PS04.17.06 CONFORMATIONAL ANALYSIS OF TRYPSIN AT ATOMIC RESOLUTION REVEALS EFFECTS OF CRYSTALLINE ENVIRONMENT. Hans D. Bartunik, Alexandre N. Popov, Max-Planck Research Unit for Structural Molecular Biology, Protein Dynamics Group, MPG-ASMB c/o DESY, Notkestraße 85, 22603 Hamburg, Germany

The crystalline environment affects the relative orientation of the two ß- barrel domains of bovine pancreatic ß-trypsin (BPT) and induces conformational strain. The comparison is based on anisotropic refinement using SHELXL-93 [1] of BPT at 5°C in two different orthorhombic crystal forms [2, 3] at 1.0-1.1 Å resolution (R=8.3%; 9.8%). The accuracy in the atomic positions is on average 0.01-0.02 Å for each structural model. When aligned on the active site region and one domain (150 residues; rmsd 0.09 Å, max. deviation 0.19 Å for the main chain atoms), the other domain exhibits relative coordinate shifts (rmsd 0.22 Å, max. 0.52 Å) as well as substantial changes in the conformational angles. The positional and conformational differences are particularly large for the ß-strands 81-90 (near the surface) and 104-108. Molecular packing interactions further induce flexibility in a number of residues (35 in the one structure, 24 in the other) that are present in discrete alternate conformations. Correlations between alternate side chain locations are observed which extend over distances up to 20 Å; in several cases, water or sulphate molecules with partial occupancies are involved. Most of the ordered solvent (ca. 2.2 waters per accessible residue - nearly all in the first coordination shells) and the degree of anisotropy in the individual atomic temperature factors are essentially not affected by the crystalline environment. In both structures, only one residue (Gln 192) is not located in well-defined electron density; the flexibility may reflect its functional role in orienting substrates. The diffraction data were measured on the synchrotron beamline BW6/DORIS.

[1] Sheldrick, G. W. (1993). SHELXL-93, Univ. Göttingen.
[2] Marquart, M. et al. (1983). Acta Crystallogr. sect. B, **39**, 480-490.
[3] Bartunik, H. D. et al. (1989). J. Mol. Biol. **210**, 813-828.

PS04.17.07 QUANTITATING CHANGES IN THE THREE DIMENSIONAL STRUCTURE OF VARIANT ENZYMES. Richard Bott and Mark Knapp, Genencor International, 180 Kimball Way, So. San Francisco, CA

Site-specific substitutions made to change performance of an enzyme toward a desired purpose often result in subtle conformational adjustments. Often there is more than one structural consequence associated with any single substitution. In order to associate these structural consequences with altered function, it would be helpful to devise means of quantitating these changes. These changes could then be correlated with altered functionality in a series of variants.

Bacillus lentus subtilisin has been modified for improved proteolytic activity. The three-dimensional structures of several variants have been determined that display increased and decreased performance. It is necessary to obtain the highest resolution data in order to detect and quantitate these subtle changes that contribute to altered performance. Currently the resolution limit of the data can exceed 1.4 Å.

Results of our efforts to employ difference distance plots and other techniques will be presented. It is important to differentiate crystal lattice effects from mutational consequences when variants crystallize in different forms.

PS04.17.08 CRYSTAL STRUCTURE ANALYSIS OF HUMAN TRANSTHYRETIN COMPLEXES WITH FLUORESCENT PROBES. Vivian Cody, Joseph R. Luft, Walter Pangborn, Hauptman-Woodward Medical Research Institute, Inc., Buffalo, NY 14203

Fluorescent probes of the N-arylaminonaphthalene sulfonate type are used to assess the hydrophobicity of protein binding sites and as a means of monitoring conformational changes in biological macromolecules. Structure-activity data show that they can also act as competitive inhibitors for thyroxine (T_4) binding to transthyretin (TTR). Fluorescence quenching studies of 8-anilino-1-naphthalene sulfonic acid (ANS) by competitive displacement of T_4 from TTR was used to determine the binding affinity of T_4 and to describe negative cooperativity in binding the hormone to the two equivalent sites on the TTR tetramer. These data supported two theoretical models for ANS quenching - one showing independent actions of the two hormone binding sites, and the other requiring interaction between the two sites. Similarly, the fluorescent probe N-(iodoacetyl)-N'-(5-sulfo-1-naphthyl)ethylenediamine (1,5-AEDANS) was shown to form a covalent bond with Cys-10 of TTR. In order to better understand negative cooperativity in hormone binding to TTR, we have carried out the X-ray crystal structure determination of human TTR co-crystallized with various fluorescent probes and report structural results for TTR complexed with ANS and 1,8-AEDANS. Both crystals diffract to 1.9Å resolution and crystallize in the orthorhombic space group $P2_12_12$ with two independent monomers in the asymmetric unit. Cell dimensions are isomorphous to previously reported lattices. Refinement of each structure was carried out to 1.9Å resolution without inhibitor contributions using the program PROLSQ. Difference (F_o-F_c) electron density maps based on these refinements reveal electron density in the center of the hormone binding domain in both data sets. In the case of 1,8-AEDANS, there is no density near Cys-10, but there is indication of a covalent link of 1,8-AEDANS to the ε-amine of Lys-15, as was obtained in the crystal structures of N-bromoacetyl-hormone derivatives. Since the reactive acetyl group is the same for 1,5-AEDANS and 1,8-AEDANS, it is not clear why there is no involvement with Cys-10. Data for the ANS complex show density in the hormone binding site which is similar to that of 1,8-AEDANS. Higher resolution data for these complexes are needed to interpret changes in TTR conformation which may explain the mechanism of negative cooperativity.

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PS04.17.09 NEW CRYSTAL FORMS OF *ESCHERICHIA COLI* P_{II} COMPLEXED WITH VARIOUS LIGANDS AND STRUCTURE SOLUTION OF P_{II}/ATP/2-KETOGLUTARATE. Karen Edwards, Peter Suffolk, Paul Carr, Eong Cheah and David Ollis. Research School of Chemistry, Australian National University, Canberra, ACT 0200, Australia

P_{II} is a signal transduction protein involved in bacterial nitrogen regulation and plays a key role in regulating both the activity and level of expression of the enzyme glutamine synthetase (GS). Regulation of GS is achieved via a number of protein-protein interactions involving P_{II}, adenylyl transferase, nitrogen regulatory proteins I and II and uridylyl transferase (UTase/UR). The function of the regulatory enzymes is reversed upon uridylation of residue Tyr51 in P_{II} which occurs in response to a drop in cellular nitrogen levels.

The structure of unliganded *E. coli* P_{II} has been solved and refined to 1.9 Å resolution (Carr *et al.*, 1996). Recent biochemical evidence suggests an important role for ligands in effecting allosteric changes in P_{II} (Kamberov *et al.*, 1995). ATP, 2-ketoglutarate and glutamate have all been shown to bind to P_{II} and

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.

Carr, P.D., Cheah, E., Suffolk, P.M., Vasudevan, S.G., Dixon, N.E. & Ollis, D.L. (1996). *Acta Cryst.* D52, 93-104.
Kamberov, E.S., Atkinson, M.R. & Ninfa, A.J. (1995). *J. Biol. Chem.* **270**, 1-11.

PS04.17.10 THE 1.8 Å RESOLUTION CRYSTAL STRUCTURE OF HGPRTASE FROM THE HUMAN PARASITE *SCHISTOSOMA MANSONI* WITH BOUND INHIBITOR
Pamela J. Focia, Douglas M. Freymann, John R. Somoza, Ching C. Wang, Robert J. Fletterick, Departments of Pharmaceutical Chemistry and Biochemistry and Biophysics, U.C.S.F.

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 WxF AND WxA MUTANTS WITH THE NATIVE PROTEIN.
Stefanie Freitag[a], Isolde Le Tronga[a], Ronald E. Stenkampa[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 ($K_a \sim 10^{13\text{-}15}$ 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 $P2_1$. Xray 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. Fujii[1], Y. Hata[1], M. Ohzeki[2], H. Moriyama[2], T. Wakagi[2], N. Tanaka[2] and T. Oshima[2]. [1]Institute for Chemical Research, Kyoto University, Uji, Kyoto 611, Japan; [2]Department 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 Cu$K\alpha$ 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 dicluster 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.