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[1], C.G. Genzor[2] and J. Sancho[2], [1]Instituto de Quimica Fisica Rocasolano. Departamento de, Cristalografia. CSIC. Serrano, 119 E-28006-Madrid, Spain. [2]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 suggest that the interaction between the FMN and the apoprotein could start at this place.

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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 watermediated hydrogen bond network in the TS reaction, by cocrystalizing 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% (Rfree=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_{free} = 26%). Differences between mutant and wild type were also studied by examination of F_o - F_o difference maps.

Estimates of coordinate error yielded expected differences from true atomic positions of 0.35 - 0.4 Å. F_o - F_o 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

the base of the J helix. The distinct atomic shifts of invariant Asp 169 away from the active site may prevent interactions between its main chain amide and the pyrimidine of the 5-fluoro-2'-deoxyuridylate inhibitor and between the Asp 169 side chain and the quinazoline ring of the cofactor. These movements could explain the decreased sensitivity of the mutant enzyme to 5-fluoro-2'-deoxyuridylate inhibition.

PS04.17.37 STRUCTURAL COMPARISON OF AMYLOIDOGENIC LIGHT CHAIN DIMER IN TWO CRYSTAL FORMS WITH NONAMYLOIDOGENIC COUNTERPARTS. Norbert Schormann and Merrill D. Benson, Dept. of Medicine, Indiana University School of Medicine, Indianapolis, IN 46202; USA.

In the majority of cases, immunocyte-derived amyloid light chain (AL) (primary) amyloidosis is associated with a nonmalignant expansion of a single plasma cell clone. The monoclonal immunoglobulin (IG) light chain product of the plasma cell clone is the precursor of the amyloid fibril subunit protein.

To investigate structural factors in amyloid fibril formation an attempt has been made to compare the variable region of amyloid proteins with nonamyloid light chain proteins with the goal of developing a structural model for the polymerization or aggregation process of IG light chain proteins.

A κI protein from an individual (BRE) with amyloidosis was completely characterized at the primary structure level. The DNA sequence coding for the variable segment of the κI light chain (κI V_L) was amplified by PCR from the patient's bone marrow DNA using the determined amino acid sequence. The DNA construct was used to express the recombinant κI V_L protein in vitro. Protein BRE was purified to homogeneity and crystallized using ammonium sulfate as precipitant. Two distinct crystal forms were obtained (Crystal form I: monoclinic, space group $P2_1$, with a=82.37Å, b=77.75Å, c=82.23Å, ß=119.97°; crystal form II: orthorhombic, space group $C222_1$, with a=82.04Å, b=142.11Å, c=77.86Å).

An effort was made to verify the original Laue group assignment, since both space groups are lower symmetry subgroups of hexagonal space group $P6_122$. The data sets were reindexed and processed using older R-Axis processing software and the newer BioteX program package. Reindexing trials and Laue checks of 'merge files' from different indexing solutions for crystal form I showed unambigously that the crystal system is correctly assigned as monoclinic. This structure was refined to 2Å. In the case of crystal form II, differences in R_{sym} and R_{merge} between indexing solutions were not as pronounced to discriminate against the hexagonal crystal system. In addition to refining the structure of BRE II in space group $C222_1$ to 1.8Å, we therefore included refinement in two possible hexagonal space groups ($P6_1$ and $P6_122$, with a=b=82.05Å, c=77.87Å, γ=120°).

The structures of both crystal forms were compared to each other and to nonamyloidogenic light chain dimers with special emphasis on domain-domain interactions. Models for amyloid fibril formation in AL amyloidosis are discussed.

PS04.17.38 STRUCTURE SOLUTION OF SOME C-REACTIVE PROTEINS. Annette K. Shrive, David Holden, Allison Metcalfe, Dean A.A. Myles, Margaret Hopkins, Ian D. Glover, David Hoole[1], Anne C. Bloomer[2] and Trevor J. Greenhough*, Dept. of Physics, and [1]Biological Sciences, Keele University, Keele, Staffs, ST5 5BG, UK. [2]MRC Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH, UK. * and CCLRC Daresbury Laboratory, Warrington WA4 4AD, UK

C-reactive protein (CRP) is a member of the pentraxin family, a conserved, phylogenetically ancient super-family of oligomeric calcium-binding proteins exhibiting high sequence homologies, often forming a cyclic pentameric assembly. Studies centred at Keele have produced the structure of Ca-free rat CRP and Ca-bound human CRP, with the structure solution of Ca-free human CRP and CRP from *Limulus polyphemus* in progress.

Human CRP is a trace plasma protein of unknown biological function that is expressed dramatically as part of the acute-phase response and as such is used almost universally as a clinical test for infection and inflammation. The generation of a pentameric model from the pentraxin serum amyloid P (SAP) has finally provided the structure solution for both the rat and human crystals. The Ca-bound human structure contains a crystal contact where the calcium-binding loop from one protomer coordinates into the calcium site of a protomer in a symmetry related pentamer, revealing the mode of binding of the principal ligand phosphocholine (PC). A striking structural cleft, on the pentameric face opposite to the PC binding site, suggests an important functional role, perhaps in complement activation.

Rat CRP, in contrast to human CRP with which it shares 65% sequence identity, is a normal component of serum, is glycosylated, has been reported to contain a disulphide-linked dimer, and exhibits comparable affinities for phosphoethanolamine (PE) and PC. Native data to 3.3Å, from a single cryocooled small crystal, and the rebuilt pentameric SAP model have provided the structure solution. Conformational changes with respect to human SAP seen in human CRP are also present in rat CRP. The proposed disulphide linkages and PC/PE binding are discussed.

PS04.17.39 CRYSTAL PACKING INTERACTIONS IN STREPTAVIDIN CRYSTALS. Ronald E. Stenkamp, Stefanie Freitag, Isolde Le Trong, Ashutosh Chilkoti and Patrick S. Stayton, Dept. of Biological Structure and Center for Bioengineering, University of Washington, Seattle, WA 98195

The high-affinity streptavidin-biotin interaction is widely used in biotechnologies such as bioseparations and diagnostics. This interaction makes streptavidin and biotin prime candidates for structural and functional studies of protein-ligand interactions, but streptavidin's ability to crystallize in a number of three- dimensional and two-dimensional crystal forms [1,2,3,4,5] might also provide a mechanism for the design of biomaterials with specific inter-molecular spacings and orientations. For example, streptavidin is being investigated as a potential two-dimensional crystalline substrate for electron microscopic studies of other biological macromolecules. To provide a basis for the design of mutated streptavidin molecules with different packing characteristics, we are examining the crystal packing interactions in the various crystal forms available to date. We will determine which residues are most commonly involved in the inter-tetramer interfaces and locate potential regions on the protein surface suitable for mutagenesis studies aimed at modulating the crystal packing and growth properties of the protein.

This work is supported by NIH grant DK49655.

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