Articles

Environmental Health Perspectives Volume 102, Number 11, November 1994

Near-Ultraviolet Mutagenesis in Superoxide Dismutase-deficient Strains of Escherichia coli

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Abstract

We compared mutagenic spectra induced by polychromatic near-ultraviolet radiation (near-UV; 300-400 nm) with superoxide anion (O_2^-) -dependent mutagenesis using a set of Escherichia coli tester strains. Near-UV radiation produced increased frequencies of G:C to A:T transitions, G:C to T:A and A:T to T:A transversions, and small increases in frameshift mutations in wild-type cells. Tester strains lacking superoxide dismutase (SOD) activity (sodAsodB double mutants) demonstrated high spontaneous mutation frequencies and increased near-UV sensitivity. The double mutants also showed increased mutations induced by near-UV compared to either isogenic wild type, sodA or sodB single mutants. Futhermore, these mutants had an unusual spontaneous mutation spectrum, with a predominance of A:T to T:A transversions, followed by G:C to T:A transversions and frameshifts generated in runs of adenines in both the +1 and -1 direction. Other frameshifts were detected to a lesser degree. The oxygen dependency and the type of mutations spontaneously induced in SOD-deficient cells indicated that this mutagenic spectrum was caused by oxidative DNA damage. However, no apparent synergistic action between near-UV radiation and an increased flux of O_2^- could be detected. From the frequency and types of mutations induced by the two agents, we speculate that near-UV-induced mutagenesis and O_2^--dependent mutagenesis involve, in part, different lesion(s) and/or mechanism(s). The nature and possible mutagenic pathways of each are discussed. **Key words:** A:T to T:A transversion, free radicals, hydroxyl radical, mutagenic specificity, near-UV radiation, superoxide anion, superoxide dismutase. **Environ Health Perspect** 102: 88-94(1994)

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We thank C. Cupples, D. Touati, and J. Imlay for kindly providing strains and for helpful discussion. This publication was made possible by grant number ES04889 from the National Institute of Environmental Health Sciences.

Received 15 July 1993; accepted 20 October 1993.

Introduction

Near-ultraviolet radiation (near-UV; 320-400 nm) has been shown to be cytotoxic, mutagenic, and weakly carcinogenic (*1-3*). Near-UV may be one of the most ubiquitous mutagens to which organisms are exposed.

Furthermore, with the depletion of the ozone filter in the stratosphere, there is increasing concern that additional near-UV radiation may impinge on the earth's surface.

Cellular death and DNA damage by near-UV occur mainly by indirect photosensitization pathways that produce reactive intermediates from intracellular chromophores such as porphyrin, flavins, and reduced nicotinamide coenzymes; however, near-UV may also damage DNA (*4*), key enzymes (*5*), and thiolated tRNA (*1*) directly. Near-UV generation of reactive oxygen species (e.g., hydroxyl radicals, hydrogen peroxide, superoxide anion, and singlet oxygen) kills cells; near-UV radiation in the absence of oxygen significantly reduces cell death (*6*). Although hydrogen peroxide (H_2O_2) resulting from normal metabolism may play an important role in spontaneous mutagenesis in *E. coli* (*7*,*8*), H_2O_2 generated by 365-nm near-UV radiation is not important in the death of some Chinese hamster ovary cell lines (*9*). It has been speculated that much of the toxicity of superoxide anion (O_2^-) and H_2O_2 in cells is due to hydroxyl radical (OH·) or some other reactive species generated by a Haber-Weiss reaction, using iron as a catalyst (*10*). Increased intracellular O_2^- concentrations resulting from near-UV irradiation (*11*) may react in such a way with H_2O_2 as to produce highly reactive OH· that damages DNA directly. On the other hand, while the reactivity of O_2^- has been questioned (*12*), there is increasing evidence that O_2^- can directly damage such molecules (*13*).

Compounds that produce oxygen free-radicals have been shown to be mutagenic (*14*,*15*), while mutagenesis by free-radical generators can be prevented by free-radical scavengers (*8*). Of particular interest, *E. coli* mutants completely lacking superoxide dismutase (SOD) have greatly enhanced mutation rates during aerobic growth, and treatments that increase the flux of O_2^- further stimulate mutagenesis in these strains (*15*). Further, near-UV radiation significantly increases the mutation frequency in SOD-deficient cells (*16*). This study (*16*) suggests that the synergistic action of near-UV and O_2^- induces premutational lesions and the enzyme exonuclease III converts these lesions to mutations. Further evidence of the importance of O_2^- in mutagenesis is provided, as an increase in the aerobic, spontaneous mutation rate has been reported in copper, zinc SOD mutants of *Saccharomyces cerevisiae* (*17*).

In *E. coli*, two forms of SOD are produced: manganese SOD (MnSOD) encoded by the *sodA* gene and inducible under increased levels of oxygen, and iron SOD (FeSOD) encoded by the *sodB* gene, which is not induced by oxidative stress but is synthesized constitutively (*18*). Despite the strong evidence of near-UV and O_2^- involvement in mutagenesis, little is known about the frequency and types of mutations induced by these agents, either individually or in some synergistic fashion.

Using a set of *E. coli* mutagenicity tester strains that can detect the six base substitutions and five specific frameshifts in the *lacZ* gene (*19*,*20*), we determined a mutagenic spectrum in cells under an increased flux of O_2 radicals and the involvement of near-UV in this mutagenicity. Because reports vary on the mutagenicity of redox cycling compounds, such as plumbagin and paraquat [drugs that are highly toxic at the concentrations required to observe significant mutagenesis (*21*,*22*)], a more direct approach to analyzing O_2^- mutagenicity was taken. Additionally, because O_2^- is generated during normal aerobic metabolism, we assessed O_2^--dependent mutagenesis in tester strains completely lacking SOD.

Materials and Methods

The *E. coli* strains used in this study are described in Table 1. Briefly, the indicator or tester strains CC101-CC111 (kindly supplied by C. Cupples, Department of Biology, Concordia University) are derivatives of the strain P90C [*ara* *(lac proB)XIII*] carrying an F′ *lacI*$^-$ *Z*′ *proB*$^+$ episome. Each strain carries a different *lacZ* mutation affecting one of two crucial active site residues of ß-galactosidase, Glu-461 or Tyr-503. A brief description of each reversion event necessary to restore the Lac$^+$ phenotype is listed in Table 2. A complete description of the nucleotide sequences involved in Lac$^+$ reversion for each of the strains is given in Cupples and Miller (*19*) and Cupples et al. (*20*).

Table 1. Bacterial strains

Strain	Genotype and description[a]	Reference or source
CC101–CC111	P90C [ara Δ(lac proB)XIII] F' lacI⁻ Z⁻ proB⁺	(*20*)
QC781	K-12 F- Δlac U169 rpsL ø(sodA::MudPR13)25 Cm^R	(*24*)
QC773	K-12 F- Δlac U169 rpsL ø(sodB-kan)1-Δ2 Km^R	(*24*)
RKCC101–RKCC111	As CC101-CC111 but ø(sodA::MudPR13)25 Cm^R ø(sodB-kan)1-Δ2 Km^R	This study[b]
AB1157	F- thr-1 leuB6 proA2 his-4 thi-1 argE3 lacY1 galK2 rpsL supE44 ara-14 xyl-15 mtl-1 tsx-33	(*7*)
JI130	As AB1157 but ø(sodA::Mud PR13)25 Cm^R	(*7*)
JI131	As AB1157 but ø(sodB-kan)1-Δ2 Km^R	(*7*)
JI132	As AB1157 but ø(sodA::Mud PR13)25 Cm^R ø(sodB-kan)1-Δ2 Km^R	(*7*)

[a] Cm^R, chloramphenicol resistance; Km^R, kanamycin resistance.
[b] P1.QC781 X CC101–CC111 select Cm^R and Lac⁻ then P1.QC773 select Km^R and Lac⁻.

Table 2. Reversion necessary to restore the Lac^+ phenotype in strains CC101–CC111

Strain	Reversion event ($Lac^- \rightarrow Lac^+$ phenotype)
CC101	A:T → C:G
CC102	G:C → A:T
CC103	G:C → C:G
CC104	G:C → T:A
CC105	A:T → T:A
CC106	A:T → G:C
CC107	+1G
CC108	-1G
CC109	-2(-C-G-)
CC110	+1A
CC111	-1A

With respect to SOD phenotype, we studied four tester strains for each mutational event: wild-type Sod^+, *sodA* and *sodB* single mutants, and *sodAsodB* double mutants. The *sodA* and *sodB* mutations were introduced into strains CC101-CC111 by generalized transduction using wild-type phage P1 (*23*). P1 phage lysates were prepared from donor strains QC781 (*sodA*) and QC773 (*sodB*) (obtained from D. Touati, Institut Jacques Monod, CNRS, Université Paris).

To further assess the Sod^- phenotypes in the constructed indicator strains, near-UV inactivation and mutagenesis were compared to other strains [JI130 (*sodA*), JI131 (*sodB*), JI132 (*sodAsodB* double mutant)] that contain the same mutations but in the AB1157 background (supplied by J. Imlay, Department of Biochemistry, Duke University).

Precultures were grown overnight with appropriate antibiotics and shaking (200 rpm in a rotary bath) at 37°C in Luria-Bertani broth [LB; 10 g/l tryptone (Difco Laboratories, Detroit, Michigan), 5 g/l yeast extract (Difco), and 10 g/l NaCl (Fisher Scientific Co., Springfield, New Jersey)] solidified as required with 15 g/l Bacto agar (Difco) (*23*). Cultures were grown with chloramphenicol (Cm) and kanamycin (Km) (Sigma Chemical Co., St. Louis, Missouri) at concentrations of 30 µg/ml and 50 µg/ml, respectively. We determined optical density (OD) of growing cultures using a Varian Cary 210 dual-beam spectrophotometer (Varian Instrument Group, Columbia, Maryland).

The *sodA* and *sodB* mutations were introduced into each tester strain, CC101- CC111, using wild-type phage P1 in generalized transduction as described (*23*). We prepared P1 phage lysates from strains QC781 (*sodA*) and QC773 (*sodB*) with transductants being scored on LB medium containing 40 µg/ml 5-bromo-4-chloro-3-indolyl-ß-d-galactosidase (X-Gal; Research Organics, Inc., Cleveland, Ohio), 0.01 M sodium citrate (Fisher), and 30 µg/ml Cm and/or 50 µg/ml Km. Successful transductants appeared as colorless, Cm^R and/or Km^R colonies. We used X-Gal in this selection to avoid any transductants that may have undergone spontaneous Lac^+ reversion.

Transduction resulted in the creation of three superoxide dismutase mutants for each indicator strain--*sodA* and *sodB* single mutants and *sodAsodB* double mutants (designated RKCC101-RKCC111).

We confirmed Sod^- phenotypes for all strains by a gel electrophoresis activity stain. Each strain was inoculated into 20 ml LB broth with appropriate antibiotics and grown to an OD_{600} of 1.5 at 37°C, with shaking. Cell suspensions were washed twice by centrifugation and resuspension in wash buffer (0.05 M Tris-HCl, pH 7.5; 0.01 M Mg^{2+}-acetate; 0.001 M EDTA; 0.0001 M dithiothreitol; and 10% glycerol). We prepared crude extracts by resuspending the washed cell pellets in 1.0 ml of sonication buffer (0.01 M Tris-HCl, pH 7.5; 0.1 M KCl; 0.01 M $MgSO_4$; 0.0001 M dithiothreitol; and 10% glycerol) and disrupting the cells by sonication as described by Carlioz and Touati (*24*).

Crude extract, or approximately 30 µg of protein, was loaded onto non-denaturing 9.5% polyacrylamide gels. We separated SOD isoenzymes by electrophoresis under 25 mA constant current and visualized them using an activity stain (*25*).

From overnight precultures grown in LB medium, a 1:20 dilution of the cultures was made into 20 ml of fresh LB with appropriate antibiotics present. Bacterial cultures were grown in 250-ml at 37°C with shaking at 200 rpm to an OD_{550} of 1.0-1.3. The late logarithmic phase cultures were sedimented by centrifugation, washed, and resuspended at a concentration of approximately 5-8 x 10^8 cells/ml in 10 ml M9 buffer (*26*) at ambient temperature (25°C). Broad-spectrum near-UV radiation was provided by eight GTE Sylvania F15T8/black-light blue-integral-filter light bulbs with emission in the 300-420 nm range and a peak at approximately 365 nm (GTE Sylvania Engineering Bulletin 0-306, GTE Sylvania, Danvers, Massachusetts). The ends of the bulbs were sealed with black tape to prevent any contamination from energy in the far-ultraviolet region. The eight bulbs were housed radially in a wooden box as described (*4*), equipped with a built-in fan for temperature regulation, a sample holder, and an air pump for mixing and aeration of cells. We irradiated the 10-ml cell suspensions for 35 min at ambient temperature in 15 x 100 mm Pyrex glass test tubes with gentle aeration. Fluence rates behind the Pyrex glass were determined to be 17.3 J/m^2/sec at the 365 nm wavelength using a Spectroline DM-365N ultraviolet meter.

To measure cell survival, 0.1-ml aliquots were removed every 5 min during irradiation, serially diluted in M9 buffer, appropriately plated onto LB medium, and incubated for 24 hr at 37°C before viable cell counts where made.

To monitor mutations occurring spontaneously as well as those induced by near-UV radiation in wild-type and SOD mutants, we used two assays. First, mutagenesis was inferred by measuring rifampicin-sensitive (Rif^S) to rifampicin-resistant (Rif^R) mutation frequency. The site of action of the antibiotic rifampicin is the ß subunit of the RNA polymerase. Rifampicin-resistant mutants accomplish transcription with an altered ß subunit due to mutations in the *rpoB* gene (*27*). It is considered that most of these mutations are base substitutions. Therefore, measuring the Rif^S to Rif^R mutation frequency is a sensitive assay for monitoring this class of mutational events. We also measured mutagenesis by assaying the frequency of thymine-requiring (Thy^-) mutants. Mutations in the thymidylate synthetase gene (*thyA*) are resistant to the drug trimethoprim and can be selected from a Thy^+ population (*23*). Assaying mutagenesis in Sod^- *E. coli* by monitoring Rif^S to Rif^R and Thy^+ to Thy^- mutation frequencies was reported in Farr et al. (*15*), and the thymidylate synthetase gene has been reported to be a useful quantitative mutation marker in Chinese hamster cells (*28*).

At 5-min intervals throughout the 35 min of near-UV irradiation, 0.1-ml aliquots were removed from the treated cell suspensions and inoculated into 5 ml of fresh LB broth. After overnight growth at 37°C with shaking, 0.1-ml aliquots of each culture were plated in duplicate directly onto LB medium containing 100 µg/ml rifampicin (Sigma). Another 0.1-ml aliquot of each culture was plated in duplicate onto LB medium containing 200 µg/ml thymine and 15 µg/ml trimethoprim. A final 0.1 ml aliquot was removed, diluted, and plated onto LB medium without trimethoprim and incubated 24 hr at 37°C to determine the titer of each overnight culture. We incubated plates containing rifampicin or thymine and trimethoprim for 36 hr at 37°C, at which time mutants were scored and the number of mutants per total viable cells were calculated for all tester strains. Strains AB1157, JI130, JI131, and

JI132 were handled in a similar manner.

To detect any mutagenic specificity occurring as a result of treatment with a mutagen, we assessed the Lac^+ reversion frequency or each indicator strain. For mutagenic specificity induced by near-UV treatment, 0.1-ml aliquots were taken from the overnight cultures described above and plated in duplicate directly onto lactose-minimal A medium (*23*). Another 0.1-ml aliquot was removed, diluted, and plated onto glucose-minimal A medium (same as above, except supplemented with 0.2% glucose instead of lactose) to again determine the titer of each overnight culture. Plates were incubated 48 hr at 37°C before viable cell counts were made and Lac^+ reversion frequencies calculated.

As a control, we tested the ability of the wild-type indicator strains to detect mutagenic specificity induced by other mutagens. Mutagenesis with *N*-methyl-*N*'-nitro-*N*-nitrosoguanidine (MNNG; Aldrich Chemical Co., Inc., Cedar Knolls, New Jersey), ethyl methanesulfonate (EMS; Aldrich), and far-ultraviolet irradiation (far-UV; 254 nm) was essentially as outlined by Cupples and Miller (*19*). Handling of cultures, plating, and scoring of Lac^+ revertants, as well as monitoring mutagenic treatments, were as described above.

Results

Figure 1 presents the inactivation data expressed as percent survival during 35 min of near-UV irradiation for strains CC101-CC111 and the corresponding SOD mutants. Figures 2-5 represent the Rif^R and Thy^- mutation frequencies induced by near-UV for wild-type and SOD mutant tester strains. With respect to SOD phenotype, all the tester strains were qualitatively identical in response to near-UV treatment. The data represent an average of three to five individual experiments for each strain (two plates per time point) and then a final average for all 11 strains. The standard error was calculated for mutational frequency data and is so indicated. Identical experiments were carried out using the AB1157-derived strains, which produced near-UV survival and mutation frequencies similar to those of the mutant indicator strains (data not shown). In general, the near-UV survival curves for the wild-type, *sodA*, or *sodB* mutant indicator strains were qualitatively similar, while all of the *sodAsodB* double mutants were more sensitive to near-UV irradiation.

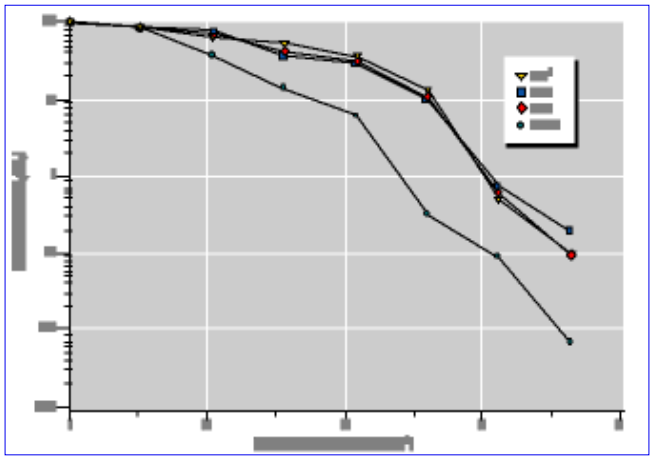

Figure 1. Comparision of near-UV sensitivity of strains containing mutations in superoxide dismutase genes with wild-type allele.

The spontaneous mutation frequency, as monitored by Rif^R, was approximately twofold greater in the *sodA* mutant indicator strains (19.2 versus 9.6) and sixfold greater in the *sodAsodB* double mutants (RKCC101-RKCC111) compared to the isogenic wild types (63 versus 9.6). A threefold increase in the spontaneous Thy^- mutation frequency was seen in the *sodA sodB* double mutant tester strains (190 versus 63), whereas only a slight increase was seen in strains with the *sodA* mutation alone. Both Rif^R and Thy^- mutation frequencies increased by approximately twofold in wild-type indicator strains after 20.8 and 26.0 kJ/m^2 (20 and 25 min, respectively) of near-UV exposure. However, mutations were not induced as a simple linear function of the time of irradiation. The

numbers of Rif^R and Thy^- mutants decreased below spontaneous mutation frequencies as the number of cells surviving near-UV treatment decreased. A similar pattern of increased mutation frequency was seen in the *sodA* mutants. However, the *sodAsodB* double mutants had even higher mutation frequencies induced by near-UV. Tester strains lacking both the MnSOD and the FeSOD had a fourfold increase in the Rif^R and almost a sixfold increase in the Thy^- mutation frequencies as a result of near-UV irradiation. Although the calculated Rif^R and Thy^- frequencies are estimates because they incorporate variations in titration of viable bacteria plated and titration of mutants among total bacteria plated, the data do suggest that there is an overall mutation increase of twofold induced by near-UV fluences between 20 and 25 kJ/m^2 in wild-type tester strains. In addition, near-UV induced a significant increase in mutations occurring in the *sodAsodB* double mutants.

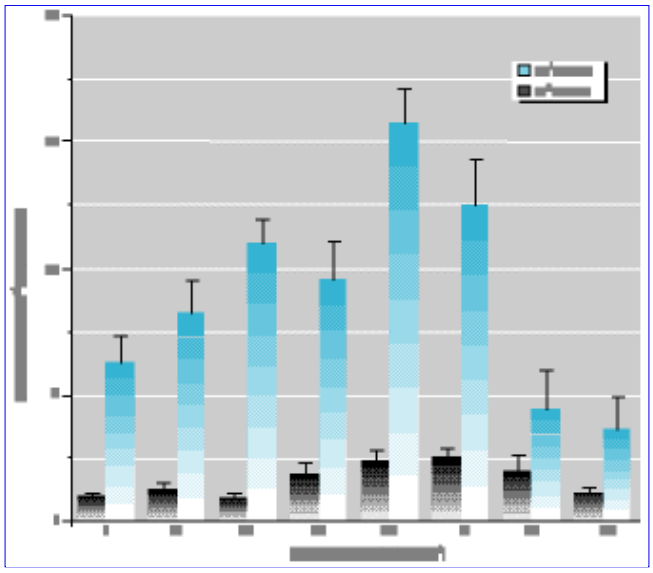

Figure 2. Mutation frequencies of wild-type allele at varying doses of near-UV, as measured by resistance to rifampicin (Rif^R) and to thymine dependency (Thy^-).

Wild-type tester strains CC101- CC111 were treated with MNNG, EMS, and far-UV to confirm their sensitivity in detecting mutagenic specificity as a result of exposure to various mutagens. Several trials using these strains verified their sensitivity, providing results qualitatively similar to those reported in Cupples and Miller (*19*) and Cupples et al. (*20*) (data not shown).

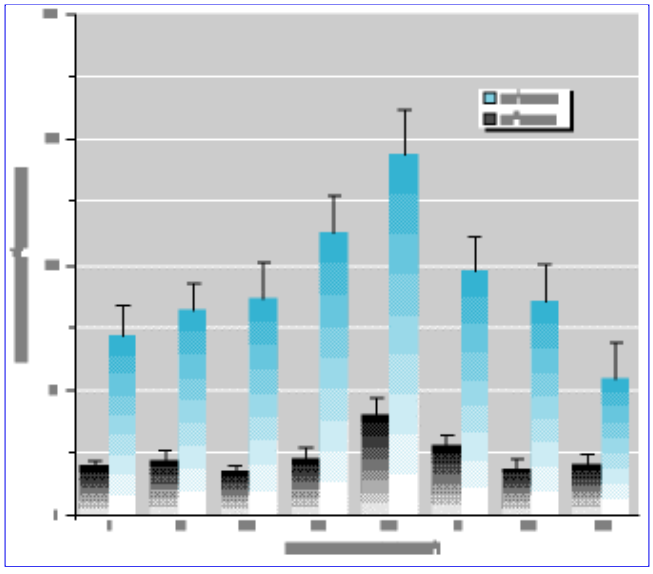

Figure 3. Mutation frequencies of mutant (sodA) strain deficient in manganese superoxide dismutase at varying doses of near-UV, as measured by resistance to rifampicin (Rif^R) and to thymine dependency (Thy^-).

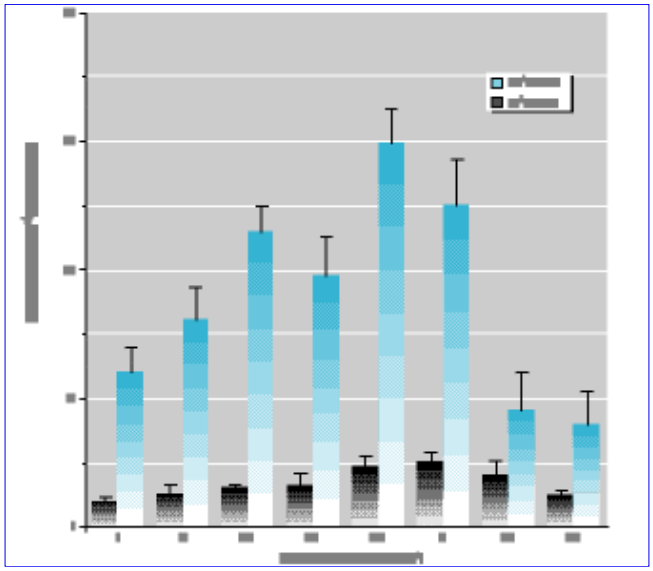

Figure 4. Mutation frequencies of mutant (sodB) strain deficient in iron superoxide dismutase at varying doses of near-UV, as measured by resistance to rifampicin (Rif^R) and to thymine dependency (Thy^-).

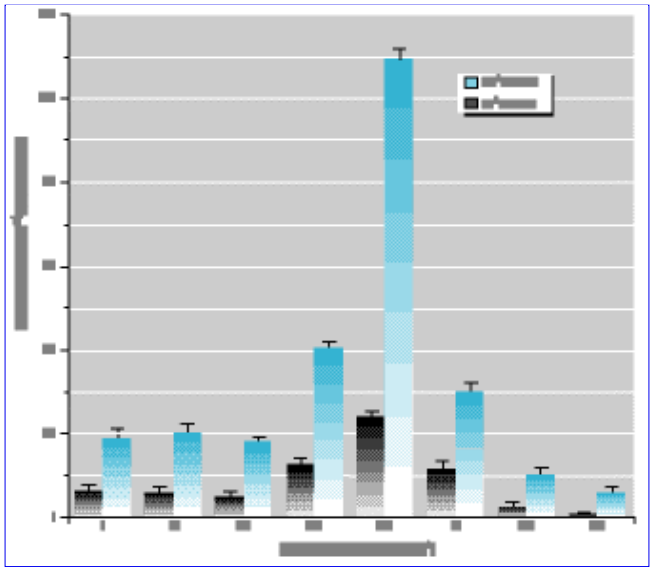

Figure 5. Mutation frequencies of double mutant (sodAsodB) strain deficient in both iron and manganese superoxide dismutase at varying doses of near-UV, as measured by resistance to rifampicin (Rif^R) and to thymine dependency (Thy^-).

Table 3 summarizes the Lac^+ reversion frequencies for all tester strains studied with standard error included. Data represent the average of three to five individual experiments for each strain. Strains CC101-CC111 containing either the single *sodA* or *sodB* mutations responded similarly to the wild-type strains, and the results were not tabulated. There was some preferential stimulation of strain CC102, the indicator for G:C to A:T transitions (33 versus 3.9), as well as of the A:T to G:C transition in strain CC106 (2.3 versus 0.3). Furthermore, tester CC104 detected some G:C to T:A transversions induced by near-UV (26.5 versus 4.8), with a weaker stimulation of the A:T to T:A transversion evidenced to a lesser extent in strain CC105 (9.1 versus 2.6). However, neither the A:T to C:G nor the G:C to C:G transversions were significantly induced by the near-UV treatment. All of the frameshift

tester strains showed weak stimulation in the number of Lac^+ revertants upon exposure to near-UV. Again, the relationship between mutagenesis and near-UV irradiation was not completely linear. The number of mutants in most cases increased and then declined with increasing near-UV exposure. Spontaneous mutation frequencies for all wild-type indicator strains were similar to those in Cupples et al. (*20*).

Table 3. Lac^+ revertants per 10^8 cells induced by near-UV irradiation in Sod^+ and *SodAB* tester strains

Near-UV fluence (kJ/m^2)	Strain and reversion event											
	CC101 (A:T to C:G)		CC102 (G:C to A:T)		CC103 (G:C to C:G)		CC104 (G:C to T:A)		CC105 (A:T to T:A)		CC106 (A:T to G:C)	
	Sod^+	Sod^-	Sod^+	Sod^-	Sod^+	Sod^-	Sod^+	Sod^-	Sod^+	Sod^-	Sod^+	Sod^-
0	2.5 (1.1)	4.1 (2.1)	3.9 (1.8)	4.2 (1.9)	3.5 (1.9)	4.0 (2.0)	4.8 (2.1)	30.0 (1.2)	2.6 (1.4)	59.8 (4.2)	0.3 (1.0)	0.95 (0.8)
5.2	2.0 (2.0)	4.0 (3.0)	4.0 (1.5)	4.9 (3.0)	3.0 (1.7)	4.2 (1.7)	6.9 (2.3)	29.7 (1.9)	3.1 (1.7)	46.1 (3.4)	0.3 (1.0)	1.1 (0.4)
10.4	5.0 (0.9)	5.4 (1.7)	7.2 (2.3)	6.0 (2.0)	1.4 (0.7)	2.7 (2.0)	7.9 (2.4)	31.0 (1.5)	1.9 (1.0)	40.4 (2.2)	1.0 (1.3)	0.9 (0.8)
15.6	3.4 (1.1)	2.0 (1.1)	15.4 (4.4)	14.6 (4.2)	1.3 (0.5)	2.1 (1.9)	14.0 (4.2)	37.4 (1.9)	9.1 (2.0)	24.1 (2.1)	0.6 (1.1)	1.6 (0.7)
20.8	4.8 (2.1)	1.9 (1.1)	24.1 (4.7)	21.2 (5.1)	3.0 (0.7)	3.2 (2.1)	26.5 (4.0)	34.1 (2.0)	2.8 (0.5)	52.3 (3.5)	2.3 (1.1)	0.95 (0.8)
26.0	5.0 (2.4)	4.2 (2.0)	33.0 (4.0)	28.7 (5.9)	1.2 (1.0)	3.0 (2.0)	6.1 (2.0)	28.4 (2.6)	2.7 (0.9)	49.0 (4.7)	1.0 (0.9)	0.4 (0.5)
31.1	0.7 (0.9)	1.2 (1.0)	11.0 (9.7)	14.3 (4.1)	2.4 (1.2)	2.9 (1.4)	2.1 (1.8)	23.5 (3.5)	2.3 (1.0)	46.4 (5.1)	0.4 (0.5)	0.4 (0.1)
36.3	0.5 (1.0)	1.01 (1.2)	2.5 (1.5)	6.7 (2.4)	2.6 (1.6)	3.0 (1.5)	2.5 (1.5)	24.1 (4.0)	2.0 (0.6)	41.3 (3.1)	0.2 (0.3)	0.3 (0.3)

	CC107 (+1G)		CC108 (-1G)		CC109 [-2(-C-G-)]		CC110 (+1A)		CC111 (-1A)	
	Sod^+	Sod^-	Sod^+	Sod^-	Sod^+	Sod^-	Sod^+	Sod^-	Sod^+	Sod^-
0	57.3 (4.2)	70.3 (4.2)	20.3 (3.2)	50.3 (4.1)	192.5 (4.5)	510.3 (5.9)	5.3 (1.0)	74.2 (4.2)	57.0 (3.2)	300.2 (5.7)
5.2	50.4 (3.5)	75.2 (4.5)	25.0 (4.1)	47.3 (3.2)	201.3 (4.2)	505.7 (6.1)	7.8 (2.3)	82.3 (4.5)	57.5 (4.1)	315.3 (6.2)
10.4	67.4 (4.9)	74.5 (4.2)	24.7 (3.2)	51.5 (3.5)	242.3 (4.9)	472.5 (7.2)	7.3 (3.1)	85.4 (5.2)	58.5 (5.1)	301.0 (6.0)
15.6	51.7 (4.7)	75.6 (4.6)	28.8 (3.8)	52.0 (3.5)	200.5 (4.5)	513.2 (7.3)	9.0 (3.2)	87.2 (6.1)	63.5 (3.1)	315.0 (4.5)
20.8	54.6 (4.4)	79.3 (4.4)	16.5 (3.5)	45.7 (5.2)	154.5 (5.3)	423.6 (9.1)	6.5 (2.9)	81.3 (5.1)	43.8 (2.5)	324.5 (5.9)
26.0	47.4 (4.6)	87.0 (4.7)	24.1 (4.1)	42.5 (4.1)	88.7 (6.2)	405.3 (4.7)	6.4 (2.4)	76.2 (5.0)	34.6 (2.6)	294.7 (4.2)
31.1	44.9 (3.8)	69.6 (5.6)	26.7 (4.0)	35.6 (4.4)	155.2 (7.2)	357.3 (4.6)	5.3 (2.1)	81.0 (4.3)	30.4 (4.5)	305.3 (7.3)
36.3	40.2 (3.7)	71.2 (5.2)	23.4 (3.5)	30.1 (4.5)	110.4 (5.1)	274.8 (5.5)	5.5 (2.3)	75.4 (4.9)	32.1 (9.2)	284.8 (5.4)

Although Lac^+ reversion frequencies induced by near-UV irradiation in Sod^- indicator strains were not significantly different from those seen in the wild types, spontaneous mutation rates in several of these strains did preferentially increase over isogenic wild types. A 20-fold increase in the number of Lac^+ spontaneous revertants was seen in strain RKCC105, the indicator for A:T to T:A transversions (59.8 versus 2.6). Also, a sixfold stimulation of the G:C to T:A transversion was detected in strain RKCC104 (30 versus 4.8). However, the spontaneous mutation rate for the other four base substitutions remained unchanged in the absence of SOD activity. In addition, the number of spontaneous Lac^+ revertants was elevated for all frameshift tester strains. Tester strains lacking superoxide dismutase had higher spontaneous rates of reversion in runs of adenines in both the +1 and -1 directions. Elevated reversion rates were stimulated in RKCC110 and RKCC111, the two strains that detect frameshifts in runs of adenines. A 15-fold increase in the spontaneous reversion rate was detected in RKCC110 (+1A) (74.2 versus 5.3), whereas a 5-fold increase was stimulated in RKCC111 (-1A) (300 versus 57). However, about a twofold stimulation occurred spontaneously in RKCC108 (-1G) and RKCC109 [-2(-C-G-)], but only a very weak stimulation was detected in RKCC107 (+1G). The Sod^- phenotype of several randomly chosen colonies scored as Lac^+ revertants was confirmed by SOD activity gels (see Materials and Methods).

Discussion

Although the tester strains used only detect mutations occurring in two target codons of the *lacZ* gene and did not necessarily detect mutational hot spots or strand specificity, the assay allowed analysis of specific DNA mutations. While near-UV radiation was weakly mutagenic in wild-type cells and four- to sixfold more mutagenic in SOD-deficient cells, the frequency and types of mutations produced by near-UV radiation were different from those spontaneously occurring in cells assumed to have an increased flux of O_2^-.

The polychromatic near-UV (300-420 nm) radiation studied preferentially induced G:C to A:T transitions most frequently, followed by the G:C to T:A transversion in wild-type *E. coli*. Furthermore, the A:T to T:A transversion

was weakly stimulated along with all of the frameshifts analyzed. The finding that G:C to A:T transitions predominate near-UV-induced base substitutions correlates with other studies of ultraviolet radiation mutagenesis (*29*,*30*). Armstrong and Kunz (*30*) reported site and strand specificity in the *SUP4-o* gene in *S. cerevisiae* after UV-B (285-320 nm) mutagenesis. Their study further suggests that mutations induced by mid-UV or UV-B (290-320 nm) radiation and far-UV or UV-C (190-290 nm) radiation involve the same lesion(s) and/or mechanism(s). Mutagenic spectra induced by both of these radiations include the predominance of G:C to A:T transitions and the preference for substitutions at the 3´ base of dipyrimidine sequences. This has been interpreted to indicate that cyclobutane pyrimidine dimers and pyrimidine-pyrimidone (6-4) photoproducts are the most important premutational lesions induced by far-UV radiation. However, cyclobutane dimers have recently been implicated as the major form of premutational DNA damage for both far-UV and mid-UV radiations (*30*). It should be noted that there is some region of overlap in the mid-UV (285-320 nm) radiation studied by Armstrong and Kunz (*30*) and the near-UV (300-420 nm) radiation used in these experiments. The involvement of cyclobutane dimer formation in this near-UV-induced mutagenesis remains a possibility. However, the prevalence of the G:C to T:A and A:T to T:A transversions suggests that other lesion(s) or mutagenic pathways may be involved in near-UV mutagenesis, as these transversions occurred infrequently in mid-UV mutagenesis. This is further supported by the noticeable absence of the other base substitutions apparently not stimulated by near-UV. Interestingly, the G:C to T:A transversion is the most common base substitution detected after the SOS system has been induced in the absence of DNA damage (*31*).

In comparison, the frequency and types of mutations occurring spontaneously in *E. coli* mutants lacking both MnSOD and FeSOD were different. Slightly higher spontaneous mutation frequencies for *sodA* mutants, but not *sodB* mutants, is in agreement with other reports (*15*). Because the high spontaneous mutation rate in SOD-deficient cells is oxygen dependent (*15*), the mutagenic specificity detected in these mutants is likely due to oxidative DNA damage. This is also supported by the observation that the predominant base substitution induced in these mutants is the A:T to T:A transversion, the base-pair change most frequently caused by oxidative mutagens. The A:T to T:A transversion spontaneously occurred 20-fold over wild-type (Sod^+) cells, followed by a 6-fold stimulation in G:C to T:A transversions. There was also significant increase of the +1A and -1A frameshift mutations in the Sod^- mutants. These results are consistent with Storz et al. (*32*), who found a similar mutation spectrum in *Salmonella typhimurium* strains containing deletions of *oxyR*, a gene that positively regulates cellular defenses against oxidative stress in both *S. typhimurium* and *E. coli*. However, Storz et al. (*32*) also reported a substantial increase in G:C to A:T transitions, a base change not detected in the *sodAsodB* double mutants of *E. coli*. Furthermore, the mutagenic spectrum was quite different from that produced by Fe^{2+}-induced oxidative DNA damage in the M13mp2 forward mutation assay, which implicates the formation of 8-hydroxyguanosine as the most frequently produced base modification, responsible for G:C to C:G transversions (*33*). However, others have found that the G:C to T:A transversion is predominantly induced (*34*).

There is evidence that mutations induced by H_2O_2 are not necessarily those induced during O_2^--dependent mutagenesis. First, O_2^- mutagenesis is independent of the SOS response (*22*), whereas the SOS response is induced by H_2O_2 (*7*). Second, although O_2^- induces synthesis of endonuclease IV, H_2O_2 does not; rather H_2O_2 mutagenesis depends heavily on the activity of exonuclease III (*35*). There is indirect proof that endonuclease IV and exonuclease III do not share the same substrate specificities (*36*). Results from this study support this conclusion. H_2O_2-dependent mutagenesis largely generates transitions, whereas the *sodAsodB E. coli* mutants examined here show elevated frequencies of two transversions, as well as frameshifts in runs of adenines in both the +1 and -1 direction. The three other frameshifts analyzed were also stimulated, although to a lesser extent.

Considering possible mutagenic pathways involved in the spontaneous mutagenesis seen in SOD mutants, it has been demonstrated that oxidizing agents can cause the disruption of the imidazole ring of purines, producing the formamidopyrimidine derivative of adenine and guanine (*37*). Generation of these derivatives in cells with increased O_2^- flux may explain, in part, some of the mutations induced. The *E. coli* formamidopyrimidine-DNA glycosylase, the *mutM* gene product, is not part of the SOS regulon, and its possible involvement in O_2^- mutagenicity should be analyzed. Alternatively, it has been shown that MutY, which is an adenine glycosylase

specific for GA mispairs, has homology to endonuclease III and may be an $(Fe\text{-}S)_4$-containing protein (*38*). Some $(Fe\text{-}S)_4$-containing proteins have been shown to be sensitive to increased concentrations of O_2^- (*39*). Furthermore, *E. coli mutY* mutants demonstrate a high stimulation of transversions (*40*,*41*). We therefore suggest a possible role of MutY in O_2^--induced mutagenesis. It is possible that the MutY protein may become inactivated in SOD-deficient mutants and play some role in O_2^--dependent mutagenesis.

Curiously, while near-UV radiation induced up to a sixfold increase in the mutation frequency in the *sodAsodB* double mutants (Sod^-), no similar increase in any of the specific mutations was detected. Specific mutations induced by near-UV were similar in both wild-type cells and SOD-less mutants. However, the different frequencies and types of mutations induced by near-UV and O_2^- suggests, in part, separate lesion(s) and/or mechanisms of mutagenesis. Although no apparent synergistic action could be interpreted from these data, further mutagenic analysis using complete target genes such as *lacI* (*29*) may resolve this issue.

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Last Update:August 18, 1998