesis frequency at abasic sites.[44,45]

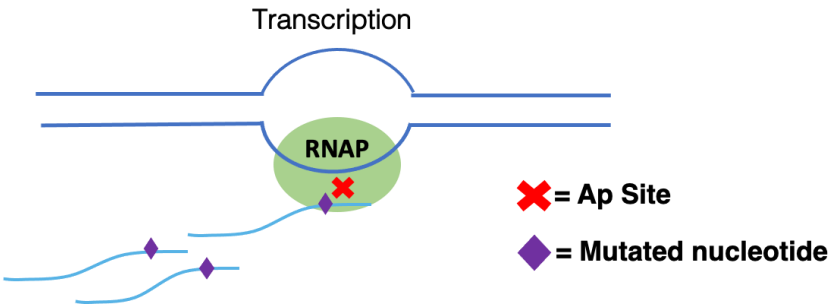
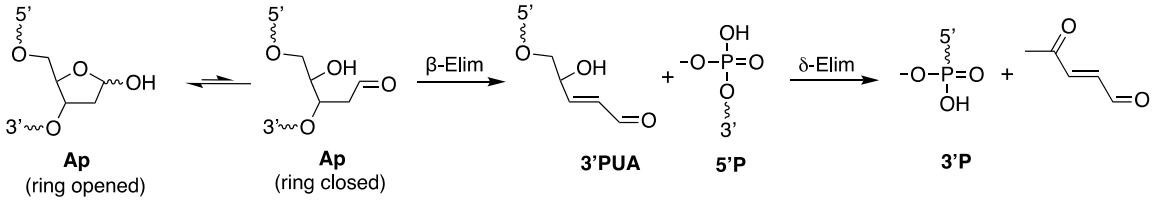


Figure 1.3.2.1. Abasic sites in DNA can cause transcriptional mutagenesis

1.3.3 Strand Breaks at Abasic site in DNA

Abasic site in DNA can exist as an equilibrium mixture of the ring-closed hemiacetal (~99%) and ring-opened aldehyde (~1%).[31,46] The ring-opened aldehyde form is electrophilic in nature and is very reactive. Abstraction of the acidic α-protons of ring-opened aldehyde form of abasic sites generates strand breaks of DNA via β-elimination of the 3′-phosphoryl group (Scheme 1.3.3.1). Under physiological conditions strand break reaction at AP site in DNA is slow ($t_{1/2}$ ~ 200-2000 h).[47–50] The rate of strand cleavage at an Ap site increases with heating, under alkaline conditions or in presence of amines.[47,51–57] The amine mediated strand cleavage is biologically important. Cellular nucleus have high concentrations (mM) of spermine.[58–61] Spermine can catalyze β-elimination at AP sites and generate strand break. Cleavage of abasic site in DNA via β-elimination generates strand breakage with 5'-Phosphoryl group (5'P) on one end and 3'-trans-α,β-unsaturated

aldehyde (3'PUA) on the other end (Scheme 1.3.3.1). 3'PUA can be eliminated in the presence of amine or under more vigorous conditions via γ,δ-elimination reaction and generates 5′P and 3′-phosphoryl (3′P) as end groups (Scheme 1.3.3.1). Amine residues in protein can also catalyze strand cleavage at the Abasic site (β-lyase reaction).[48,54,62–66] The 3'PUA has been detected in human cells.[67] Studies have shown that adducts arising from strand breaks at an abasic site in DNA are mutagenic and potentially more harmful than parent abasic sites.[68–70] 3'PUA generated from spermine catalyzed strand break at an abasic site can lead to DNA interstrand crosslink, which will be discussed in detail in next section.

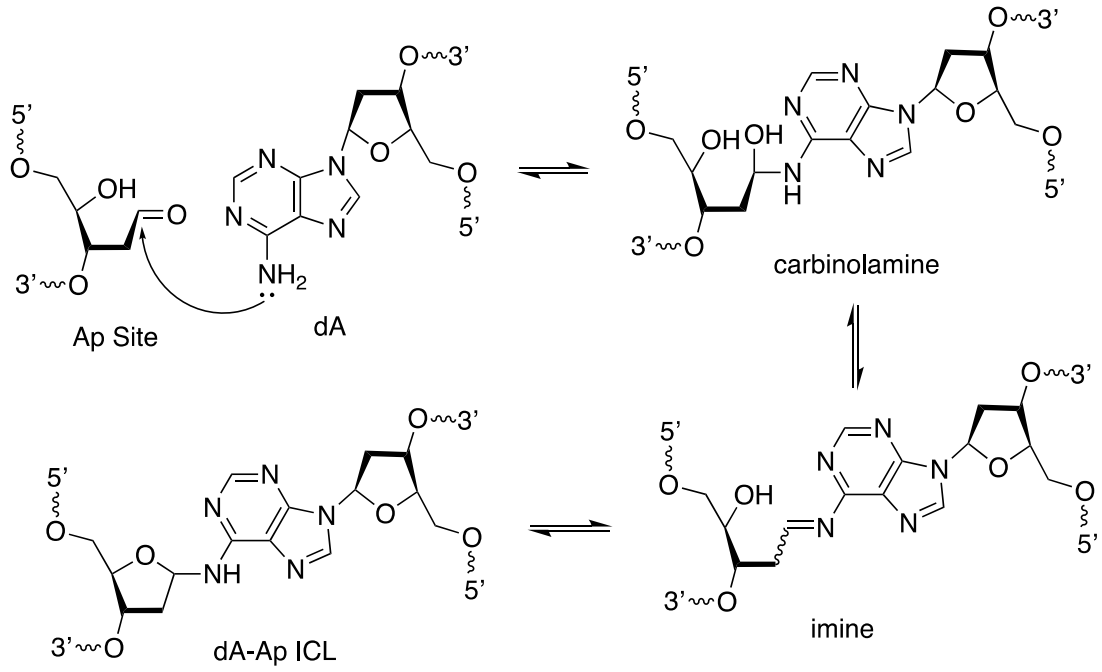


Scheme 1.3.3.1 Strand cleavage at an abasic site in DNA

1.3.4 DNA Interstrand Crosslink

Abasic sites can form highly cytotoxic DNA Interstrand crosslinks. Abasic site exists as an equilibrium mixture of ring ring-closed hemiacetal form (99%) and the ring-opened aldehyde form (1%).[31] The electrophilic ring-opened aldehyde form is highly reactive and can react with exocyclic amines of adenine and guanine nucleobases on the opposing strand of double-stranded DNA and generate DNA Interstrand Crosslinks (ICLs) (Scheme 1.3.4.1).[17,71,72] Crosslink between an abasic site and adenine base on opposing strand (dA-AP ICL) has been detected in 15-70% yield depending on the sequence in synthetic oligonucleotide.[71] The dG-AP ICL forms comparatively in lower yield (2–3%).[72]

AP derived crosslinks showed good stability after formation. Half-life for dissociation of dA-AP ICL is 60-90 h and dG-AP ICL is ~160 h under physiological condition.[71,72]



Scheme 1.3.4.1. Formation of dA-Ap ICL.

Crosslinks derived from abasic site can stall replication by blocking DNA polymerase and lead to cytotoxicity if not repaired.[73–75] Interestingly recent findings shows that dA-Ap crosslink can be repaired by unhooking of ICL by NEIL3.[76] Further repair of the abasic site generated from unhooking of dA-Ap ICL via translesion synthesis can be mutagenic.[76] If the NEIL3 repair pathway is inhibited, then the ICL gets repaired by Fanconi anemia pathway. During this repair pathway bypass of the AP-ICL-derived adenine mono-adduct can also lead to mutation (Figure 1.3.4.1).[76]

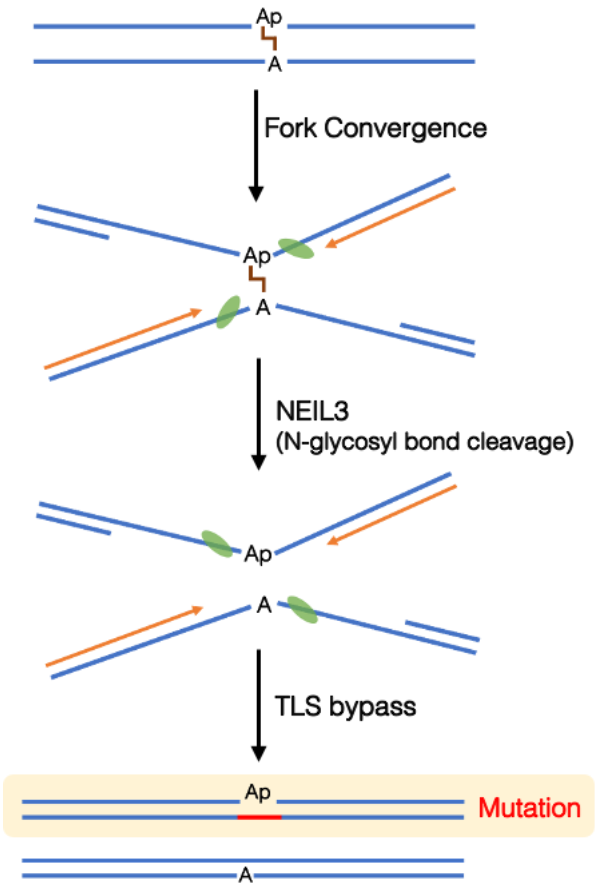
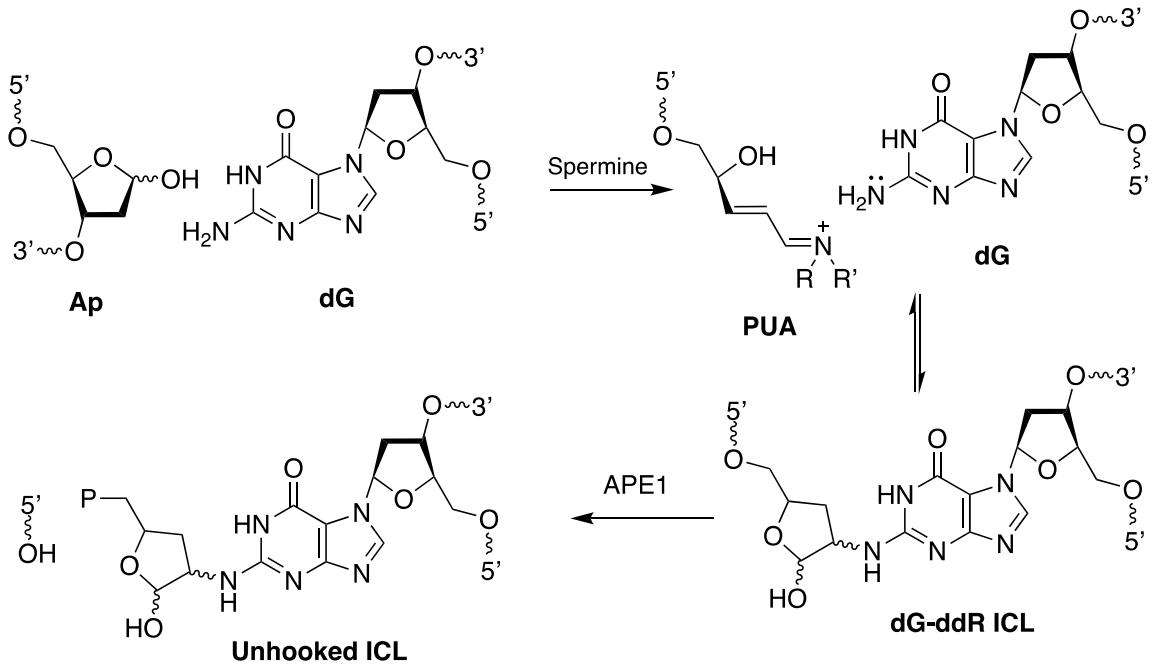


Figure 1.3.4.1. Repair of dA-Ap interstrand crosslink can be mutagenic

Cells contain millimolar concentration of spermine.[58–61] Spermine can catalyze strand cleavage at AP site generate α,β-unsaturated iminium ions which can lead to formation of interstrand crosslink with the exocyclic amine groups of DNA bases on the opposing strand (Scheme 1.3.4.3).[60,77] Guanine and adenine have shown capability of forming interstrand crosslink with spermine catalyzed strand break at an abasic site. The dA-ddR and dG-ddR can block DNA replication by ϕ29 DNA polymerase.[78] Studies have shown that the dG-ddR crosslink can be repaired by 3'exonuclease activity of APE1 (Scheme 1.3.4.3).[77]

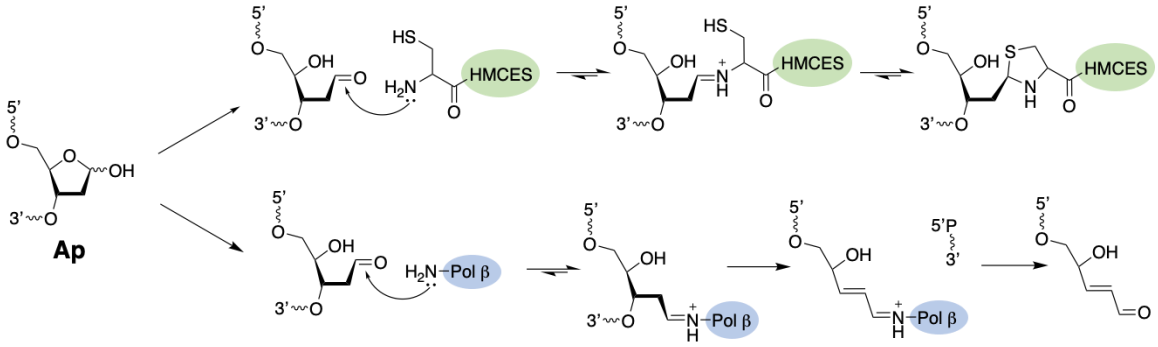


Scheme 1.3.4.3. Formation and repair of dG-ddR ICL.

1.3.5 DNA Protein Crosslink

Highly reactive ring-opened aldehyde forms of abasic sites can form DNA-Protein crosslinks (DPCs). DNA proteins can be either transient Schiff-base intermediates or stable. Stable DNA protein crosslinks are deleterious towards genomic stability. Repair enzymes like Pol β, PARP and KU form transient Schiff-base DPCs with AP sites.[18,79–82] These DPCs are unstable, and the enzymes get released by β-elimination. The Schiff base intermediates can be trapped by reductive amination with NaBH4. Oxidized AP site derivative, 2-deoxyribonolactone can make a stable DPC with Pol β. Recent findings show that a new enzyme, HMCES (5-hydroxymethylcytosine embryonic stem cells specific) can recognize abasic site and can generate a stable DPC.[83–85] The amino-terminal cysteine residue of HMCES with α-amino and sulfhydryl substituents can react with ring-open

aldehyde form of abasic site and forms a stable thiazolidine bond. The HMCES-DPC strand protects abasic site from strand cleavage and reduces mutation.[86] But the HMCES-DPC repair pathway is still unclear.



Scheme 1.3.4.3. Formation of DNA protein crosslinks by HMCES and Pol β.

1.4. Mutagenic repair pathways of AP Sites

1.4.1 Base Excision Repair

In double stranded DNA abasic sites get repaired by base excision repair (BER) pathways.[13,87–89] First, abasic sites get excisioned by AP endonucleases or AP lyases. Then it can further get repaired via either short or long patch repair pathways (Figure 1.4.1.1). Short patch repair is a more common pathway. APE1 cleaves abasic site and generates strand breaks with 3' hydroxyl (3'OH) and 5' dRP ends. Ap lyases also can cleave AP sites via β-elemination and generates 3'PUA and 3'P end groups. To ligate the DNA strand with the correct nucleotide it must have a 3'OH end and 5'P end. The cleaning of end groups can be done by Pol β, APE1 or PNKP. The gap is filled with the correct nucleotide by DNA polymerases (Pol β, Pol λ). And finally, the nick gets sealed by DNA ligase. In long patch

synthesis DNA polymerase (Pol δ, ε, and β) removes 2-10 nucleotides by displacing the strand. The 5' flap generated from strand displacements gets removed by Flap endonuclease 1. Finally, DNA ligase ligates the strand. Preference over a short or long patch repair depends on the organism, cell type, type of damaged lesion and the cell cycle stage.[90] A recent study has shown that in mammalian cells the BER intermediates can be mutagenic. Mutation frequency of 5'dRP and 3'PUA is high and comparable to parent abasic sites. Preference in insertion of adenine is observed across 5'dRP and 3'PUA similar to abasic site.[68]

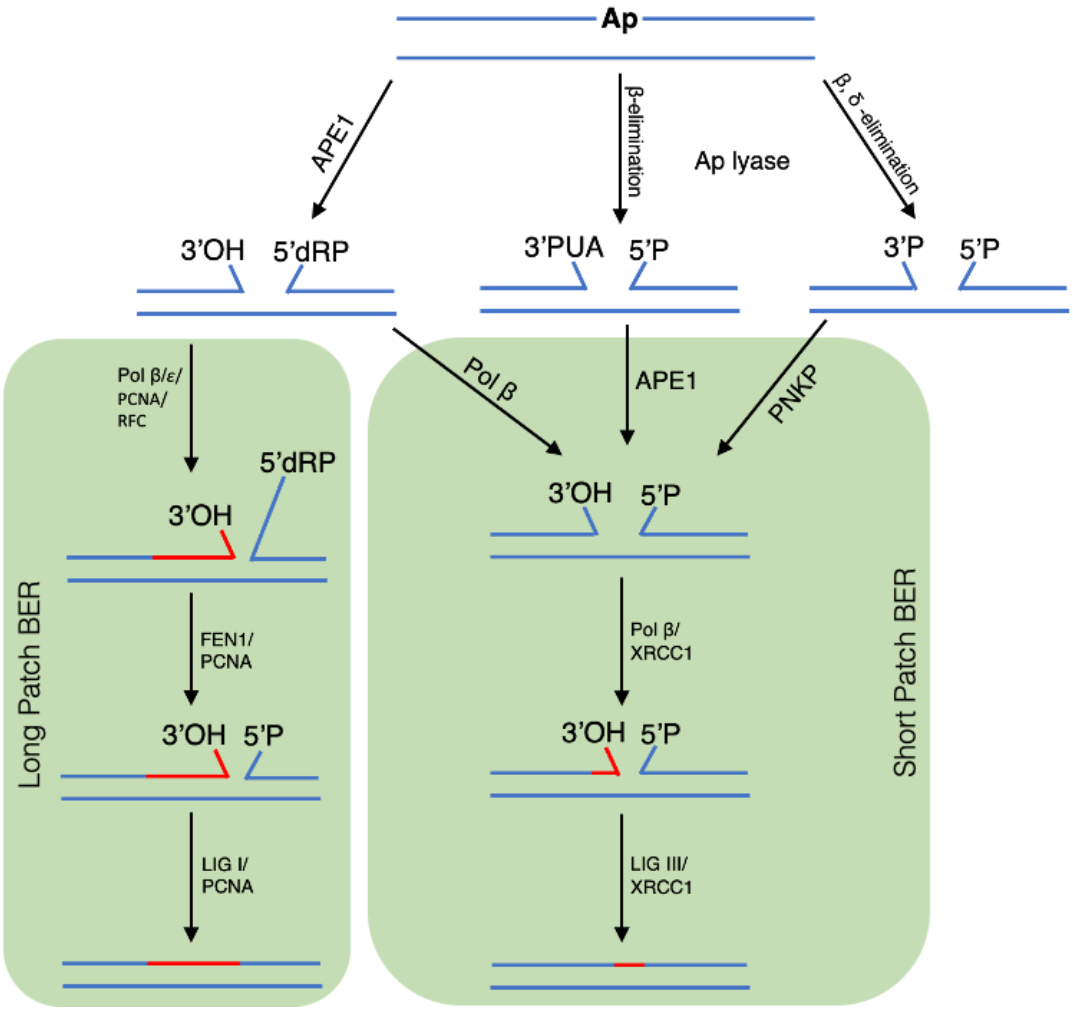


Figure 1.4.1.1 Repair of AP sites by Base excision repair pathway

1.4.2 Translesion synthesis (TLS) repair

At replication fork, transcription bubble and at telomeres DNA exist as single-strands because of unwinding of the duplex.[4] Abasic site in single-stranded DNA cannot be repaired by the BER pathway because in the BER pathway the DNA backbone gets cleaved and there is no template for repair. Also, AP endonucleases cannot work on single-stranded AP sites.[91] In this scenario, Abasic site in single-stranded DNA gets repaired by the error prone Translesion synthesis (TLS) pathway.[92,93] TLS polymerases bypass the abasic site and resume extension. The TLS repair pathway is mutagenic because there is a lack of coding information available on the template strand of TLS polymerase which can easily insert a wrong base across the Ap site. In Saccharomyces cerevisiae, Pol eta and Rev1 bypass the AP Site.[94] In humans Polη, Polι, Polκ, and Rev1 serve this role.[95] Insertion of base opposite of abasic site is done by the Y family of polymerases.[96] Preferentiality of nucleotide insertion across the abasic site depends on the cell type and organism.

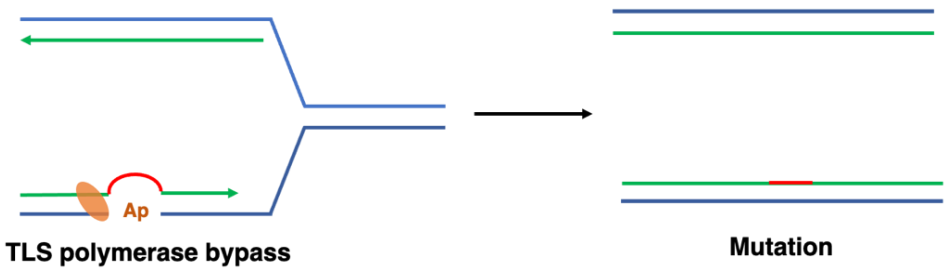


Figure 1.4.2.1 Mutagenic Translesion synthesis (TLS) bypass of AP site in single stranded DNA

1.5 Non-Mutagenic repair pathways of AP sites

Abasic site in single stranded DNA can be repaired by error-free pathways like homologous recombination, fork reversal and template switching.[97–99] Polymerases stalled by abasic sites during replication lead to accumulation of abasic sites in DNA and can initiate homologous recombination and template switching pathways. Studies have shown that in BER and TLS deficient cells rate of AP site repair by homologous recombination increased.[98] In the template switching repair pathway the strands get realigned and DNA gets synthesized using an undamaged alternative template, which makes it an error free mechanism.[100] In the fork reversal pathway a four-way chicken foot structure gets formed by template switching of nascent DNA strands.[101] Later the abasic sites get repaired by the BER pathway. These pathways are non-mutagenic.

1.6 Conclusion

Abasic sites are the most common form of DNA damage and have profound biological consequences. AP sites can generate spontaneously by hydrolysis of N-glycosidic bonds, enzymatically as an intermediate of BER pathway and loss of destabilized nucleobases. Unrepaired abasic sites in DNA can lead to cytotoxicity by strand cleavage, formation of DNA interstrand crosslinks and DNA-protein crosslinks. Repair of these lesions can be mutagenic. Genomic functions like replication and transcription can get stalled by abasic sites. Bypassing abasic sites in DNA causes mutation. Abasic site in cellular DNA can get multiple pathways depending on the situation. In double-stranded DNA, AP sites get repaired by the BER pathway. The intermediates of the BER pathway are mutagenic. At replication fork or transcription bubble abasic site can exist in single-

stranded DNA, which can get repaired by mutagenic TLS pathway or non-mutagenic pathways like homologous recombination, fork reversal and template switching. Understanding the formation and consequences of abasic site in cellular DNA is extremely important.

1.7 References

(1) Watson, J. D.; Crick, F. H. C. A Structure for Deoxyribose Nucleic Acid. *Nature* **1953**, *171*, 737.

(2) Todd, A. R. Chemical Structure of the Nucleic Acids. *Proc. Natl. Acad. Sci. U.S.A.* **1954**, *40*, 748.

(3) Alberts, B.; Johnson, A.; Lewis, J.; Raff, M.; Roberts, K.; Walter, P. *Molecular Biology of the Cell*; 4, Ed.; 2002.

(4) Alberts, B. DNA Replication and Recombination. *Nature* **2003**, *421*, 431.

(5) Zhou, B.-B. S.; Elledge, S. J. The DNA Damage Response: Putting Checkpoints in Perspective. *Nature* **2000**, *408*, 433.

(6) Norbury, C. J.; Hickson, I. D. Cellular Responses to DNA Damage. *Annu. Rev. Pharmacol. Toxicol.* **2001**, *41*, 367.

(7) Rouse, J.; Jackson, S. P. Interfaces between the Detection, Signaling, and Repair of DNA Damage. *Science* **2002**, *297*, 547.

(8) Lindahl, T.; Nyberg, B. Rate of Depurination of Native Deoxyribonucleic Acid. *Biochemistry* **1972**, *11*, 3610.

(9) Lindahl, T.; Karlstrom, O. Heat-Induced Depyrimidination of Deoxyribonucleic Acid in Solution. *Biochemistry* **1973**, *12*, 5151.

(10) Lawley, P.; Brookes, P. Further Studies on the Alkylation of Nucleic Acids and Their Constituent Nucleotides. *Biochemical Journal* **1963**, *89* (1), 127.

(11) Nooner, T.; Dutta, S.; Gates, K. S. Chemical Properties of the Leinamycin–Guanine Adduct in DNA. *Chem. Res. Toxicol.* **2004**, *17*, 942.

(12) Loeb, L. A.; Preston, B. D. Mutagenesis by Apurinic/Apyrimidinic Sites. *Annual review of genetics* **1986**, *20* (1), 201–230.

(13) Krokan, H. E.; Bjoras, M. Base Excision Repair. *Cold Spring Harbor Perspect. Biol.* **2013**, *5*, a012583.

(14) Tomilin, N.; Aprelikova, O. Uracil-DNA Glycosylases and DNA Uracil Repair. *International review of cytology* **1989**, *114*, 125–179.

(15) Lockhart, M.; Deutsch, J.; Yamaura, I.; Cavalieri, L.; Rosenberg, B. Termination of DNA Synthesis in Vitro at Apurinic Sites but Not at Ethyl Adducts on the Template. *Chemico-Biological Interactions* **1982**, *42* (1), 85–95.

(16) Sagher, D.; Strauss, B. Insertion of Nucleotides Opposite Apurinic Apyrimidinic Sites in Deoxyribonucleic Acid during in Vitro Synthesis: Uniqueness of Adenine Nucleotides. *Biochemistry* **1983**, *22* (19), 4518–4526.

(17) Dutta, S.; Chowdhury, G.; Gates, K. S. Interstrand Crosslinks Generated by Abasic Sites in Duplex DNA. *J. Am. Chem. Soc.* **2007**, *129*, 1852.

(18) Prasad, R.; Horton, J. K.; Chastain, P. D.; Gassman, N. R.; Freudenthal, B. D.; Hou, E. W.; Wilson, S. H. Suicidal Cross-Linking of PARP-1 to AP Site Intermediates in Cells Undergoing Base Excision Repair. *Nucleic acids research* **2014**, *42* (10), 6337–6351.

(19) Talpaert-Borlè, M. Formation, Detection and Repair of AP Sites. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis* **1987**, *181* (1), 45–56.

(20) Stivers, J. T.; Jiang, Y. L. A Mechanistic Perspective on the Chemistry of DNA Repair Glycosylases. *Chem. Rev.* **2003**, *103*, 2729.

(21) Zoltewicz, J. A.; Clark, D. F.; Sharpless, T. W.; Grahe, G. Kinetics and Mechanism of Acid-Catalyzed Hydrolysis of Some Purine Nucleosides. *J. Am. Chem. Soc.* **1970**, *92*, 1741.

(22) Shapiro, R.; Danzig, M. Acidic Hydrolysis of Deoxycytidine and Deoxyuridine Derivatives. The General Mechanism of Deoxyribonucleoside Hydrolysis. *Biochemistry* **1972**, *11*, 23.

(23) Shapiro, R.; Klein, R. S. The Deamination of Cytidine and Cytosine by Acidic Buffer Solutions. Mutagenic Implications. *Biochemistry* **1966**, *5*, 2358.

(24) Garrett, E. R.; Tsau, J. Solvolyses of Cytosine and Cytidine. *J. Pharm. Sci.* **1972**, *61*, 1052.

(25) Shen, J.-C.; Rideout, W. M.; Jones, P. A. The Rate of Hydrolytic Deamination of 5-Methylcytosine in Double-Stranded DNA. *Nucleic Acids Res.* **1994**, *22*, 972.

(26) Frederico, L. A.; Kunkel, T. A.; Shaw, B. R. A Sensitive Genetic Asssay for Detection of Cytosine Deamination: Determination of Rate Constants and the Activation Energy. *Biochemistry* **1990**, *29*, 2532.

(27) Frederico, L. A.; Kunkel, T. A.; Shaw, B. R. Cytosine Deamination in Mismatched Base Pairs. *Biochemistry* **1993**, *32*, 6523.

(28) Joyce, C. M. Choosing the Right Sugar: How Polymerases Select a Nucleotide Substrate. *Proceedings of the National Academy of Sciences* **1997**, *94* (5), 1619–1622.

(29) Cadet, J.; Wagner, J. R. DNA Base Damage by Reactive Oxygen Species, Oxidizing Agents, and UV Radiation. *Cold Spring Harbor perspectives in biology* **2013**, *5* (2), a012559.

(30) Teoule, R. Radiation-Induced DNA Damage and Its Repair. *International Journal of Radiation Biology and Related Studies in Physics, Chemistry and Medicine* **1987**, *51* (4), 573–589.

(31) Gates, K. S. An Overview of Chemical Processes That Damage Cellular DNA: Spontaneous Hydrolysis, Alkylation, and Reactions with Radicals. *Chem. Res. Toxicol.* **2009**, *22*, 1747.

(32) Beranek, D. T. Distribution of Methyl and Ethyl Adducts Following Alkylation with Monofunctional Alkylating Agents. *Mutat. Res.* **1990**, *231*, 11.

(33) Schaaper, R. M.; Kunkel, T. A.; Loeb, L. A. Infidelity of DNA Synthesis Associated with Bypass of Apurinic Sites. *Proceedings of the National Academy of Sciences* **1983**, *80* (2), 487–491.

(34) Strauss, B. S. The "A" Rule Revisited: Polymerases as Determinants of Mutational Specificity. *DNA repair* **2002**, *1* (2), 125–135.

(35) Gibbs, P. E.; Lawrence, C. W. Novel Mutagenic Properties of Abasic Sites InSaccharomyces Cerevisiae. *Journal of molecular biology* **1995**, *251* (2), 229–236.

(36) Gentil, A.; Renault, G.; Madzak, C.; Margot, A.; Cabral-Neto, J.; Vasseur, J.; Rayner, B.; Imbach, J.; Sarasin, A. Mutagenic Properties of a Unique Abasic Site in Mammalian Cells. *Biochemical and biophysical research communications* **1990**, *173* (2), 704–710.

(37) Klinedinst, D. K.; Drinkwater, N. R. Mutagenesis by Apurinic Sites in Normal and Ataxia Telangiectasia Human Lymphoblastoid Cells. *Molecular carcinogenesis* **1992**, *6* (1), 32–42.

(38) Neto, J. B. C.; Cabral, R. E. C.; Margot, A.; Le Page, F.; Sarasin, A.; Gentil, A. Coding Properties of a Unique Apurinic/Apyrimidinic Site Replicated in Mammalian Cells. *Journal of molecular biology* **1994**, *240* (5), 416–420.

(39) Otsuka, C.; Sanadai, S.; Hata, Y.; Okuto, H.; Noskov, V. N.; Loakes, D.; Negishi, K. Difference between Deoxyribose-and Tetrahydrofuran-type Abasic Sites in the in Vivo Mutagenic Responses in Yeast. *Nucleic acids research* **2002**, *30* (23), 5129–5135.

(40) Tornaletti, S.; Maeda, L. S.; Hanawalt, P. C. Transcription Arrest at an Abasic Site in the Transcribed Strand of Template DNA. *Chemical research in toxicology* **2006**, *19* (9), 1215–1220.

(41) Yu, S.-L.; Lee, S.-K.; Johnson, R. E.; Prakash, L.; Prakash, S. The Stalling of Transcription at Abasic Sites Is Highly Mutagenic. *Molecular and cellular biology* **2003**, *23* (1), 382–388.

(42) Wang, W.; Walmacq, C.; Chong, J.; Kashlev, M.; Wang, D. Structural Basis of Transcriptional Stalling and Bypass of Abasic DNA Lesion by RNA Polymerase II. *Proceedings of the National Academy of Sciences* **2018**, *115* (11), E2538–E2545.

(43) Kitsera, N.; Rodriguez-Alvarez, M.; Emmert, S.; Carell, T.; Khobta, A. Nucleotide Excision Repair of Abasic DNA Lesions. *Nucleic acids research* **2019**, *47* (16), 8537–8547.

(44) Kim, N.; Jinks-Robertson, S. Abasic Sites in the Transcribed Strand of Yeast DNA Are Removed by Transcription-Coupled Nucleotide Excision Repair. *Molecular and cellular biology* **2010**, *30* (13), 3206–3215.

(45) Clauson, C. L.; Oestreich, K. J.; Austin, J. W.; Doetsch, P. W. Abasic Sites and Strand Breaks in DNA Cause Transcriptional Mutagenesis in Escherichia Coli. *Proceedings of the National Academy of Sciences* **2010**, *107* (8), 3657–3662.

(46) Wilde, J. A.; Bolton, P. H.; Mazumder, A.; Manoharan, M.; Gerlt, J. A. Characterization of the Equilibrating Forms of the Abasic Site in Duplex DNA Using 17O-NMR. *J. Am. Chem. Soc.* **1989**, *111*, 1894.

(47) Lindahl, T.; Andersson, A. Rate of Chain Breakage at Apurinic Sites in Double-Stranded Deoxyribonucleic Acid. *Biochemistry* **1972**, *11*, 3618.

(48) Zhou, C.; Sczepanski, J. T.; Greenberg, M. M. Mechanistic Studies on Histone Catalyzed Cleavage of Apyrimidinic/Apurinic Sites in Nucleosome Core Particles. *J. Am. Chem. Soc.* **2012**, *134*, 16734.

(49) Lawley, P. D.; Lethbridge, J.; Edwards, P. A.; Shooter, K. Inactivation of Bacteriophage T7 by Mono- and Difunctional Sulphur Mustards in Relation to Cross-Linking and Depurination of Bacteriophage DNA. *J. Mol. Biol.* **1969**, *39*, 181.

(50) Laurence, D. J. R. Chain Breakage of Deoxyribonucleic Acid Following Treatment with Low Doses of Sulphur Mustard. *Proc. R. Soc. A* **1963**, *271*, 520.

(51) Gates, K. S.; Nooner, T.; Dutta, S. Biologically Relevant Chemical Reactions of N7-Alkyl-2′-Deoxyguanosine Adducts in DNA. *Chem. Res. Toxicol.* **2004**, *17*, 839.

(52) Sugiyama, H.; Fujiwara, T.; Ura, A.; Tashiro, T.; Yamamoto, K.; Kawanishi, S.; Saito, I. Chemistry of Thermal Degradation of Abasic Sites in DNA. Mechanistic Investigation on Thermal DNA Strand Cleavage of Alkylated DNA. *Chem. Res. Toxicol.* **1994**, *7*, 673.

(53) Bailly, V.; Verly, W. G. Possible Roles of β-Elimination and δ-Elimination Reactions in the Repair of DNA Containing AP (Apurinic/Apyrimidinic) Sites in Mammalian Cells. *Biochem. J.* **1988**, *253*, 553.

(54) Mazumder, A.; Gerlt, J. A.; Absalon, M. J.; Stubbe, J.; Cunningham, R. P.; Withka, J.; Bolton, P. H. Stereochemical Studies of the B-Elimination Reactions at Aldehydic Abasic Sites in DNA: Endonuclease III from Escherichia Coli, Sodium Hydroxide, and Lys-Trp-Lys. *Biochemistry* **1991**, *30*, 1119.

(55) McHugh, P. J.; Knowland, J. Novel Reagents for Chemical Cleavage at Abasic Sites and UV Photoproducts in DNA. *Nucleic Acids Res.* **1995**, *23*, 1664.

(56) Liu, M.; Bandaru, V.; Bond, J. P.; Jaruga, P.; Zhao, X.; Christov, P. P.; Burrows, C. J.; Rizzo, C. J.; Dizdaroglu, M.; Wallace, S. S. The Mouse Ortholog of NEIL3 Is a Functional DNA Glycosylase in Vitro and in Vivo. *Proc. Natl. Acad. Sci. U.S.A.* **2010**, *107*, 4925.

(57) Imani-Nejad, M.; Price, N. E.; Haldar, T.; Lewis, C.; Wang, Y.; Gates, K. S. Interstrand DNA Cross-Links Derived from Reaction of a 2-Aminopurine Residue with an Abasic Site. *ACS Chem. Biol.* **2019**, *14*, 1481.

(58) Male, R.; Fosse, V. M.; Kleppe, K. Polyamine-Induced Hydrolysis of Apurinic Sites in DNA and Nucleosomes. *Nucleic Acid Res.* **1982**, *10*, 6305.

(59) Wallace, H. M.; Fraser, A. V.; Hughes, A. A Perspective of Polyamine Metabolism. *Biochem. J.* **2003**, *376*, 1.

(60) Yang, Z.; Price, N. E.; Johnson, K. M.; Wang, Y.; Gates, K. S. Interstrand Cross-Links Arising from Strand Breaks at True Abasic Sites in Duplex DNA. *Nucleic Acids Res.* **2017**, *45*, 6275.

(61) Raspaud, E.; Chaperon, I.; Leforestier, A.; Livolant, F. Spermine-Induced Aggregation of DNA, Nucleosome, and Chromatin. *Biophys. J.* **1999**, *77*, 1547.

(62) Brooks, S. C.; Adhikary, S.; Rubinson, E. H.; Eichman, B. F. Recent Advances in the Structural Mechanisms of DNA Glycosylases. *Biochim. Biophys. Acta* **2013**, *1834*, 247.

(63) Kim, J.; Linn, S. The Mechanism of Action of E. Coli Endonuclease III and T7 UV Endonuclease (Endonuclease V) on AP Sites. *Nucleic Acids Res.* **1988**, *16*, 1135.

(64) Sczepanski, J. T.; Wong, R. S.; McKnight, J. N.; Bowman, G. D.; Greenberg, M. M. Rapid DNA-Protein Cross-Linking and Strand Scission by an Abasic Site in a Nucleosome Core Particle. *Proc. Natl. Acad. Sci. U.S.A.* **2010**, *107*, 22475.

(65) McCullough, A. K.; Sanchez, A.; Dodson, M. L.; Marapaka, P.; Taylor, J.-S.; Lloyd, R. S. The Reaction Mechanism of DNA Glycosylase/AP Lyases at Abasic Sites. *Biochemistry* **2001**, *40*, 561.

(66) Fromme, J. C.; Verdine, G. L. Structure of a Trapped Endonuclease III-DNA Covalent Intermediate. *EMBO J.* **2003**, *22*, 3461.

(67) Rahimoff, R.; Kosmatchev, O.; Kirchner, A.; Pfaffeneder, T.; Spada, F.; Brantl, V.; Müller, M.; Carell, T. 5-Formyl- and 5-Carboxydeoxycytidines Do Not Cause Accumulation of Harmful Repair Intermediates in Stem Cells. *J. Am. Chem. Soc.* **2017**, *139*, 10359.

(68) Simonelli, V.; Narciso, L.; Dogliotti, E.; Fortini, P. Base Excision Repair Intermediates Are Mutagenic in Mammalian Cells. *Nucleic Acids Res.* **2005**, *33*, 4404.

(69) Boiteux, S.; Guillet, M. Abasic Sites in DNA: Repair and Biological Consequences in Saccaromyces Cerevisiae. *DNA Repair* **2004**, *3*, 1.

(70) Ma, W.; Westmoreland, J. W.; Gordenin, D. A.; Resnick, M. A. Alkylation Base Damage Is Converted into Repairable Double-Strand Breaks and Complex Intermediates in G2 Cells Lacking AP Endonuclease. *PLoS Genet.* **2011**, *7*, e1002059.

(71) Price, N. E.; Johnson, K. M.; Wang, J.; Fekry, M. I.; Wang, Y.; Gates, K. S. Interstrand DNA–DNA Cross-Link Formation between Adenine Residues and Abasic Sites in Duplex DNA. *Journal of the American Chemical Society* **2014**, *136* (9), 3483–3490.

(72) Johnson, K. M.; Price, N. E.; Wang, J.; Fekry, M. I.; Dutta, S.; Seiner, D. R.; Wang, Y.; Gates, K. S. On the Formation and Properties of Interstrand DNA-DNA Cross-Links Forged by Reaction of an Abasic Site With the Opposing Guanine Residue of 5'-CAp Sequences in Duplex DNA. *J. Am. Chem. Soc.* **2013**, *135*, 1015.

(73) Schärer, O. D. DNA Interstrand Crosslinks: Natural and Drug-Induced DNA Adducts That Induce Unique Cellular Responses. *ChemBioChem* **2005**, *6*, 27.

(74) Noll, D. M.; Mason, T. M.; Miller, P. S. Formation and Repair of Interstrand Crosslinks in DNA. *Chem. Rev.* **2006**, *106*, 277.

(75) Rajski, S. R.; Williams, R. M. DNA Cross-Linking Agents as Antitumor Drugs. *Chem. Rev.* **1998**, *98*, 2723.

(76) Semlow, D. R.; Zhang, J.; Budzowska, M.; Drohat, A. C.; Walter, J. C. Replication-Dependent Unhooking of DNA Interstrand Cross-Links by the NEIL3 Glycosylase. *Cell* **2016**, *167* (2), 498–511.

(77) Housh, K.; Jha, J. S.; Yang, Z.; Haldar, T.; Johnson, K. M.; Yin, J.; Wang, Y.; Gates, K. S. Formation and Repair of an Interstrand DNA Cross-Link Arising from a Common Endogenous Lesion. *J. Am. Chem. Soc.* **2021**, *143*, 15344.

(78) Yang, Z.; Price, N. E.; Johnson, K. M.; Gates, K. S. Characterization of Interstrand DNA–DNA Cross-Links Derived from Abasic Sites Using Bacteriophage Φ29 DNA Polymerase. *Biochemistry* **2015**, *54* (27), 4259–4266. https://doi.org/10.1021/acs.biochem.5b00482.

(79) Khodyreva, S.; Prasad, R.; Ilina, E.; Sukhanova, M.; Kutuzov, M.; Liu, Y.; Hou, E.; Wilson, S.; Lavrik, O. Apurinic/Apyrimidinic (AP) Site Recognition by the 5′-DRP/AP Lyase in Poly (ADP-Ribose) Polymerase-1 (PARP-1). *Proceedings of the National Academy of Sciences* **2010**, *107* (51), 22090–22095.

(80) DeMott, M. S.; Beyret, E.; Wong, D.; Bales, B. C.; Hwang, J.-T.; Greenberg, M. M.; Demple, B. Covalent Trapping of Human DNA Polymerase β by the Oxidative DNA Lesion 2-Deoxyribonolactone. *Journal of Biological Chemistry* **2002**, *277* (10), 7637–7640.

(81) Quiñones, J. L.; Thapar, U.; Yu, K.; Fang, Q.; Sobol, R. W.; Demple, B. Enzyme Mechanism-Based, Oxidative DNA–Protein Cross-Links Formed with DNA Polymerase β in Vivo. *Proceedings of the National Academy of Sciences* **2015**, *112* (28), 8602–8607.

(82) Roberts, S. A.; Strande, N.; Burkhalter, M. D.; Strom, C.; Havener, J. M.; Hasty, P.; Ramsden, D. A. Ku Is a 5′-DRP/AP Lyase That Excises Nucleotide Damage near Broken Ends. *Nature* **2010**, *464* (7292), 1214–1217.

(83) Thompson, P. S.; Amidon, K. M.; Mohni, K. N.; Cortez, D.; Eichman, B. F. Protection of Abasic Sites during DNA Replication by a Stable Thiazolidine Protein-DNA Cross-Link. *Nature structural & molecular biology* **2019**, *26* (7), 613–618.

(84) Halabelian, L.; Ravichandran, M.; Li, Y.; Zeng, H.; Rao, A.; Aravind, L.; Arrowsmith, C. H. Strutural Basis of HMCES Interactions with Abasic DNA and Multivalent Substrate Recognition. *Nat. Struct. Mol. Biol.* **2019**, *26*, 607.

(85) Wang, N.; Bao, H.; Chen, L.; Liu, Y.; Li, Y.; Wu, B.; Huang, H. Molecular Basis of Abasic Site Sensing in Single-Stranded DNA by the SRAP Domain of E. Coli YedK. *Nucleic acids research* **2019**, *47* (19), 10388–10399.

(86) Mohni, K. N.; Wessel, S. R.; Zhao, R.; Wojciechowski, A. C.; Luzwick, J. W.; Layden, H.; Eichman, B. F.; Thompson, P. S.; Mehta, K. P. M.; Cortez, D. HMCES Maintains Genome Integrity by Shielding Abasic Sites in Single-Strand DNA. *Cell* **2019**, *176*, 144.

(87) David, S. S.; O'Shea, V. L.; Kundu, S. Base-Excision Repair of Oxidative DNA Damage. *Nature* **2007**, *447* (7147), 941–950.

(88) Wallace, S. S. Base Excision Repair: A Critical Player in Many Games. *DNA repair* **2014**, *19*, 14–26.

(89) Kim, Y.-J.; M Wilson III, D. Overview of Base Excision Repair Biochemistry. *Current molecular pharmacology* **2012**, *5* (1), 3–13.

(90) Fortini, P.; Dogliotti, E. Base Damage and Single-Strand Break Repair: Mechanisms and Functional Significance of Short-and Long-Patch Repair Subpathways. *DNA repair* **2007**, *6* (4), 398–409.

(91) Marenstein, D. R.; Wilson III, D. M.; Teebor, G. W. Human AP Endonuclease (APE1) Demonstrates Endonucleolytic Activity against AP Sites in Single-Stranded DNA. *DNA repair* **2004**, *3* (5), 527–533.

(92) Prakash, S.; Johnson, R. E.; Prakash, L. Eukaryotic Translesion Synthesis DNA Polymerases: Specificity of Structure and Function. *Annu. Rev. Biochem.* **2005**, *74*, 317–353.

(93) Waters, L. S.; Minesinger, B. K.; Wiltrout, M. E.; D'Souza, S.; Woodruff, R. V.; Walker, G. C. Eukaryotic Translesion Polymerases and Their Roles and Regulation in DNA Damage Tolerance. *Microbiology and Molecular Biology Reviews* **2009**, *73* (1), 134–154.

(94) Gibbs, P. E. M.; McDonald, J.; Woodgate, R.; Lawrence, C. W. The Relative Roles in Vivo of Saccharomyces Cerevisiae Pol η, Pol ζ, Rev1 Protein and Pol32 in the Bypass and Mutation Induction of an Abasic Site, T-T (6-4) Photoadduct and T-T Cis-Syn Cyclobutane Dimer. *Genetics* **2005**, *169* (2), 575–582. https://doi.org/10.1534/genetics.104.034611.

(95) Chan, K.; Resnick, M. A.; Gordenin, D. A. The Choice of Nucleotide Inserted Opposite Abasic Sites Formed within Chromosomal DNA Reveals the Polymerase Activities Participating in Translesion DNA Synthesis. *DNA repair* **2013**, *12* (11), 878–889.

(96) Choi, J.-Y.; Lim, S.; Kim, E.-J.; Jo, A.; Guengerich, F. P. Translesion Synthesis across Abasic Lesions by Human B-Family and Y-Family DNA Polymerases α, δ, η, ι, κ, and REV1. *Journal of molecular biology* **2010**, *404* (1), 34–44.

(97) Otterlei, M.; Kavli, B.; Standal, R.; Skjelbred, C.; Bharati, S.; Krokan, H. E. Repair of Chromosomal Abasic Sites in Vivo Involves at Least Three Different Repair Pathways. *The EMBO journal* **2000**, *19* (20), 5542–5551.

(98) Swanson, R. L.; Morey, N. J.; Doetsch, P. W.; Jinks-Robertson, S. Overlapping Specificities of Base Excision Repair, Nucleotide Excision Repair, Recombination, and Translesion Synthesis Pathways for DNA Base Damage in Saccharomyces Cerevisiae. *Molecular and cellular biology* **1999**, *19* (4), 2929–2935.

(99) Adar, S.; Izhar, L.; Hendel, A.; Geacintov, N.; Livneh, Z. Repair of Gaps Opposite Lesions by Homologous Recombination in Mammalian Cells. *Nucleic acids research* **2009**, *37* (17), 5737–5748.

(100) Izhar, L.; Goldsmith, M.; Dahan, R.; Geacintov, N.; Lloyd, R. G.; Livneh, Z. Analysis of Strand Transfer and Template Switching Mechanisms of DNA Gap Repair by Homologous Recombination in Escherichia Coli: Predominance of Strand Transfer. *Journal of molecular biology* **2008**, *381* (4), 803–809.

(101) Bhat, K. P.; Cortez, D. RPA and RAD51: Fork Reversal, Fork Protection, and Genome Stability. *Nature structural & molecular biology* **2018**, *25* (6), 446–453.

2 Chapter 2: Unexpected Complexity in the Products Arising from NaOH, Heat, Amine, and Glycosylase-Induced Strand Cleavage at an Abasic Site in DNA

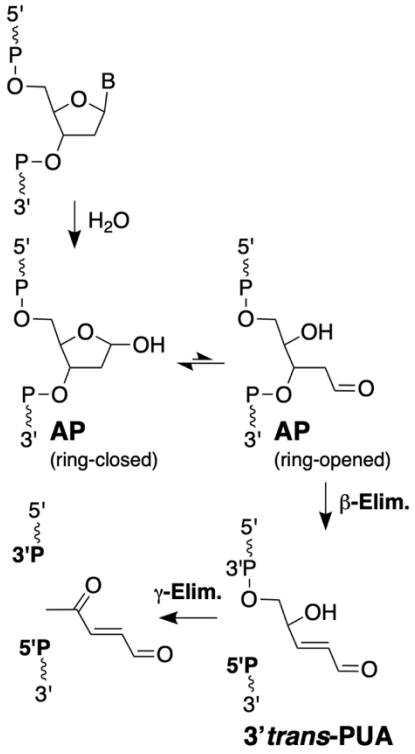
2.1 Introduction

Apurinic/apyrimidinic sites (AP, Scheme 2.1.1) are generated by Spontaneous[1–3] and enzyme catalyzed[4–7] hydrolysis of the glycosidic bonds connecting nucleobases (B in Scheme 2.1.1) to the deoxyribose backbone of DNA. Chemical modification of the DNA bases also can accelerate hydrolysis of the glycosidic bonds to generate AP sites.[3,8,9] These processes combine to make AP sites among the most common unavoidable lesions found in both cellular and synthetic DNA.[8,10–14]

AP sites exist as an equilibrium mixture of the ring-closed hemiacetal alongside small amounts (~1%) of the ring-opened aldehyde (Scheme 2.1.1).[15,16] Much of the interesting chemistry associated with AP residues in DNA, as is the case for all aldose sugars, stems from equilibrium amounts of the reactive, ring-opened aldehyde. The electrophilic nature of the AP aldehyde residue enables generation of secondary lesions including DNA-DNA interstrand crosslinks,[17–22] and DNA protein cross-links.[23–26] In addition, the acidic nature of the α-protons[27] of the ring-opened AP aldehyde facilitates the generation of DNA strand breaks via β-elimination of the 3'-phosphoryl group (Scheme 2.2.1).[8,28–33]

In neutral aqueous buffers at 37 ˚C, AP sites in DNA are converted into strand breaks rather slowly, with half-times in the range of 200-2000 h.[30,34–36] However, the rate of strand cleavage at AP sites in DNA increases substantially with heating, under alkaline

conditions, or in the presence of amines.[30,37–43] Amine-catalyzed DNA strand cleavage may be biologically important because the cell nucleus is rich in low molecular weight polyamines such as spermine that efficiently catalyze β-elimination at AP sites.[30,31,41,44–48] In addition, amine residues in peptides, histones, and various DNA repair proteins also can catalyze strand cleavage at AP sites (in enzymology, this is classified as a β-lyase reaction[49]).



Scheme 2.1.1 The canonical products arising from strand cleavage at an AP site in DNA are the 5'-phosphoryl terminus (5'P) and the *trans*-α,β-unsaturated aldehyde (3'PUA) sugar remnant generated by an initial β-elimination reaction, and the 3'phosphoryl terminus (3'P) generated by subsequent γ,δ-elimination.[a]

[a] The wavy lines annotated with 5' and 3' labels represent DNA strands. The P in this Scheme represents either a DNA phosphodiester linkage or a terminal phosphoryl group.

A series of seminal studies established that β-elimination of phosphate from an AP site in DNA induced by heat, NaOH, or amine catalysts initially generates a strand break with a phosphoryl group on the 5'-terminus (5'P) and a *trans*-α,β-unsaturated aldehyde residue on the 3'-terminus (Scheme 2.1.1).[31,32,37,40,41,50,51] This 3'-sugar remnant has been referred to by a variety of names including *trans*-4-hydroxy-2-pentenal 5-phosphate,[52,53] *trans*-2-hydroxy-5-oxopent-3-enyl,[54] βE (β-elimination product),[55] 3'dRP (3'deoxyribose phosphate),[56] 3'ddR5P (2,3-didehydro-2,3-dideoxy-ribose-5phosphate),[48,57] and 3'PUA (phospho-α,β-unsaturated aldehyde).[58,59] Here we will use the 3'PUA nomenclature. Under more vigorous conditions, the 3'*trans*-PUA sugar remnant is removed from DNA altogether by a γ,δ-elimination reaction that generates a single nucleotide gap flanked by 5'P and 3'-phosphoryl (3'P) groups (Scheme 2.1.1).[8,28–30,41,50] The 3'*trans*-PUA sugar remnant has been detected in the DNA of cultured human cells[55] and there is evidence from studies involving dysregulated base excision repair that AP-derived strand breaks are more toxic to cells than the parent abasic site.[60–63]

A small number of studies have described product mixtures derived from the cleavage of AP sites in DNA that are different, and in some cases more complex, than the canonical 3'*trans*-PUA, 3'P, and 5'P cleavage products. Noncanonical 3'-sugar remnants reported to arise from β-elimination at an AP site include: 3'*cis*PUA,[37,64,65] 3'deoxyribose (3'dR),[37,64,66–69] 3',4'-cyclized deoxyribose,[28,30,41] and adducts arising from conjugate addition of nitrogen nucleophiles[70–72] or thiols[39,51,73] to the 3'*trans*-PUA group (Figure 2.1.1).

It is important to determine the secondary products arising from abundant lesions such as the AP site in synthetic and cellular DNA. With this in mind, we investigated the products arising from strand cleavage induced by treatment of an AP-containing DNA oligonucleotide with heat, NaOH, piperidine, spermine, and the base excision repair glycosylases Fpg and Endo III. Under multiple conditions, we found that noncanonical sugar remnants were generated on the 3'-terminus of AP-derived strand breaks in a DNA oligomer. Our results suggest that the products of strand cleavage at AP sites in DNA often may be more complex than commonly expected.

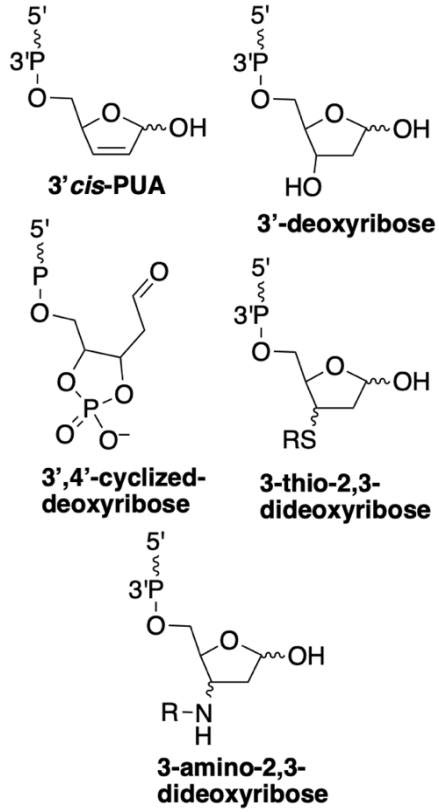
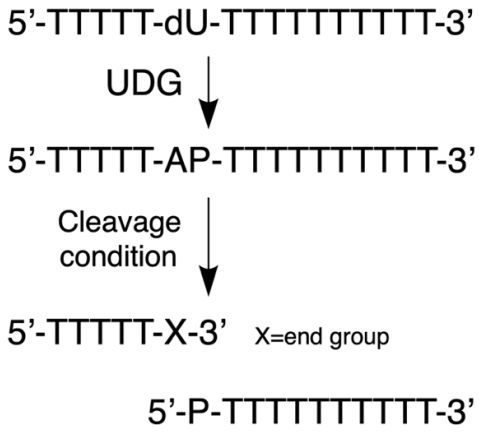


Figure 2.1.1 Possible noncanonical 3′end groups generated by cleavage at an AP site in DNA. The wavy lines annotated with 5′ and 3′ labels represent DNA strands. The P represents a DNA phosphodiester.

2.2 Generation of the AP-containing 2'-oligodeoxynucleotide.

We examined the products arising from strand cleavage at an AP site embedded in a polythymidine oligodeoxynucleotide (Scheme 2.2.1). The AP site was installed 5 nucleotides from the 5'end and 10 nucleotides from the 3'-end by treatment of a 2'-deoxyuridine-containing precursor oligodeoxynucleotide with the enzyme uracil DNA glycosylase (UDG, Scheme 2.2.1).[19,74–76] The cleavage products generated under various conditions were characterized by reverse-phase HPLC, nanospray ESI-QTOF mass spectrometry, comparison to authentic synthetic standards, and by diagnostic chemical reactions (e.g. conjugate addition of 2mercaptoethanol). The exact retention times varied slightly from run-to-run, but the products consistently eluted in the relative order: 3'P, 3'dR, 3'*trans*-PUA, 3'*cis*-PUA, 3'PUA-thiol adducts, 5'P, AP-oligo, and dU-oligo (early to late, Figure 2.2.1).



Scheme 2.2.1 The AP-containing oligodeoxynucleotide was generated by treatment of a 2'deoxyuridine (dU) containing precursor oligodeoxynucleotide with the enzyme uracil DNA glycosylase (UDG).[a]

[a] In the strand cleavage product shown here, X corresponds to the 3'-end groups generated under various cleavage conditions and P corresponds to a terminal phosphoryl group.

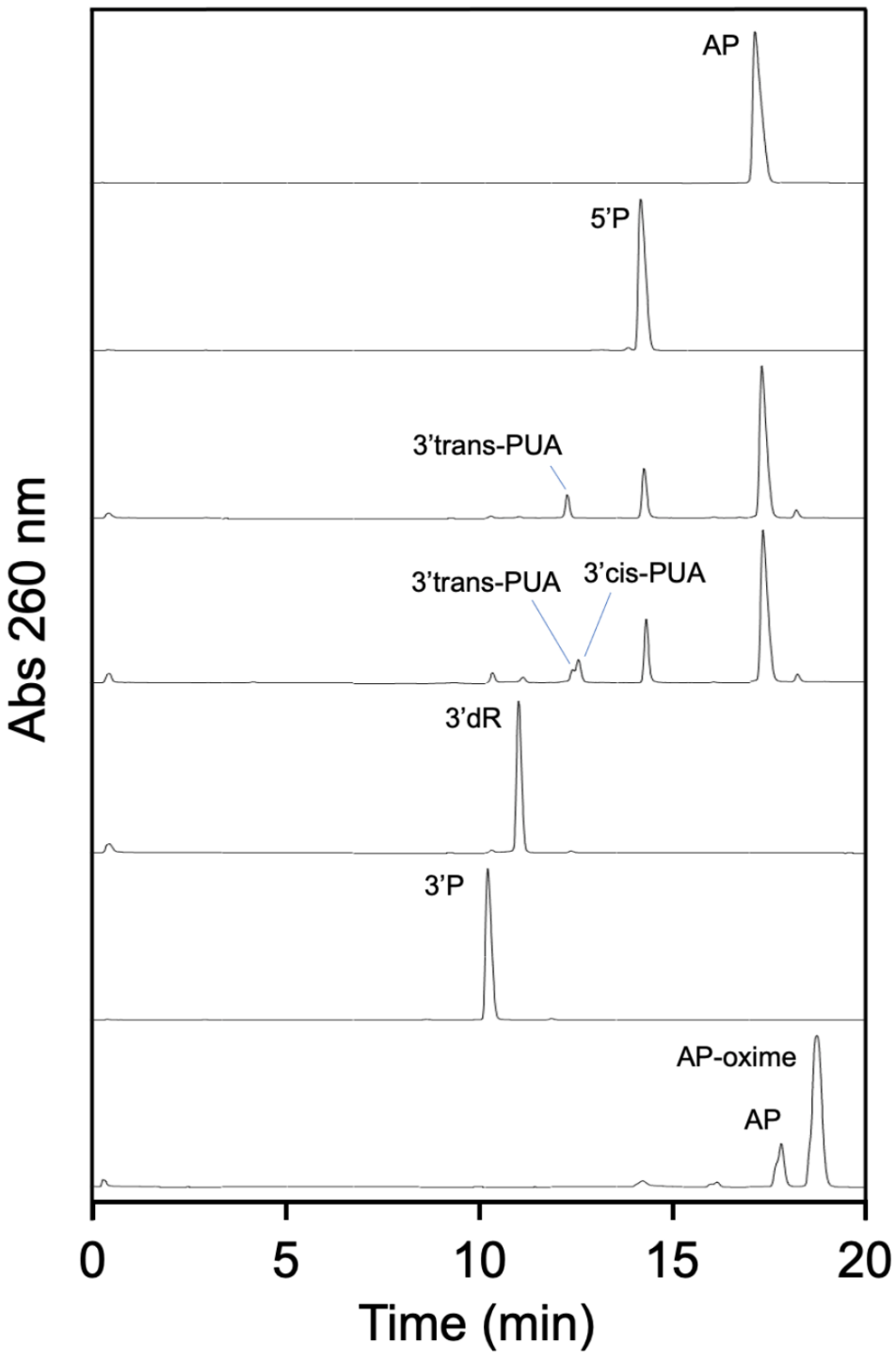


Figure 2.2.1. HPLC retention times of authentic standards for AP-oligonucleotide, 5'P, 3'*cis*- and *trans*-PUA, 3'dR, 3'P and AP-oxime. From top: the AP-containing oligonucleotide (5'- TTTTTXTTTTTTTTT, where X=AP) was generated by treatment

of the corresponding dU containing oligomer with UDG. The 5'-P product (5'-PTTTTTTTTTT, where P = a phosphoryl group) and 3'P (5'-TTTTTP-3') were purchased from IDT. The 3'*trans*-PUA product was generated by heating the AP-oligo at 85℃ in HEPES buffer (50 mM, pH 7.4) containing NaCl (100 mM) for 15 min.[37] The mixture of 3'*cis*- PUA and 3'*trans*-PUA was generated by incubation of the AP-containing oligo in Tris-borate buffer according to the method of Kushida et al.[64] The 3'dR product (5'-TTTTT-dR-3') was generated by acid-catalyzed depurination of the adenine residue in the precursor 5'-TTTTTA-3' according to the conditions of Bailly and Verly (10 mM HCl, 65℃, 1 h).[39] For comparison, the AP-oxime was generated by treatment of the AP-oligo with $CH_3ONH_2 \cdot HCl$ (2 mM) at 37 °C in HEPES buffer (50 mM, pH 7) containing NaCl (100 mM) for 2 h.

2.3 Thermal cleavage of the AP-oligonucleotide generates 3'PUA and 3'P.

Sugiyama showed that thermal treatment of an AP-containing trinucleotide at 90 ˚C in pH 7 sodium cacodylate buffer generated 3'*trans*-PUA as the major early product (20 min).[37] At longer reaction times (1 h), 10-20% yields of the 3'*cis*-PUA and 3'dR products were observed alongside the 3'*trans*-PUA product, while after extended heating (>1.5 h) the 3'P product dominated (see Scheme 2.1.1 and Figure 2.1.1 for structures of these products).

We found that heating the AP-containing oligonucleotide at 85 ˚C in HEPES buffer (50 mM, pH 7.4) containing NaCl (100 mM) for 15 min generated two major cleavage products eluting at 12.4 and 14.4 min in reverse-phase HPLC analysis (Figure 2.3.1, HPLC trace c). The 14.4 min peak corresponded to the 5'$P(T)_{10}$ product derived from β-

elimination at the AP site. Based on Sugiyama's work, we suspected that the 12.4 min peak corresponded to the 3'*trans*-PUA cleavage product.[37] Evidence for the nature of the 12.4 min peak was provided by investigating its reaction with 2-mercaptoethanol. Thiols readily undergo conjugate addition to α,β-unsaturated aldehydes in neutral aqueous solution.[77–80] More specifically, our recent work along with earlier precedents showed that thiols readily add to the 3'*trans*-PUA group to generate diastereomeric 3-thio-2,3-dideoxyribose (3-thio-ddR) adducts.[39,51,73,81] Indeed, we found that addition of 2-mercaptoethanol (5 mM) to the thermolysis reaction mixture, followed by incubation for 15 min at 37 ˚C, led to disappearance of the putative 3'*trans*-PUA group at 12.4 min, with concomitant appearance of two new peaks eluting at approximately 13.0 and 13.5 min (Figure 2.3.2). We ascribed these new peaks to the expected diastereomeric 3-thio-ddR products on the 3'-terminus of the strand break, arising from conjugate addition of the thiol to the 3'*trans*-PUA group (Figure 2.3.2). Similar results were observed using other thiols (Figure 2.3.3).

The 3'*trans*-PUA and 3-thio-ddR end products were stable in pH 7.4 buffer at 37 ˚C. Specifically, incubation of the 3'*trans*-PUA product for 6-12 h led to production of small amounts of the 3'P product (Figure 2.3.4). The 3'*cis*-PUA product was not observed (see below for further discussion of the 3'*cis*-PUA). The diastereomeric 3-thio-2,3-ddR products on the 3'-terminus showed no decomposition over the course of 24 h (Figure 2.3.5).

Extended heating for 45 min at 85 ˚C led to complete cleavage of the AP oligonucleotide, with generation of the 5'P and 3'P products, eluting at 10.4 and 14.4 min respectively (Figure 2.3.1, HPLC trace d). A very small amount of the 3'dR product may be seen eluting near 11 min (marked by an asterisk in Figure 2.3.1, HPLC trace d).

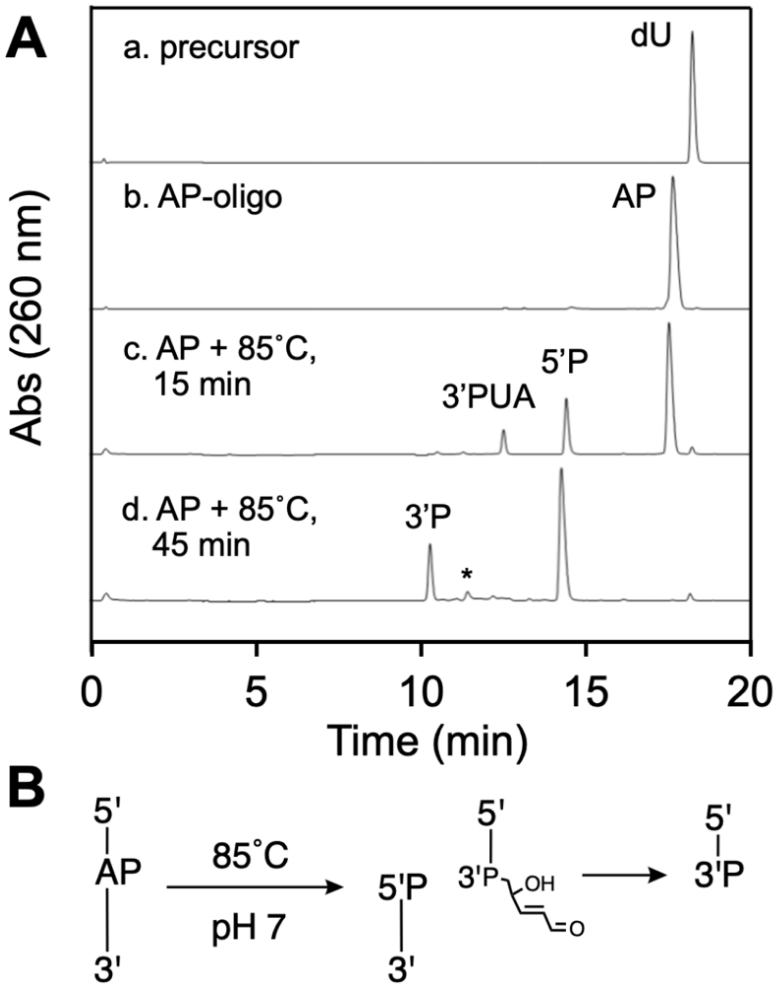


Figure 2.3.1. HPLC analysis of thermal cleavage of an AP site in DNA (panel A) and a schematic depiction of the product structures, where P represents a terminal phosphoryl group or a phosphodiester linkage (panel B).

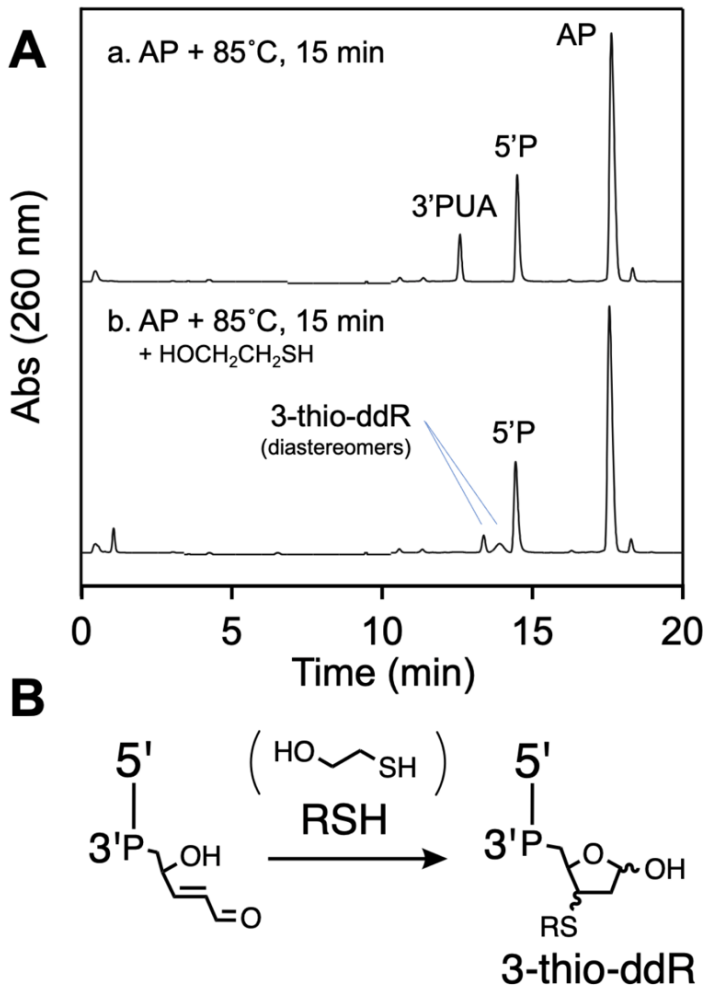


Figure 2.3.2 HPLC analysis of the products arising from reaction of 2mercaptoethanol with the 3'PUA thermal cleavage product (panel A) to generate the diastereomeric mixture of 3-alkylthio-2,3-dideoxyribose (3-thio-ddR) products on the 3'terminus of the strand break with structures depicted in panel B, where P represents a phosphodiester linkage.

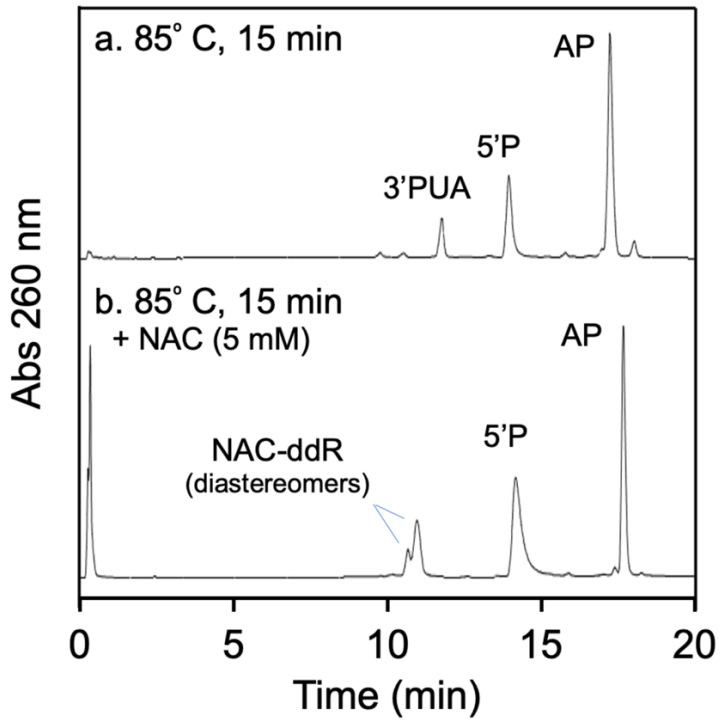


Figure 2.3.3. HPLC analysis of the reaction of *N*-acetylcysteine (NAC) with 3'*trans*-PUA generated by thermal treatment of the AP-containing oligodeoxynucleotide. Panel a. Heating the AP-containing oligonucleotide in HEPES buffer (50 mM, pH 7.4) containing NaCl (100 mM) at 85℃ for 15 min generated two major cleavage products, 3'*trans*-PUA and 5'P. Panel b. Heating the AP-containing oligonucleotide in HEPES buffer (50 mM, pH 7.4) containing NaCl (100 mM) at 85℃ for 15 min, followed by addition of NAC (5 mM) and incubation for 15 min at 37℃, caused disappearance of the 3'*trans*-PUA peak and appearance of two new peaks, assigned as a diastereomeric mixture of 3-alkylthio-2,3-dideoxyribose (NAC-ddR) products on the 3'- terminus of the strand break. Note that the NAC-ddR products elutes at a different time (retention time near 11 min) than the diastereomeric mixture of 3-alkylthio-2,3-dideoxyribose products generated by 2-mercaptoethanol (with retention times near 13 min under these chromatographic conditions)

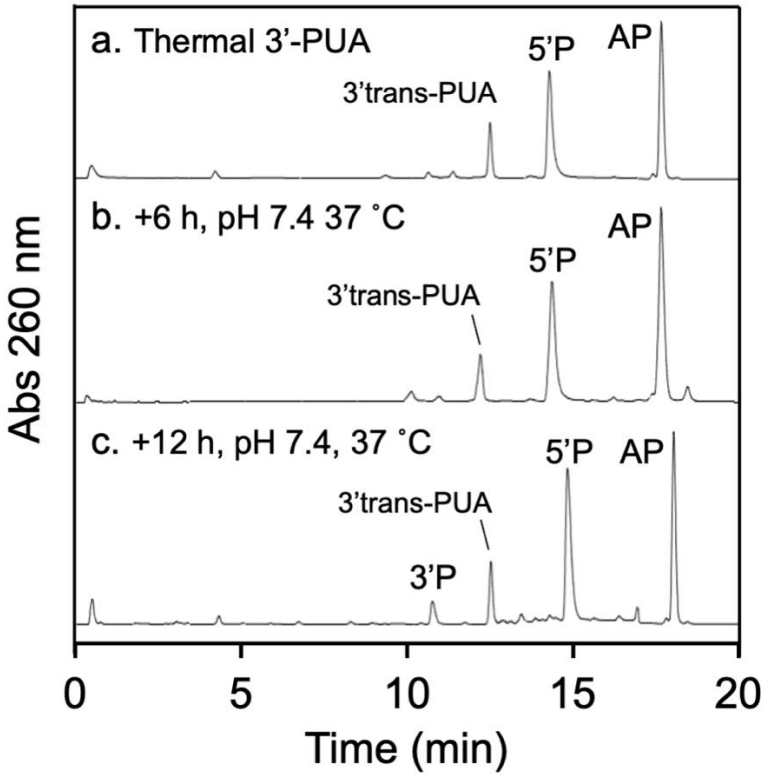


Figure 2.3.4. 3'*trans*-PUA is stable in pH 7.4 buffer (in the absence of amines and thiol). Panel a. 3'trans-PUA was generated by incubation of the AP-containing oligonucleotide at 85℃ in HEPES buffer (50 mM, pH 7.4) containing NaCl (100 mM) for 15 min. Panel b. The product was then incubated at 37℃ for 6 h prior to HPLC analysis. Panel c. Incubation of 3'*trans*-PUA for 12 h. The 3'trans-PUA end group is relatively stable, with only small amounts of the 3'P product produced by g-elimination over the course of 12 h.

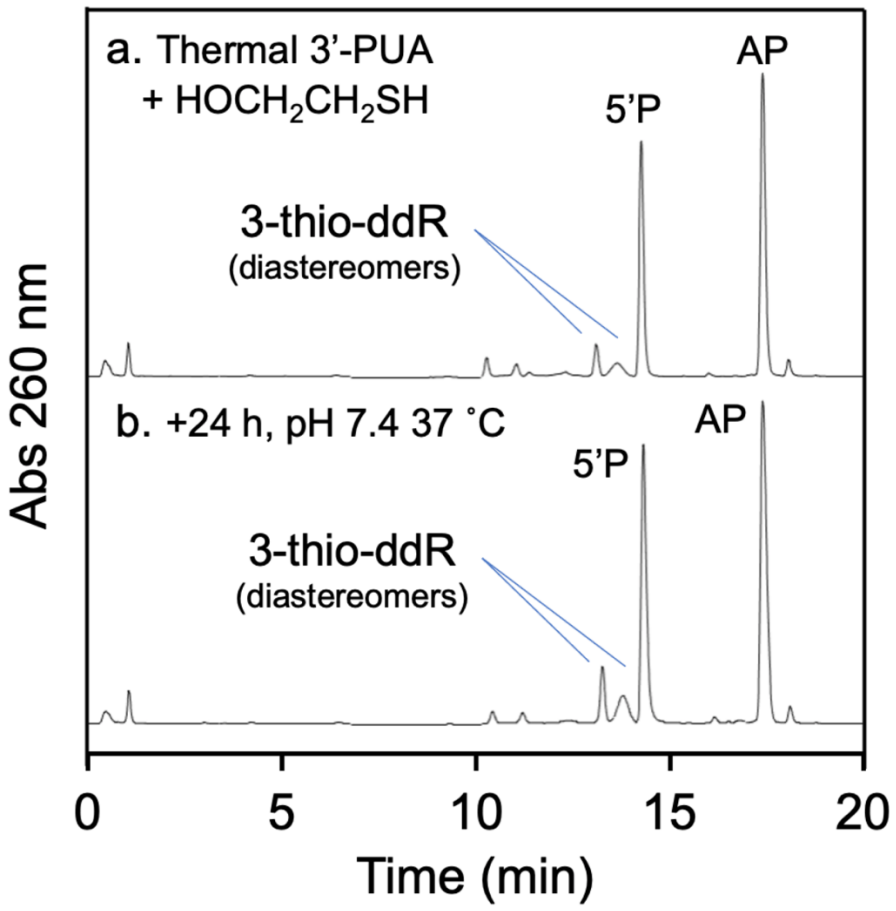


Figure 2.3.5. The 3-thio-2,3-dideoxyribose thiol adducts on the 3'-terminus in pH 7.4 buffer are stable (in the absence of amines and thiol). Panel a. Thermal generation of the 3'trans-PUA cleavage product, followed by addition of 2-mercaptoethanol (5 mM) and incubation at 37 ℃ for 15 min generated a diastereomeric mixture of 3-alkylthio-2,3-dideoxyribose (3-thio-ddR) products on the 3'-terminus of the strand break. Panel b. Incubation at 37 ℃ for 24 h did not cause significant degradation of the of the 3-alkylthio-2,3-dideoxyribose (3-thio-ddR) products.

Nanospray ESI-TOF-MS analysis provided support for the product assignments described above (Figures 2.3.1 and 2.3.2). The reaction mixture at 15 min revealed strong signals consistent with the starting AP oligodeoxynucleotide, the 5'P product, the 3'*trans*-PUA product, the 3'P product, and a weak, but distinct, signal corresponding to the 3'dR product (Figure 2.3.6). The observed *m*/*z* values and relative signal intensities in the isotope clusters closely matched those calculated for the proposed product structures (Figure 2.3.6). The reaction mixture generated by heating the AP-oligodeoxynucleotide at 85 ˚C for 45 min showed signals for the 5'P product and the 3'P product consistent with the HPLC data (Figure 2.3.7). Mass spectrometric analysis of the mixture generated by thermolysis of the AP-oligonucleotide followed by reaction with 2-mercaptoethanol showed a strong signal consistent with the 3-thio-ddR products on the 3'-terminus of the strand break (Figure 2.3.8).

Overall, the data indicate that heating the AP-containing oligonucleotide at 85 ˚C in pH 7.4 buffer induces β-elimination that generates the 3'*trans*-PUA cleavage product, as expected.[37] Extended heating generated the 3'P product via δ-elimination of the sugar remnant. Under our reaction conditions, there was no evidence that the 3'*cis*-PUA product was formed, and we observed only a trace of the 3'dR product.

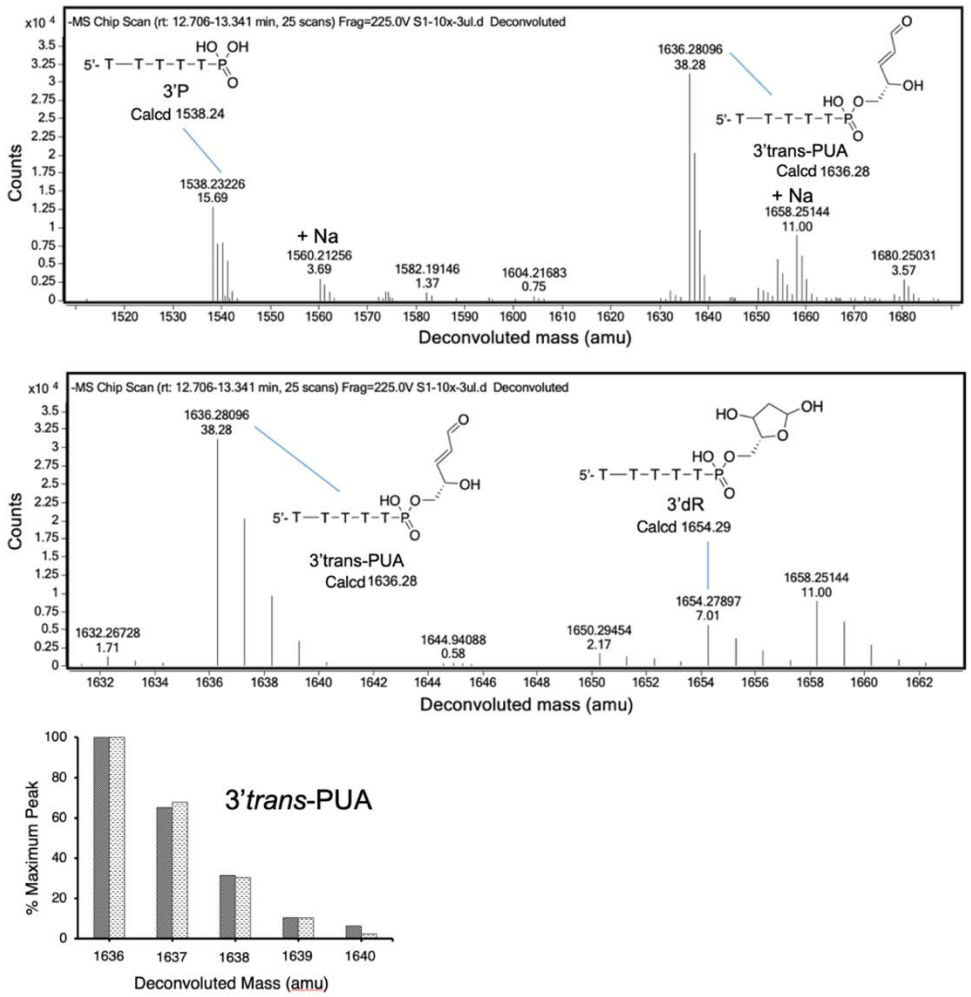


Figure 2.3.6. ESI(-)-QTOF-LC-MS analysis of the products generated by thermolysis of the AP-containing oligonucleotide after 15 min. Upper panel. Mass spectrometric analysis revealed deconvoluted neutral masses for the cleavage products 3'trans-PUA, 3'dR (minor) and 3'P generated by thermal cleavage of the AP-containing oligo in HEPES buffer (50 mM, pH 7.4) containing NaCl (100 mM) at 85℃ for 15 min. Lower panel. Comparison of experimentally measured intensities for each peak in the isotope cluster (bars on the left side of each pair) and expected intensity (bars on the right side of each pair) calculated from the molecular formula of the 3'trans-PUA cleavage product.

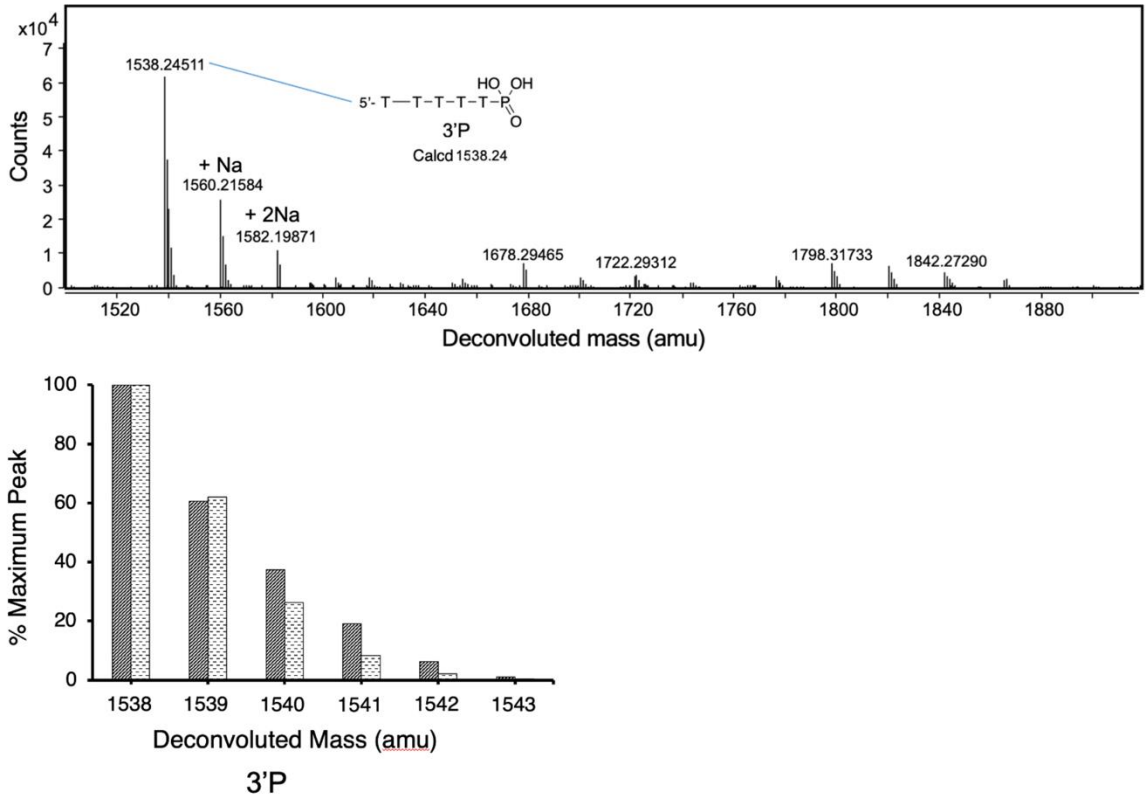


Figure 2.3.7. ESI(-)-QTOF-LC-MS analysis of the products generated by thermolysis of the AP-containing oligonucleotide after 45 min. Upper panel. Mass spectrometric analysis revealed deconvoluted neutral masses for the cleavage product 3'P generated by thermal cleavage of the AP-containing oligo in HEPES buffer (50 mM, pH 7.4) containing NaCl (100 mM) at 85 ℃ for 45 min. Lower panel. Comparison of experimentally measured intensities for each peak in the isotope cluster (bars on the left side of each pair) and expected intensity (bars on the right side of each pair) calculated from the molecular formula of the 3'P cleavage product.

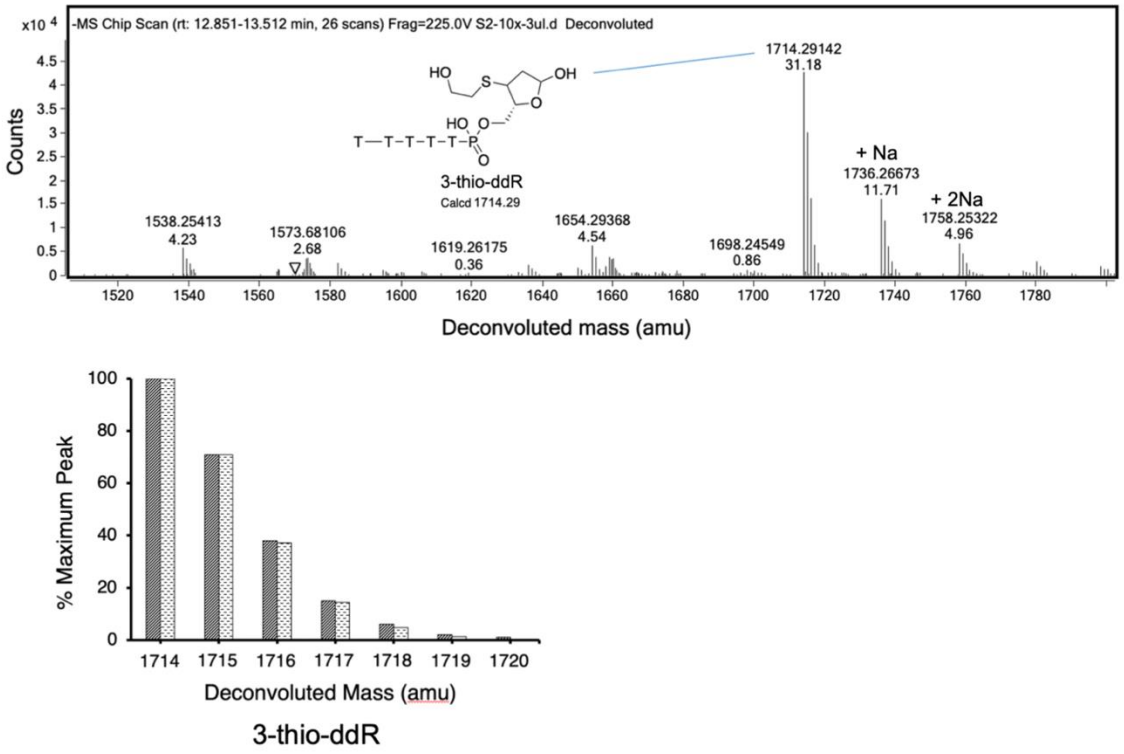


Figure 2.3.8. ESI(-)-QTOF-LC-MS analysis of products generated by thermolysis of the AP- containing oligonucleotide in the presence of 2-mercaptoethanol. Upper panel. Mass spectrometric analysis revealed deconvoluted neutral masses for the cleavage products 3-alkylthio- 2,3-dideoxyribose (3-thio-ddR) products on the 3'-terminus of the strand break generated by thermal cleavage of the AP-containing oligo in HEPES buffer (50 mM, pH 7.4) containing NaCl (100 mM) at 85 ℃ for 15 min, followed by incubation with 2-mercaptoethanol (5 mM) for 15 min. Lower panel. Comparison of experimentally measured intensities for each peak in the isotope cluster (bars on the left side of each pair) and expected intensity (bars on the right side of each pair) calculated from the molecular formula of the cleavage product bearing the 3-thio-ddR end group on the 3'-terminus of the strand break.

2.4 Cleavage of the AP Oligonucleotide with NaOH Generates 3′trans-PUA, 3′P, and the Noncanonical 3′dR Product.

Sodium hydroxide is often used to cleave DNA AP sites in biochemical assays. For example, assays designed to measure the activity of monofunctional DNA glycosylases often feature a NaOH workup.[42,43] The general expectation is that mild NaOH treatment generates the 3'trans-PUA group by b-elimination at the AP sites.[8] For example, Mazumdar et al. reported (based on HPLC retention time) that the 3'transPUA group was generated by mild NaOH cleavage of a single-stranded, AP-containing, undecameric oligodeoxynucleotide (100 mM NaOH, pH 13, 4 ˚C).[40] More vigorous NaOH treatments reportedly generated the 5'P and 3'P products via sequential α,β- and γ,δ-elimination reactions (Scheme 2.1.1).[38–40]

We characterized the products generated by NaOH-mediated cleavage of the AP-containing oligodeoxynucleotide under several different conditions. We found that a very mild treatment of the AP-containing oligonucleotide with NaOH (5 mM, 37 ˚C, 1 h) generated low yields of strand cleavage with the 3'*trans*-PUA (12.4 min) and 5'P (14.4 min) termini at the strand break (Figure 2.4.1, HPLC trace d). Interestingly, a somewhat more vigorous NaOH treatment (200 mM, 37 ˚C, 20 min) generated a product eluting at 11.2 min, clearly distinct from the 3'*trans*-PUA product (Figure 2.4.1, HPLC trace c). We assigned the material eluting at 11.2 min as the 3'dR product, based on its co-elution with an authentic standard of 5'-TTTTT-dR-3' prepared by acid depurination of a 5'TTTTTA-3' precursor according to the method of Bailly and Verly (10 mM HCl, 1 h, 65 ˚C).[39]

To the best of our knowledge, generation of the 3'dR terminus via NaOH mediated cleavage of an AP site in DNA has not been reported previously. However, a chemical precedent for the formation of this product can be found in the conjugate addition of water

to the low molecular weight α,β-unsaturated aldehyde, acrolein, in neutral aqueous solution.[82] Accordingly, we suspected that the 3'dR product generated by treatment of the AP oligonucleotide with 5 mM NaOH arose via addition of water to the 3'*trans*-PUA cleavage product. Consistent with this idea, a control experiment showed that an authentic sample of the 3'*trans*-PUA product generated by thermolysis of the AP-oligonucleotide was, indeed, converted to a mixture of 3'P and 3'dR upon treatment with 5 mM NaOH, 37 ˚C for 6 h (Figure 2.4.2)

Treatment of the AP-containing DNA with 200 mM NaOH at 37 ˚C for 5 h cleanly generated the 5'P and 3'P products resulting from sequential α,β- and γ,δ-elimination reactions (Figure 2.4.1, HPLC trace b). We also examined an NaOH workup commonly used in the study of monofunctional base excision repair DNA glycosylases,[42] involving addition of an equal volume of 500 mM NaOH to the AP-oligonucleotide in 20 mM HEPES pH 7.4 containing 100 mM NaCl, followed by heating at 95 ˚C for 2 min. As expected, this procedure gave the expected 5'P and 3'P cleavage products (Figure 2.4.1, HPLC trace a).

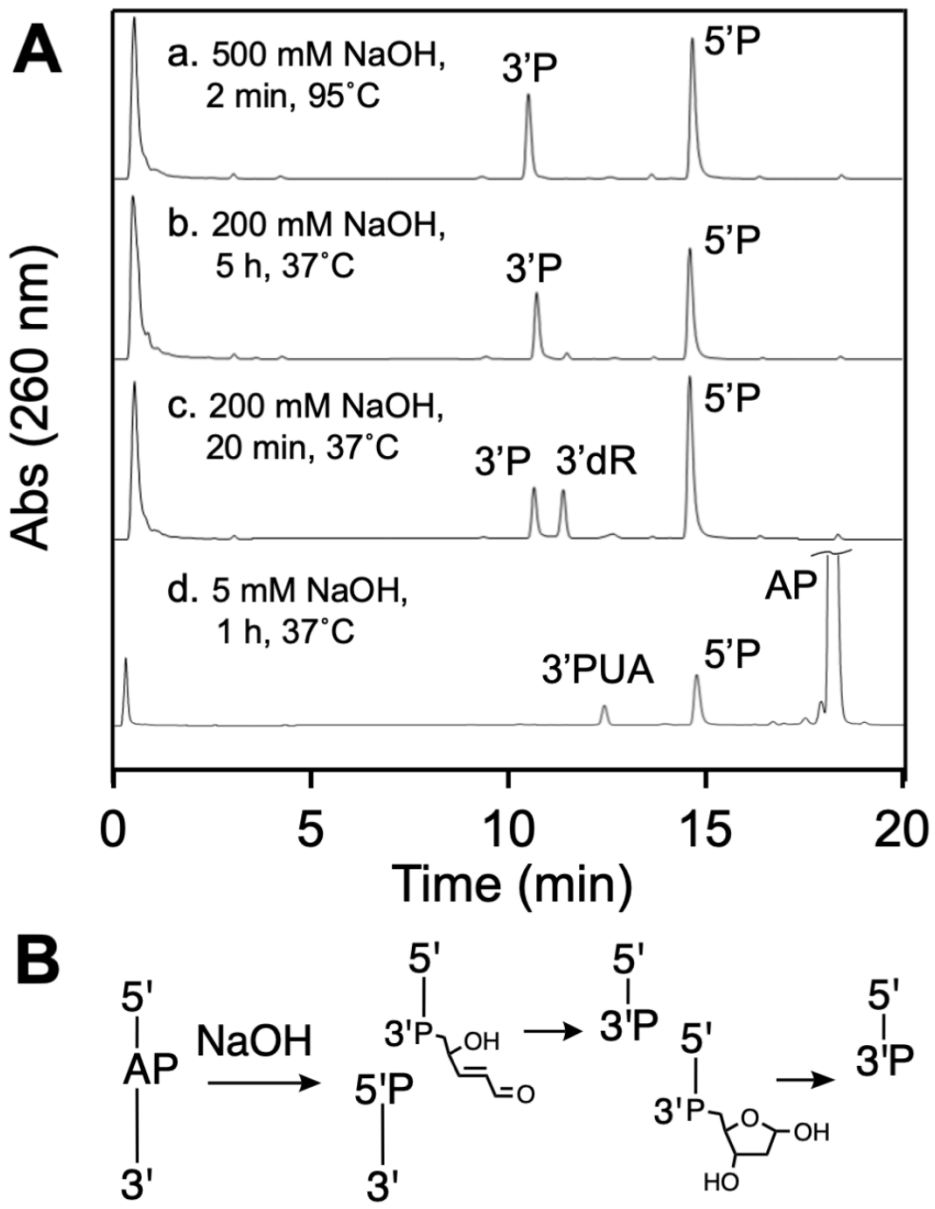


Figure 2.4.1. HPLC analysis of the products generated by the NaOH- mediated strand cleavage of an AP site in DNA (A) and a schematic depiction of the product structures, where P represents a terminal phosphoryl group or a phosphodiester linkage (B).

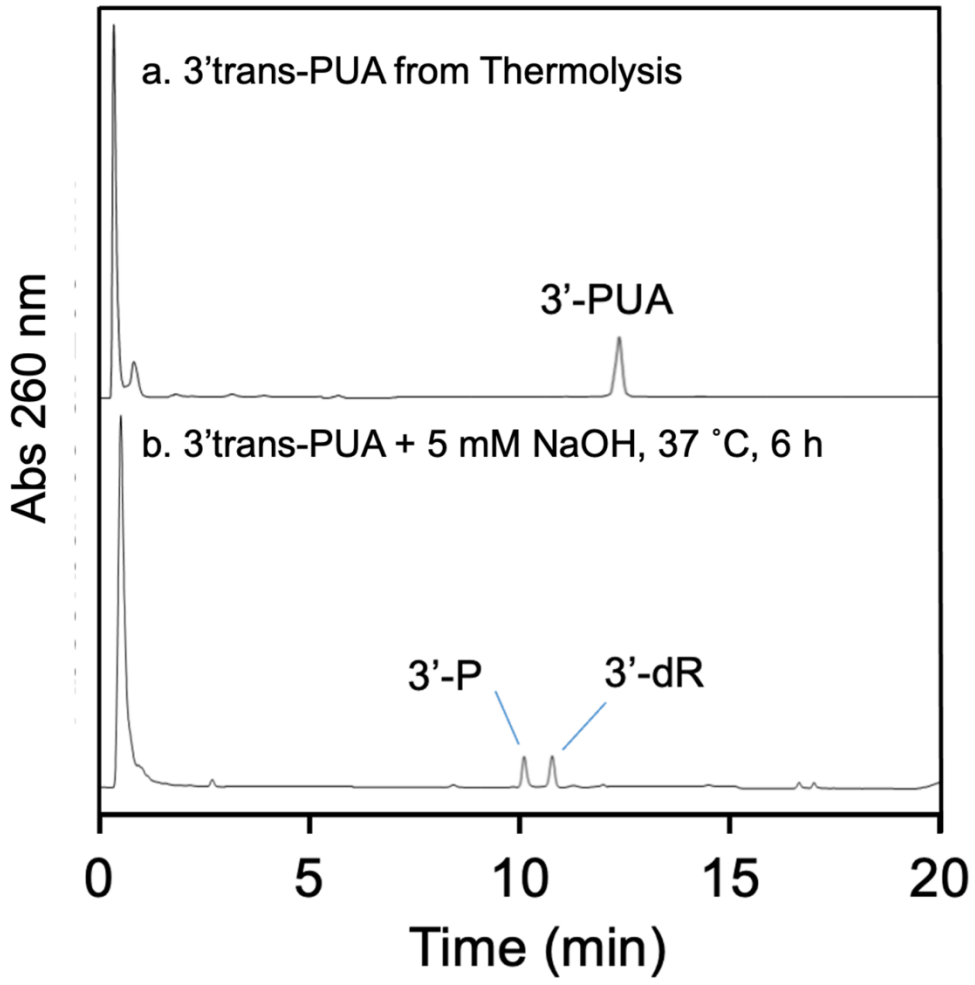


Figure 2.4.2. 3'trans-PUA is converted to 3'dR by treatment with 5 mM NaOH. The 3'trans- PUA product isolated from the thermolysis of the AP-containing oligonucleotide (upper panel) was converted to a mixture of 3'P and 3'dR by treatment with 5 mM NaOH, 37 ℃ for 6 h (lower panel).

Mass spectrometric analyses supported the structural assignments described above. The mixture generated by mild NaOH treatment (5 mM, 1 h, 37 ˚C) showed strong signals for the starting AP-oligonucleotide, the 5'P product, and the 3'*trans*-PUA product (Figure 2.4.3). Analysis of the AP-oligodeoxynucleotide treated with 200 mM NaOH, 20 min, 37 ˚C revealed strong signals consistent with 5'P, 3'dR, and 3'P (Figure 2.4.4). The product mixture generated by addition of 500 mM NaOH to the AP oligonucleotide in 20 mM HEPES pH 7.4 containing 100 mM NaCl, followed by heating at 95 ˚C for 2 min gave strong signals for the 5'P and 3'P cleavage products (Figure 2.4.5).

Overall, the results show that treatment of an AP-containing oligodeoxynucleotide with very mild NaOH (5 mM NaOH, 1 h, 37 ˚C) generates low yields of strand cleavage with 5'P and 3'*trans*-PUA at the termini of the break. Slightly more vigorous conditions (200 mM NaOH, 20 min, 37 ˚C) gave complete cleavage of the AP site and, unexpectedly, generated a mixture of the 3'dR and 3'P products. An NaOH workup commonly used to detect AP sites in typical DNA glycosylase assays cleanly generated the 3'P cleavage product.

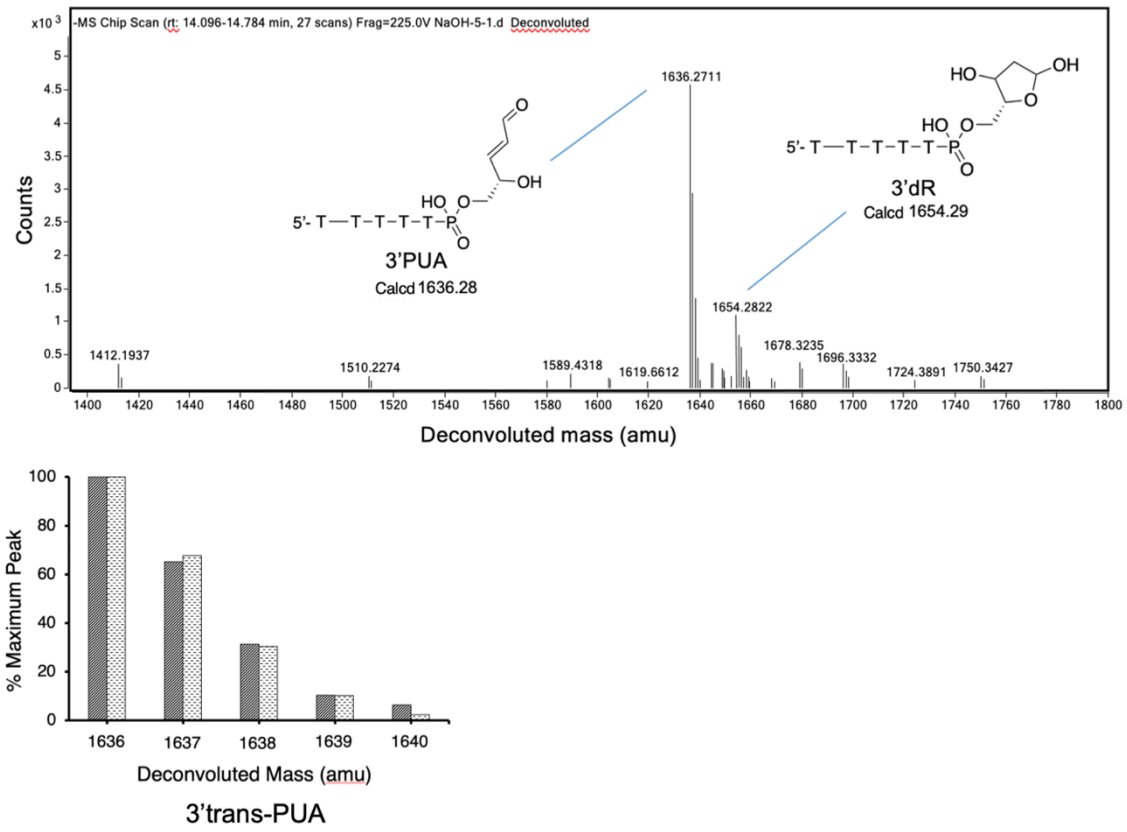


Figure 2.4.3. ESI(-)-QTOF-LC-MS analysis of products generated treatment of the AP- containing oligonucleotide with 5 mM NaOH. Upper panel. Mass spectrometric analysis revealed deconvoluted neutral masses for the cleavage products 3'trans-PUA (major) and 3'dR (minor) generated by treatment of the AP-containing oligonucleotide with mild NaOH (5 mM, 1 h, 37 ℃). Lower panel. Comparison of experimentally measured intensities for each peak in the isotope cluster (bars on the left side of each pair) and expected intensity (bars on the right side of each pair) calculated from the molecular formula of the 3'trans-PUA cleavage product.

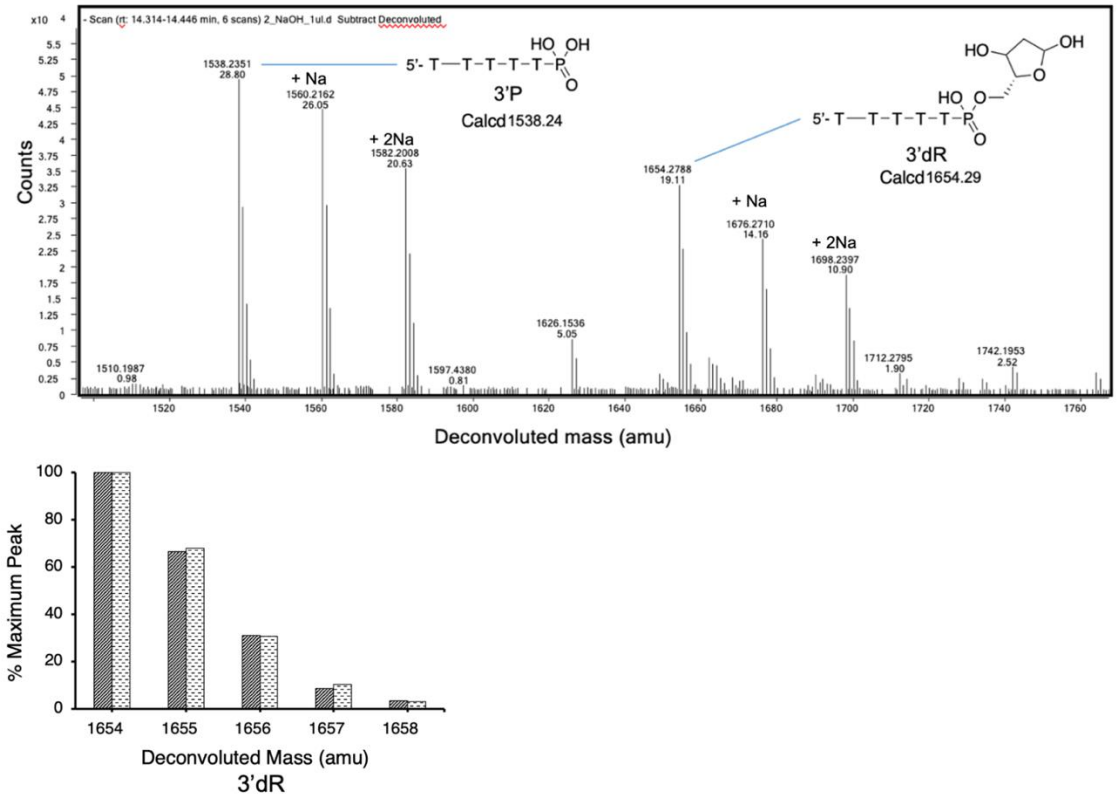


Figure 2.4.4. ESI(-)-QTOF-LC-MS analysis of products generated treatment of the AP- containing oligonucleotide with NaOH (200 mM). Upper panel. Mass spectrometric analysis revealed deconvoluted neutral masses for the cleavage products 3'dR and 3'P generated by treatment of the AP-containing oligonucleotide with NaOH (200 mM, 20 min, 37 ℃). Lower panel. Comparison of experimentally measured intensities for each peak in the isotope cluster (bars on the left side of each pair) and expected intensity (bars on the right side of each pair) calculated from the molecular formula of the 3'dR cleavage product.

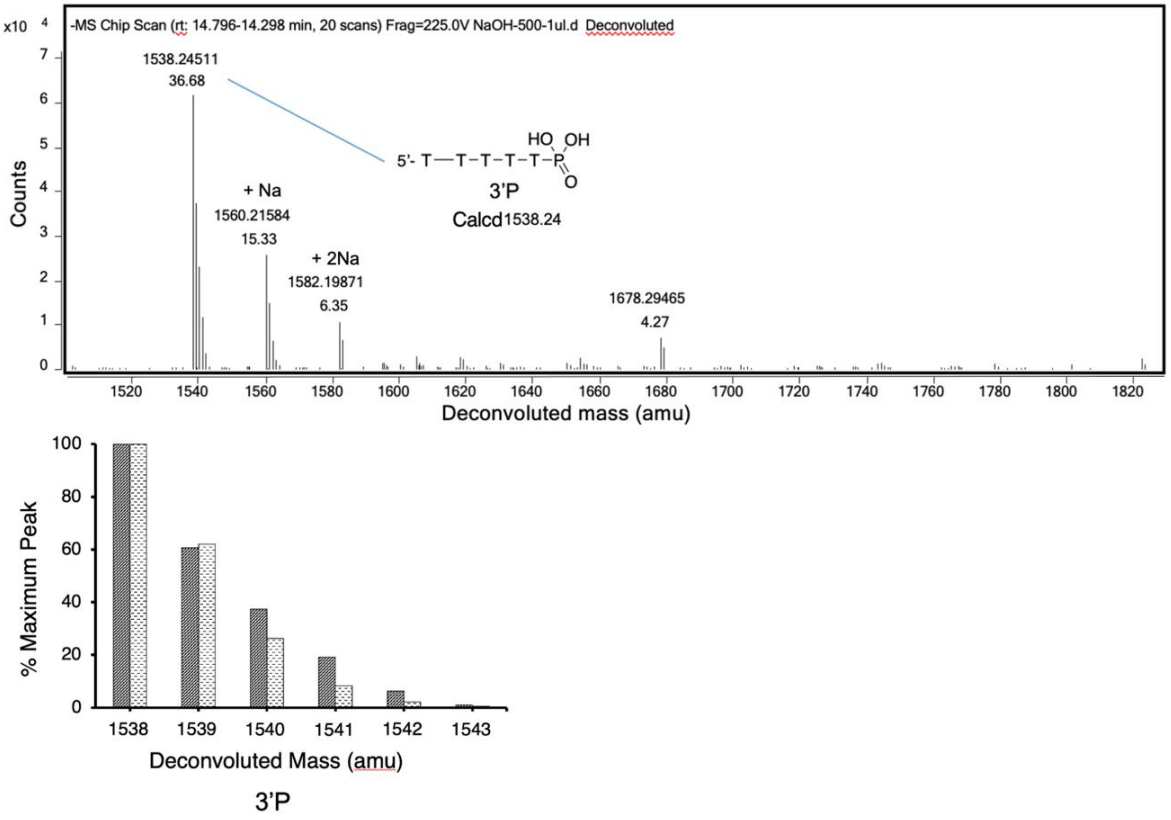


Figure 2.4.5. ESI(-)-QTOF-LC-MS analysis of products generated by addition of an equal volume of 500 mM NaOH to the AP-oligonucleotide in 20 mM HEPES pH 7.4 containing 100 mM NaCl, followed by heating at 95 ℃ for 2 min. Upper panel. Mass spectrometric analysis revealed deconvoluted neutral masses for the cleavage product 3'P generated by treatment of the AP-containing oligonucleotide with NaOH (500 mM, 2 min, 95 ℃). Lower panel. Comparison of experimentally measured intensities for each peak in the isotope cluster (bars on the left side of each pair) and expected intensity (bars on the right side of each pair) calculated from the molecular formula of the 3'P cleavage product.

2.5 Cleavage of the AP oligonucleotide with piperidine generates 3'*trans*-PUA, 3'P, and a noncanonical 3'-piperidinyl adduct.

Treatment with hot piperidine (0.1-1 M, 90-95 ˚C, 15-30 min) is a standard method to convert various DNA modifications into strand cleavages that can easily be detected using gel electrophoresis.[3,83,84] For example, piperidine workup is employed in classical Maxam-Gilbert sequencing reactions.[85] Piperidine workup of a DNA oligonucleotide containing an AP site is expected to generate a strand break with 5'P and 3'P termini via sequential α,β- and γ,δ-elimination reactions.[3,41,83–85]

We examined the products generated by treatment of the AP-containing oligodeoxynucleotide with piperidine under several different conditions. We found that heating the AP-oligonucleotide under standard Maxam-Gilbert conditions[85] involving piperidine (1 M) at 95 ˚C for 30 min in either HEPES (50 mM, pH 7.4 containing 100 mM NaCl) or water cleanly generated the expected 3'P and 5'P cleavage products eluting at 10.4 and 14.4 min, respectively (Figure 2.5.1, HPLC trace a). Electrospray mass spectrometric analysis of the mixtures generated under these conditions showed the expected signals for the 5'P and 3'P products (Figure 2.5.2). For comparison, treatment of the AP-oligonucleotide under the same conditions (95 ˚C for 30 min) without piperidine generated a cleavage mixture composed of 3'*trans*-PUA, 3'dR, and 3'P (Figure 2.5.3).

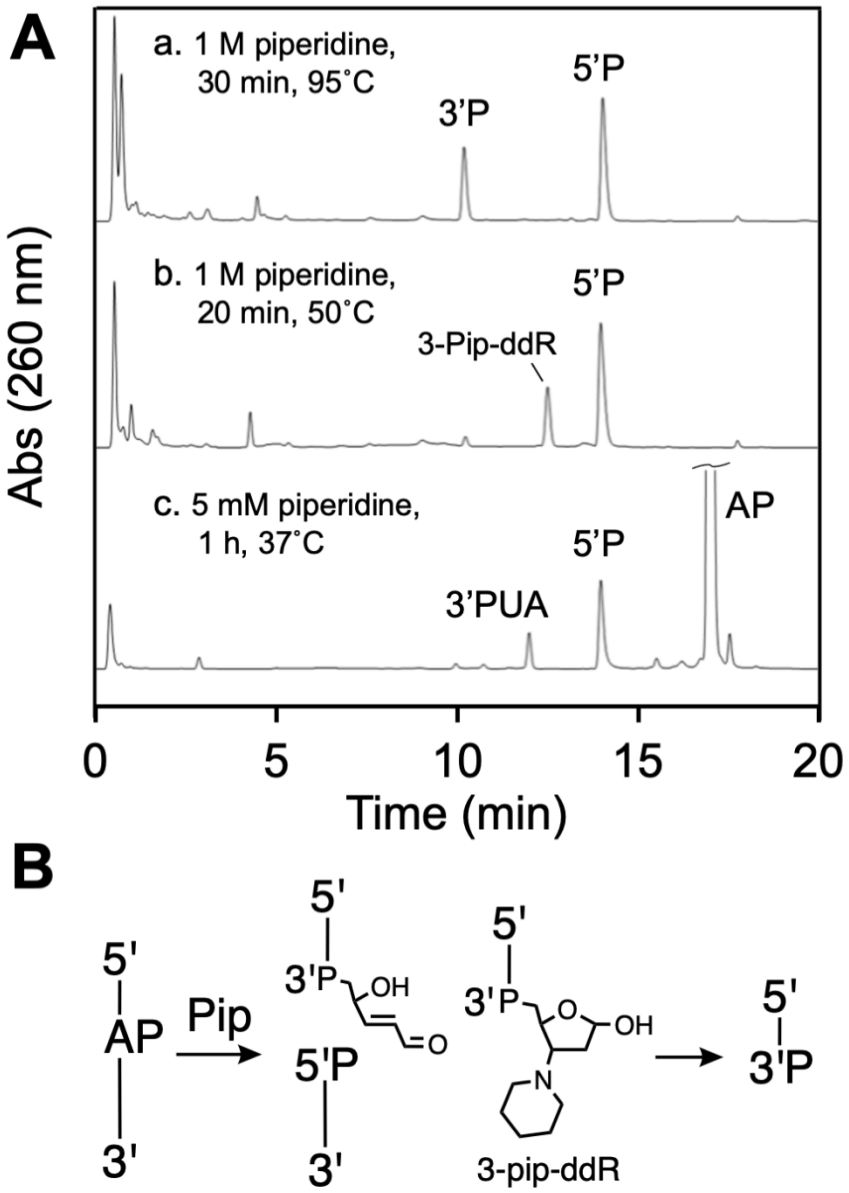


Figure 2.5.1. HPLC analysis of the products generated by piperidine-mediated strand cleavage of an AP in DNA (panel A) and schematic depiction of the product structures, where P represents a terminal phosphoryl group or a phosphodiester linkage (panel B).

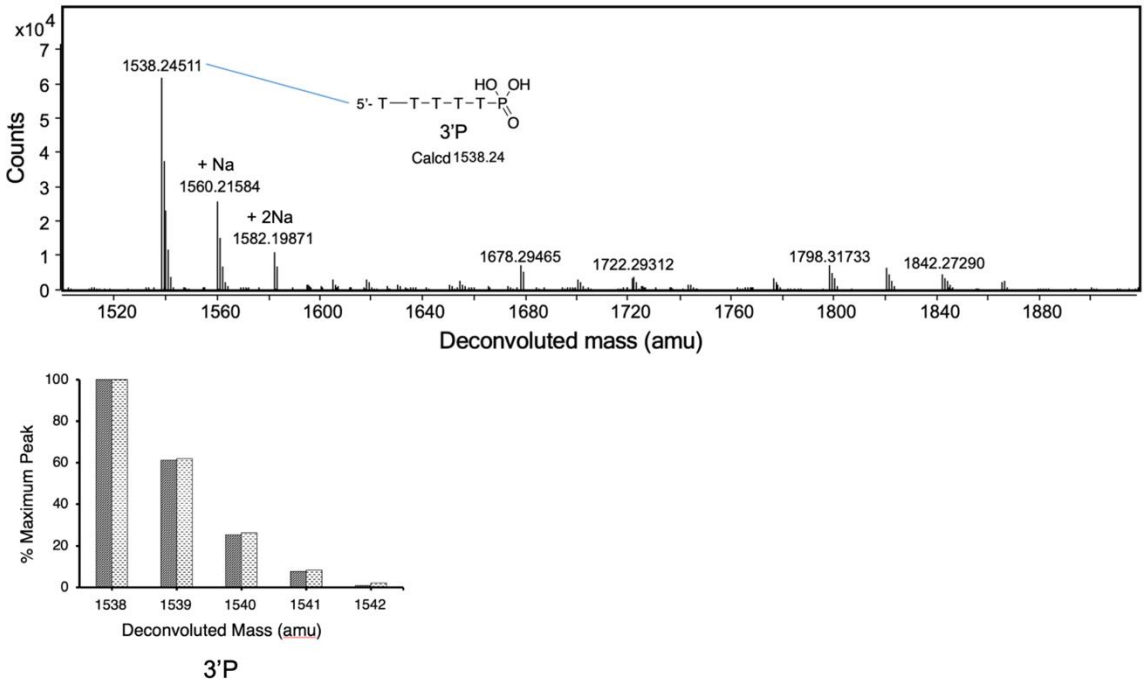


Figure 2.5.2. ESI(-)-QTOF-LC-MS analysis of products generated by treatment of the AP- oligonucleotide with piperidine (1 M) at 95 ℃ for 30 min (Maxam-Gilbert workup). Upper panel. Mass spectrometric analysis revealed deconvoluted neutral masses for the cleavage products 3'P generated by treatment of the AP-containing oligonucleotide with piperidine (1 M) at 95 ℃ for 30 min. Lower panel. Comparison of experimentally measured intensities for each peak in the isotope cluster (bars on the left side of each pair) and expected intensity (bars on the right side of each pair) calculated from the molecular formula of the 3'P cleavage product.

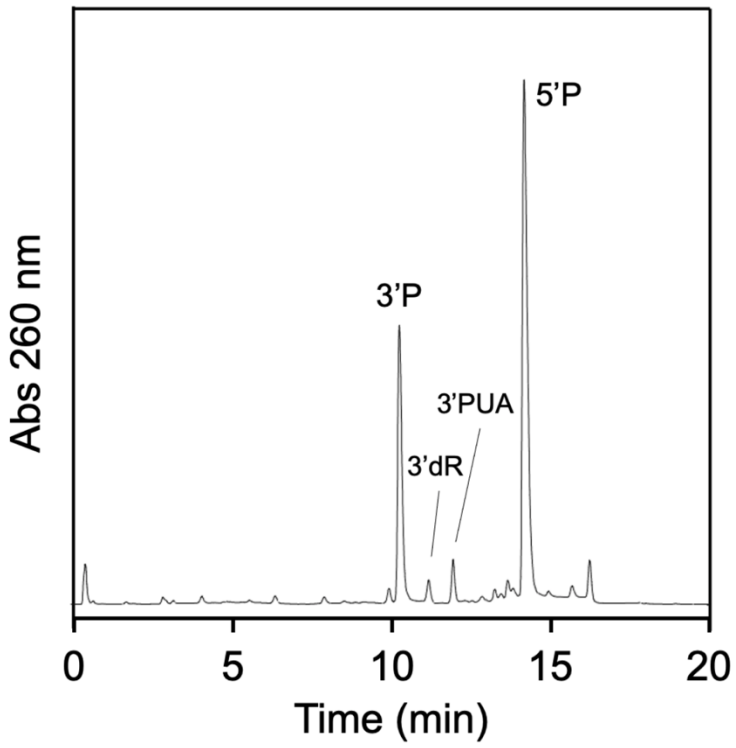


Figure 2.5.3. HPLC analysis of a control reaction involving heating the AP oligonucleotide at 95℃ for 30 min (no piperidine). Heating the AP-containing DNA oligomer at 95℃ for 30 min in water without piperidine generated a mixture of 3'PUA, 3'dR, and 3'P.

A milder condition involving treatment of the AP-containing oligonucleotide with 1 M piperidine in HEPES (50 mM) and NaCl (100 mM) at 50 ˚C for 20 min gave complete cleavage of the AP-oligonucleotide but generated an unexpected product alongside the typical 5'P elimination product (Figure 2.5.1, HPLC trace b). The retention time of the unknown product was clearly distinct from that of either the 3'P or 3'*trans*PUA products, eluting at about 12.9 min, slightly, but consistently, later than 3'*trans*PUA. The retention time of this material was not altered by addition of 2mercaptoethanol (5 mM, 15 min),

indicating that the product was not an α,β-unsaturated aldehyde or iminium ion. Nanospray ESI-TOF mass spectrometric analysis of the reaction products showed a strong signal consistent with a cleavage product bearing a 3-piperidinyl-2,3-dideoxyribose adduct on the 3'-terminus (3-pip-ddR, Figure 2.5.1, Figure 2.5.4). We recently characterized the analogous product generated by a nucleoside model system that mimics amine-catalyzed strand cleavage.[81] In addition, similar 1,4-addition products previously have been proposed to arise from the putrescine- and 9-aminoellipticine-mediated cleavage of AP-containing oligonucleotides.[70,72]

A very mild piperidine workup (5 mM piperidine, 1 h, 37 ˚C) produced low yields of cleavage at the AP site, with 5'P and 3'*trans*-PUA groups at the termini of the strand break (Figure 2.5.1, HPLC trace c).

Overall, the results show that typical Maxam-Gilbert workup of the AP-containing oligodeoxynucleotide (1 M, 95 ˚C, 30 min) generates a strand break with the expected 3'P and 5'P termini at the gap. Less vigorous conditions (1 M, 50 ˚C, 20 min), induced cleavage at the AP site, with generation of an unexpected 3-piperidino-2,3dideoxyribose adduct (3-pip-ddR) on the 3'-terminus of the strand break.

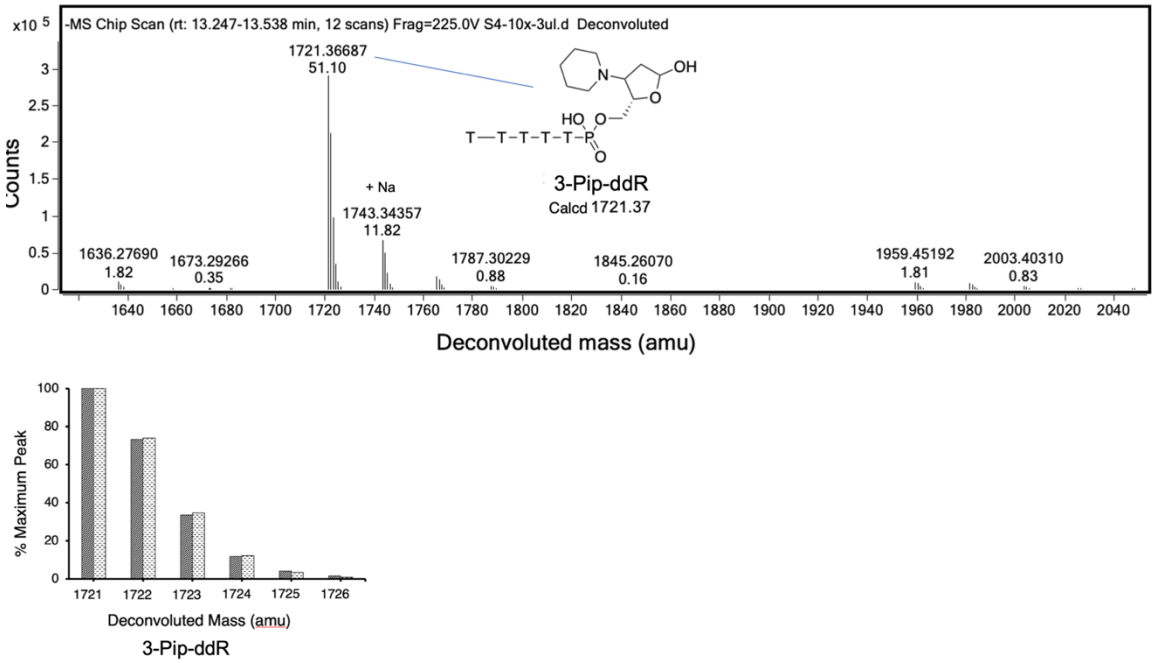
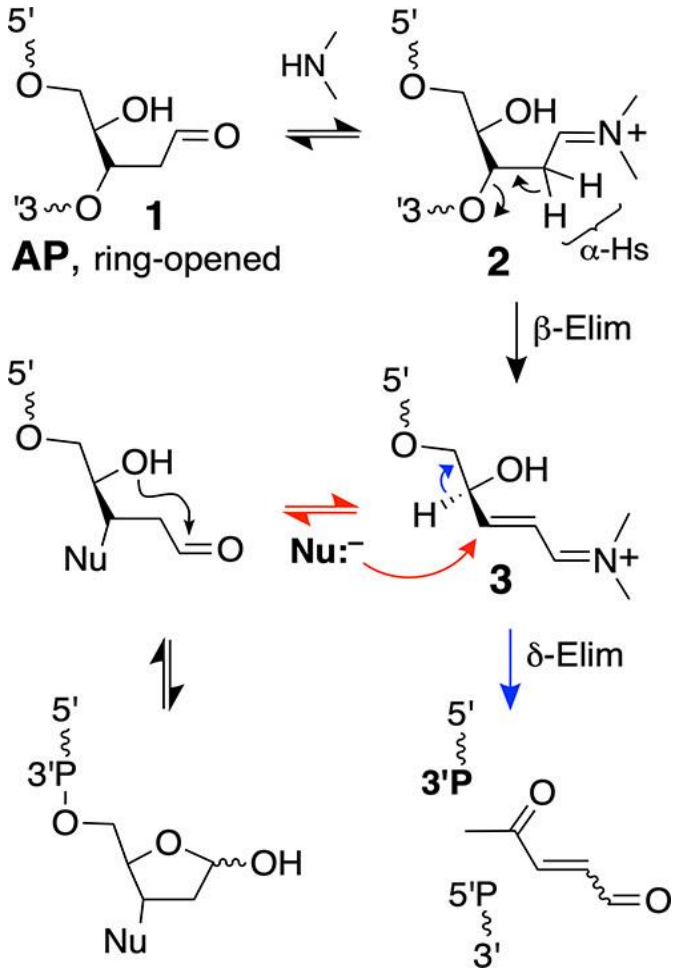


Figure 2.5.4. ESI(-)-QTOF-LC-MS analysis products generated by treatment of the AP oligonucleotide with piperidine (1 M) at 50℃ for 20 min. Upper panel. Mass spectrometric analysis revealed deconvoluted neutral masses for the cleavage product bearing a 3-piperidinyl- 2,3-dideoxyribose adduct (3-pip-ddR) on the 3'-terminus of the strand break generated by treatment of the AP-containing oligonucleotide with piperidine (1 M) at 50 ℃ for 30 min. Lower panel. Comparison of experimentally measured intensities for each peak in the isotope cluster (bars on the left side of each pair) and expected intensity (bars on the right side of each pair) calculated from the molecular formula of the 3-pip-ddR cleavage product.

2.6 Spermine-mediated cleavage of the AP-oligonucleotide generates a dynamic mixture of 3'*trans*-PUA, 3'*cis*-PUA, and 3'dR end products that ultimately converge on 3'P as the final cleavage product.

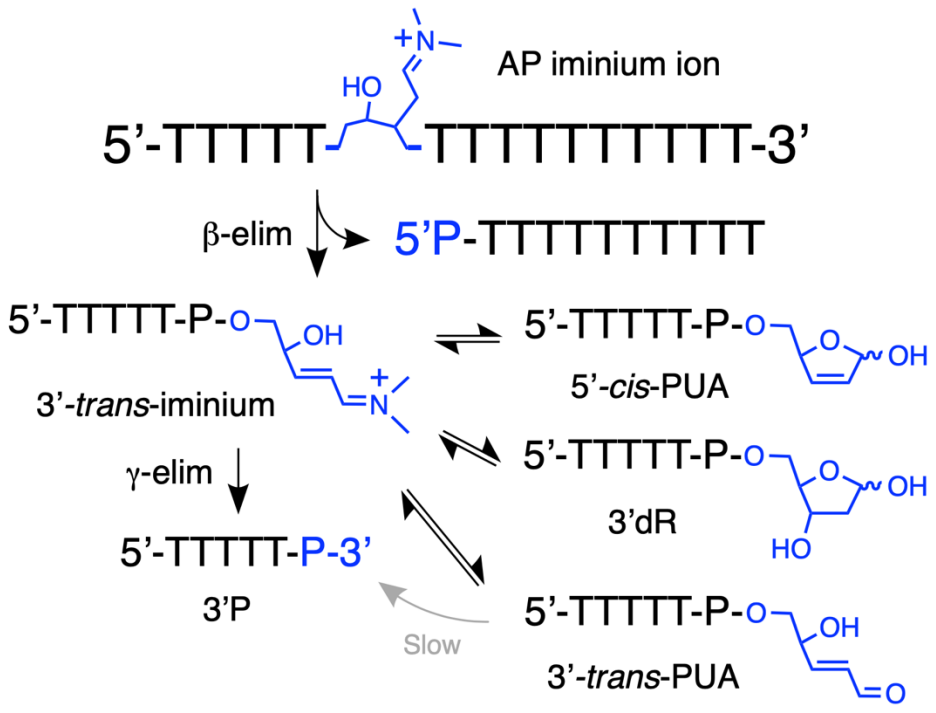
Spermine is a biogenic amine that is present in the cell at millimolar concentrations.[44] This polyamine efficiently catalyzes strand cleavage at AP sites in DNA via conversion of the AP aldehyde to the corresponding iminium ion.[38,39,45,48,86] Elimination of the 3'-phosphoryl group is facilitated by the dramatic increase in the acidity of the α-protons of the iminium ion compared to those of the corresponding aldehyde (Scheme 2.6.1).[87,88] The β-elimination reaction generates an α,β-unsaturated iminium ion intermediate that is substantially more reactive than the corresponding α,β-unsaturated aldehyde, with respect to both the conjugate addition of nucleophiles and the acidity of the γ-proton (Scheme 2.6.1).[89–91]

The products generated by spermine-catalyzed cleavage at an AP in DNA have not previously been characterized by any means other than their gel electrophoretic mobility.[38,39,41,48,86] Early work provided evidence that the amine-containing tripeptide, Lys-Trp-Lys, generates the 3'*trans*-PUA and 3'P products.[40] However, our recent results obtained using a nucleoside model system suggested that amine-catalyzed strand cleavage at an AP site has the potential to generate complex mixtures including the 3'*trans*-PUA, 3'*cis*-PUA, and 3'dR products (Scheme 2.6.2).[81]



Scheme 2.6.1. Conversion of the AP aldehyde residue to the corresponding iminium ion facilitates elimination via increased acidity of the α-Protons.[a]

[a] The resulting α,β-unsaturated iminium has the potential to undergo conjugate addition of water (or other nucleophiles, red arrow) or γ,δ-elimination (blue arrow) to generate the 3′P end group. For brevity, the amine catalyst is shown as a simple dialkylamine (the actual structure of spermine is shown in Figure 2.6.1). The wavy lines annotated with 5′ and 3′ labels represent DNA strands. The P in this scheme represents either a DNA phosphodiester linkage or a terminal phosphoryl group.



Scheme 2.6.2. Proposed equilibria involved in the evolution of 3′-end groups following spermine-catalyzed cleavage of an AP site in DNA.[a]

[a] All of the reactions and equilibria shown here are subject to catalysis by amines. For brevity, the amine catalyst is shown as a simple dialkylamine (the actual structure of spermine is shown in Figure 2.6.1).

Indeed, we found that spermine-induced cleavage of the AP-containing oligodeoxynucleotide under physiologically relevant conditions (5 mM spermine, pH 7.4 buffer, 37 °C) generated a complex mixture of products that evolves over time. At early times (15 min), we observed the intact AP-containing oligodeoxynucleotide, the 5′P elimination product, and two closely spaced peaks eluting near 12.5 min in the HPLC chromatogram (Figure 2.6.1, HPLC trace a). Based upon our recent work,[81] we suspected that these two closely spaced peaks might correspond to the 3′trans-PUA and 3′cis-PUA isomers.

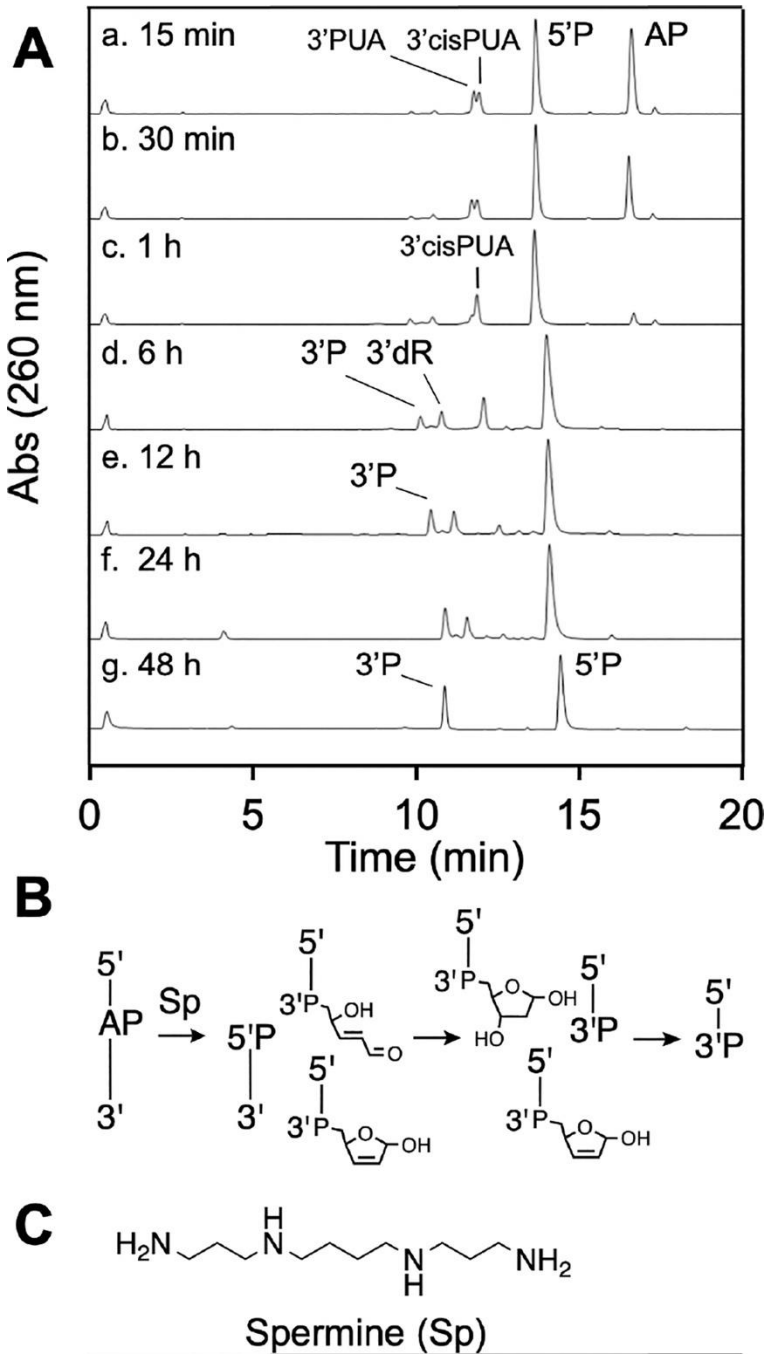


Figure 2.6.1. HPLC analysis of products generated from spermine-mediated strand cleavage at an AP site in DNA (panel A) and a schematic depiction of the product structures, where P represents a terminal phosphoryl group or a phosphodiester linkage (panel B). The chemical structure of spermine is shown in (panel C).

Addition of 2-mercaptoethanol to this early product mixture caused disappearance of the earlier peak in the doublet, with concomitant generation of two new peaks corresponding to the diastereomeric mixture of 3-thio-2,3-ddR isomers arising from conjugate addition of the 2-mercaptoethanol to the 3'*trans*-PUA group (Figure 2.6.2). This suggested that the first peak in the doublet was the 3' *trans*-PUA product. The later-eluting peak in the doublet was unaffected by addition of 2-mercaptoethanol, consistent with the less reactive nature of the 3'*cis*-PUA group.[81] Supporting the idea that 3'*cis*-PUA arises from amine-catalyzed isomerization of the initially-formed 3'*trans*-PUA, a separate experiment showed that the authentic 3'*trans*-PUA product generated by thermolysis of the AP-oligonucleotide was converted to the doublet of HPLC peaks corresponding to the *cis*- and *trans*-PUA isomers upon treatment with spermine (Figure 2.6.3).

ESI-MS analysis of the reaction mixture generated by spermine-catalyzed cleavage of the AP oligonucleotide at early times supported these product assignments. Specifically, when the reaction was allowed to proceed for 30 min, followed by workup with 2-mercaptoethanol (5 mM, 15 min), we observed strong signals consistent with the intact AP oligonucleotide, 5′P, the 3-thio-ddR isomers, and the 3′cis-PUA product. Note that the 3′cis-PUA product is isobaric with 3′trans-PUA but is resistant to reaction with the added thiol under these conditions (Figure 2.6.4).

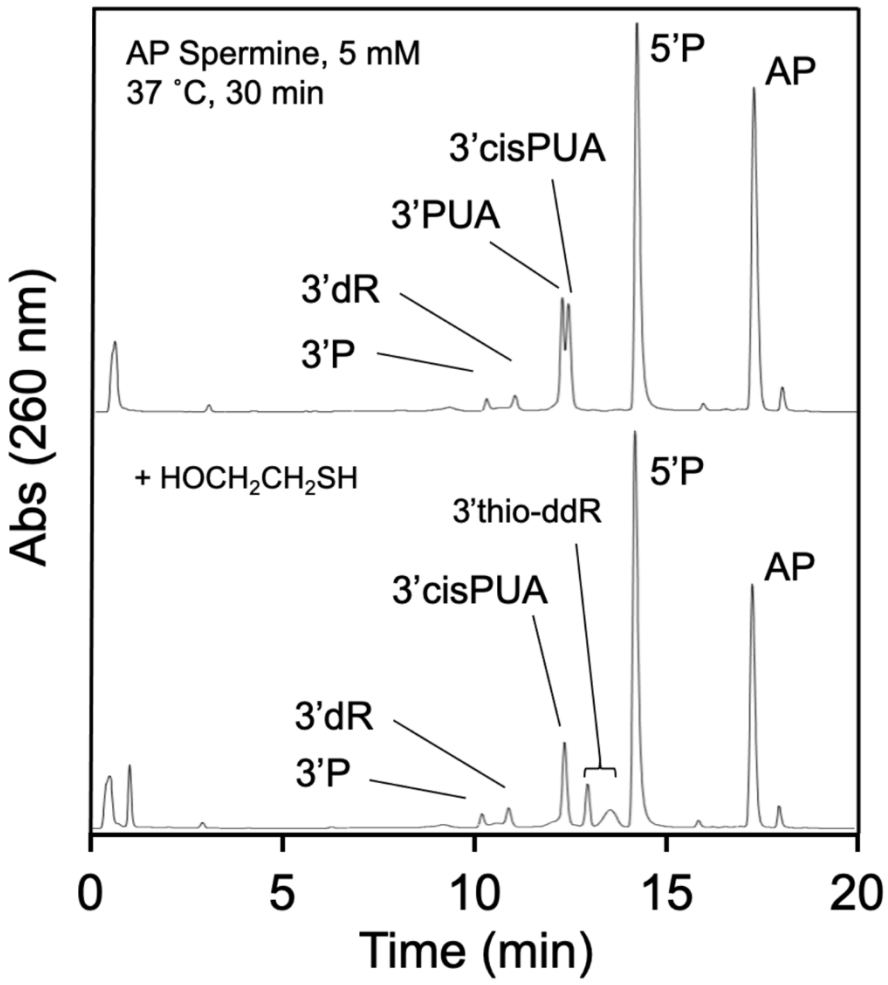


Figure 2.6.2. Addition of 2-mercaptoethanol to the product mixture generated by the spermine-catalyzed cleavage of an AP-containing oligodeoxynucleotide leads to the disappearance of one component of the doublet eluting at approximately 12.5 min, with concomitant generation of the 3-thio-ddR adduct on the 3′-terminus.

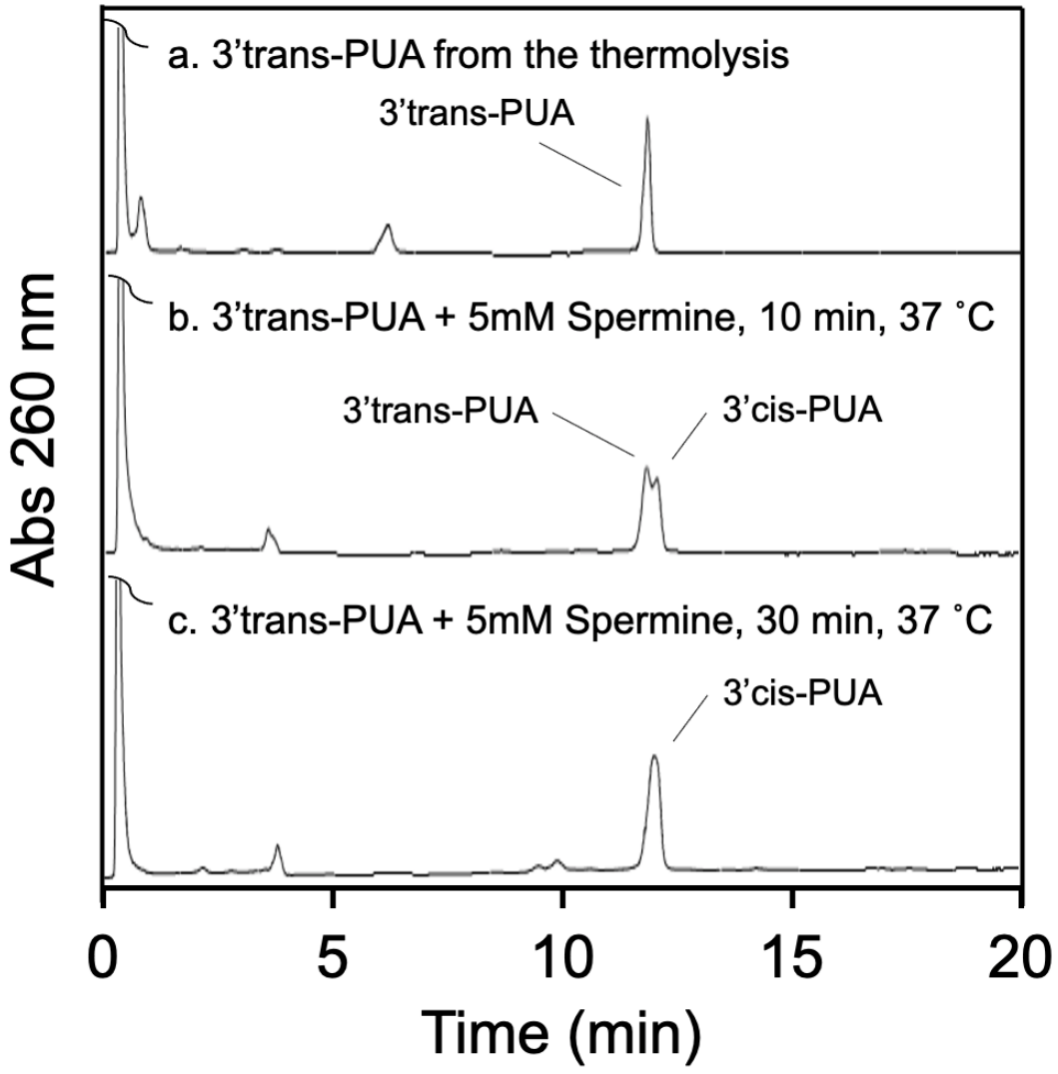


Figure 2.6.3. 3'trans-PUA is converted to a mixture of 3'trans-PUA and 3'cis-PUA by treatment with spermine (5 mM). The 3'trans-PUA product isolated from the thermolysis of the AP-containing DNA oligomer (panel a) was converted to a mixture of 3'trans-PUA and 3'cis- PUA by treatment with 5 mM Spermine, 37 ℃ for 10 min (panel b) and to a single peak ascribed to the 3'cis-PUA cleavage product after 30 min at 37 ℃ (panel c).

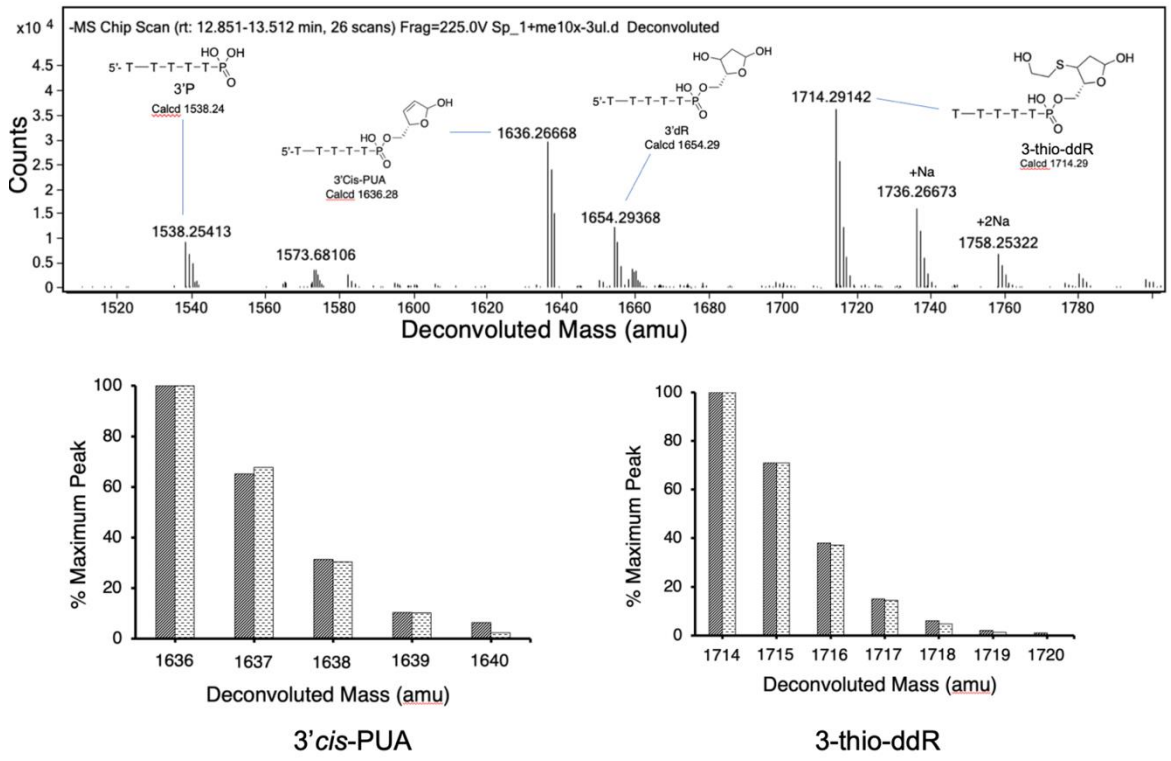


Figure 2.6.4. ESI(-)-QTOF-LC-MS analysis of the products generated by spermine-mediated cleavage of the AP oligonucleotide in the presence of 2-mercaptoethanol. Mass spectrometric analysis revealed deconvoluted neutral masses for the cleavage products 3'P, 3'cis-PUA, 3'dR, and 3-thio-ddR generated by treatment of the AP-containing DNA oligomer with spermine (5 mM) in HEPES buffer (50 mM, pH 7.4) containing NaCl (100 mM) at 37 ℃ for 30 min, followed by addition of 2-mercaptoethanol (5 mM) and incubation for 15 min. Lower panel left side. Comparison of experimentally measured peak intensities for each peak in the isotope cluster (bars on the left side of each pair) and expected intensity (bars on the right side of each pair) calculated from the molecular formula of the 3'cis-PUA cleavage product. Lower panel right side. Comparison of experimentally measured intensities for each peak in the isotope cluster (bars on the left

side of each pair) and expected intensity (bars on the right side of each pair) calculated from the molecular formula of the 3-thio-ddR adduct.

To further support our assignment that the closely-spaced peaks eluting near 12.5 minutes in our HPLC analyses were the *cis*- and *trans*-PUA cleavage products we used a literature protocol to generate an authentic sample of these products. Specifically, Kushida et al. showed that heating an AP-containing oligodeoxynucleotide in Tris-borate buffer (pH 7.5) generated a 4:1 mixture of the *cis*- and *trans*-PUA cleavage products, alongside the 3'dR cleavage product.[64] In our hands, HPLC analysis of the product mixture generated by heating our AP-containing oligonucleotide using the Kushida conditions did indeed reveal a closely-spaced doublet of peaks eluting near 12.5 min in which the later-eluting (3'*cis*-PUA) product dominated. The 3'dR cleavage product, eluting near 11.5 min, was also evident in this reaction mixture, as expected. Addition of 2-mercaptoethanol to this product mixture caused disappearance of the early eluting (3'*trans*-PUA) component of the doublet along with the appearance of characteristic peaks corresponding to the mixture of 3-thio-ddR isomers arising from conjugate addition of the thiol to the 3'*trans*-PUA group (Figure 2.6.5). Again, the later eluting 3'*cis*-PUA component of the doublet, with its aldehyde residue masked as a cyclic hemiacetal, was resistant to reaction with 2-mercaptoethanol.[81] The results support our conclusion that the early products of spermine-mediated strand cleavage of the AP-oligonucleotide are an approximately 1:1 mixture of 3'*cis*- and 3'*trans*-PUA end groups.

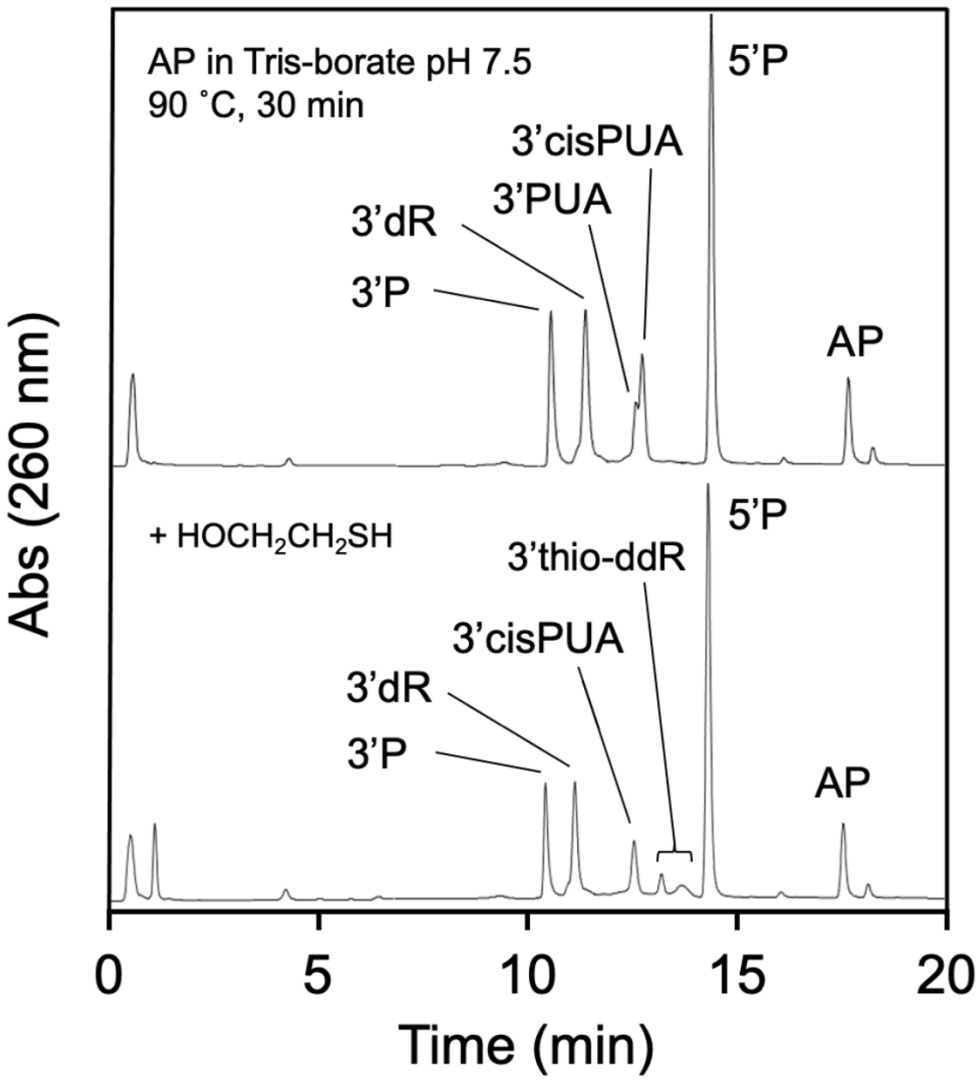


Figure 2.6.5. HPLC analysis of authentic cis- and trans-PUA. Upper HPLC trace shows an authentic mixture of 3'P, 3'dR, 3'PUA, and 3'cis-PUA generated by incubation of the AP-containing oligonucleotide in Tris-borate buffer according to the method of Kushida et al.[64] The lower HPLC trace shows that 2- mercaptoethanol reacts with the later-eluting component 3'trans-PUA to generate a characteristic 3-thio-2,3-ddR residue on the 3'-terminus, while the early-eluting 3'cis- PUA is resistant to reaction with the thiol.

The products generated by spermine-mediated strand cleavage of the AP-containing DNA oligomer continued to evolve into more complex mixtures at intermediate reaction times. After 1-6 h, the amount of the 3'*trans*-PUA product decreased, 3'*cis*-PUA persisted, and the amounts of the 3'dR and 3'P products increased (Figure 2.6.1, HPLC traces c and d). The identity of the 3'dR product eluting at 11.2 min was confirmed based on co-elution with an authentic standard of the 5'TTTTT-dR-3' prepared by acid treatment of a 5'-TTTTTA-3' precursor (10 mM HCl, 1 h, 65 ˚C).[39] Similarly, the identity of the 3'P product was confirmed based on co-elution with an authentic sample of the material.

From 12-24 h 3'dR, and 3'P were the major products (Figure 2.6.1, HPLC traces e and f) and, by 48 h, the 3'P and 5'P were the only products remaining from the spermine-mediated strand cleavage process (Figure 2.6.1, HPLC trace g). In separate experiment modeling the final stages of this product evolution, we showed that an authentic sample of 3'dR is stable in pH 7.4 buffer (in the absence of amines) but is converted to the 3'P product by incubation with spermine (5 mM) for 24 h at 37 ˚ C (Figure 2.6.6). ESI-MS analysis of the reaction at 24 h supported the product assignments shown in Figure 2.6.1 (Figure 2.6.7).

Scheme 2.6.2 shows the putative equilibria involved in the evolution of the product mixture generated by the spermine-catalyzed cleavage of the AP-containing DNA oligomer. Iminium ion catalysis is central to the formation, interconversion, and ultimate disappearance of the various 3′-sugar remnants observed in this experiment (illustrated for the reversible formation of 3′dR and its ultimate conversion to 3′P in Scheme 2.6.3).

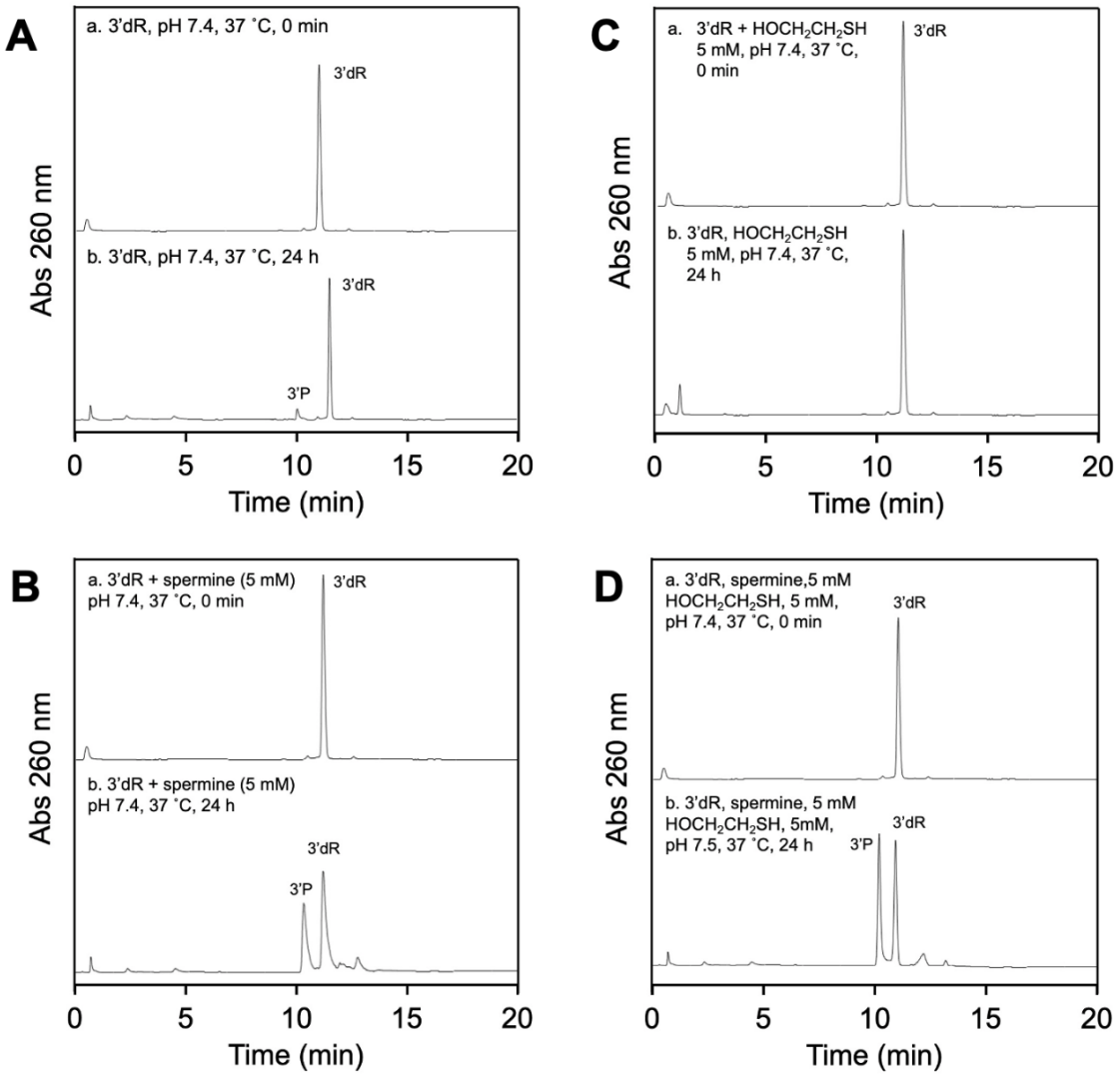


Figure 2.6.6. Reactivity of 3'dR under various conditions. Panel A, upper: Stability of authentic 3'dR in pH 7.4 buffer. The authentic 3'dR material was generated from by acid-catalyzed depurination (10 mM HCl, 65 ℃, 1 h) of the adenine residue in the precursor DNA oligomer 5'- TTTTTA. Panel A, lower: Incubation of 3'dR at 37 ℃ in pH 7.4 HEPES buffer (50 mM, containing 100 mM NaCl) for 24 h showed generation of only a small amount of the 3'P product. Panel B: Incubation of 3'dR at 37 ℃ in pH 7.4 HEPES buffer (50 mM, with 100 mM NaCl) containing spermine (5 mM) for 24 h showed significant

conversion of 3’dR to the 3’P product. Panel C: Incubation of 3’dR at 37 ℃ in pH 7.4 HEPES buffer (50 mM, with 100 mM NaCl) containing 2-mercaptoethanol (5 mM) for 24 h, showed no significant conversion of 3’dR to new products. Panel D: Incubation of 3’dR at 37 ℃ in pH 7.4 HEPES buffer (50 mM, with 100 mM NaCl) containing 2-mercaptoethanol (5 mM) and spermine (5 mM) for 24 h, showed significant conversion of 3’dR to the 3’P product.

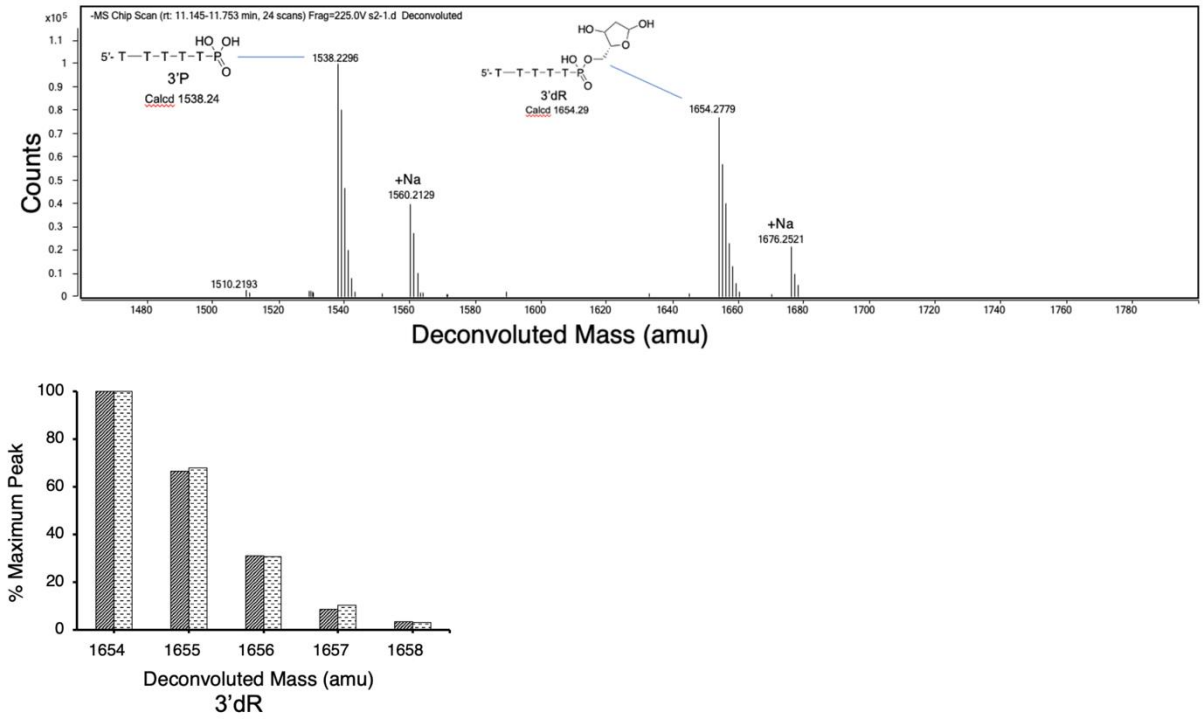
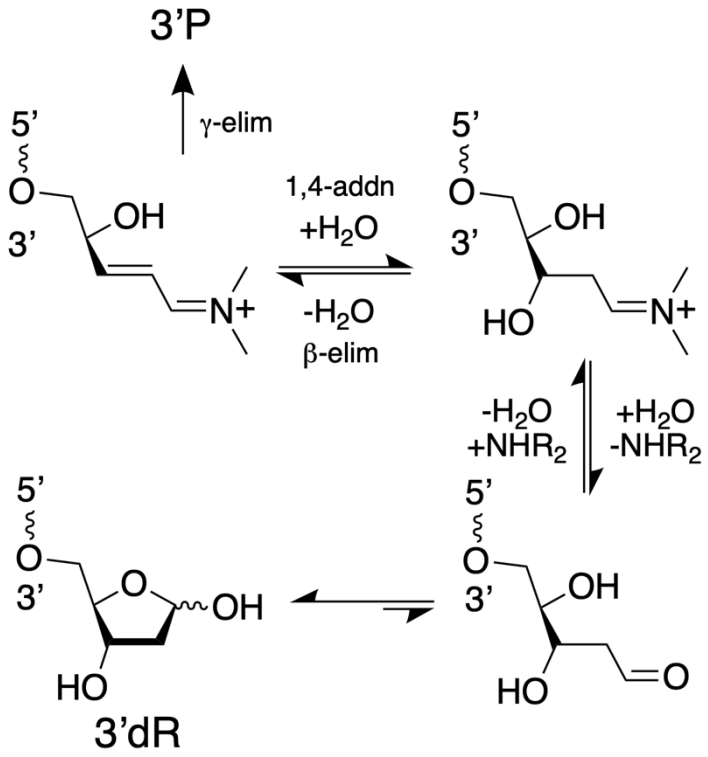


Figure 2.6.7. ESI(-)-QTOF-LC-MS analysis products generated by spermine-mediated cleavage of the AP-containing oligonucleotide at 24 h. Upper panel. Mass spectrometric analysis revealed deconvoluted neutral masses for the cleavage products 3’dR and 3’P generated by treatment of the AP-containing DNA oligomer in HEPES buffer (50 mM, pH 7.4) containing NaCl (100 mM) with spermine (5 mM) at 37 ℃ for 24 h. Lower panel. Comparison of experimentally measured intensities for each peak in the

isotope cluster (bars on the left side of each pair) and expected intensity (bars on the right side of each pair) calculated from the molecular formula of the 3'dR cleavage product.



Scheme 2.6.3. Iminium Ion Intermediates Are Central to the Formation, Decomposition, and Interconversion of Various 3′-Sugar Remnants Generated in Amine-Catalyzed Strand Cleavage.[a]

[a] Reactions involved in the amine-catalyzed reversible formation of various 3′-sugar remnants are shown here in the context of the 3′dR end group. For brevity, the amine catalyst is shown as a simple dialkylamine. The wavy lines annotated with 5′ and 3′ labels represent DNA strands.

2.7 Modeling Cleavage of an AP Site in DNA under Cellular Conditions: Amine-Catalyzed Strand Cleavage in the Presence of Thiol

Cells contain millimolar concentrations of thiols including protein thiols and the low-molecular-weight thiol, glutathione.[92–95] Accordingly, we felt that it would be interesting to examine spermine-mediated cleavage of the AP oligonucleotide in the presence of thiol. These reactions may model one of the possible chemical fates of DNA AP sites in the cellular environment.

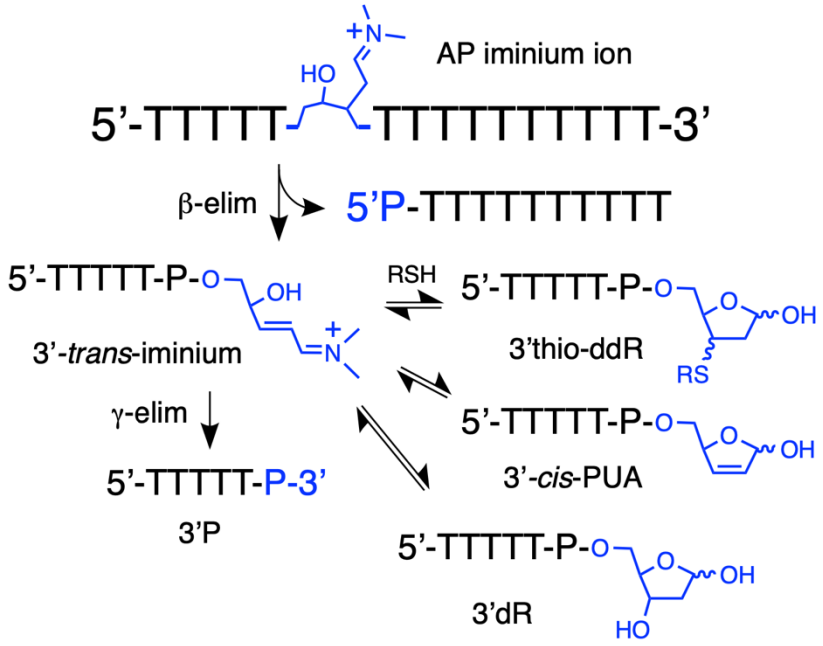
We treated the AP-containing oligodeoxynucleotide with spermine (5 mM) in the presence of 2-mercaptoethanol (5 mM) in pH 7.4 HEPES buffer (50 mM, containing 100 mM NaCl). At an early reaction time (1 h), the only products observed were 5′P and the characteristic adducts resulting from the addition of 2-mercaptoethanol to the 3′-α,β-unsaturated iminium ion (Scheme 2.7.1, Figure 2.7.1).

By 24 h, the product mixture contained a subset of the diastereomeric thiol adducts, along with 3′cis-PUA, 3′dR, and 3′P. We infer that the species eluting near 12.5 min must be the 3′cis-PUA cleavage product because the 3′trans-PUA does not persist in the presence of thiols. It is uncertain why a subset of the diastereomeric thiol adducts is more persistent than the others (only one of the two HPLC peaks corresponding to the diastereomeric thiol adducts remains at 24 h).

At 48 h, the only significant products remaining were 3′dR and 3′P end products. By 72 h, the mixture evolved to 3′P and 5′P as the final, stable end products. In a control experiment designed to model the final stages of product evolution under these conditions, we showed that an authentic sample of the 3′dR cleavage product was stable in pH 7.4 buffer containing 2-mercaptoethanol but does, in fact, generate the 3′P end group as the

major product when incubated in the presence of spermine (5 mM) and 2-mercaptoethanol (5 mM) for 24 h (Figure 2.6.6, Scheme 2.6.3).

It is noteworthy that the presence of thiol in the reaction mixture substantially slows the generation of the final 3′P product relative to the same conditions without the thiol (compare Figure 2.6.1 and Figure 2.7.1). Scheme 2.7.1 shows the putative equilibria involved in the evolution of products generated by the spermine-catalyzed cleavage of the DNA AP site in the presence of thiol.



Scheme 2.7.1. Proposed Equilibria Involved in the Evolution of Various 3′-End Groups Following Spermine-Mediated Cleavage of an AP Site in the Presence of Thiol.[a]

[a] All of the reactions and equilibria shown are subject to amine catalysis. For brevity, the amine catalyst is shown as a simple dialkylamine (the actual structure of spermine is shown in Figure 2.6.1).

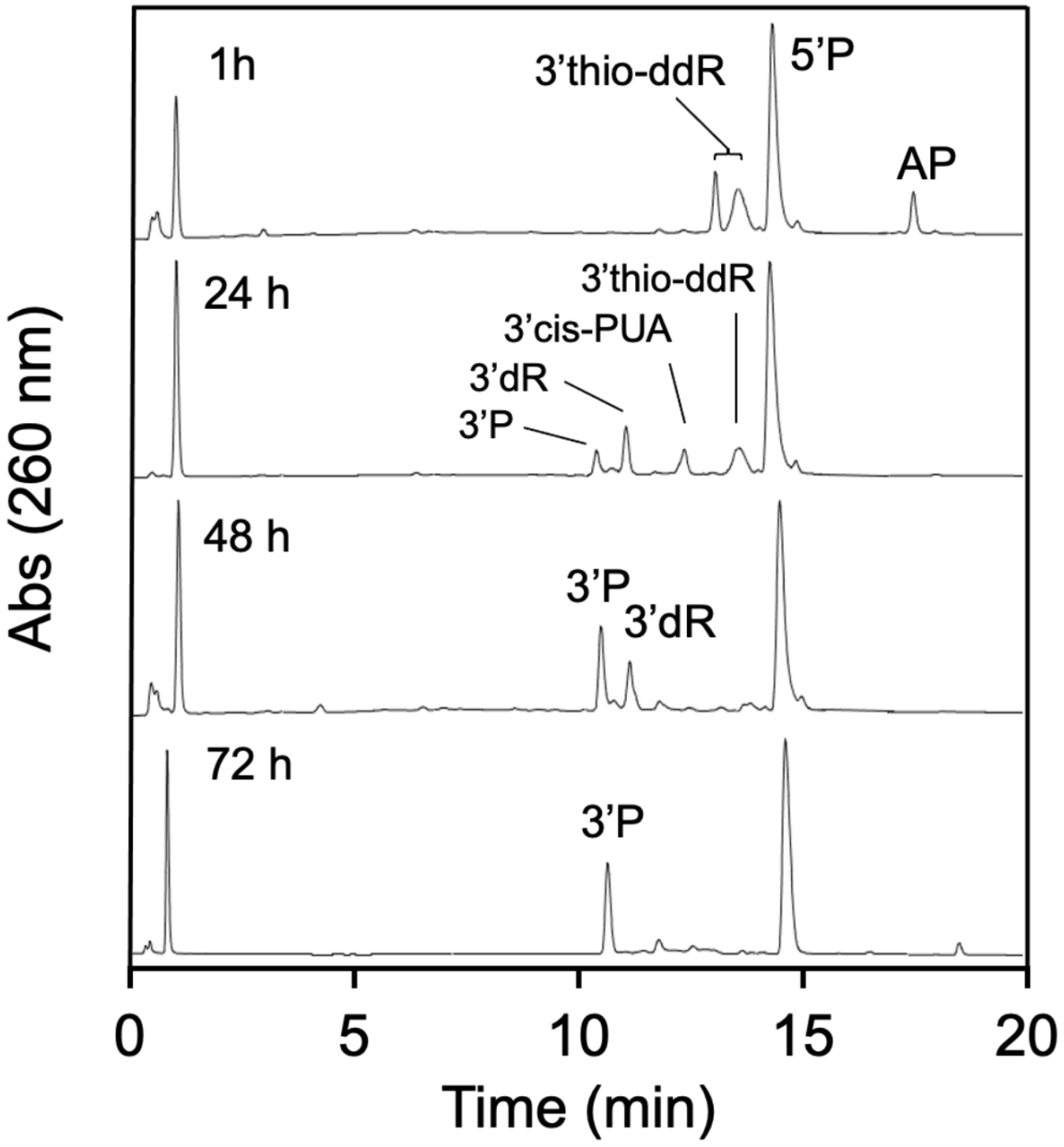
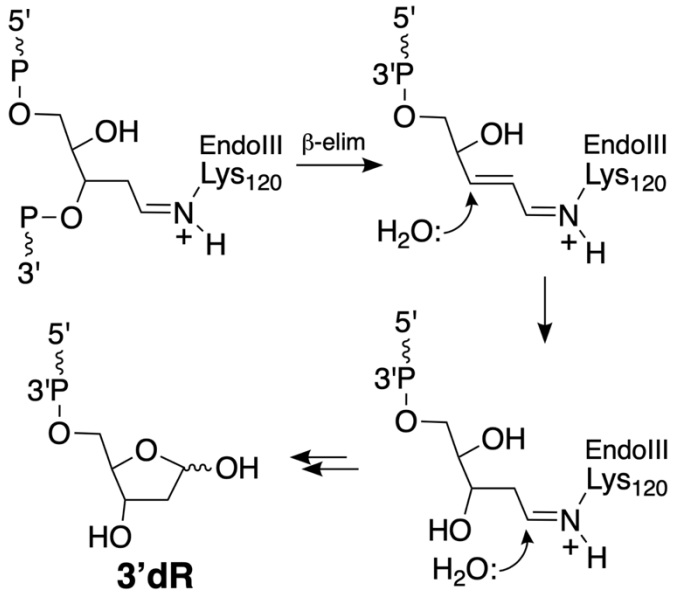


Figure 2.7.1. HPLC analysis of the time course for the evolution of products resulting from the cleavage of the AP-containing oligodeoxynucleotide in the pH 7.4 buffer induced by spermine (5 mM) in the presence of 2-mercaptoethanol (5 mM).

2.8 AP Lyase Activity of the DNA Glycosylase Endo III Generates 3′dR (Not 3′PUA) as the Major Strand-Cleavage Product

The AP-lyase activity of bifunctional DNA glycosylase enzymes induces strand cleavage at AP sites and may be important in cellular DNA repair.[96–98] The typical expectation is that these lyase reactions generate either the 3′trans-PUA product via β-elimination or the 3′P product by sequential α,β- and γ,δ-elimination reactions.[4,40,50,51,99–101] Here, we examined the AP lyase activity of two well-studied glycosylases, Fpg and Endo III.

The lyase activity of Fpg on AP-containing DNA was expected to generate a one nucleotide gap flanked by 3'P and 5'P groups via sequential α,β- and γ,δ-elimination reactions.[4,102] On the other hand, the expectations surrounding the lyase activity of Endo III were somewhat less clear. It is widely believed that Endo III generates the 3'*trans*-PUA product. For example, the New England Biolabs catalog notes that Endo III "cleaves 3' to the AP site leaving a 5'-phosphate and a 3'phospho-α,β-unsaturated aldehyde" (p. 109, 2019-20 catalog).[103] This expectation was born in the early reports characterizing the enzyme[31,32,40,104,105] and is now well entrenched in the literature of base excision repair. Interestingly, however, the Sowers and Cadet groups independently presented MALDI-MS evidence indicating that the combined glycosylase-AP lyase action of *E. coli* Endo III generates the 3'dR cleavage product – not 3'*trans*-PUA – following removal of 5,6-dihydrothymine, 5hydroxycytosine, or 5-fluorouracil from DNA oligomers.[66,67] In addition, more recent studies showed that the lyase action of Endo III on AP-containing duplexes generates the 3'dR cleavage product.[68,69]



Scheme 2.8.1 AP-Lyase Action of the DNA Glycosylase Endo III Generates 3′dR─Not 3′PUA─as the Major Product.[a]

[a]The wavy lines annotated with 5′ and 3′ labels represent DNA strands and P represents a phosphodiester linkage.

The amplified reactivity of the 3'PUA-lysine-120 iminium ion intermediate generated in the catalytic cycle of Endo III may enable conjugate addition of water that produces 3'dR (Scheme 2.8.1). In this regard, formation of the 3'dR cleavage product by Endo III may be mechanistically analogous to the spermine-catalyzed generation of 3'dR described above. The exact reasons for the apparently discordant results in the literature regarding the nature of the 3'-end group generated by the lyase action of *E. coli* Endo III are not completely clear but may arise from difficulties in separating the 3'dR and 3'PUA cleavage products by gel electrophoretic and chromatographic methods.[68,69] For example, when analyzed using denaturing 20% polyacrylamide gels, the 3'dR and 3'PUA products on a 5'-Cy3-labeled 11 mer displayed only slightly different mobilities[69] while in a 5'-^{32}P-

phosphorylated 17 mer, no clear resolution of these products was observed.[68] In addition, the ability of the 3'dR and 3'PUA end groups to interconvert under some conditions (as seen in Figure 2.4.2 and 2.6.6) may present a confounding factor in some analyses.[105]

We found that the treatment of an AP-containing duplex with Fpg cleanly generated the expected 3′P and 5′P cleavage products (Figure 2.8.1, HPLC trace c). The treatment of an AP-containing duplex for 15 min with Endo III primarily generated the 3′dR product alongside a small amount of a 3′PUA product (Figure 2.8.1, HPLC trace a). Mass spectrometric analysis of the mixture supported these structural assignments (Figure 2.8.2). When the AP oligonucleotide was incubated for 2 h with Endo III in the presence of 2-mercaptoethanol (5 mM), 3′dR was still the major product (Figure 2.8.3). This control reaction provided evidence that 3′dR is produced directly by the AP-lyase activity "on the enzyme" rather than by the conversion of an initially generated 3′trans-PUA product to 3′dR. At 2 h, 3′dR is the only product observed (Figure 2.8.1, HPLC trace b). It may be interesting to note that the products generated by the AP-lyase hydration reactions catalyzed by spermine and Endo III are formally equivalent to those resulting from the hydrolysis of the phosphoryl group on the 3'side of the AP site.

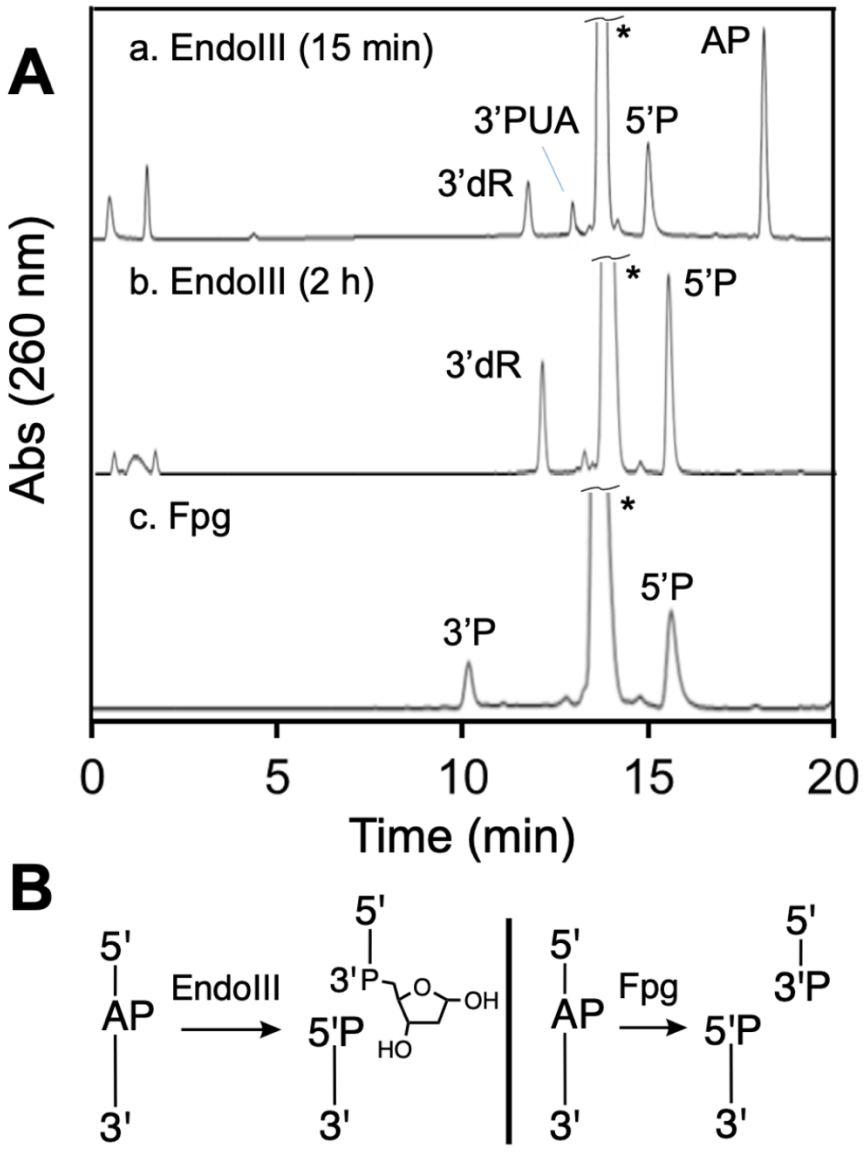


Figure 2.8.1. HPLC analysis of products generated by Fpg- and Endo III- mediated strand cleavage of an AP site in duplex DNA (panel A). The AP-containing strand is 5'TTTTTXTTTTTTTTTT3', where X=AP. The large peak labeled with (*) corresponds to the complementary strand 5'TTA$_{16}$TT. Panel B provides a schematic depiction of the product structures, where P represents a terminal phosphoryl group or a phosphodiester linkage.

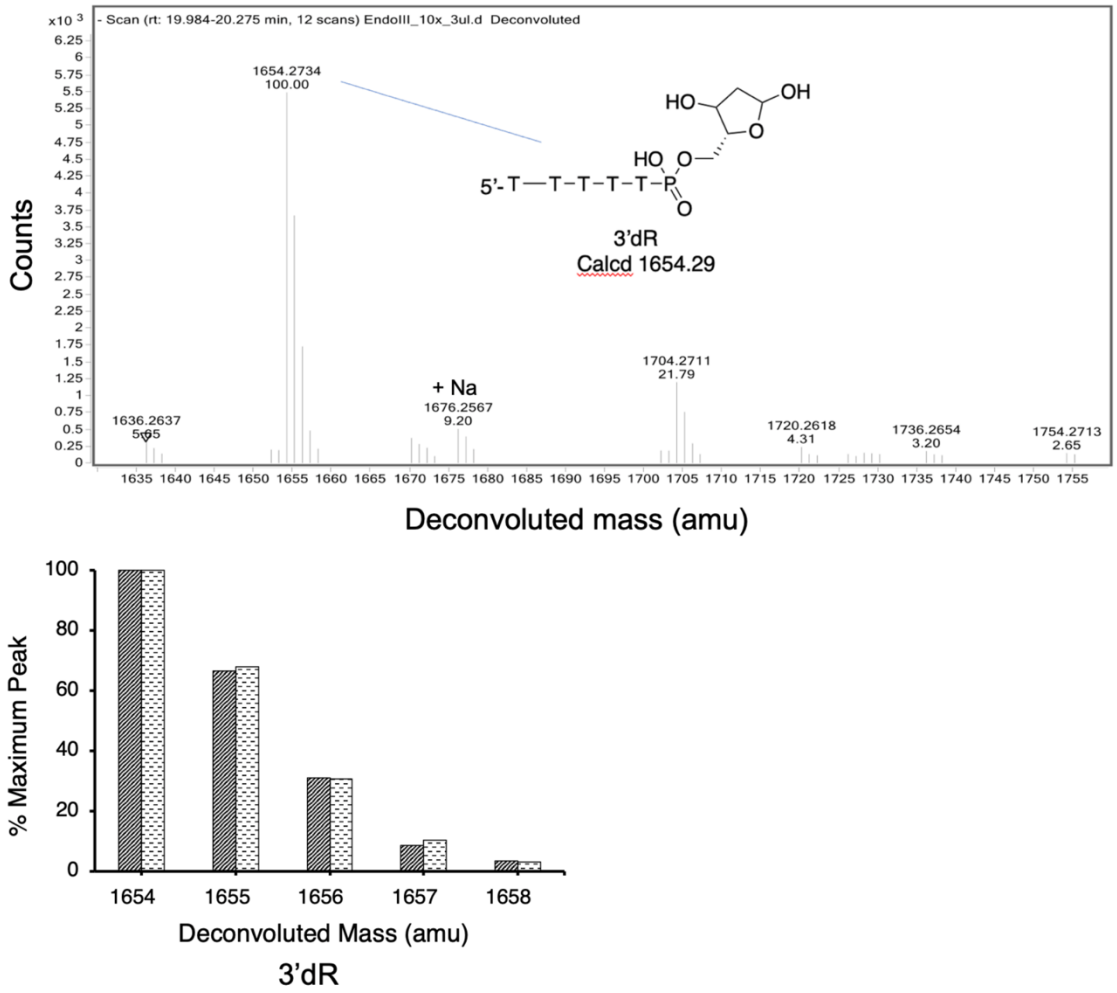


Figure 2.8.2. ESI(-)-QTOF-LC-MS analysis of the products generated by cleavage of the AP- containing oligonucleotide duplex by Endo III. Upper panel. Mass spectrometric analysis revealed deconvoluted neutral masses for the cleavage products 3'dR and 3'P generated by treatment of the AP-containing DNA duplex in Endo III (20 units) in 20 mM Tris-HCl, 1 mM EDTA, 1 mM DTT, at 37 ℃ for 2 h. Lower panel. Comparison of experimentally measured intensities for each peak in the isotope cluster (bars on the left side of each pair) and expected intensity (bars on the right side of each pair) calculated from the molecular formula of the 3'dR cleavage product. The duplex was composed of an AP-containing strand 5'TTTTTXTTTTTTTTTT3', where X=AP and the complementary strand, 5'$TTA_{16}TT$.

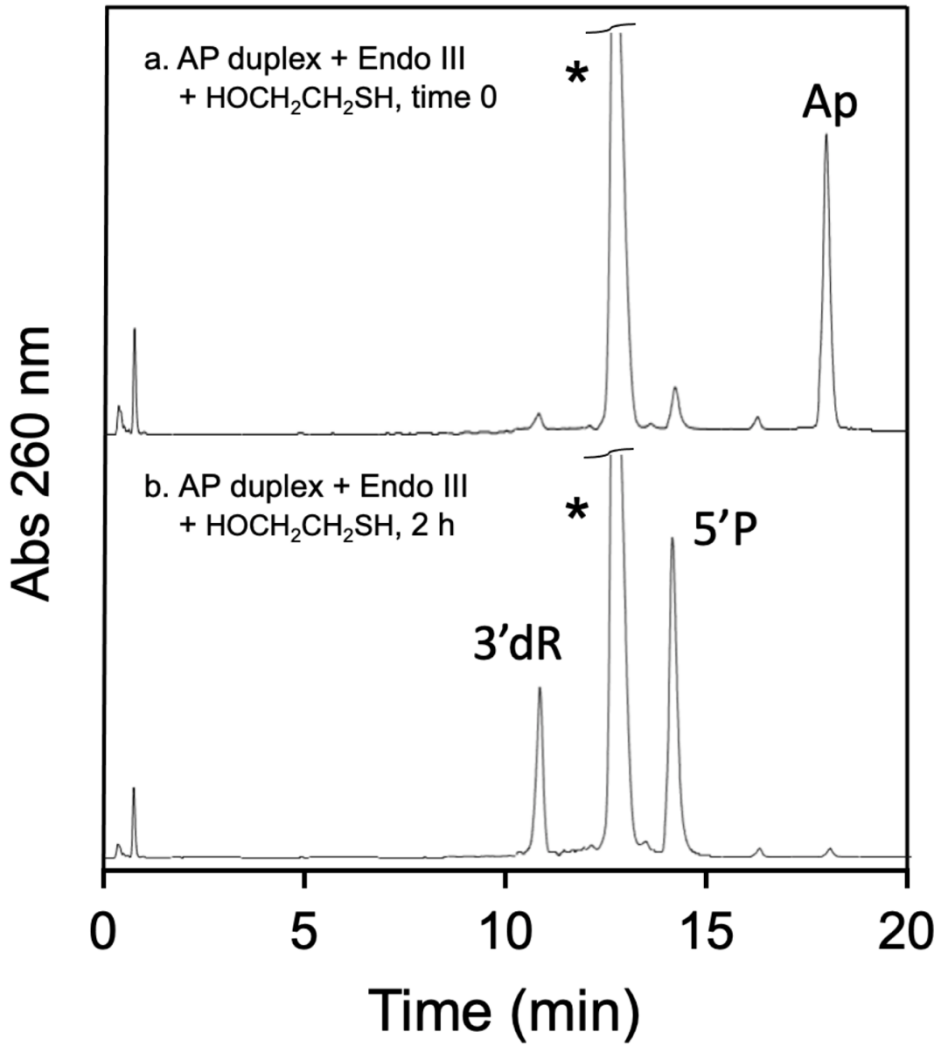


Figure 2.8.3. HPLC analysis of products generated by cleavage of the AP-containing oligonucleotide by Endo III in presence of 2-mercaptoethanol. When the AP-containing DNA duplex was incubated with Endo III in the presence of 2-mercaptoethanol for 2 h, 3'dR was observed as the major product. No significant amounts of 3'PUA or 3-thio-ddR products were seen. The AP-containing strand is 5'TTTTTXTTTTTTTTTT3', where X=AP and the large peak labeled with (*) corresponds to the complementary strand, 5'TTA$_{16}$TT.

2.9 Conclusions

In the work described here, we characterized the strand cleavage products arising from the treatment of an AP-containing DNA oligonucleotide with heat, NaOH, hot piperidine, spermine, and the base-excision repair glycosylases Fpg and Endo III (Table 2.9.1). Our findings mesh well with the existing literature, but also identify unexpected products generated under many of the strand-cleavage conditions examined.

condition	*cis*-PUA	*trans*-PUA	3′dR	3′P
85 °C, 15 min		√		
85 °C, 45 min		√		√
NaOH, 500 mM, 2 min, 95 °C				√
NaOH, 200 mM, 5 h, 37 °C				√
NaOH, 200 mM, 20 min, 37 °C			√	√
NaOH, 5 mM, 1 h, 37 °C		√		√
piperidine, 1 M, 30 min, 95 °C				√
piperidine, 1 M, 1 h, 37 °C		√		√
spermine, 5 mM				
37 °C, 30 min	√	√		
1 h	√			
12 h			√	√
48 h				√
Fpg				√
Endo III			√	

Table 2.9.1. Products Generated by the Cleavage of an AP-Containing Oligodeoxynucleotide under Various Conditions (HPLC Data Is Shown in the Figures, and Exact Conditions Are Discussed in the Text)

Thermal treatment of the AP-containing DNA oligomer (85 °C, 15 min) in pH 7.4 buffer produced the 3′trans-PUA product as expected.[37] The 3′trans-PUA product was rather stable in the pH 7.4 buffer at 37 °C (in the absence of an amine catalyst), decomposing via a slow δ-elimination process to produce small amounts of the 3′P product over 12 h. In the presence of thiol, however, the 3′trans-PUA sugar remnant was rapidly converted to a diastereomeric mixture of 3-thio-2,3-ddR adducts on the 3′-end of the strand break. The thiol adducts were stable in the absence of an amine catalyst. Our reactions, carried out at 85 °C, did not produce significant amounts of the 3′dR or 3′cis-PUA end groups that were observed previously when an AP-containing trinucleotide was heated at 90 °C in pH 7 buffer.[37]

We found that a mild NaOH treatment (5 mM, 1 h, 37 °C) of the AP oligonucleotide gave the expected 3′trans-PUA cleavage product. A more vigorous NaOH treatment (500 mM, 2 min, 95 °C) cleanly generated the expected 5′P and 3′P products. Interestingly, a NaOH treatment of intermediate intensity (200 mM, 20 min, 37 °C) generated significant amounts of the 3′dR product. To the best of our knowledge, the 3′dR cleavage product has not previously been observed to arise from the NaOH treatment of an AP site in DNA.

We found that a standard piperidine workup (1 M, 30 min, 95 °C)[85] completely cleaved the AP-containing oligonucleotide, with the generation of the expected 5′P and 3′P termini at the strand break. Very mild conditions (5 mM, 1 h, 37 °C) gave a mixture of 3′P and 3′trans-PUA groups. When the AP oligonucleotide was subjected to a piperidine workup of intermediate intensity (1 M, 20 min, 50 °C), we observed an unprecedented piperidinyl adduct on the 3′-terminus of the strand break, arising from the conjugate addition of the amine to the α,β-unsaturated iminium ion intermediate.

Perhaps the most interesting reactions reported here, both from the perspective of biological significance and unexpected complexity, involved spermine-catalyzed cleavage of the AP-containing oligodeoxynucleotide. The cell nucleus is rich in spermine and other polyamines,[45] making this process of likely significance in cellular DNA. The results show that amine-catalyzed cleavage of an AP site in DNA under physiologically relevant conditions generates complex, interconverting mixtures that include several noncanonical sugar remnants on the 3′-terminus of the strand break. Iminium ion catalysis underlies the formation, interconversion, and ultimate removal of various sugar remnants on the 3′-terminus of AP-derived strand breaks (illustrated in Schemes 2.6.1 and 2.6.3).

We found that spermine-catalyzed strand cleavage initially generated a mixture of the 3′cis- and 3′trans-PUA isomers on the 3′-terminus of the strand break. The 3′trans-PUA isomer gave way to 3′dR and 3′P products, while the 3′cis-PUA isomer persisted. The 3′cis-PUA is relatively unreactive because the aldehyde group of this isomer exists predominantly in the masked, hemiacetal form. After 1 h, the AP-containing oligodeoxynucleotide was almost completely consumed and the 3′cis-PUA product was the major cleavage product. At 12–24 h, 3′dR was the only remaining 3′-sugar remnant and, finally, 3′P was generated as the ultimate, chemically stable end product. It is striking that two noncanonical cleavage products, the 3′cis-PUA and 3′dR, are the major sugar remnants on the 3′-terminus of the AP-derived strand break during the central portion of the reaction time course. It may be useful to point out that in gel electrophoretic experiments commonly used to characterize DNA-cleavage reactions, the noncanonical cleavage of 3′cis-PUA and 3′dR could remain unnoticed because they may not be clearly distinguished from the expected 3′trans-PUA cleavage product.

Cells contain millimolar concentrations of thiols including the tripeptide glutathione and protein thiols.[92–95] Thus, our experiments examining the spermine-catalyzed cleavage of the AP-containing oligonucleotide in the presence of thiol may model the chemical fate of an AP site in cellular DNA. At early reaction times, the only products observed under these conditions were the 5′P elimination product and the diastereomeric 3-thio-ddR adducts on the 3′-terminus arising from the conjugate addition of thiol to the α,β-unsaturated iminium ion intermediate (Scheme 2.7.1). The thiol adducts gave way to a mixture of the 3′dR and 3′cis-PUA at 48 h and, finally, to the 3′P product by 72 h. Notably, the canonical 3′trans-PUA product was not observed in the presence of thiol.

These results suggests that in the glutathione-rich environment of the cell,[92–95] a diastereomeric mixture of 3-glutathionyl-2,3-dideoxyribose end groups will be the major product arising from cleavage at AP sites in DNA. Furthermore, our results support the early report of and Bailly and Verly, who noted that the formation of thiol adducts generated by the amine-catalyzed cleavage of an AP site in the presence of thiol significantly slowed the subsequent amine-catalyzed γ,δ-elimination reaction that generates the final 3′P product.[38,39]

Finally, our findings reinforce earlier reports,[66–69] indicating that the AP-lyase activity of E. coli Endo III generates 3′dR─and not 3′PUA─as the major product. This enzymatic process is mechanistically analogous to the generation of 3′dR by the low-molecular-weight amine spermine, likely involving the conjugate addition of water to an electrophilic α,β-unsaturated iminium ion intermediate attached to the active-site lysine (Scheme 2.8.1).[106] Generation of 3′dR by the spermine- and Endo III-catalyzed cleavage of AP sites in DNA may have important functional consequences as these processes evade

the production of the more electrophilic (and potentially genotoxic[63] or cytotoxic[48,68]) α,β-unsaturated aldehyde residue on the 3′-terminus of an AP-derived strand break. This could be especially important to the extent that this enzyme and its homologs function in APE-independent base excision repair pathways.[56,97,107–109] It may be interesting to determine whether the lyase action of the human and Saccharomyces cerevisiae Endo III homologs, hNth and Ntg1/2p, similarly generates 3′dR.

Overall, our results reveal that the products arising from strand cleavage at an AP site in DNA can be more complex than commonly expected. Our results offer a deeper understanding of the possible degradation products arising from synthetic oligodeoxynucleotides used in molecular biology, materials science, and medicine. In addition, the results expand our understanding of 3′-blocked ends that must be chemically or enzymatically removed as part of cellular single-strand break repair and base-excision repair pathways.[110,111] Finally, it is significant that some of these 3′-sugar remnants have the potential to generate interstrand cross-links at AP-derived strand breaks in duplex DNA.[48,68]

2.10 Experimental Section

Materials

Reagents were purchased from the following suppliers and were of the highest purity available: oligonucleotides were from Integrated DNA Technologies (IDT, Coralville, IA); uracil DNA glycosylase (UDG), endonuclease III (Nth, Endo III), and Fpg were from New England Biolabs (Ipswich, MA); BS poly-prep columns were obtained from Bio-Rad (Hercules, CA, USA); and other reagents, salts, and buffers were purchased from Sigma-Aldrich (St. Louis, MO). Dimethylbutylammonium acetate (DMBAA)

solutions used in the electrospray ionization–mass spectrometry (ESI-MS) experiments were prepared as follows: a stock of N,N-dimethylbutyl amine (7.125 M) was diluted to 100 mM with water and adjusted to pH 7.1 with glacial acetic acid.

Enzymatic Generation of the AP Site in DNA Oligonucleotides

The 2′-deoxyuridine-containing precursor oligonucleotide (5′TTTTTdUTTTTTTTTTT-3′, 5 μL of a stock solution containing 1 nmol/μL, and 5 nmol total) was mixed with a stock solution of buffer (10 μL of 250 mM HEPES pH 7.4, containing 500 mM NaCl), followed by deionized water (28 μL) and the enzyme UDG (7 μL of a 5000 U/mL solution, 35 U) to give a solution containing 100 μM of the dU-containing oligonucleotide in 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffer (50 mM, pH 7.4, containing 100 mM NaCl), and 0.7 U/μL UDG in a total volume of 50 μL. After incubating for 2 h at 37 °C, the enzyme was removed by phenol–chloroform extraction, the DNA ethanol precipitated, and the pellet was washed with cold 80% ethanol in water. The resulting AP-containing DNA oligomer 5′TTTTTXTTTTTTTTT (where X = AP) was dried in a SpeedVac concentrator and stored at −20 °C until use.

Nonenzymatic Generation of the DNA Oligomer 5′-TTTTT-dR-3′ Containing a 3'-Terminal AP Site (3'dR) by the Acid Treatment of 5′-TTTTTA-3′

The 2′-deoxyadenosine-containing precursor oligonucleotide 5′-TTTTTA-3′ (5 μL of a stock solution containing 1 nmol/μL, 5 nmol) was mixed with an aqueous solution of HCl (2 μL of a 100 mM stock solution), followed by dilution with deionized water (13 μL) to give a solution containing 250 μM of the 5′-TTTTTA oligodeoxynucleotide in 20 μL of

10 mM HCl. The microcentrifuge tube containing the mixture was heated at 65 °C for 1 h in a thermostat-controlled aluminum dry-block system to induce depurination of the adenine residue. The reaction mixture was then passed through a Sephadex spin column prepared using G-25 beads swollen in 50 mM HEPES, pH 7.4, and 100 mM NaCl. The resulting solution of 5′-TTTTT-dR-3′ was frozen and stored at −20 °C until use.

Thermal Cleavage of the AP-Containing Oligonucleotide

The AP-containing oligodeoxynucleotide pellet from the UDG reaction described above was dissolved in 10 μL of 250 mM HEPES, pH 7.4, 500 mM NaCl, and deionized water (40 μL) to give a solution containing approximately 100 μM AP oligonucleotide and HEPES pH 7.4 (50 mM, containing 100 mM NaCl) in a total volume of 50 μL. The mixture was incubated in a thermostat-controlled aluminum dry-block system held at 85 °C. At selected times (0, 15, and 45 min), aliquots of the reaction mixture (10 μL) were removed and subjected to HPLC analysis.

Cleavage of the AP Oligonucleotide with NaOH

Four different conditions were examined. (1) 5 mM NaOH, 37 °C, 1 h. Approximately 1 nmol of the AP-containing oligonucleotide was dissolved in 18 μL of deionized water and was mixed with 2 μL of a 50 mM stock solution of NaOH in water. The solution was heated at 37 °C for 1 h and then subjected to HPLC analysis. (2) 200 mM NaOH, 37 °C, 20 min. Approximately 1 nmol of the AP-containing oligonucleotide was dissolved in 16 μL of deionized water and was mixed with 4 μL of a 1 M stock solution of NaOH in water. The solution was heated at 37 °C for 15 min and then subjected to HPLC analysis. (3) 200 mM NaOH, 37 °C, 5 h. Approximately 1 nmol of the AP-

containing oligonucleotide was dissolved in 16 μL of deionized water and was mixed with 4 μL of a 1 M stock solution of NaOH in water. The solution was heated at 37 °C for 5 h and then subjected to HPLC analysis. (4) Typical workup for the monofunctional base excision repair glycosylase assay. Approximately 1 nmol of the AP-containing oligonucleotide in 10 μL of 20 mM HEPES buffer (pH 7.4 containing 100 mM NaCl) was mixed with 10 μL of a stock solution of 1 M NaOH in water. The solution was heated at 95 °C for 2 min and then subjected to HPLC analysis.

Cleavage of the AP Oligonucleotide with Piperidine

Four different conditions were examined. (1) 5 mM piperidine, 37 °C, 1 h. Approximately 1 nmol of the AP oligonucleotide in 18 μL of HEPES (50 mM, pH 7.4, containing 100 mM NaCl) was mixed with a 2 μL of a stock solution of 50 mM piperidine in water. The solution was heated at 37 °C for 1 h and then subjected to HPLC analysis. (2) 1 M piperidine, 50 °C, 20 min. Approximately 1 nmol of the AP oligonucleotide in 10 μL of deionized water was mixed with a 40 μL of a stock solution of 1.25 M piperidine in water. The microcentrifuge tube containing the solution was heated in a 50 °C hot block for 20 min and then subjected to HPLC analysis. (3) 1 M piperidine, 95 °C, 30 min. Approximately 1 nmol of the AP oligonucleotide in 10 μL of deionized water was mixed with a 40 μL of a stock solution of 1.25 M piperidine in water. The microcentrifuge tube containing the solution was heated in a 95 °C hot block for 30 min and then subjected to HPLC analysis. (4) A control reaction by heating the AP oligonucleotide at 95 °C for 30 min, in the absence of piperidine, was carried out. A microcentrifuge tube containing approximately 1 nmol of the AP oligonucleotide in 50 μL of deionized water was heated in a 95 °C hot block for 30 min and then subjected to HPLC analysis.

Cleavage of the AP Oligonucleotide with Spermine

Approximately 10 nmol of the AP-containing DNA oligomer from the UDG reaction described above was dissolved in 20 μL of 250 mM HEPES, pH 7.4, containing 500 mM NaCl and mixed with deionized water (70 μL) and spermine (10 μL of a 50 mM stock solution in water, with the pH adjusted to 7.4) to give a final mixture containing 100 μM of the AP oligonucleotide and 5 mM spermine in HEPES buffer (50 mM, pH 7.4, containing 100 mM NaCl). The mixture was incubated at 37 °C, and aliquots of the reaction mixture (10 μL) were removed at 5 min, 30 min, 1 h, 6 h, 12 h, 24 h, and 48 h and subjected to HPLC analysis. The experiment examining the cleavage of the AP oligonucleotide induced by spermine in the presence of 2-mercaptoethanol was carried out in a similar manner by dissolving approximately 5 nmol of the AP-containing DNA oligomer from the UDG reaction described above in 10 μL of HEPES buffer (250 mM, pH 7.4, containing 500 mM NaCl), deionized water (30 μL), spermine (5 μL of a 50 mM stock solution in water, with the pH adjusted to 7.4), and 2-mercaptoethanol (5 μL of a 50 mM stock solution in water).

Generation of a Mixture Containing 3′*cis*-PUA, 3′*trans*-PUA, and 3′dR Cleavage Products Using the Conditions of Kushida et al.[64]

The AP-containing oligodeoxynucleotide pellet from the UDG reaction described above was dissolved in 5 μL of Tris-borate buffer (200 mM, pH 7.5) and deionized water (45 μL) to give a final mixture containing approximately 100 μM of the AP oligonucleotide in 50 μL of Tris-borate buffer (20 mM, pH 7.5). The mixture was incubated in a thermostat-controlled aluminum dry block system held at 90 °C for 30 min, and a 10 μL aliquot of the reaction mixture was removed and subjected to HPLC analysis.

Reactions of 2-Mercaptoethanol with the Products Arising from Cleavage of the AP Oligonucleotide

To an aliquot (10 μL) containing 1 nmol of DNA derived from cleavage of the AP-containing oligonucleotide was added 1.1 μL of a 50 mM stock solution of 2-mercaptoethanol in water. The resulting reaction mixtures, containing a 5 mM final concentration of 2-mercaptoethanol and 0.91× concentrations of the buffer and salt found in the original cleavage reaction mixtures, were incubated at 37 °C for 15 min and subjected to HPLC analysis.

Cleavage of an AP-Containing Duplex with Endonuclease III

Cleavage of an AP-containing duplex by the base-excision repair glycosylase Endo III was carried out by mixing 2 nmol of the AP-containing oligonucleotide 5′TTTTTXTTTTTTTTTT (where X = AP) with 2 nmol of the complementary strand 5′$dT_2A_{16}T_2$ in Tris-HCl buffer (20 mM, pH 8.4) containing ethylenediaminetetraacetic acid (1 mM), DTT (1 mM), and the enzyme Endo III (20 units). The mixture was incubated at 37 °C. Aliquots (10 μL) were removed at 15 min and 2 h and subjected to HPLC analysis.

Cleavage of an AP-Containing Duplex by Fpg

Cleavage of an AP-containing duplex by the base-excision repair glycosylase Fpg was carried out by mixing 1 nmol of the AP-containing oligonucleotide 5′TTTTTXTTTTTTTTTT (where X = AP) with 1 nmol of the complementary strand 5′$dT_2A_{16}T_2$ in bis-Tris-propane-HCl (pH 7.0), $MgCl_2$ (10 mM), DTT (1 mM), and the

enzyme Fpg (16 units). The mixture was incubated at 37 °C for 6 h and subjected to HPLC analysis.

HPLC Analysis of the Products Arising from the Cleavage of the AP-Containing Oligonucleotide

HPLC analyses were conducted using a reverse-phase column at 24 °C (Agilent AdvanceBio, C18, 4.6 × 50 mm, 2.7 μm) eluted with a linear gradient of 6–14% acetonitrile in aqueous 0.1 M triethylammonium acetate pH 7.0 over 20 min at a flow rate of 0.6 mL/min. The products were monitored by their absorbance at 260 nm.

ESI-QTOF-LC–MS Analysis of AP-Derived Cleavage Products

The samples for mass spectrometric analysis were prepared using 5 nmol of the AP-containing oligonucleotide. Liquid chromatography (LC)–MS data were acquired on an Agilent Technologies 6520A accurate mass quadrupole time-of-flight (QTOF) system. The samples were analyzed according to the protocol of Studzińska and Buszewski[112] with slight modifications as outlined. The sample was injected onto a C8 trap column (Michrom Bioresources Captrap) at a flow rate of 5 μL/min of 10 mM DMBAA, pH 7.1, over 4 min and separated by isocratic elution (either 80 or 42.5% methanol, 15 mM DMBAA, pH 7.1) at a flow rate of 0.4 μL/min on a 10 cm × 75 μm C8 analytical column (fused silica packed with Michrom Bioresources C8, 3.5 μm particles). Following the 4 min trap load, separation on the trap/analytical columns continued for 16 min under isocratic elution conditions. The total run time was 20 min. The mass spectra were acquired using the following parameters: negative-ion mode; VCap 2500 V; mass range 290–3200 *m*/*z*; 0.63 spectra/s; fragmentor at 300 V (250 V for IDT oligo); the internal MS recalibration was

achieved using the K/Na adducted Hexakis 1221 Chip Cube High Mass Reference compound (*m*/*z* 1279.99). The samples were loaded in sequence as follows: blank (10 mM DMBAA), sample, and blank. Multiply charged DNA peaks were deconvoluted using the maximum entropy algorithm in Qualitative Analysis software (version B.07.00 Agilent Technologies) with the following parameters: adduct = proton loss; *m*/*z* range = 600–1500 *m*/*z*; mass range = expected mass ± 2 kDa; peak height to calculate mass = 25%. The *m*/*z* values reported are neutral deconvoluted masses.

2.11 References

(1) Lindahl, T.; Karlstrom, O. Heat-Induced Depyrimidination of Deoxyribonucleic Acid in Solution. *Biochemistry* **1973**, *12*, 5151.

(2) Lindahl, T.; Nyberg, B. Rate of Depurination of Native Deoxyribonucleic Acid. *Biochemistry* **1972**, *11*, 3610.

(3) Gates, K. S.; Nooner, T.; Dutta, S. Biologically Relevant Chemical Reactions of N7-Alkyl-2′-Deoxyguanosine Adducts in DNA. *Chem Res Toxicol* **2004**, *17*, 839.

(4) David, S. S.; Williams, S. D. Chemistry of Glycosylases and Endonucleases Involved in Base-Excision Repair. *Chem Rev* **1998**, *98*, 1221.

(5) Stivers, J. T.; Jiang, Y. L. A Mechanistic Perspective on the Chemistry of DNA Repair Glycosylases. *Chem Rev* **2003**, *103*, 2729.

(6) Brooks, P. J.; Enoch, M.-A.; Goldman, D.; Li, T.-K.; Yokoyama, A. The Alcohol Flushing Response: An Unrecognized Risk Factor for Esophageal Cancer from Alcohol Consumption. *PLoS Med.* **2009**, *6* (3), e1000050. https://doi.org/10.1371/journal.pmed.1000050.

(7) Imai, K.; Slupphaug, G.; Lee, W.-I.; Revy, P.; Nonoyama, S.; Catalan, N.; Yel, L.; Forveille, M.; Kavli, B.; Krokan, H. E.; Ochs, H. D.; Fischer, A.; Durandy, A. Human Uracil-DNA Glycosylase Deficiency Associated with Profoundly Impaired Immunoglobulin Class-Switch Recombination. *Nat Immunol* **2003**, *4*, 1023.

(8) Gates, K. S. An Overview of Chemical Processes That Damage Cellular DNA: Spontaneous Hydrolysis, Alkylation, and Reactions with Radicals. *Chem Res Toxicol* **2009**, *22*, 1747.

(9) Nooner, T.; Dutta, S.; Gates, K. S. Chemical Properties of the Leinamycin–Guanine Adduct in DNA. *Chem Res Toxicol* **2004**, *17*, 942.

(10) Nakamura, J.; Swenberg, J. A. Endogenous Apurinic/Apyrimidinic Sites in Genomic DNA of Mammalian Tissues. *Cancer Res* **1999**, *59*, 2522.

(11) De Bont, R.; van Larebeke, N. Endogenous DNA Damage in Humans: A Review of Quantitative Data. *Mutagenesis* **2004**, *19*, 169.

(12) Swenberg, J. A.; Lu, K.; Moeller, B. C.; Gao, L.; Upton, P. B.; Nakamura, J.; Starr, T. B. Endogenous versus Exogenous DNA Adducts: Their Role in Carginogenesis, Epidemiology, and Risk Assessment. *Toxicol Sci* **2011**, *120*, S130.

(13) Septak, M. Kinetic Studies on Depurination and Detritylation of CPG-Bound Intermediates during Oligonucleotide Synthesis. *Nucleic Acids Res* **1996**, *24*, 3053.

(14) Chen, H.; Yao, L.; Brown, C.; Rizzo, C. J.; Turesky, R. J. Quantitation of Apurinic/Apyrimidinic Sites in Isolated DNA and in Mammalian Tissue with a Reduced Level of Artifacts. *Anal Chem* **2019**, *91*, 7403.

(15) Wilde, J. A.; Bolton, P. H.; Mazumder, A.; Manoharan, M.; Gerlt, J. A. Characterization of the Equilibrating Forms of the Abasic Site in Duplex DNA Using 17O-NMR. *J Am Chem Soc* **1989**, *111*, 1894.

(16) Overend, W. G. Deoxy-Sugars. Part XIII. Some Observations on the Feulgen Nucleal Reaction. *J Chem Soc* **1950**, 2769.

(17) Imani-Nejad, M.; Price, N. E.; Haldar, T.; Lewis, C.; Wang, Y.; Gates, K. S. Interstrand DNA Cross-Links Derived from Reaction of a 2-Aminopurine Residue with an Abasic Site. *ACS Chem Biol* **2019**, *14*, 1481.

(18) Varela, J. G.; Gates, K. S. A Simple, High-Yield Synthesis of DNA Duplexes Containing a Covalent, Thermally-Reversible Interstrand Cross-Link At a Defined Location. *Angew Chem Int Ed* **2015**, *54*, 7666.

(19) Johnson, K. M.; Price, N. E.; Wang, J.; Fekry, M. I.; Dutta, S.; Seiner, D. R.; Wang, Y.; Gates, K. S. On the Formation and Properties of Interstrand DNA-DNA Cross-Links Forged by Reaction of an Abasic Site With the Opposing Guanine Residue of 5'-CAp Sequences in Duplex DNA. *J Am Chem Soc* **2013**, *135*, 1015.

(20) Price, N. E.; Johnson, K. M.; Wang, J.; Fekry, M. I.; Gates, K. S.; Gates, K. S. Interstrand DNA–DNA Cross-Link Formation Between Adenine Residues and Abasic Sites in Duplex DNA. *J Am Chem Soc* **2014**, *136*, 3483.

(21) Catalano, M. J.; Price, N. E.; Gates, K. S. Effective Molarity in a Nucleic Acid Controlled Reaction. *Bioorg Med Chem Lett* **2016**, *26*, 2627.

(22) Varela, J. G.; Pierce, L. E.; Guo, X.; Price, N. E.; Johnson, K. M.; Yang, Z.; Wang, Y.; Gates, K. S. Interstrand Cross-Link Formation Involving Reaction of a Mispaired Cytosine Residue with an Abasic Site in Duplex DNA. *Chem Res Toxicol* **2021**, *34*, 1124.

(23) Mohni, K. N.; Wessel, S. R.; Zhao, R.; Wojciechowski, A. C.; Luzwick, J. W.; Layden, H.; Eichman, B. F.; Thompson, P. S.; Mehta, K. P. M.; Cortez, D. HMCES Maintains Genome Integrity by Shielding Abasic Sites in Single-Strand DNA. *Cell* **2019**, *176*, 144.

(24) Thompson, P. S.; Amidon, K. M.; Mohni, K. N.; Cortez, D.; Eichman, B. F. Protection of Abasic Sites during DNA Replication by a Stable Thiazolidine Protein-DNA Cross-Link. *Nat Struct Mol Biol* **2019**, *26*, 613.

(25) Halabelian, L.; Ravichandran, M.; Li, Y.; Zeng, H.; Rao, A.; Aravind, L.; Arrowsmith, C. H. Strutural Basis of HMCES Interactions with Abasic DNA and Multivalent Substrate Recognition. *Nat Struct Mol Biol* **2019**, *26*, 607.

(26) Chan, W.; Ham, Y.-H.; Jin, L.; Chan, H. W.; Wong, Y.-L.; Chan, C.-K.; Chung, P.-Y. Quantification of a Novel DNA-Protein Cross-Link Product Formed by Reacting Apurinc/Apyrimidinic Sites in DNA with Cysteine Residues in Protein by Liquid Chromotography-Tandem Mass Spectrometry Coupled with the Stable Isotope-Dilution Method. *Anal Chem* **2019**, *91*, 4987.

(27) Guthrie, J. P.; Cossar, J.; Klym, A. Halogenation of Acetone. A Method for Determining PKas of Ketones in Aqueous Solution, with an Examination of the Thermodynamics and Kinetics of Alkaline Halogenation and a Discussion of the Best Value for the Rate Constant for a Diffusion-Controlled Reaction. Energetic Requirements for a Diffusion-Controlled Reaction Involving Heavy-Atom Bond Formation. *J Am Chem Soc* **1984**, *106*, 1351.

(28) Bayley, C. R.; Brammer, K. W.; Jones, A. S. The Nucleotide Sequence in Deoxyribonucleic Acids. Part V. The Alkaline Degradation of Apurinic Sites. *J Chem Soc* **1961**, 1903.

(29) Crine, P.; Verly, W. G. A Study of DNA Spontaneous Degradation. *Biochim Biophys Acta* **1976**, *442*, 50.

(30) Lindahl, T.; Andersson, A. Rate of Chain Breakage at Apurinic Sites in Double-Stranded Deoxyribonucleic Acid. *Biochemistry* **1972**, *11*, 3618.

(31) Bailly, V.; Verly, W. G. Escherichia Coli Endonuclease III Is Not an Endonuclease but a Beta-Elimination Catalyst. *Biochem J* **1987**, *242*, 565.

(32) Kim, J.; Linn, S. The Mechanism of Action of E. Coli Endonuclease III and T7 UV Endonuclease (Endonuclease V) on AP Sites. *Nucleic Acids Res* **1988**, *16*, 1135.

(33) Brown, D. M.; Todd, A. R. Nucleic Acids. *Annu Rev Biochem* **1955**, *24*, 311.

(34) Zhou, C.; Sczepanski, J. T.; Greenberg, M. M. Mechanistic Studies on Histone Catalyzed Cleavage of Apyrimidinic/Apurinic Sites in Nucleosome Core Particles. *J Am Chem Soc* **2012**, *134*, 16734.

(35) Lawley, P. D.; Lethbridge, J.; Edwards, P. A.; Shooter, K. Inactivation of Bacteriophage T7 by Mono- and Difunctional Sulphur Mustards in Relation to Cross-Linking and Depurination of Bacteriophage DNA. *J Mol Biol* **1969**, *39*, 181.

(36) Laurence, D. J. R. Chain Breakage of Deoxyribonucleic Acid Following Treatment with Low Doses of Sulphur Mustard. *Proc R Soc A* **1963**, *271*, 520.

(37) Sugiyama, H.; Fujiwara, T.; Ura, A.; Tashiro, T.; Yamamoto, K.; Kawanishi, S.; Saito, I. Chemistry of Thermal Degradation of Abasic Sites in DNA. Mechanistic Investigation on Thermal DNA Strand Cleavage of Alkylated DNA. *Chem Res Toxicol* **1994**, *7*, 673.

(38) Bailly, V.; Derydt, M.; Verly, W. G. δ-Elimination in the Repair of AP (Apurinic/Apyrimidinic) Sites in DNA. *Biochem J* **1989**, *261*, 707.

(39) Bailly, V.; Verly, W. G. Importance of Thiols in the Repair Mechanisms of DNA Containing AP (Apurinic/Apyrimidinic) Sites. *Nucleic Acids Res* **1988**, *16*, 9489.

(40) Mazumder, A.; Gerlt, J. A.; Absalon, M. J.; Stubbe, J.; Cunningham, R. P.; Withka, J.; Bolton, P. H. Stereochemical Studies of the B-Elimination Reactions at Aldehydic Abasic Sites in DNA: Endonuclease III from Escherichia Coli, Sodium Hydroxide, and Lys-Trp-Lys. *Biochemistry* **1991**, *30*, 1119.

(41) McHugh, P. J.; Knowland, J. Novel Reagents for Chemical Cleavage at Abasic Sites and UV Photoproducts in DNA. *Nucleic Acids Res* **1995**, *23*, 1664.

(42) Liu, M.; Bandaru, V.; Bond, J. P.; Jaruga, P.; Zhao, X.; Christov, P. P.; Burrows, C. J.; Rizzo, C. J.; Dizdaroglu, M.; Wallace, S. S. The Mouse Ortholog of NEIL3 Is a Functional DNA Glycosylase in Vitro and in Vivo. *Proc Natl Acad Sci USA* **2010**, *107*, 4925.

(43) Imani-Nejad, M.; Housh, K.; Rodriguez, A. A.; Haldar, T.; Kathe, S.; Wallace, S. S.; Eichman, B. F.; Gates, K. S. Unhooking of an Interstrand Cross-Link at DNA Fork Structures by the DNA Glycosylase NEIL3. *DNA Repair* **2020**, *86*, 102752.

(44) Sarhan, S.; Seiler, N. On the Subcellular Localization of the Polyamines. *Biol Chem Hoppe-Seyler* **1989**, *370*, 1279.

(45) Male, R.; Fosse, V. M.; Kleppe, K. Polyamine-Induced Hydrolysis of Apurinic Sites in DNA and Nucleosomes. *Nucleic Acid Res* **1982**, *10*, 6305.

(46) Raspaud, E.; Chaperon, I.; Leforestier, A.; Livolant, F. Spermine-Induced Aggregation of DNA, Nucleosome, and Chromatin. *Biophys J* **1999**, *77*, 1547.

(47) Wallace, H. M.; Fraser, A. V.; Hughes, A. A Perspective of Polyamine Metabolism. *Biochem J* **2003**, *376*, 1.

(48) Yang, Z.; Price, N. E.; Johnson, K. M.; Wang, Y.; Gates, K. S. Interstrand Cross-Links Arising from Strand Breaks at True Abasic Sites in Duplex DNA. *Nucleic Acids Res* **2017**, *45*, 6275.

(49) Cornish-Bowden, A. Current IUBMB Recommendations on Enzyme Nomenclature and Kinetics. *Perspect Sci* **2014**, *1*, 74.

(50) Mazumder, A.; Gerlt, J. A.; Rabow, L.; Absalon, M. J.; Stubbe, J.; Bolton, P. H. UV Endonuclease V from Bacteriophage T4 Catalyzes DNA Strand Cleavage at Aldehydic Abasic Sites by a Syn Beta-Elimination Reaction. *J Am Chem Soc* **1989**, *111*, 8029.

(51) Manoharan, M.; Mazumder, A.; Ransom, S. C.; Gerlt, J. A.; Bolton, P. H. Mechanism of UV Endonuclease V Cleavage of Abasic Sites in DNA Determined by 13C Labeling. *J Am Chem Soc* **1988**, *110*, 2690.

(52) Piersen, C. E.; McCullough, A. K.; Lloyd, R. S. AP Lyases and DRPases: Commonality of Mechanism. *Mutat Res* **2000**, *459*, 43.

(53) Sandigursky, M.; Yacoub, A.; Kelley, M. R.; Xu, Y.; Franklin, W. A.; Deutsch, W. A. The Yeast 8-Oxoguanine DNA Glycosylase (Ogg1) Contains a DNA Deoxyribophosphodiesterase (DRpase) Activity. *Nucleic Acids Res* **1997**, *25*, 4557.

(54) Golan, G.; Zharkov, D. O.; Grollman, A. P.; Dodson, M. L.; McCullough, A. K.; Lloyd, R. S.; Shoham, G. Structure of T4 Pyrimidine Dimer Glycosylase in a Reduced Imine Covalent Complex with Abasic Site-Containing DNA. *J Mol Biol* **2006**, *362*, 241.

(55) Rahimoff, R.; Kosmatchev, O.; Kirchner, A.; Pfaffeneder, T.; Spada, F.; Brantl, V.; Müller, M.; Carell, T. 5-Formyl- and 5-Carboxydeoxycytidines Do Not Cause Accumulation of Harmful Repair Intermediates in Stem Cells. *J Am Chem Soc* **2017**, *139*, 10359.

(56) Wiederhold, L.; Leppard, J. B.; Kedar, P.; Karimi-Busheri, F.; Rasouli-Nia, A.; Weinfeld, M.; Tomkinson, A. E.; Izumi, T.; Prasad, R.; Wilson, S. H.; Mitra, S.; Hazra, T. K. AP Endonuclease-Independent DNA Base Excision Repair in Human Cells. *Mol Cell* **2004**, *15*, 209.

(57) Levin, J. D.; Johnson, A. W.; Demple, B. Homogeneous Escherichia Coli Endonuclease IV. Characterization of an Enzyme That Recognizes Oxidative Damage in DNA. *J Biol Chem* **1988**, *263*, 8066.

(58) Ren, M.; Shang, M.; Wang, H.; Xi, Z.; Zhou, C. Histones Participate in Base Excision Repair of 8-OxodGuo by Transiently Cross-Linking with Active Repair Intermediates in Nucleosome Core Particles. *Nucleic Acids Res* **2021**, *49*, 257.

(59) Wilson, D. M. Processing of Nonconventional DNA Strand Break Ends. *Env. Mol Mutagen* **2007**, *48*, 772.

(60) Ma, W.; Westmoreland, J. W.; Gordenin, D. A.; Resnick, M. A. Alkylation Base Damage Is Converted into Repairable Double-Strand Breaks and Complex Intermediates in G2 Cells Lacking AP Endonuclease. *PLoS Genet* **2011**, *7*, e1002059.

(61) Guillet, M.; Bioteux, S. Endogenous DNA Abasic Sites Cause Cell Death in the Absence of Apn1, Apn2, and Rad1/Rad10 in Saccaromyces Cerevisae. *EMBO J* **2002**, *21*, 2833.

(62) Boiteux, S.; Guillet, M. Abasic Sites in DNA: Repair and Biological Consequences in Saccaromyces Cerevisiae. *DNA Repair* **2004**, *3*, 1.

(63) Simonelli, V.; Narciso, L.; Dogliotti, E.; Fortini, P. Base Excision Repair Intermediates Are Mutagenic in Mammalian Cells. *Nucleic Acids Res* **2005**, *33*, 4404.

(64) Kushida, T.; Uesugi, M.; Sugiura, Y.; Kigoshi, H.; Tanaka, H.; Hirokawa, J.; Ojika, M.; Yamada, K. DNA Damage by Ptaquiloside, a Potent Bracken Carcinogen: Detection of Selective Strand Breaks and Identification of DNA-Cleavage Products. *J Am Chem Soc* **1994**, *116*, 479.

(65) Chung, S. J.; Verdine, G. L. Structures of End Products Resulting from Lesion Processing by a DNA Glycosylase/Lyase. *Chem Biol* **2004**, *11*, 1643.

(66) Darwanto, A.; Farrel, A.; Rogstad, D. K.; Sowers, L. C. Characterization of DNA Glycosylase Activity by Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry. *Anal Biochem* **2009**, *394*, 13.

(67) D'Ham, C.; Romieu, A.; Jaquinod, M.; Gasparutto, D.; Cadet, J. Excision of 5,6-Dihydroxy-5,6-Dihydrothymine, 5,6-Dihydrothymine, and 5-Hydroxycytosine from Define Sequence Oligonucleotides by Escherichia Coli Endonuclease III and Fpg Proteins: Kinetic and Mechanistic Aspects. *Biochemistry* **1999**, *38*, 3335.

(68) Housh, K.; Jha, J. S.; Yang, Z.; Haldar, T.; Johnson, K. M.; Yin, J.; Wang, Y.; Gates, K. S. Formation and Repair of an Interstrand DNA Cross-Link Arising from a Common Endogenous Lesion. *J Am Chem Soc* **2021**, *143*, 15344.

(69) Alexeeva, M.; Moen, M. N.; Grøsvik, K.; Tesfahun, A. N.; Xu, X. M.; Muruzábal-Lecumberri, I.; Olsen, K. M.; Rasmussen, A.; Ruoff, P.; Kirpekar, F.; Klungland, A.; Bjelland, S. Excision of Uracil from DNA by HSMUG1 Includes Strand Incision and Processing. *Nucleic Acids Res* **2019**, *47*, 779.

(70) Shishkina, I. G.; Johnson, F. A New Method for the Postsynthetic Generation of Abasic Sites in Oligomeric DNA. *Chem Res Toxicol* **2000**, *13*, 907.

(71) Pruss, D.; Gavin, I. M.; Melnik, S.; Bavykin, S. G. DNA-Protein Cross-Linking Applications for Chromatin Studies in Vitro and in Vivo. *Methods Enzym.* **1999**, *304*, 516.

(72) Vasseur, J. J.; Rayner, B.; Imbach, J. L.; Verma, S.; McCloskey, J. A.; Lee, M.; Chang, D. K.; Lown, J. W. Structure of the Adduct Formed between 3-Aminocarbazole and the Apurinic Site Oligonucleotide Model d[Tp(Ap)PT]. *J Org Chem* **1987**, *52*, 4994.

(73) Bricteux-Grégoire, S.; Verly, W. G. The Use of Thioglycolate to Distinguish between 3′ AP (Apurinic/Apyrimidinic) Endonucleases and AP Lyases. *Nucleic Acids Res* **1989**, *17*, 6269.

(74) Lindahl, T.; Ljungquist, S.; Siegert, W.; Nyberg, B.; Sperens, B. DNA N-Glycosidases: Properties of Uracil-DNA Glycosidase from Escherichia Coli. *J Biol Chem* **1977**, *252*, 3286.

(75) Varshney, U.; van de Sande, J. H. Specificities and Kinetics of Uracil Excision from Uracil-Containing DNA Oligomers by Escherichia Coli Uracil DNA Glycosylase. *Biochemistry* **1991**, *30*, 4055.

(76) Stuart, G. R.; Chambers, R. W. Synthesis and Properties of Oligodeoxynucleotides with an AP Site at a Preselected Position. *Nucleic Acids Res* **1987**, *15*, 7451.

(77) Seiner, D. R.; LaButti, J. N.; Gates, K. S. Kinetics and Mechanism of Protein Tyrosine Phosphatase 1B Inactivation by Acrolein. *Chem Res Toxicol* **2007**, *20*, 1315.

(78) Esterbauer, H.; Ertl, A.; Scholz, N. The Reaction of Cysteine with α,β-Unsaturated Aldehydes. *Tetrahedron* **1976**, *32*, 285.

(79) Esterbauer, H.; Zöllner, H.; Scholz, N. Reaction of Glutathione with Conjugated Carbonyls. *Z Naturforsch* **1975**, *30*, 466.

(80) Wakita, C.; Maeshima, T.; Yamazaki, A.; Shibata, T.; Ito, S.; Akagawa, M.; Ojika, M.; Yodoi, J.; Uchida, K. Stereochemical Configuration of 4-Hydroxy-2-Nonenal-Cysteine Adducts and Their Stereoselective Formation in a Redox-Regulated Protein. *J Biol Chem* **2009**, *284*, 28810.

(81) Jha, J. S.; Nel, C.; Haldar, T.; Peters, D.; Housh, K.; Gates, K. S. Products Generated by Amine-Catalyzed Strand Cleavage at Apurinic/Apyrimidinic Sites in DNA: New Insights from a Biomimetic Nucleoside Model System. *Chem Res Toxicol* **2021**.

(82) Engels, C.; Schwab, C.; Zhang, J.; Stevens, M. J. A.; Bieri, C.; Ebert, M.-O.; McNeill, K.; Sturla, S. J.; Lacroix, C. Acrolein Contributes Strongly to Antimicrobial and Heterocyclic Amine Transformation Activities of Reuterin. *Sci Rep* **2016**, *6*, 36246.

(83) Mattes, W. B.; Hartley, J. A.; Kohn, K. W. Mechanism of DNA Strand Breakage by Piperidine at Sites of N7 Alkylation. *Biochim Biophys Acta* **1986**, *868*, 71.

(84) Zang, H.; Gates, K. S. Sequence Specificity of DNA Alkylation by the Antitumor Natural Product Leinamycin. *Chem Res Toxicol* **2003**, *16*, 1539.

(85) Maxam, A. M.; Gilbert, W. Sequencing End-Labeled DNA with Base-Specific Chemical Cleavages. *Methods Enzym.* **1980**, *65*, 499.

(86) Minko, I. G.; Jacobs, A. C.; de Leon, A. R.; Gruppi, F.; Donley, N.; Harris, T. M.; Rizzo, C. J.; McCullough, A. K.; Lloyd, R. S. Catalysts of DNA Strand Cleavage at Apurinic/Apyrmidinic Sites. *Sci Rep* **2016**, *6*, 28894.

(87) Bender, M. L.; Williams, A. Ketimine Intermediates in Amine-Catalyzed Enolization of Acetone. *J Am Chem Soc* **1966**, *88*, 2502.

(88) Gallopo, A. R.; Cleland, W. W. Properties of 3-Hydroxypropionaldehyde 3-Phosphate. *Arch Biochem Biophys* **1979**, *195*, 152.

(89) Langenbeck, W.; Sauerbier, R. Organic Catalysts. XVII. The Hydration of Crotonaldehyde to Aldol. *Chem Ber* **1937**, *70*, 1540.

(90) Paras, N. A.; MacMillan, D. W. C. The Enantioselective Organocatalytic 1,4-Addition of Electron-Rich Benzenes to Alpha,Beta-Unsaturated Aldehydes. *J Am Chem Soc* **2002**, *124*, 7894.

(91) Enders, D.; Wang, C.; Liebich, J. X. Organocatalytic Asymmetric Aza-Michael Additions. *Chem─Eur J* **2009**, *15*, 11058.

(92) Meister, A.; Anderson, M. E. Glutathione. *Annu Rev Biochem* **1983**, *52*, 711.

(93) Szapacs, M. E.; Riggins, J. N.; Zimmerman, L. J.; Liebler, D. C. Covalent Adduction of Human Serum Albumin by 4-Hydroxy-2-Nonenal: Kinetic Analysis of Competing Alkylation Reactions. *Biochemistry* **2006**, *45*, 10521.

(94) Chen, Y.; Cong, Y.; Quan, B.; Lan, T.; Chu, X.; Ye, Z.; Hou, X.; Wang, C. Chemoproteomic Profiling of Targets of Lipid-Derived Electrophiles by Bioorthogonal Aminooxy Probe. *Redox Biol* **2017**, *12*, 712.

(95) Hansen, R. E.; Roth, D.; Winther, J. R. Quantifying the Global Cellular Thiol-Disulfide Status. *Proc Natl Acad Sci USA* **2009**, *106*, 422.

(96) Maher, R. L.; Wallace, S. S.; Pederson, D. S. The Lyase Activity of Bifunctional DNA Glycosylases and the 3'-Phosphodiesterase Activity of APE1 Contribute to the Repair of Oxidized Bases in Nucleosomes. *Nucleic Acids Res* **2019**, *47*, 2922.

(97) Meadows, K. L.; Song, B.; Doetsch, P. W. Characterization of AP Lyase Activities of Saccharomyces Cerevisiae Ntg1p and Ntg2p: Implications for Biological Function. *Nucleic Acids Res* **2003**, *31*, 5560.

(98) Alseth, I.; Korvald, H.; Osman, F.; Seeberg, E.; Bjoras, M. A General Role of the DNA Glycosylase Nth1 in the Abasic Sites Cleavage Step of Base Excision Repair in Schizosaccharomyces Pombe. *Nucleic Acids Res* **2004**, *32*, 5119.

(99) Brooks, S. C.; Adhikary, S.; Rubinson, E. H.; Eichman, B. F. Recent Advances in the Structural Mechanisms of DNA Glycosylases. *Biochim Biophys Acta* **2013**, *1834*, 247.

(100) Krokan, H. E.; Bjoras, M. Base Excision Repair. *Cold Spring Harb. Perspect Biol* **2013**, *5*, a012583.

(101) Mullins, E. A.; Rodriguez, A. A.; Bradley, N. P.; Eichman, B. F. Emerging Roles of DNA Glycosylases and the Base Excision Repair Pathway. *Trends Biochem Sci* **2019**, *44*, 765.

(102) Bhagwat, M.; Gerlt, J. A. 3'- and 5'-Strand Cleavage Reactions Catalyzed by the Fpg Protein from Escherichia Coli Occur via Successive β- and δ-Elimination Mechanisms, Respectively. *Biochemistry* **1996**, *35*, 659.

(103) Endonuclease III (Nth) | NEB https://www.neb.com/products/m0268-endonuclease-iii-nth#Product%20Information (accessed 2022 -04 -17).

(104) Kow, Y. W.; Wallace, S. S. Mechanism of Action of Escherichia Coli Endonuclease III. *Biochemistry* **1987**, *26*, 8200.

(105) Sandigursky, M.; Lalezari, I.; Franklin, W. A. Excision of Sugar-Phosphate Products at Apurinic/Apyrimidinic Sites by DNA Deoxyribophosphodiesterase of Escherichia Coli. *Radiat Res* **1992**, *131*, 332.

(106) Fromme, J. C.; Verdine, G. L. Structure of a Trapped Endonuclease III-DNA Covalent Intermediate. *EMBO J* **2003**, *22*, 3461.

(107) Silhan, J.; Nagorska, K.; Zhao, Q.; Jensen, K.; Freemont, P. S.; Tang, C. M.; Baldwin, G. S. Specialization of an Exonuclease III Family Enzyme in the Repair of 3' DNA Lesions during Base Excision Repair in the Human Pathogen Neisseria Meningitidis. *Nucleic Acids Res* **2012**, *40*, 2065.

(108) Nilsen, L.; Forstrøm, R. J.; Bjørås, M.; Alseth, I. AP Endonuclease Independent Repair of Abasic Sites in Schizosaccharomyces Pombe. *Nucleic Acids Res* **2012**, *40*, 2000.

(109) Barbado, C.; Córdoba-Cañero, D.; Ariza, R. R.; Roldán-Arjona, T. Nonenzymatic Release of N7-Methylguanine Channels Repair of Abasic Sites into an AP Endonuclease-Independent Pathway in Arabidopsis. *Proc Natl Acad Sci USA* **2018**, *115*, E916.

(110) Caldecott, K. W. Single-Strand Break Repair and Genetic Disease. *Nat Rev Genet* **2008**, *9*, 619.

(111) Hegde, M. L.; Hazra, T. K.; Mitra, S. Early Steps in the DNA Base Excision/Single-Strand Interruption Repair Pathway in Mammalian Cells. *Cell Res* **2008**, *18*, 27.

(112) Studzińska, S.; Buszewski, B. Evaluation of Ultra High-Performance [Corrected] Liquid Chromatography Columns for the Analysis of Unmodified and Antisense Oligonucleotides. *Anal Bioanal Chem* **2014**, *406*, 7127.

3 Chapter 3: Deoxyguanosine Triphosphate (dGTP) Adduct Generated by Spermine Mediated Strand Cleavage at a DNA Abasic Site

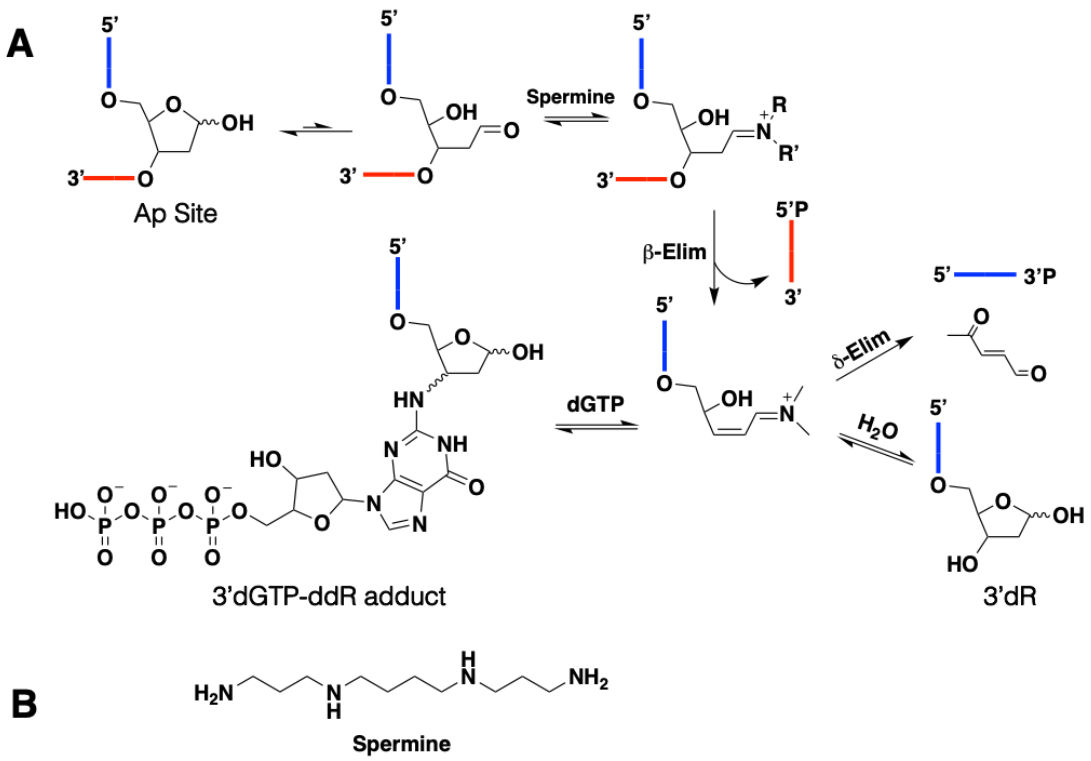
3.1 Introduction

DNA carries the genetic information for every organism.[1–3] Every function of DNA requires unwinding of the two strands of DNA and accurate reading of the genetic code.[4,5] Therefore, any chemical modification to DNA is detrimental towards survival of the organism.[6–8] But DNA is under constant threat of unavoidable chemical damage by exogenous and endogenous factors like reactive oxygen species, metabolites, UV light etc.[9] The N-glycosidic bond which holds the nucleobases to the deoxyribose sugar is a weak point in DNA. Loss of nucleobase via spontaneous hydrolysis of the glycosidic bonds generates an abasic site (AP site).[9,10] Abasic sites are the most common form of DNA damage.[10] Alkylation of DNA bases can destabilize the N-glycosidic bond and lead to abasic site.[11–13] Abasic site can form during base excision repair (BER) intermediate by removal of a damaged base by DNA Glycosylases.[14,15] Abasic site can exist as a ring closed alcohol (~99%) and ring opened aldehyde (~1%).[16,17] Abstraction of the α-proton of the ring-open aldehyde form of an abasic site can lead to spontaneous strand cleavage of DNA via β-elimination of the 3'-phosphate residues.[9,18–22] Strand breaks in DNA can lead to cytotoxicity by double strand breaks and blocks of replication if not repaired.[23–26] Studies have shown that defects in repair proteins for DNA strand break cause neurodegenerative diseases.[27–29] The nucleus of a cell has a high concentration of polyamines like spermine.[30,31] The stand cleavage of DNA at an abasic site via β-elimination can be catalyzed by spermine.[20,32–35] Spermine can react with the ring-open aldehyde of abasic

site and generate an iminium ion and make the α-protons more acidic compared to the α-protons in aldehyde form. Amine residues in DNA repair and DNA binding proteins can catalyze β-elimination and lead to strand cleavage (β-lyase reaction).[36–39,39,40] Strand break at an abasic site in DNA via β-elimination generates 3'-α,β-unsaturated aldehyde (3'PUA) end group and 5'-Phosphoryl group (5'P).[41,42]

Nucleotides are building blocks for DNA and RNA.[43] Nucleotides also serve an important role in several biological pathways like metabolism, providing cellular energy and cell signaling.[44] Deoxyguanosine Triphosphate (dGTP) is one of the monomeric nucleotide precursors for DNA synthesis. dGTP generates as a metabolite in purine metabolism, thioguanine action pathways, azathioprine action pathway and mercaptopurine action pathway.[45] The synthesis of deoxynucleotides by the enzyme ribonucleotide reductase enzyme is regulated by the substrate dGTP.[46] For tetrameric SAMHD1 deoxynucleoside triphosphate triphosphohydrolase enzyme dGTP acts as an allosteric activator by binding to the active site of the enzyme and changing conformation.[47] The cell contains ~5 μM of Deoxyguanosine Triphosphate.[48,49] The exocyclic amine of dGTP can react with α,β-unsaturated aldehyde of 3'trans-PUA generated from amine catalyzed strand cleavage at AP site in DNA under physiological condition (Scheme 3.1.1.).

In this work, we characterized a new DNA adduct generated from AP site in presence of dGTP. We provide evidence for generation of 3-dGTP-ddR adduct under physiological conditions. Formation of this unrecognized DNA adduct can be cytotoxic or mutagenic.



Scheme 3.1.1. Generation of 3'dGTP-ddR adduct from spermine catalyzed strand cleavage at an AP site in DNA in presence of deoxyguanosine triphosphate (dGTP) (Panel A). The chemical structure of spermine is shown in (panel B). In this Scheme, the blue and red lines represent DNA strands and P represents a phosphodiester linkage or a terminal phosphoryl group.

3.2 Identification and Characterization of a Novel DNA-Cleavage Adduct Arising from spermine catalyzed β-Elimination at an Abasic Site in the Presence of Deoxyguanosine Triphosphate (dGTP).

We examined the products arising from strand cleavage catalyzed by spermine at an AP site in presence and absence of Guanosine. Two different 15-mer oligodeoxynucleotide (Sequence A and B, Figure 3.2.1) has been used in this study where the AP site is generated at a defined location by treatment of a 2'-deoxyuridine-containing precursor oligodeoxynucleotide with the enzyme uracil DNA glycosylase (UDG). The

cleavage products and DNA-dGTP adducts were characterized by reverse-phase HPLC, gel-electrophoresis and nanospray ESI-QTOF mass spectrometry.

A 5′-TTTTTApTTTTTTTTTT-3′

B 5′-Cy5-ATAGATGACApAAGAG-3′

Figure 3.2.1. DNA sequences used in these studies. AP-containing duplexes were generated by the action of UDG on the corresponding dU-containing duplex. Sequence B has a fluorescent Cy5 group labeled at 5'end.

Spermine, a polyamine, is present in cells at millimolar concentration. It can catalyze β-elimination of abasic site by converting the AP aldehyde to a more reactive iminium ion and leads to strand cleavage. The β-elimination of AP site generates an α,β-unsaturated iminium ion intermediate in presence of spremine, which is more reactive towards the conjugate addition than the corresponding α,β-unsaturated aldehyde.

The 15-mer with a fluorescent Cy5 group labeled at the 5' end AP containing oligonucleotide A was prepared from the corresponding 2'-deoxyuridine-containing strand by treatment with uracil DNA glycosylase (UDG). To confirm the successful installation of AP site in oligonucleotide A was treated with piperidine (1 M, 95 ˚C, 25 min) and analyzed by electrophoresis on a denaturing 20% polyacrylamide gel (Figure 3.2.2). The treatment with hot piperidine induced strand cleavage at AP to give the expected fast migrating band for 3'P product. Incubation with NaOH (200 mM, 37 ˚C, 20 min) also induced strand cleavage at AP site and generated fast migrating bands 3'dR and 3'P.

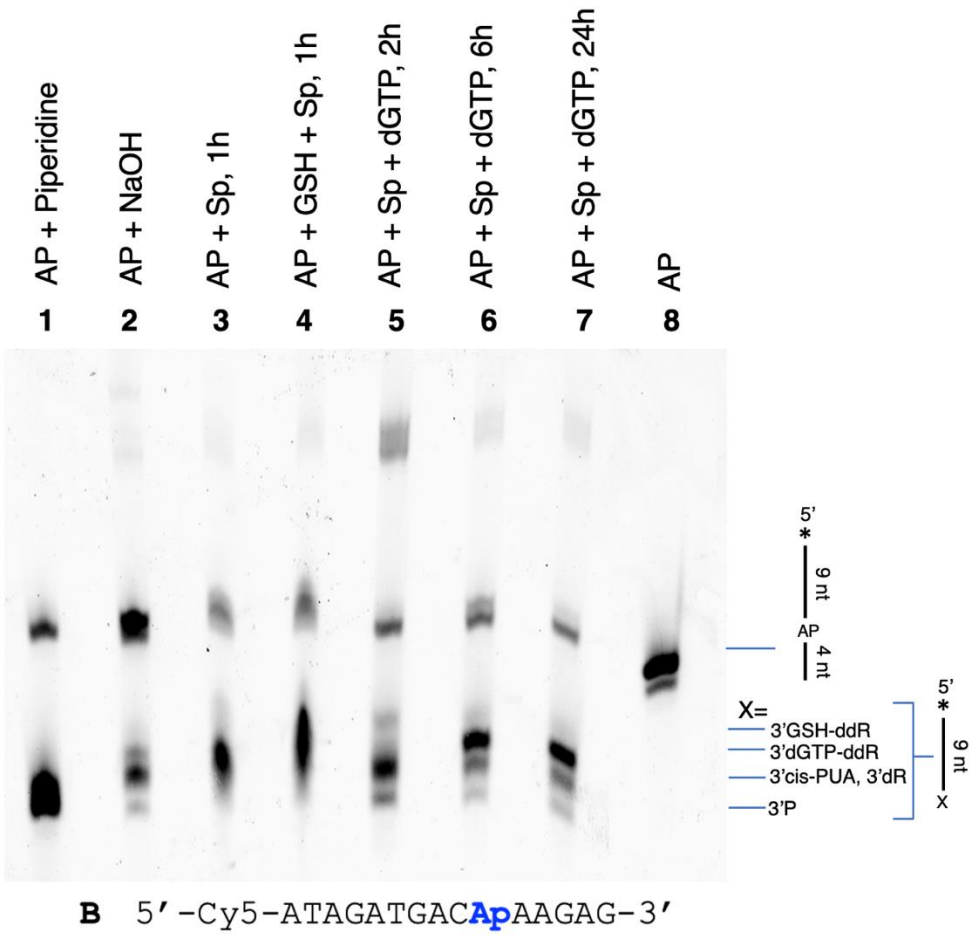


Figure 3.2.2. Gel electrophoretic evidence for generation of 3'dGTP-ddR adduct arising from spermine catalyzed strand cleavage at an AP site in the presence of dGTP. These experiments employed the 5'-Cy5-labeled sequence B shown in the Figure. Labeled DNA fragments were resolved by denaturing 20% polyacrylamide gel electrophoresis and visualized by fluorescence imaging. Lane 1: Treatment of the AP-containing oligonucleotide with piperidine (1 M, 95 ˚C, 25 min) generated the 3'P cleavage product. Lane 2: Treatment of the AP-containing oligonucleotide with NaOH (200 mM, 37 ˚C, 20 min) generated the 3'dR and 3'P cleavage products. Lane 3: Treatment of the AP-containing oligonucleotide with spermine (1 mM) in HEPES buffer (100 mM, pH 7.4) containing NaCl (200 mM) at 37 ˚C for 1 h generated 3'cis-PUA. Lane 4: Treatment of the

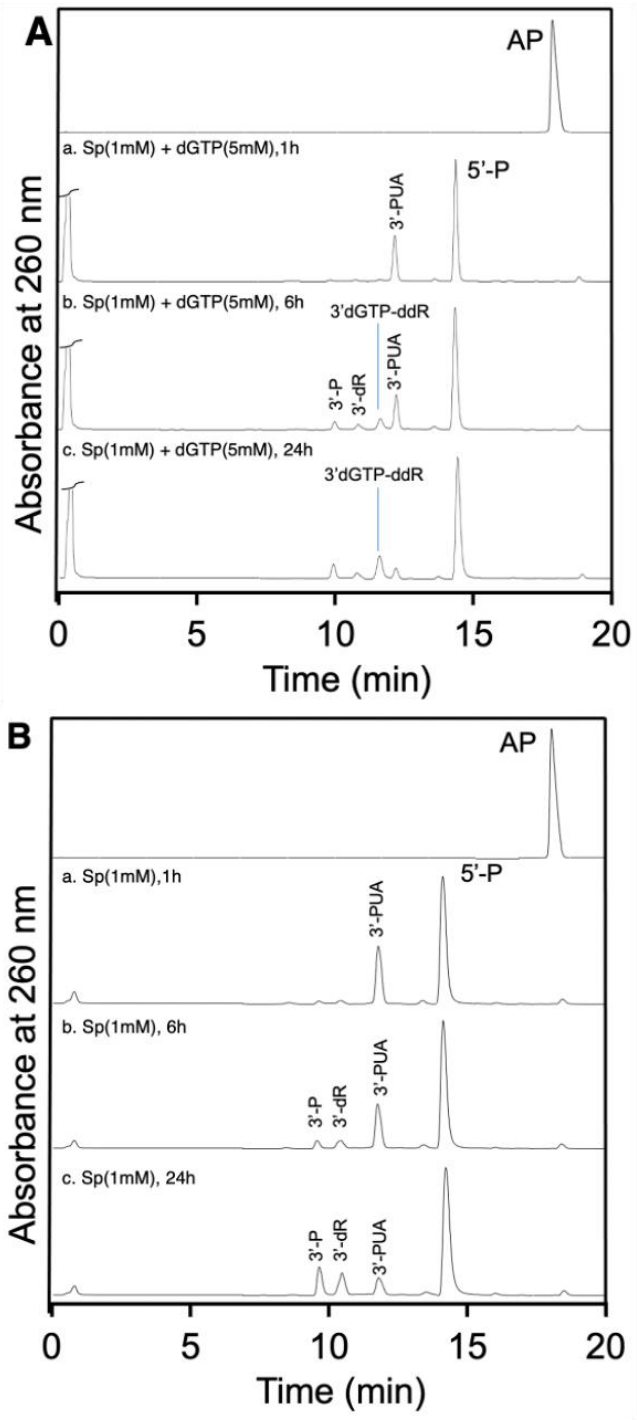
AP-containing oligonucleotide with spermine (1 mM) and GSH (5mM) in HEPES buffer (100 mM, pH 7.4) containing NaCl (200 mM) at 37 ˚C for 1 h generated 3'GS-ddR adduct. Lane 5: Treatment of the AP-containing oligonucleotide with spermine (1 mM) and dGTP (5mM) in HEPES buffer (100 mM, pH 7.4) containing NaCl (200 mM) at 37 ˚C for 2 h generated 3'cis-PUA, 3'dR and minor amount of 3'GS-ddR adduct. Lane 6: Treatment of the AP-containing oligonucleotide with spermine (1 mM) and dGTP (5mM) in HEPES buffer (100 mM, pH 7.4) containing NaCl (200 mM) at 37 ˚C for 6 h generated major amount of 3'GS-ddR adduct and minor 3'cis-PUA, 3'dR and 3'P. Lane 7: Treatment of the AP-containing oligonucleotide with spermine (1 mM) and dGTP (5mM) in HEPES buffer (100 mM, pH 7.4) containing NaCl (200 mM) at 37 ˚C for 24 h generated major amount of 3'GS-ddR adduct and minor 3'cis-PUA, 3'dR and 3'P. Lane 8: The AP-containing oligonucleotide.

To examine the formation of the 3'dGTP-ddR adduct, AP containing oligonucleotide was cleaved using Spermine (1 mM, 37 ˚C, 1h) in pH 7.4 HEPES buffer (100 mM) containing NaCl (200 mM). A fast migrating band for 3' cis-PUA was observed. When the spermine mediated cleavage reaction was done in presence of GSH (5mM, 37 ˚C, 1h) a slower migrating band compared to 3'cis-PUA for 3'GS-ddR was seen. Incubation of AP containing oligonucleotide with Spermine (1 mM) and dGTP (5mM) for 24 h showed generation of a different product.

The new product is suspected to be generated from the conjugate addition of dGTP to the α,β-unsaturated aldehyde iminium ion on the 3'-end of the Ap-derive strand cleavage (Scheme 3.1.1.). To the best of our knowledge the 3'dGTP-ddR product has not been

reported previously. The dGTP adduct migrated slower than 3'PUA in gel, presumably due to the large size of dGTP added on the 3' end of the cleavage product.

We have also used reverse-phase HPLC to characterize the new 3'dGTP-ddR adduct. We have used an AP containing 15 mer polythymidine oligodeoxynucleotide. AP site was installed by treatment with uracil DNA glycosylase (UDG) from the corresponding 2'-deoxyuridine-containing strand. AP containing oligodeoxynucleotide was treated with spermine (1 mM) in the presence of dGTP (5mM) in pH 7.4 HEPES buffer (50 mM, containing 100 mM NaCl) (Figure 3.2.2, panel A). At an early time (1h) the only products observed was 3'cis-PUA and 5'P. By 6 h a new peak was observed between the already characterized product 3'PUA and 3'dR. By 24 h the new peak becomes the major product compared to other 3' end products (3'PUA, 3'dR and 3'P). We infer that the new species eluting near 11.9 min corresponds to the 3'dGTP-ddR adduct. In a control experiment where the AP containing oligodeoxynucleotide was treated with spermine (1 mM) in absence of dGTP in pH 7.4 HEPES buffer (50 mM, containing 100 mM NaCl) for 24 h, the 3'dGTP-ddR peak was not seen (Figure 3.2.2, panel B). That proves that the new peak in Figure is the 3'dGTP-ddR adduct.



A 5′-TTTTTApTTTTTTTTTT-3′

Figure 3.2.3. HPLC analysis of products generated from spermine-mediated strand cleavage in presence of dGTP at an AP site in DNA (panel A) and in absence of dGTP at an AP site in DNA (panel B).

To further characterize the product formed by spermine (1 mM) mediated strand cleavage of AP site in presence of dGTP (5mM, 24 h, 37 ˚C) LC-ESI-TOF-MS was used. To facilitate mass spectrometric characterization, we used the unlabeled AP-containing oligonucleotide, sequence B (5'-ATAGATGACApAAGAG-3'). The major signals observed in the mass spectrum were consistent with that expected for the 3'dGTP-ddR, 3'dR and 3'P cleavage products.

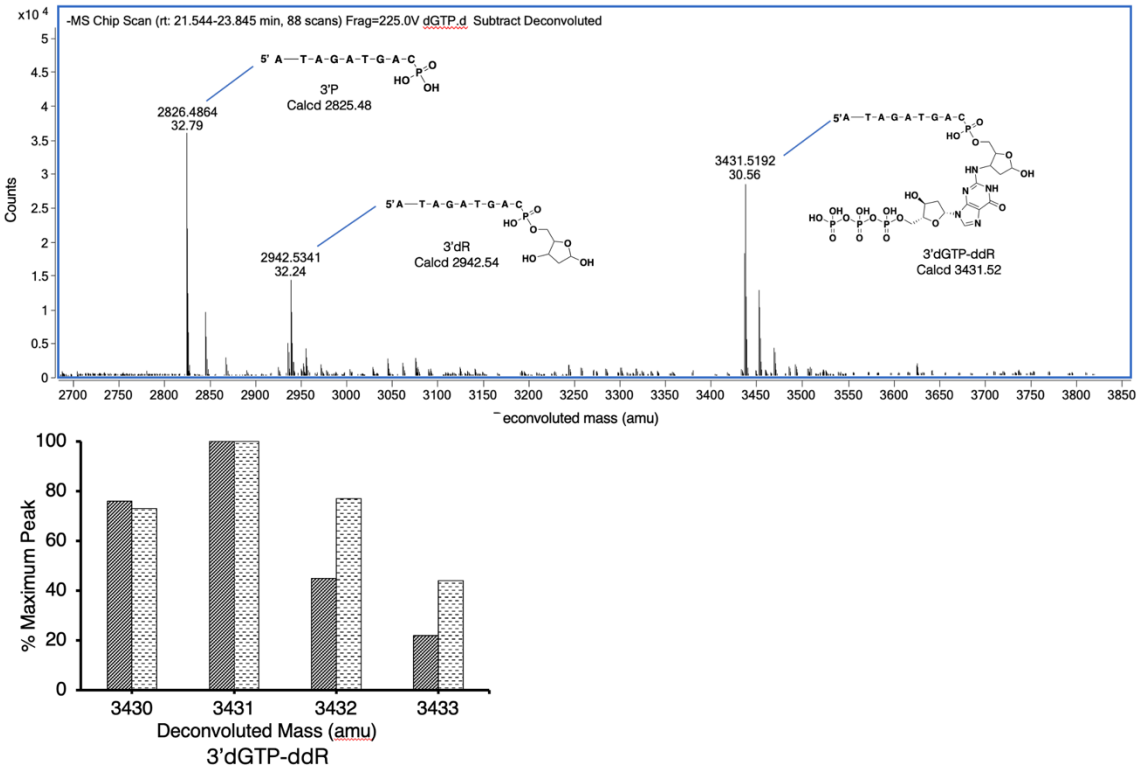


Figure 3.2.4. ESI(-)-QTOF-LC-MS analysis of products generated by spermine (1mM) mediated strand cleavage of AP site in presence of dGTP (5mM). Upper panel. Mass spectrometric analysis revealed deconvoluted neutral masses for the 3'dGTP-ddR adduct and cleavage products 3'dR and 3'P generated by by spermine (1mM) mediated strand cleavage of AP site in presence of dGTP (5mM) (24 h, 37 ˚C). Lower panel.

Comparison of experimentally measured intensities for each peak in the isotope cluster (bars on the left side of each pair) and expected intensity (bars on the right side of each pair) calculated from the molecular formula of the 3'dR cleavage product.

Results described above provide evidence for a previously uncharacterized DNA cleavage adduct generated from biologically relevant spermine catalyzed β-elimination of AP site. The evidence suggests that the conjugate addition of dGTP to the α,β-unsaturated aldehyde iminium ion on the 3'-end of the Ap-derive strand breaks (Scheme 3.1.1.).

3.3 Conclusion

Abasic site is the most common form of DNA damage. Abasic site can lead to strand cleavage which has significant biological consequences. Cells contain millimolar concentration of spermine, which can induce strand cleavage at an abasic site in DNA. Abasic site can exist as a ring-open aldehyde which can react with spermine and generate more reactive iminium ion. Because of the acidic nature of α-proton it can undergo β-elimination and form an α,β-unsaturated aldehyde iminium ion. dGTP is a building block of DNA. It is also a source of energy in some biological pathways. dGTP also plays an important role in cell signaling. The exocyclic amine of dGTP can react with the α,β-unsaturated aldehyde iminium ion of the on the 3'-end of the Ap-derive strand cleavage and generates a previously uncharacterized 3'dGTP-ddR adduct. Further study will be required for understanding of repair of this DNA adduct.

3.4 Experimental Section

Materials

Reagents were purchased from the following suppliers and were of the highest purity available: oligonucleotides were from Integrated DNA Technologies (IDT, Coralville, IA); uracil DNA glycosylase (UDG) was from New England Biolabs (Ipswich, MA); BS poly-prep columns were obtained from Bio-Rad (Hercules, CA, USA); and other reagents, salts, and buffers were purchased from Sigma-Aldrich (St. Louis, MO). Dimethylbutylammonium acetate (DMBAA) solutions used in the electrospray ionization–mass spectrometry (ESI-MS) experiments were prepared as follows: a stock of N,N-dimethylbutyl amine (7.125 M) was diluted to 100 mM with water and adjusted to pH 7.1 with glacial acetic acid.

Enzymatic generation of the AP site in DNA oligonucleotides

The 2′-deoxyuridine-containing precursor oligonucleotide (5 μL of a stock solution containing 1 nmol/μL, and 5 nmol total) was mixed with a stock solution of buffer (10 μL of 250 mM HEPES pH 7.4, containing 500 mM NaCl), followed by deionized water (28 μL) and the enzyme UDG (7 μL of a 5000 U/mL solution, 35 U) to give a solution containing 100 μM of the dU-containing oligonucleotide in 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffer (50 mM, pH 7.4, containing 100 mM NaCl), and 0.7 U/μL UDG in a total volume of 50 μL. After incubating for 2 h at 37 °C, the enzyme was removed by phenol–chloroform extraction, the DNA ethanol precipitated, and the pellet was washed with cold 80% ethanol in water. The resulting AP-containing DNA oligomer was dried in a SpeedVac concentrator and stored at −20 °C until use.

Generation of 3'dGTP-ddR cleavage product by spermine-mediated cleavage of an AP site in the presence of GTP.

The AP-containing DNA was redissolved in HEPES buffer (100 mM, pH 7.4) containing NaCl (200 mM), dGTP (5 mM), and spermine (1 mM), followed by incubation for at 37 ˚C for 24h. Aliquots of the reaction mixture were removed at prescribed time points and stored at –20 ˚C prior to HPLC analysis or gel electrophoretic analysis as described below.

Gel electrophoretic analysis of the 3'dGTP-ddR cleavage product Arising from the cleavage of the AP-containing oligonucleotide.

Aliquots of reaction mixture (10 μL) was subjected to gel electrophoresis analysis at selected time points. Samples were ethanol precipitated before gel electrophoretic analysis then redissolved in formamide loading buffer, loaded onto a 0.4 mm thick, denaturing 20% polyacrylamide gel, and electrophoresed for 15 h at 500 V. The labeled products resolved by electrophoresis were quantitatively visualized by fluorescence imaging.

HPLC analysis of the 3'dGTP-ddR cleavage product Arising from the cleavage of the AP-containing oligonucleotide.

Aliquots of reaction mixture (10 μL) was subjected to HPLC analysis at selected time points. HPLC analyses were conducted using a reverse-phase column at 24 °C (Agilent AdvanceBio, C18, 4.6 × 50 mm, 2.7 μm) eluted with a linear gradient of 6–14% acetonitrile in aqueous 0.1 M triethylammonium acetate pH 7.0 over 20 min at a flow rate of 0.6 mL/min. The products were monitored by their absorbance at 260 nm.

ESI-QTOF-LC-MS analysis of 3'dGTP-ddR cleavage product.

Samples for mass spectrometric analysis were prepared using 5 nmol of the AP-containing oligonucleotide. The 3'dGTP-ddR cleavage product was generated by spermine-mediated cleavage of the AP-containing oligonucleotide in the presence of dGTP as described above. LC-MS data were acquired on an Agilent Technologies 6520A Accurate Mass QTOF. Samples were analyzed according to the protocol of Studzinska and Buszewski, with slight modifications as outlined.[Studzińska, 2014 #4458] Sample was injected onto a C8 trap column (Michrom Bioresources Captrap) at a flow rate of 5 μL/min of 10 mM DMBAA pH 7.1 over 4 min. and separated by isocratic elution (either 80% or 42.5% methanol, 15 mM DMBAA, pH 7.1) at a flow rate of 0.4 μL/min on a 10 cm × 75 μm C8 analytical column (fused silica packed with Michrom Bioresources C8, 3.5 μm particles). Following the 4-min sample loading to trap column, separation on the trap/analytical columns continued for 16 min, under isocratic elution conditions. Total run time was 20 min. Mass spectra were acquired using the following parameters: negative-ion mode; VCap 2500 V; mass range 290-3200 m/z; 0.63 spectra/second; fragmentor at 300 V (250 V for IDT oligo); internal MS recalibration was achieved using the K/Na adducted Hexakis 1221 Chip Cube High Mass Reference compound (m/z 1279.99). Samples were loaded in sequence as follows: blank (10 mM DMBAA), sample, and blank. Multiply-charged DNA peaks were deconvoluted using the maximum entropy algorithm in Qualitative Analysis software (version B.07.00 Agilent Technologies) with the following parameters: adduct = proton-loss; m/z range = 600-1500 m/z; mass range = expected mass ±2 kDa; peak height to calculate mass = 25%. The m/z values reported are neutral deconvoluted masses.

3.5 References

(1) Todd, A. R. Chemical Structure of the Nucleic Acids. *Proc Natl Acad Sci USA* **1954**, *40*, 748.

(2) Watson, J. D.; Crick, F. H. C. A Structure for Deoxyribose Nucleic Acid. *Nature* **1953**, *171*, 737.

(3) Venter, J. C. The Sequence of the Human Genome. *Science* **2001**, *291*, 1304.

(4) Alberts, B. DNA Replication and Recombination. *Nature* **2003**, *421*, 431.

(5) Alberts, B.; Johnson, A.; Lewis, J.; Raff, M.; Roberts, K.; Walter, P. *Molecular Biology of the Cell*; 4, Ed.; 2002.

(6) Zhou, B.-B. S.; Elledge, S. J. The DNA Damage Response: Putting Checkpoints in Perspective. *Nature* **2000**, *408*, 433.

(7) Norbury, C. J.; Hickson, I. D. Cellular Responses to DNA Damage. *Annu Rev Pharmacol Toxicol* **2001**, *41*, 367.

(8) Rouse, J.; Jackson, S. P. Interfaces between the Detection, Signaling, and Repair of DNA Damage. *Science* **2002**, *297*, 547.

(9) Gates, K. S. An Overview of Chemical Processes That Damage Cellular DNA: Spontaneous Hydrolysis, Alkylation, and Reactions with Radicals. *Chem Res Toxicol* **2009**, *22*, 1747.

(10) Lindahl, T.; Nyberg, B. Rate of Depurination of Native Deoxyribonucleic Acid. *Biochemistry* **1972**, *11*, 3610.

(11) Gates, K. S.; Nooner, T.; Dutta, S. Biologically Relevant Chemical Reactions of N7-Alkyl-2′-Deoxyguanosine Adducts in DNA. *Chem Res Toxicol* **2004**, *17*, 839.

(12) Moschel, R. C.; Hudgins, W. R.; Dipple, A. Substituent-Induced Effects on the Stability of Benzylated Guanosines: Model Systems for the Factors Influencing the Stability of Carcinogen-Modified Nucleic Acids. *J Org Chem* **1984**, *49*, 363.

(13) Muller, N.; Eisenbrand, G. The Influence of N7-Substitutents on the Stability of N7-Alkylated Guanosines. *Chem-Biol Interact* **1985**, *53*, 173.

(14) Kim, Y.-J.; M Wilson III, D. Overview of Base Excision Repair Biochemistry. *Curr. Mol. Pharmacol.* **2012**, *5* (1), 3–13.

(15) Tomilin, N.; Aprelikova, O. Uracil-DNA Glycosylases and DNA Uracil Repair. *Int. Rev. Cytol.* **1989**, *114*, 125–179.

(16) Wilde, J. A.; Bolton, P. H.; Mazumder, A.; Manoharan, M.; Gerlt, J. A. Characterization of the Equilibrating Forms of the Abasic Site in Duplex DNA Using 17O-NMR. *J Am Chem Soc* **1989**, *111*, 1894.

(17) Overend, W. G. Deoxy-Sugars. Part XIII. Some Observations on the Feulgen Nucleal Reaction. *J Chem Soc* **1950**, 2769.

(18) Bayley, C. R.; Brammer, K. W.; Jones, A. S. The Nucleotide Sequence in Deoxyribonucleic Acids. Part V. The Alkaline Degradation of Apurinic Sites. *J Chem Soc* **1961**, 1903.

(19) Crine, P.; Verly, W. G. A Study of DNA Spontaneous Degradation. *Biochim Biophys Acta* **1976**, *442*, 50.

(20) Lindahl, T.; Andersson, A. Rate of Chain Breakage at Apurinic Sites in Double-Stranded Deoxyribonucleic Acid. *Biochemistry* **1972**, *11*, 3618.

(21) Bailly, V.; Verly, W. G. Escherichia Coli Endonuclease III Is Not an Endonuclease but a Beta-Elimination Catalyst. *Biochem J* **1987**, *242*, 565.

(22) Brown, D. M.; Todd, A. R. Nucleic Acids. *Annu Rev Biochem* **1955**, *24*, 311.

(23) Ma, W.; Westmoreland, J. W.; Gordenin, D. A.; Resnick, M. A. Alkylation Base Damage Is Converted into Repairable Double-Strand Breaks and Complex Intermediates in G2 Cells Lacking AP Endonuclease. *PLoS Genet* **2011**, *7*, e1002059.

(24) Guillet, M.; Bioteux, S. Endogenous DNA Abasic Sites Cause Cell Death in the Absence of Apn1, Apn2, and Rad1/Rad10 in Saccaromyces Cerevisae. *EMBO J* **2002**, *21*, 2833.

(25) Boiteux, S.; Guillet, M. Abasic Sites in DNA: Repair and Biological Consequences in Saccaromyces Cerevisiae. *DNA Repair* **2004**, *3*, 1.

(26) Simonelli, V.; Narciso, L.; Dogliotti, E.; Fortini, P. Base Excision Repair Intermediates Are Mutagenic in Mammalian Cells. *Nucleic Acids Res* **2005**, *33*, 4404.

(27) Caldecott, K. W. Single-Strand Break Repair and Genetic Disease. *Nat Rev Genet* **2008**, *9*, 619.

(28) Ahel, I.; Rass, U.; El-Khamisy, S. F.; Katyal, S.; Clements, P. M.; McKinnon, P. J.; Caldecott, K. W.; West, S. C. The Neurodegenerative Disease Protein Aprataxin Resolves Abortive DNA Ligation Intermediates. *Nature* **2006**, *443* (7112), 713–716. https://doi.org/10.1038/nature05164.

(29) Kalasova, I.; Hanzlikova, H.; Gupta, N.; Li, Y.; Altmüller, J.; Reynolds, J. J.; Stewart, G. S.; Wollnik, B.; Yigit, G.; Caldecott, K. W. Novel PNKP Mutations Causing Defective DNA Strand Break Repair and PARP1 Hyperactivity in MCSZ. *Neurol. Genet.* **2019**, *5* (2). https://doi.org/10.1212/NXG.0000000000000320.

(30) Sarhan, S.; Seiler, N. On the Subcellular Localization of the Polyamines. *Biol Chem Hoppe-Seyler* **1989**, *370*, 1279.

(31) Raspaud, E.; Chaperon, I.; Leforestier, A.; Livolant, F. Spermine-Induced Aggregation of DNA, Nucleosome, and Chromatin. *Biophys J* **1999**, *77*, 1547.

(32) Male, R.; Fosse, V. M.; Kleppe, K. Polyamine-Induced Hydrolysis of Apurinic Sites in DNA and Nucleosomes. *Nucleic Acid Res* **1982**, *10*, 6305.

(33) Wallace, H. M.; Fraser, A. V.; Hughes, A. A Perspective of Polyamine Metabolism. *Biochem J* **2003**, *376*, 1.

(34) Yang, Z.; Price, N. E.; Johnson, K. M.; Wang, Y.; Gates, K. S. Interstrand Cross-Links Arising from Strand Breaks at True Abasic Sites in Duplex DNA. *Nucleic Acids Res* **2017**, *45*, 6275.

(35) McHugh, P. J.; Knowland, J. Novel Reagents for Chemical Cleavage at Abasic Sites and UV Photoproducts in DNA. *Nucleic Acids Res* **1995**, *23*, 1664.

(36) Brooks, S. C.; Adhikary, S.; Rubinson, E. H.; Eichman, B. F. Recent Advances in the Structural Mechanisms of DNA Glycosylases. *Biochim Biophys Acta* **2013**, *1834*, 247.

(37) Kim, J.; Linn, S. The Mechanism of Action of E. Coli Endonuclease III and T7 UV Endonuclease (Endonuclease V) on AP Sites. *Nucleic Acids Res* **1988**, *16*, 1135.

(38) Zhou, C.; Sczepanski, J. T.; Greenberg, M. M. Mechanistic Studies on Histone Catalyzed Cleavage of Apyrimidinic/Apurinic Sites in Nucleosome Core Particles. *J Am Chem Soc* **2012**, *134*, 16734.

(39) Mazumder, A.; Gerlt, J. A.; Absalon, M. J.; Stubbe, J.; Cunningham, R. P.; Withka, J.; Bolton, P. H. Stereochemical Studies of the B-Elimination Reactions at Aldehydic Abasic Sites in DNA: Endonuclease III from Escherichia Coli, Sodium Hydroxide, and Lys-Trp-Lys. *Biochemistry* **1991**, *30*, 1119.

(40) Sczepanski, J. T.; Wong, R. S.; McKnight, J. N.; Bowman, G. D.; Greenberg, M. M. Rapid DNA-Protein Cross-Linking and Strand Scission by an Abasic Site in a Nucleosome Core Particle. *Proc Natl Acad Sci USA* **2010**, *107*, 22475.

(41) Jha, J. S.; Nel, C.; Haldar, T.; Peters, D.; Housh, K.; Gates, K. S. Products Generated by Amine-Catalyzed Strand Cleavage at Apurinic/Apyrimidinic Sites in DNA: New Insights from a Biomimetic Nucleoside Model System. *Chem Res Toxicol* **2021**.

(42) Haldar, T.; Jha, J. S.; Yang, Z.; Nel, C.; Housh, K.; Cassidy, O. J.; Gates, K. S. Unexpected Complexity in the Products Arising from NaOH-, Heat-, Amine-, and Glycosylase-Induced Strand Cleavage at an Abasic Site in DNA. *Chem. Res. Toxicol.* **2022**, *35* (2), 218–232. https://doi.org/10.1021/acs.chemrestox.1c00409.

(43) Nucleotide https://www.genome.gov/genetics-glossary/Nucleotide (accessed 2022 -04 -19).

(44) Mendz, G. L. Nucleotide Metabolism. In *Helicobacter pylori: Physiology and Genetics*; Mobley, H. L., Mendz, G. L., Hazell, S. L., Eds.; ASM Press: Washington (DC), 2001.

(45) Human Metabolome Database: Showing metabocard for dGTP (HMDB0001440) https://hmdb.ca/metabolites/HMDB0001440 (accessed 2022 -04 -19).

(46) Cory, J. G.; Mansell, M. M.; Whitford, T. W. Control of Ribonucleotide Reductase in Mammalian Cells. *Adv. Enzyme Regul.* **1976**, *14*, 45–62. https://doi.org/10.1016/0065-2571(76)90007-8.

(47) Ji, X.; Wu, Y.; Yan, J.; Mehrens, J.; Yang, H.; DeLucia, M.; Hao, C.; Gronenborn, A. M.; Skowronski, J.; Ahn, J.; Xiong, Y. Mechanism of Allosteric Activation of SAMHD1 by DGTP. *Nat. Struct. Mol. Biol.* **2013**, *20* (11), 1304–1309. https://doi.org/10.1038/nsmb.2692.

(48) Traut, T. W. Physiological Concentrations of Purines and Pyrimidines. *Mol. Cell. Biochem.* **1994**, *140* (1), 1–22. https://doi.org/10.1007/BF00928361.

(49) Dean, P.; Hirt, R. P.; Embley, T. M. Microsporidia: Why Make Nucleotides If You Can Steal Them? *PLoS Pathog.* **2016**, *12* (11), e1005870. https://doi.org/10.1371/journal.ppat.1005870.

VITA

Tuhin Subhra Haldar was born in Kolkata, India. After graduating from Diamond Harbor High School in May 2011, he attended National Institute of Science Education and Research (NISER), Bhubaneswar, India where he earned his Integrated M.Sc. in Chemistry in 2016. While at NISER, he did his master's thesis research on mechanochemistry under supervision of Dr. Prasenjit Mal. After graduating from NISER he worked as a research fellow at Regional Centre for Biotechnology (RCB), Faridabad, India for 1 year under Dr. Tushar K Maiti. In fall of 2017 he was accepted into the PhD program for Chemistry in University of Missouri-Columbia. He joined Professor Gates' research group, in spring, 2018 where he did his doctoral research on abasic sites in DNA. After graduating with a PhD in Chemistry in May 2022, he took a position as a Process Development Scientist for Eurofins Genomics in Louisville, Kentucky.