Public Abstract
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Title: Structural Studies of Glyceraldehyde-3-Phosphate Dehydrogenase Complexes and the *E. coli* PutA DNA Binding Domain

Glyceraldehyde-3-phospahte dehydrogenase (GAPDH) is a glycolytic enzyme that catalyzes the formation of 1,3-bisphosphoglycerate (BPGA) from glyceraldehyde-3-phosphate (GAP). 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 the recently discovered NO–S-nitrosylation–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. Plausible binding sites are identified in the adenosine pocket of the NAD^+-binding site and in a hydrophobic channel located in the center of the protein. 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. We also explore the contributions these structures make to the ongoing debate as to the location of the substrate phosphate during catalytic cycle of GAPDH.

Finally, Proline utilization A (PutA) is a large, membrane-associated bi-functional enzyme that catalyzes the sequential two-step oxidation of proline to glutamate. As part of our ongoing studies of PutA structure and function, we report the first crystal structures of a PutA DNA-binding domain. Crystals of this domain were obtained from a polypeptide corresponding to *E. coli* PutA residues 1-52 (PutA52).The PutA52 structures show that PutA belongs to the ribbon-helix-helix family (RHH), which establishes PutA as the largest RHH family member. Based on analysis of the structure and comparison to other RHH proteins, we propose several residues that may be important for dimerization and DNA recognition. We tested one of these predictions by mutating Lys9 to Met in full-length PutA, which abrogated binding to DNA. These structures provide key information for understanding structure-function relationships in PutA and new insights into the functional domain arrangement of PutA.