

**PS04.17.29 A FORMAL ANALYSIS OF HINGING IN ACTIN.** Rebecca Page, Princeton University, Uno Lindberg, Stockholm University and C.E.Schutt, Princeton University.

A formal analysis of the current atomic models of the protein actin reveals that changes in actin conformation are localized to only a few regions of the actin polypeptide. These regions function as hinges, about which the actin domains rotate as rigid bodies. Specifically, the crystal structures of the actin monomer, including the  $\alpha$ -actin [2,3] and  $\beta$ -actin [4,5] structures, were systematically compared, using methods for studying alternative conformations of the same protein developed by Chothia, Lesk and Gerstein[5]. The results of this analysis indicate that the conformational changes observed in actin can be best described as a combination of small hinging and sheering motions between actin domains. The motions are due to structural changes in loops and helices connecting the actin domains. This analysis confirms predictions made about mechanisms of actin domain motions based on tertiary structure[5], and extends the observations made by Schutt *et al.* in 1993.

The mechanisms of conformational change observed crystallographically serve as strong constraints on plausible models of the actin filament. Thus, the above analysis was extended to include the present models of the actin filament [6,7]. Tertiary and secondary structural elements of the two f-actin models differ significantly from the conformations observed in the actin crystal structures. These changes require a higher energetic cost than those observed crystallographically, which are classified as low energy transitions[4].

The  $\beta$ -actin monomers form polymeric actin ribbons in the profilin: $\beta$ -actin crystals. It has been proposed that the atomic structure of the actin filament may be structurally related to the actin:actin contacts observed in the ribbon[2]. The results described above are being used as constraints in the development of an alternative model of f-actin. In this model, the actin:actin ribbon contacts are preserved and the conformation of the monomer is related to the crystallographic structures by changes in the hinges described above.

[1] Kabsch, *et al.* (1990) *Nature*, 347, 37-44. [2] McLaughlin *et al.* (1993) *Nature*, 364, 685-692. [3] Schutt *et al.* (1993) *Nature*, 365, 810-816. [4] Chik *et al.* (1996) submitted. [5] Gerstein *et al.* (1994) *Biochemistry*, 33, 6739-6749. [6] Lorenz *et al.* (1993) *J.Mol.Biol.*, 234, 826-836. [7] Tirion *et al.* (1995) *Biophys.J.*, 68, 5-12.

**PS04.17.30 STRUCTURE OF HEVEIN, A LECTIN-LIKE FROM HEVEA BRASILIENSIS AT 1.9Å RESOLUTION.** Kaliyamoorthy Panneerselvam and Manuel Soriano-García. Instituto de Química, Universidad Nacional Autónoma de México, Circuito Exterior, Ciudad Universitaria, Delegación Coyoacán 04510. México, D.F.

Hevein is a small, single-chain protein of 43 amino acids and rich in cysteine and glycine. Hevein is a lectin-like protein and experimental evidence indicates that this protein is involved in the coagulation of latex by bridging together rubber particles<sup>1</sup>. We solved the crystal structure of hevein by means of molecular replacement techniques using the PROTEIN program and refined the structure at 3.0Å resolution<sup>2</sup>. However, the resolution limit of the diffraction data collected could be improved and prompted us to remeasure a new native data-set up to 1.9 Å resolution. The crystals are orthorhombic with space group  $P2_12_12_1$  with cell dimensions  $a=21.59(2)$ ,  $b=31.60(3)$  and  $c=51.21(5)$ Å,  $V=34931(1)$  Å<sup>3</sup> and  $Z=4$ . The three-dimensional structure of hevein has been refined with XPLOR and SHELXL93 programs. A total of 324 protein atoms, 20 ordered water sites and 23 disorder water sites refined to a final R-factor of 0.16 at a resolution of 1.9Å and an average

B-value of 15.1Å<sup>2</sup>, using 73% (2326) of the total possible number of reflections in the range 10 to 1.9 Å with  $I \geq 2\sigma(I)$ . The final structure has r.m.s deviations from ideal bond distances and angles of 0.017Å and 2.7°, respectively. The standard deviation of atomic position estimated by Luzzati plot was 0.15Å. The protein comprised of four-stranded  $\beta$ -sheet, five turns and a short  $\alpha$ -helix located at the C-terminal, all of these is required to accomplish the hevein-lectin like fold. The final structure of hevein in the solid state displays a folding similar to one determined by NMR techniques and also to that of domain C of wheat germ agglutinin.

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[1]Gidrol, X., et al., (1994). *J. Biol. Chem.* 269, 9278-9283.

[2]Rodríguez-Romero, A., et al., (1991)*FEBS Lett.*291,307-309.

**PS04.17.31 ANALYSIS OF C-H...O HYDROGEN BONDS IN BETA SHEETS.** Vasantha Pattabhi, G. Felcy Fabiola & S.Krishnaswamy+ Department of Biophysics, University of Madras, Guindy Campus, Madras-600025, INDIA and +Bioinformatics Centre, School of Biotechnology, Madurai Kamaraj University, Madurai-625021, INDIA

C-H...O hydrogen bonds stabilising organic structures have been thoroughly studied and well documented. However, the observation of contiguous C-H...O hydrogen bonds in the beta sheet region of one of our peptide structures prompted us to analyse the beta sheet regions of proteins whose high resolution structures are known. Our analysis shows that C-H...O hydrogen bond geometry calculated using the PDB coordinates is well within the acceptable limits. As for the sequence specificity, tryptophan is the major contributor (23%) to C-H...O hydrogen bonds in antiparallel region whereas Val, Leu and Lys contribute equally to the parallel sheet region. On the whole residues other than the nonpolar ones are poor contributors to C-H...O in parallel sheets, though lysine is an exception. In the side chain main chain interactions, charged residues like Arg, Gln, His have a major role as compared to others. Twist angle of the beta sheets do not seem to affect the formation of C-H...O hydrogen bonds in a systematic way. However C-H...O and N-H...O hydrogen bond strengths appear to be correlated.

**PS04.17.32 HOW TO AVOID OVERFITTING IN REFINEMENT OF STRUCTURES WITH APPROXIMATE NON-CRYSTALLOGRAPHIC SYMMETRY. HIGH RESOLUTION STRUCTURE REFINEMENT OF GLUTAMINE SYNTHETASE REVEALS EVIDENCE FOR HOMOTROPIC COOPERATIVE BINDING.** Gaston M.U. Pfluegl, Harindarpal Gill & David Eisenberg. Molecular Biology Institute, UCLA, Los Angeles, CA 90095-1570, USA.

Glutamine Synthetase (GS) is a key enzyme in nitrogen metabolism. GS from *S.typhimurium* is a stable complex of large size, consisting of 12 identical chains assembled into a dodecamer with 62 symmetry ( $M_r \sim 620$  KDa; 12 times 468 amino acid residues). In the presence of ADP, GS crystallizes in space group C2 with 1 dodecamer per asymmetric unit. From the association constant of ADP for GS, about two ADP should be bound per dodecamer under crystallization conditions.

The previous 2.8 Å model for GS has been refined with strict non-crystallographic symmetry restraints (12-fold averaging). In this model, three regions were invisible and some of the active site residues showed disorder. No density was found for ADP in this model.

The present work is based on a 2.5 Å cryo synchrotron data set from a single crystal (98% complete; 200,000 reflections; 10 times redundancy). The new model was refined with 12 independent but constrained subunits and shows density for two bound ADP molecules in two adjacent active sites. This density was not

visible in the 12-fold averaged structure. Due to selective binding of ADP within the dodecamer, the GS molecule deviates from exact 62 symmetry. This explains the preference of the C2 crystal form over hexagonal crystal forms which are observed for higher and lower ADP concentrations.

The use of R-free to monitor data overfitting when releasing strict non-crystallographic symmetry will be discussed and data for the choice of the reflections for the TEST-set (not well established for structures with non-crystallographic symmetry) will be presented.

Binding of two ADP molecules in an up-down fashion in the dodecamer sandwich, as seen in the new model, supports the biochemical finding of homotropic cooperative binding of substrate, for which kinetic data will be presented along with the crystallographic data.

**PS04.17.33 THE BINDING SITES OF KRYPTON AND XENON IN PROTEINS : A SURVEY OF TEN COMPLEXES.** Thierry Prangé, Marc Schiltz and Roger Fourme. LURE, Bât. 209d, Université Paris-Sud, 91405 Orsay Cedex, France.

It is now well established that the noble gases xenon and krypton bind to numerous proteins through weak Van der Waals interactions. In about fifty percent of the examined cases, the sites are sufficiently well defined (in terms of occupancy factors and phasing power) to be used as highly isomorphous heavy atom derivatives in MIR or SIRAS techniques (several examples will be presented during this meeting). The binding site environments of a set of ten proteins including elastase, cutinase, subtilisin, collagenase, lysozyme, urate oxidase, etc. are analysed in terms of polar/hydrophobic interactions and close contacts to feature out a general prediction for the binding under moderate gas pressure (4 to 40 bar).

**PS04.17.34 X-RAY STRUCTURE ANALYSIS OF APOFLAVODOXIN FROM ANABAENA PCC 7119.** A. Romero<sup>1</sup>, C.G. Genzor<sup>2</sup> and J. Sancho<sup>2</sup>, <sup>1</sup>Instituto de Química Física Rocasolano. Departamento de Cristalografía. CSIC. Serrano, 119 E-28006-Madrid, Spain. <sup>2</sup>Departamento de Bioquímica y Biología, Molecular y Celular Facultad de Ciencias, Universidad de Zaragoza, E-50009 Zaragoza, Spain

Many biological reactions are catalysed by flavoproteins, a large group of proteins carrying a flavin cofactor (either FMN or FAD). The redox properties of flavoproteins arise from the interaction between the apoprotein and the redox cofactor. Although the structures of many holoflavoproteins are known, there is no single apoflavoprotein of known three-dimensional structure.

We report here the X-ray structure of apoflavodoxin from Anabaena PCC 7119 at 2.0 Å resolution. Apoflavodoxin is a compact, well-folded protein with the same overall fold as holoflavodoxin (1). The transient cavity formed on removal of the FMN cofactor is filled by the indole ring of Trp57 that penetrates into the depression previously occupied by the pyrimidine portion of the isoalloxazine and by the ribityl. The closure of this aromatic gate disorganises the isoalloxazine and ribityl binding sites but leave the phosphate binding site intact. This suggests that the interaction between the FMN and the apoprotein could start at this place.

(1) Rao, S.T., Shaffie, F., Yu, C., Satyshur, K.A., Stockman, B.J., Markey, J.L. & Sundaralingam, M. Protein Science, 1, 1413-1427 (1992)

**PS04.17.35 SHORT-CIRCUITING A WATER-MEDIATED ENZYME REACTION.** Carleton R. Sage, Earl E. Rutenber, Thomas J. Stout and Robert M. Stroud, Department of Biochemistry and Biophysics, University of California, San Francisco, San Francisco, CA 94143-0448 USA

A water-mediated hydrogen bond network coordinated by glutamate 58 has been proposed to play an important role in the thymidylate synthase (TS) reaction mechanism. We have addressed the role of glutamate 58 in coordinating the water-mediated hydrogen bond network in the TS reaction, by cocrystallizing the *E. coli* TS mutant E58(60)Q with dUMP and the cofactor analog CB3717, and solving the X-Ray crystal structure to 2.5 Å resolution with a final R-factor of 15.3% (R<sub>free</sub>=23.7%). Using a difference Fourier analysis we were able to analyze directly the changes that occur between the wild-type and mutant structures. In the structure of the mutant enzyme the coordinated hydrogen bond network has been "short-circuited," providing an atomic resolution explanation for the disruption of the TS reaction by the E58Q mutant as well as confirming the proposal that the role of E58 in the TS reaction is to coordinate this conserved hydrogen bond network. The structure also shows an previously unobserved conformation for the cofactor analog, CB3717, which has implications for structure-based drug design and sheds light on the controversy surrounding the previously observed enzymatic nonidentity between the monomers of the TS dimer.

**PS04.17.36 STRUCTURAL BASIS OF CHEMOTHERAPY RESISTANCE MEDIATED BY THYMIDYLATE SYNTHASE** Peter H. Sayre, Carleton R. Sage, Robert M. Stroud, Dept. of Biochemistry & Biophysics, University of California, San Francisco 94143

The structure of a mutant thymidylate synthase (TS) representative of a chemotherapy-resistant enzyme reveals distinct atomic shifts away from the active site of absolutely conserved residues critical for ligand binding.

TS inhibition is an important mechanism for the chemotherapy agent 5-fluorouracil (5-FU), which prolongs life in patients with stage III colon cancer. Cells containing a well-characterized mutant form of the human enzyme (Tyr 33 -> His) exhibit relative resistance to the 5-FU metabolite 5-fluoro-2'-deoxyuridine. To investigate the structural correlates of this chemotherapy resistance, we took advantage of the extensive collection of wild type and mutant structures of *E. coli* TS built so far in this laboratory and used site-directed mutagenesis to generate the corresponding mutation in *E. coli* TS (Tyr 4 -> His). The Tyr 4 -> His mutant *E. coli* TS was expressed in bacteria, purified by ion exchange chromatography and crystallized alone or in the presence of substrate and cofactor ligands. The structure of the mutant molecule was refined to a crystallographic R factor of 22% (R<sub>free</sub> = 26%). Differences between mutant and wild type were also studied by examination of F<sub>o</sub> - F<sub>o</sub> difference maps.

Estimates of coordinate error yielded expected differences from true atomic positions of 0.35 - 0.4 Å. F<sub>o</sub> - F<sub>o</sub> difference maps allowed smaller changes between mutant and wild type structures to be discerned. These differences are concentrated in the region between the N-terminal A helix and the large central active site. The difference maps clearly reveal the loss of electron density around the position of the tyrosine hydroxyl group absent in the mutant enzyme. The hydrogen-bonding network linking the N-terminal A helix to the J helix that intervenes between the A helix and active site cavity is disrupted, since the Tyr 4 hydroxyl is no longer available to interact with side chain carbonyl of Val 170. A concerted movement of atoms away from the active site toward the mutant histidine residue propagates across