

are necessary for the uridylylation of P_{II} by UTase/UR.

In order to elucidate the potential structural changes which may occur upon ligand binding, P_{II} has been crystallised with a number of effector ligands including 2-ketoglutarate (2-KG), glutamate and ATP, and ATP in combination with 2-ketoglutarate and glutamate. We anticipate that these structure determinations will provide us with new insights into the way P_{II} influences the enzymes involved in nitrogen regulation and how its interaction with various ligands plays a crucial role in signal transduction.

A molecular replacement solution for the P_{II} /ATP/2-KG crystals has been obtained and refinement of the structure is in progress. Details of the crystallisation, structure solution and current model will be presented.

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PS04.17.10 THE 1.8 Å RESOLUTION CRYSTAL STRUCTURE OF HGPRTASE FROM THE HUMAN PARASITE SCHISTOSOMA MANSONI WITH BOUND INHIBITOR
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The crystal structure of an enzyme crucial for nucleotide metabolism in the human parasite *Schistosoma mansoni* has been solved and compared to the human homolog. The differences between the two structures will guide structure based drug design efforts against the parasite.

Schistosomiasis affects over 300 million people in tropical countries who become infected with the parasite through fresh water contact. The parasite, *S. mansoni*, has been found to lack *de novo* nucleotide biosynthesis, and relies on recycling the host's nucleotide precursors for cellular metabolism. The purines guanine and hypoxanthine are recycled via the salvage pathway by hypoxanthine-guanine phosphoribosyltransferase, HGPRTase, to form the ribonucleotides, GMP and IMP. *S. mansoni* also lacks adenine-guanine interconversion enzymes, thus HGPRTase is the parasite's sole source of guanine nucleotides and a critical metabolic enzyme.

The structure of *S. mansoni* HGPRTase was solved by molecular replacement using a modified human HGPRTase as the search model. The structure has been refined to 1.8 Å resolution against X-ray data collected from a single cryofrozen crystal.

PS04.17.11 COMPARISON OF STREPTAVIDIN W_xF AND W_xA MUTANTS WITH THE NATIVE PROTEIN. Stefanie Freitag^a, Isolde Le Trong^a, Ronald E. Stenkamp^a, Ashutosh Chilkoti^b, Patrick S. Stayton^b. Dept. of Biological Structure^a and Center of Bioengineering^b, University of Washington, Seattle, WA 98195

The high affinity binding of biotin to streptavidin ($K_a \sim 10^{13-15} M^{-1}$) is hypothesized to be highly dependent on three different binding motifs [1]. These three classes of interactions include four tryptophan side-chains that mediate aromatic contacts to biotin, an extensive hydrogen-binding network, and a flexible binding loop that becomes ordered upon biotin association. Single site-directed mutants replacing Trp residues with Phe and Ala at positions 79, 108 and 120 have been prepared, and their binding properties studied by ELISA experiments, isothermal titrating calorimetry, and kinetic analysis [2].

Four of the mutants and the native protein crystallize in the monoclinic space group $P2_1$. X-ray crystallographic analyses of the unliganded mutants as well as their complexes formed with

biotin and HABA have been carried out. Diffraction data were collected to high resolution limits between 1.7 and 2.0 Å. Refinement of the structural models were performed with the programs XPLOR and SHELXL-93. Our current structural models showing the interactions giving rise to biotin binding will be presented. (This work is supported by NIH grant DK49655.)

[1] a) Hendrickson et al., Proc. Natl. Acad. Sci. USA 86, 1989, 2190 - 2194;
b) Weber et al., Science 243, 1989, 85 - 88.

[2] A. Chilkoti and P. S. Stayton, JACS 117, 1995, 10622 - 10628.

PS04.17.12 REFINED CRYSTAL STRUCTURE OF FERREDOXIN FROM THERMOACIDOPHILIC ARCHAEON. T. Fujii¹, Y. Hata¹, M. Ohzeki², H. Moriyama², T. Wakagi², N. Tanaka² and T. Oshima². ¹Institute for Chemical Research, Kyoto University, Uji, Kyoto 611, Japan; ²Department of Life Science, Faculty of Bioscience and Biotechnology, Tokyo Institute of Technology, Nagatsuta, Yokohama, Kanagawa 226, Japan.

The crystal structure of the ferredoxin from thermoacidophilic archaeon *Sulfolobus* sp. strain 7 was determined by a multiple isomorphous replacement method and refined at 2.0 Å resolution to a crystallographic *R*-factor of 17.3 %. Archaea are classified into a third kingdom of biological world different from Bacteria and Eukarya, and many studies on the evolution of archaeon have been performed from the biochemical and biophysical aspects. In order to elucidate the molecular evolution and thermostability of thermoacidophilic archaeal ferredoxin, we have determined the crystal structure of the archaeal ferredoxin.

Crystals belong to space group $P4_32_12$ with unit cell dimensions $a = b = 50.12$ Å and $c = 69.52$ Å. Intensity data of the native and two heavy-atom derivative crystals were collected on an R-AXIS IIC using $CuK\alpha$ radiation. Phase angles were determined at 2.0 Å resolution by a multiple isomorphous replacement method supplemented with anomalous effects from iron atoms of the iron-sulfur clusters. An electron density map calculated with these phase angles were improved by a solvent-flattening method. The density map enabled us to build the model. Under constructing the model, one high peak remained in the density map, which was tetrahedrally coordinated by four amino acid residues. The peak was identified to a zinc ion by Bijvoet difference Fourier maps using data collected with X-rays of wavelength 1.275 Å and 1.290 Å produced by the synchrotron radiation source of Photon Factory, KEK, Japan.

This molecule consists of two parts: the core part and the N-terminal extended part. The core part has common folding among bacterial dicycloster type ferredoxins. The N-terminal extended part is mainly constructed from three β -strands. The zinc atom is tetrahedrally ligated by four amino acid residues, and is placed at the interface between the core part and the N-terminal extended part. The present analysis gives the first example of archaeal ferredoxin which possesses a structural zinc.