

STRUCTURAL STUDIES OF GLYCERALDEHYDE-3-PHOSPHATE
DEHYDROGENASE COMPLEXES AND THE *E. COLI* PutA DNA BINDING
DOMAIN

Jermaine Jenkins

Dr. John J. Tanner, Dissertation Supervisor

ABSTRACT

Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) is a glycolytic enzyme that catalyzes the formation of 1,3-bisphosphoglycerate from glyceraldehyde-3-phosphate. We have solved a high-resolution (1.75 Å) structure of a human GAPDH. Human GAPDH and the E3 ubiquitin ligase Siah1 have been found to interact as part of recently discovered NO/GAPDH/Siah1 apoptosis cascade. The structure is used in a computational ligand-docking study of the small-molecule compound CGP-3466, which inhibits apoptosis by preventing GAPDH binding to Siah1. The structure is also used to build a qualitative model of the complex between GAPDH–Siah1.

We have also solved three crystal structures of *Thermus aquaticus* GAPDH corresponding to phosphate concentrations of 0 (1.65 Å), 50 mM (1.85 Å), and 100 mM (2.23 Å). In these structures the binding of phosphate results in two conformations of the phosphate binding loop and the dual occupancy of both the new and classical P_i-sites.

Finally, Proline utilization A (PutA) is a membrane-associated bi-functional enzyme that catalyzes the sequential two-step oxidation of proline to glutamate. Here we report the first crystal structure of a PutA DNA-binding domain along with functional analysis of a mutant PutA defective in DNA-binding.