structure was refined to 2.2Å resolution with R=0.212 and R(free)=0.256; the cell parameters are *a*=47.2Å, *b*=58.6Å, *c*=43.4Å, α=95.3°, β=103.2°, γ=93.6°. Details on each of the structural determinations and results will be discussed.

PS04.15.31 METAL-BINDING IN SUPERANTIGENS. E.M. Schad[1], M.Dohlsten[2], Per Björk[2], L.A. Svensson[1]. Molecular Biophysics, Chemical Center, Lund University, P.O. Box 124, 221 00 Lund, Sweden[1], Pharmacia Oncology Immunology, Lund, Sweden[2].

One of the most striking aspects of the structure of SEA is the unusual octahedral metal-coordination geometry. In addition, SEA displays a N-terminal coordination to the metal-ion (Schad et al., 1995). The coordinating residues of Zn^{2+} are the same ligands as those found for Cd^{2+} including the N-terminal coordination. The unusual ligand coordination by the N-terminal serine residue observed is comparable to the coordination found in the structure of phospholipase C (Hough et al., 1989; Hansen et al., 1992). In SEA, the metal-ion is coordinated by a primary bidentate formed by ligands His 225 and Asp 227. These two ligands are separated by a short spacer that according to Vaillee & Auld (1990) provides localized and overall stabilization to the protein. A longer spacer provided by His 187 donates flexibility to the coordination site. It should be noted that zinc binding proteins commonly have a tetrahedral geometry with this short spacer-long spacer ligand composition (Vallee & Auld, 1990). Alanine substitution of His 225 and Asp 227 resulted in a more than 1000fold reduced MHC class II binding affinity, whereas the His 187 mutation displayed only a 100-fold reduced binding affinity (Abrahmsen et al., 1995). This suggests that modification of the short spacer bidentate formed by His 225 and Asp 227 severely affects the MHC class II binding to domain II. In contrast, mutations of the longer spacer His 187 have less of an effect on the other metal ligands in retaining significant MHC class II binding in this region. This is further supported by the varying temperature factors observed in the metal coordination site mentioned previously.

PS04.15.32 CRYSTAL STRUCTURE OF THE MHC CLASS IB MOLECULE H2-M3 WITH FOUR DIFFERENT FORMYLATED-PEPTIDES. San Tai Shen[a], Chyung-Ru Wang[b], Kirsten Fischer Lindahl[b,c], Johann Deisenhofer[a,b], Dept. of Biochemistry[a], Howard Hughes Medical Institute[b], Dept. of Microbiology[c], University of Texas Southwestern Medical Center, Dallas, Texas 75235-9050

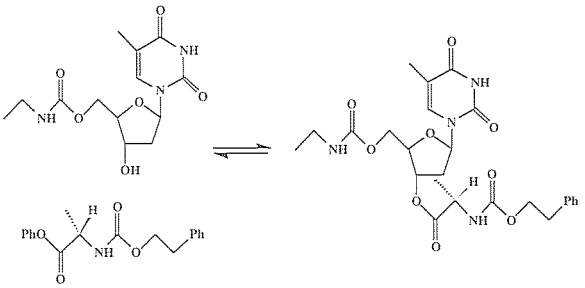
H2-M3 is a major histocompatibility complex (MHC) Ib molecule of mouse with a 10^4-fold preference for binding N-formylated peptides. H2-M3 associates with β2-microglobulin (β2m) to present a polymorphic endogenous peptide derived from the mitochondrially encoded ND1 protein to CD^{8+} cytotoxic T cells. The crystal structure of H2-M3 with a bound formylated 9-mer peptide derived from rat ND1 protein was solved in our laboratory (Wang C-R *et al.*, Cell, 70: 215-223, 1995). The overall structure of H2-M3 resembles MHC class Ia, such as HLA-A2 or H2-Kb, but the peptide-binding groove is different. The formyl group is coordinated by His-9 and a bound water molecule, and the side chain of the polymorphic residue which determines the antigen specificity of the bound peptide is buried.

To examine whether there is any conformational change in the MHC-peptide complex depending on the identity of the polymorphic residue, we intend to cocrystallize H2-M3 with each of four ND1 7-mer peptides that differ in the polymorphic residue (Ire, Ala, Val, and Thr). We have used molecular replacement to solve the crystal structures of H2-M3 bound with two of these peptides. The crystal strcuture determinations for the other two are still on progress.

PS04.15.33 CRYSTAL STRUCTURE OF AN AMINOACYLATION CATALYTIC ANTIBODY. Ben Spiller*, B.D. Santarsiero, Linda Hsieh, Raymond Stevens, Department of Molecular and Cell Biology, University of California, Berkeley CA 94720 USA

Many hydrolytic catalytic antibodies have made by raising antibodies against phosphate esters. Bimolecular addition reactions go through the same transition states as hydrolysis reactions and, with appropriate leaving groups, can be catalyzed by antibodies raised against phosphate esters.

Here, the first high resolution crystal structure of an antibody that catalyzes an addition reaction, aminoacylation, is presented. This antibody catalyzes the reaction shown. The antibody was generated by immunization with a transition state analog in which the reactive carbon ester is replaced by a phosphate ester with a phenol leaving group.



The FAB fragment was crystallized in space group $P4_32_12$ with cell parameters a=60, c= 281. Data were collected on an RaxisII and the structure was determined to 2.6 Angstroms by molecular replacement.

The aminoacylation catalytic antibody is amongst the fastest catalytic antibodies, with K_{cat}/K_m equal to 5.4×10^4 M^{-1} min^{-1}(the uncatalyzed rate is 2.6×10^{-4} M^{-1} min^{-1}). Remarkably, the antibody binds hapten with a K_d of 240 pM while K_m's for acyl acceptor and donor are 770uM and 260uM respectively. Thus the transition state analog is bound six orders of magnitude more tightly than the ground state. The antibody efficiently transfers an acyl group to an alcohol in aqueous solution.

PS04.15.34 STRUCTURE AND COMPARISON OF HIV-1 GP120 PEPTIDES IN COMPLEX WITH HIV-1 NEUTRALIZING FABS. R. L. Stanfield, J. B. Ghiara, J. M. Rini, E. A. Stura, A. C. Satterthwait, I. A. Wilson, The Scripps Research Institute, 10666 N. Torrey Pines Road, La Jolla, CA 92037

Crystal structures have been determined for three different HIV-1 neutralizing antibody Fab fragments in complex with several linear and cyclic peptides. The Fabs were all raised against the same 40-amino acid disulfide linked peptide, corresponding in sequence to the principal neutralizing determinant (PND) loop from HIV-1 gp120 (MN isolate). The complexes studied include Fab 50.1 (MN specific) with linear peptide, Fab 59.1 (broadly specific) with two linear peptides, and Fab 58.2 (potent and broadly specific) in complex with one linear and three cyclic peptides. The three different antibodies recognize overlapping epitopes on the PND loop (50.1-CKRIHIGPG, 59.1-HIGPGRAFYT, 58.2-RIHIGPGRAFY). The peptides bound to 50.1 and 59.1 are very similar, but differ from peptides bound to 58.2 around the GPGR region. Information from the early Fab- peptide complex structures has been used in the design of constrained peptides. These peptides have an Aib (α-aminoisobutyric acid) residue in the place of an Ala residue involved in a helical turn. The Aib containing

peptides all bind to Fab 58.2 more tightly than their non-Aib containing counterparts, and unpublished NMR work has shown that the Aib residue confers additional structure on the peptides in solution. The addition of the Aib residue to the peptides does not significantly change their conformation while bound to Fab. The structures for the peptides as bound to the different Fabs will be compared and contrasted.

PS04.15.35 THE THREE DIMENSIONAL STRUCTURE OF STAPHYLOCOCCAL ENTEROTOXIN C2 FROM TWO CRYSTAL FORMS. S. Swaminathan, W. Furey, J. Pletcher and M. Sax, Biocrystallography Laboratory, VA Medical Center, University Drive C, PO Box 12055, Pittsburgh, PA 15240 & Department of Crystallography, University of Pittsburgh, Pittsburgh, PA 15260 USA.

Bacterial superantigens induce massive T cell proliferation when presented by major histocompatibility complex class II (MHCII) molecules. These superantigens induce all T cells bearing particular types of Vb elements irrespective of other variable elements present in the T cell receptor by forming a ternary complex with MHCII and T cell receptor (TCR). Staphylococcal enterotoxins produced by *Staphylococcus aureus* are both toxins and superantigens. As toxins they cause vomiting and diarrhea in humans. There are five distinct serotypes of staphylococcal enterotoxins which are labeled A through E. SEC is further subdivided into SEC1-3 due to minor epitope variations. Even though all staph enterotoxins possess a common SE-fold the mode of association of these with MHCII molecule appear to be different. Further, in spite of very high sequence homology the Vb specificity of these also differ, though there is some overlap. The crystal structure of SEC2 was determined to better understand the reasons for the differences in the mode of association and Vb specificities.

SEC2 crystallizes in two forms. The monoclinic form is in space group P21 with cell dimensions a = 43.43, b = 69.92, c = 42.22Å and β = 90.1° and has two molecules in the unit cell . The tetragonal form is in space group $P4_32_12$ and has cell dimensions a = b = 42.98 and c = 289.3Å . The crystal structure determination of these two forms by the molecular replacement method will be presented. The differences in the structures of SEB and SEC2 will also be discussed.

PS04.15.36 CRYSTAL STRUCTURE OF ANTI-P-GLYCOPROTEIN FAB MRK-16 IN COMPLEX WITH ITS PEPTIDE EPITOPE. S. Vasudevan, K. Johns and D.R. Rose, Ontario Cancer Institute and Department of Medical Biophysics, University of Toronto, Toronto, M5G 2M9 Canada

Cancer cells undergoing chemotherapy can develop multidrug resistance, one form of which is associated with the overexpression of a membrane protein, P-glycoprotein (pgp). Pgp is an ATP-binding cassette (ABC) transporter that consists of two putative membrane-spanning domains and two cytoplasmic ATPase domains. Pgp has been shown to participate in energy-dependent efflux of a wide range of common anti-cancer drugs as well as other substrates. Inhibition of pgp function can improve the effectiveness of chemotherapy.

Monoclonal antibody MRK-16 binds to a discontinuous epitope consisting of two extracellular loops distant in the amino-acid sequence of pgp. It has been used as an adjuvant in anti-cancer treatments. We are studying MRK-16 firstly to understand its mode of interaction with pgp with the possible goal of improved pgp inhibitors, and secondly as part of a broad strategy to use antibodies as tools towards the structure of pgp itself. We report here the crystal structure of the MRK-16 Fab. Crystals were grown in the presence of a synthetic peptide representing one of the epitope loops. The space group is P2(1) with unit cell dimensions(a,b,c) of 54.5, 67.8, 117.2Å, β=97.6deg., and there are two Fab's per asymmetric unit. The structure was determined by standard molecular replacement techniques and refined to 2.8Å resolution with x-plor. Due to crystal packing, only one of the Fab's is complexed with peptide, permitting a comparison of liganded and unliganded structures in the same crystal. The elbow angles of the two copies of the Fab differ by about 7 degrees and there are some intriguing differences in the conformations of some of the complementarity-determining loops that make up the binding site. Conclusions based on the structure reported here, in the light of information on other pgp inhibitors, will be discussed.

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PS04.15.37 STRUCTURE OF AN IMMUNODOMINANT 38-kDa PROTEIN ANTIGEN b (Pab) FROM *MYCOBACTERIUM TUBERCULOSIS*. Nand K. Vyas, Meenakshi N. Vyas, Abha Choudhary, Zengyi Chang and Florante A. Quiocho, Department of Biochemistry and Howard Hughes Medical Institute. Baylor College of Medicine. Houston, TX-77030.

Mycobacterium tuberculosis, an etiological agent of tuberculosis, infects about one-third of the world's human population. Tuberculosis remains the largest cause of deaths (3 million each year) from a single infectious agent. Among well characterized secreted protein antigens, the 38-kDa protein antigen b (Pab) from *M. tuberculosis* is of great current interest in the immunology of tuberculosis because its B and T cell epitopes are species-specific and immunodominant. Amino acids sequence of the 38kDa protein has 30 % similarity with the periplasmic phosphahe-binding protein (PBP) from *Escherichia coli* (*E. coli*). The 38-kDa gene from *M. tuberculosis* has been subcloned and overexpressed in the nonpathogenic *E. coli* for structure-function studies. We have determined an X-ray structure of the recombinant 38-kDa at 3.0 Å resolution by the MIR method. Results of the 38-kDa structure determination and further refinement will be presented. The structure of the 38-kDa will be used for topographic mapping of known B and T cell epitopes to understand cooperation between B and T cells in immune responses. In addition, the 38-kDa structure will be used for comparison with the known structure of the PBP (*E. coli*). Crystallization of the 38- kDa by vapor diffusion and repeated seeding methods produced crystals in two distinct forms. The orthorhombic form, space group $P2_12_12$, has cell dimensions: a= 125.45 Å b= 72.27 Å and c= 73.43 Å, whereas monoclinic form, space group $P2_1$, has cell dimensions: a= 67.42 Å b= 113.38 Å, c= 42.68 Å and ß= 108.53°. Asymmetric unit of each crystal form contains two molecules of the 38-kDa. Both crystal forms diffract X-rays to 2.0 Å resolution.