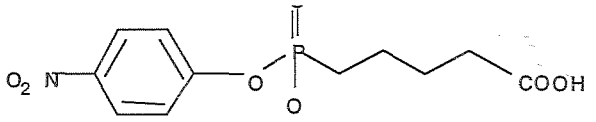
PS04.15.38 THE CRYSTAL STRUCTURES OF SEVERAL 48G7 CATALYTIC ANTIBODIES WITH ESTEROLYTIC ACTIVITY. Gary J. Wedemayer*, Leo Wang & Ray C. Stevens, Chemistry Department, University of California, Berkeley 94720 USA

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