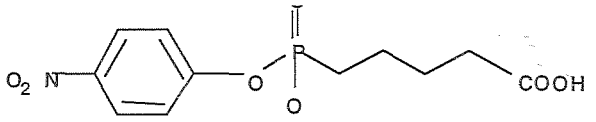
PS04.15.38 THE CRYSTAL STRUCTURES OF SEVERAL 48G7 CATALYTIC ANTIBODIES WITH ESTEROLYTIC ACTIVITY. Gary J. Wedemayer*, Leo Wang & Ray C. Stevens, Chemistry Department, University of California, Berkeley 94720 USA

Catalytic antibodies (CA's) are immunological proteins which have been selected to catalyze specific chemical reactions. The CA 48G7, which was raised against the para-nitrophenyl phosphonate transition state shown below,



catalyzes the hydrolysis of the corresponding para- nitrophenyl ester and carbonate with acceleration rates in excess of 10^4 (Patten et al., Science (in press) 1996). Working with *E. coli* expressed protein, we have studied affinity-matured and germline antibodies. X-ray diffraction data was collected on Fab-only and on Fab-hapten crystals. Processing of this data led to four crystal structures, the best of which had a resolution of 1.95 Å. A picture of both 48G7 hapten binding and affinity maturation has emerged from this study. Of particular interest is that of the nine replacement mutations fixed during affinity maturation of this CA, none is in the active site in contact with the transition state analog. This suggests that the 10^4-fold increase in hapten binding seen in the mature antibody as compared to the germline results not from direct substitutions at the active site but rather from more distant replacements which alter active site geometry or conformational flexibility. Also of interest is the lack of active site serines, which are reported to play important mechanistic roles in the catalysis of other esterolytic antibodies (Zhou et al., *Science* **265**, 1059). Instead, catalysis in the 48G7 CA appears to proceed through activated waters which are bound in the active site.

PS04.15.39 CRYSTALLIZATION OF MURINE T-CELL RECEPTORS. D. Williams, A. Heine, E.A. Stura, J. Snook and I.A. Wilson, The Scripps Research Institute, Department of Molecular Biology, 10666 North Torrey Pines Road, La Jolla, CA 92037

T-cell receptors (TcR) in association with CD3 are the central component of the T-cell signal transduction complex responsible for specific antigenic recognition under the restricting elements of the major histocompatibility complex class I and class II molecules. Activation of T-cells through this multimeric complex by their TcR is central to the regulation of the immune system and maintenance of host defense surveillance in higher organisms.

The structure determination of this receptor is key to the elucidation of how T-cell receptors differentiate between self and non-self molecules regulating T-cell activation. We have cloned several independent TcR's from insulin specific murine T-cell hybridomas(1) and expressed the alpha/beta heterodimers in both bacterial and Drosophila melanogaster(2) systems. Expressed protein was initially isolated by Ni-NTA column chromatography followed by purification by ion perfusion chromatography using a PerSeptive BIOCAD work station. Protein was subsequently used for crystallization screening trials which identified several conditions leading to crystal formation. Crystallization conditions and progress towards a structure determination will be presented.

1) D.B. Williams et.al Characterization of the Insulin A-Chain Major Immunogenic Determinant Presented by MHC Class II I-A^d Molecules. J.I. vol. 151, 3627-3637, 1993

2) M.R. Jackson et. al Empty and Peptide-Containing Conformers of Class I Major Histocompatibility Complex Molecules Expressed in Drosophila melanogaster Cells. PNAS vol. 89, pp.12117-12121, 1992

Protein Folding & Extremophiles

MS04.16.01 STRUCTURAL AND MUTATIONAL ANALYSIS OF PROTEIN STABILITY. Brian W. Matthews, Xuejun Zhang, Jian Xu and Walter A. Baase, Institute of Molecular Biology, Howard Hughes Medical Institute, University of Oregon, Eugene, OR 97403

Information on the structural basis for protein stability has come from two different sources. (1) Comparisons of the structures of proteins isolated from thermophiles with their counterparts in mesophiles. (2) Analysis of changes in the structure and stability of a given protein associated with point mutations. Conclusions from Source (1) have, however, been inconsistent. Some investigators have suggested that thermostability can come from a variety of sources including the hydrophobic effect, disulfide bridges, hydrogen bonds, metal binding and electrostatic interactions. Others have suggested that one type of interaction or effect (in particular, salt bridges) is key. The use of experiments from Source (2) to attempt to resolve these apparent discrepancies will be discussed.

MS04.16.02 CITRATE SYNTHASE FROM A MESOPHILE, THERMOPHILE AND HYPERTHERMOPHILE. G.L.Taylor, R.J.M.Russell, D.W.Hough & M.J.Danson., School of Biology and Biochemistry, University of Bath, BA2 7AY, U.K.

We have determined the crystal structures of dimeric citrate synthase from two Archaea: *Thermoplasma acidophilum*, whose habitat is around 55°C, and *Pyrococcus furiosus*, whose habitat can be up to 110°C. Comparison of these structures with citrate synthase from pig, a mesophile, reveals certain structural trends which correlate with increasing thermostability. These include: (i) a reduction in thermolabile residues and an increase in isoleucines and glutamic acids, (ii) an increase in compactness, achieved by shortening of loops and a reduction in internal cavities, (iii) an increase in complex ion-pair networks, and (iv) variations in the nature of dimer formation. Some of these features are being analyzed by site-directed mutagenesis, to gain an insight into their relative contributions to overall thermostability. The structures of several other thermostable proteins have appeared recently, some of which point to the importance of ion-pairs in conferring hyperthermostability and some of which point to compactness. We will compare and contrast our findings on citrate synthase with these studies in an attempt to discover how many roads there are to thermostability. Can we hope to emulate what Nature has spent several million years perfecting ?

1. Russell, R.J.M, Hough, D.W., Danson, M.J. & Taylor , G.L. *Structure* **2**, 1157-1167 (1994).

2. Muir, J.M., Russell, R.J.M., Hough, D.W. & Danson, M.J. *Prot. Eng.* **8**, 583-592 (1995)

MS04.16.03 THE STRUCTURE OF THE THERMOPHILIC GLUTAMATE DEHYDROGENASE FROM *THERMOCOCCUS* ANI. C. A. Smith, G. E. Norris, E. N. Baker, Department of Biochemistry, Massey University, Palmerston North, New Zealand

How thermostable enzymes maintain their structural integrity and functionality at elevated temperatures (> 90°C) remains a key question in structural biology today. In addition to their stability at high temperatures, these heat-stable enzymes generally exhibit less flexibility at lower temperatures and are much less likely to be denatured compared with mesophilic enzymes. These properties make thermostable enzymes potentially important in industry and medicine, and the ability to be able to predict which features are the most important for thermostability will enable important enzymes to be engineered for higher stability. To this end, we have undertaken the structural analysis of the highly thermostable

glutamate dehydrogenase (GDH) ($t_{1/2}$ at 90°C = 12.5 h) from the thermo-philic sulfur-reducing archaeon *Thermococcus* ANI. GDH catalyses the oxidative deamination of L-glutamate to 2-oxyglutarate and ammonia, and provides an important link between carbon and nitrogen metabolism.

The *Thermococcus* GDH has been crystallized in two orthorhombic forms; form A, a primitive cell with dimensions 155.3 x 115.1 x 173.4Å, and form B, a C centred cell with dimensions 97.6 x 247.5 x 146.4Å. Two data sets have been collected from form A crystals using an in-house rotating anode (to 3.0Å) and a synchrotron source (to 2.4Å). The V_M was 2.75 Å^3/Da, assuming 6 monomers (M_r 47,000 Da) per asymmetric unit.

The structure was solved by automated molecular replacement using AMoRe, with a search model derived from the homohexameric *Pyrococcus furiosus* GDH. The four primitive orthorhombic spacegroups were searched for two independent occurences of one *P. furiosus* trimer, with the most significant solution observed in $P2_12_12_1$ (correlation 60.2 and R-factor 34.4%). The structure has been refined using molecular dynamics (XPLOR) and restrained least-squares methods (TNT), employing strict 6-fold non-crystallographic symmetry constraints.

The structure consists of two trimers arranged "back-to-back", the interface forming an extended β-sheet. The arrangement of each trimer is similar to that observed in the *P. furiosus* and *C. symbiosum* structures, and a structural comparison, along with the identification of potential structural determinants of enzyme thermostability will be discussed.

MS04.16.04 ONE SEQUENCE, TWO FOLDS: CRYSTAL STRUCTURE OF A MISFOLDED, METASTABLE FORM OF CD2. R. Leo Brady and Alison J. Murray, Department of Biochemistry, University of Bristol, Bristol BS8 1TD U.K.

We have recently determined the crystal structure of a misfolded, metastable form of the NH_2-terminal domain of the lymphocyte cell adhesion molecule CD2. When expressed as part of a glutathione-S-transferase fusion protein the polypeptide sequence for domain 1 of CD2 can fold in two different ways. The major (85%) monomeric component forms a familiar immunoglobulin superfamily fold as previously demonstrated by both X-ray crystallography and NMR spectroscopy. We now describe the structure of a second, dimeric form present in about 15% of recombinant CD2 molecules. After denaturation and refolding in the absence of the fusion partner dimeric CD2 is converted to monomer, illustrating the dimeric form represents a metastable folded state.

The crystal structure of this dimeric form, refined to 2.0 Å resolution, reveals two domains with overall similarity to the IgSF fold found in the monomer. However, in the dimer each domain is formed by the intercalation of two polypeptide chains. Hence each domain represents a distinct folding unit that can assemble in two different ways. In the dimer the two domains fold around a hydrophilic interface believed to mimic the cell adhesion interaction at the cell surface, and we demonstrate formation of dimer can be regulated by mutating single residues at this interface. This unusual misfolded form of the protein, which appears to result from inter- rather than intramolecular interactions being favoured by an intermediate structure formed during the folding process, illustrates that evolution of protein oligomers is possible from the sequence for a single protein domain. We suggest this exploitation of a finely-balanced folding intermediate capable of developing to two distinct folds may pre-date "domain swapping" as a mechanism for the evolution of protein oligomers. A biological role for the alternative fold cannot be ruled out, as in this state the adhesion interface is buried and effectively switched "off".

Analyses of a series of deletion and single-site mutations of CD2 support the proposed folding pathway for immunoglobulin superfamily domains implied by this structure. Identifying domain features important in directing folding pathways has important implications for protein design and engineering.

MS04.16.05 STRUCTURAL INSIGHT INTO THE FUNCTION OF GroEL/GroES/ADP COMPLEX. Zhaohui Xu, Arthur Horwich, Paul B. Sigler, Yale University and the Howard Hughes Medical Institute, 295 Congress Ave., New Haven, CT 06510

GroEL is a chaperonin, composed of 14 60-kD subunits arranged as two sevenfold rotationally symmetric rings stacked back-to-back with dyed symmetry. With the aid of Mg^{2+}, ATP and GroES [a 7-subunit (10kD each) co-chaperonin], GroEL enhances the protein-folding process by preventing the formation of irreversibly misfolded and/or aggregated non-native "off pathway" intermediates. Non-native polypeptides are bound avidly by GroEL primarily through hydrophobic contacts inside the opening of the central cavity. In an ATP-consuming cycle, the bound peptide is released to achieve a folded state or rebind to the chaperonin.

We have crystallized and solved the structure of a complex of GroEL/GroES/ADP to 3.0Å. While still under refinement, the structure has shown seven ADP molecules and one GroES 7-mer ring bind to the same GroEL ring where major structural rearrangement has occurred. The elucidation of the stereochemical details of this complex will give us insight into the mechanism by which GroEL-assisted folding is accomplished.

MS04.16.06 AN UNUSUAL ROUTE TO THERMOSTABILITY IN PYROPHOSPHATASES Adrian Goldman*, Tiina Salminen*, Alexei Teplyakov‡, Barry Cooperman† and Reijo Lahti*. *University of Turku, Turku, Finland; ‡European Molecular Biology Laboratory, Hamburg, Germany; †University of Pennsylvania, Philadelphia, USA.

Rigid-body motions of entire monomers to produce tighter oligomers may be yet another way in which proteins can be made thermophilic. By comparing the structures of *Thermus thermophilus* and *E. coli* PPases (T- and E-PPases) we showed that the chief determinant of the increased thermostability of T-PPase appears to be a change in the oligomerisation and oligomeric interfaces of these hexameric enzymes. Their sequences are 47% identical and the monomer structures superimpose extremely well (rmsd of 1 Å per Cα). However, the hexamers superimpose poorly (rmsd of 2.2 Å per Cα) because of a complex rigid-body motion: with respect to the E-PPase hexamer, each T-PPase monomer in the hexamer is skewed by about 1 Å in the xy plane, is 0.3 Å closer to the centre of the hexamer in the z-direction, and is rotated by about 7° about its centre of gravity. The rigid-body translation accounts for most of the difference between the hexamer and monomer superpositions. In T-PPase the hexamer is packed more tightly than in E-PPase: the amount of surface area buried upon oligomerisation increases by 16%, in particular at one of the trimer-trimer interfaces. Three small loops involved in oligomerisation adopt different conformations. Consequently, the number of hydrogen-bonding and ionic interactions almost doubles, which allows the tighter-packing to occur. The new interactions interlock across all the oligomeric interfaces in the hexamer.

Bacterial PPases, only 170 residues long, contain large active sites (≈15 active site residues; ≈12 Å across) and have little in the way of a hydrophobic core. Much of the protein, therefore, does multiple duty: for instance, one helix contributes to the hydrophobic core of the protein, provides active-site residues and is a key part of the trimer-trimer interfaces. (Thermo)stability and activity appear to be intimately linked. Although these enzymes are not allosteric, solution data on E-PPase variant proteins show that oligomerisation stabilises the conformation of the enzyme that binds substrate and *vice versa*. The D_3 bacterial inorganic pyrophosphatases seem to be a minimalist protein design.