PS04.15.27 THE 2.5 Å STRUCTURE OF FAB JEL42-HPR COMPLEX L. Prasad, J.S. Lee, E.B. Waygood and L.T.J. Delbaere. Department of Biochemistry,University of Saskatchewan, 107 Wiggins Road, Saskatoon, Saskatchewan, S7N 5E5 CANADA

We have reported the comparison of epitope predictions from site-directed mutagenesis experiments with those from the 2.8 Å structure of the Fab Jel42 antibody-HPr complex (Prasad et al. 1993. J. Biol. Chem., 268, 10705-10708). We now report the 2.5 Å structure of the Jel42-HPr complex which provides further details of antibody-antigen recognition.

The refinement of the structure was extended to 2.5 Å with x-ray intensity data collected on a large crystal (1.2 x 0.4 x 0.2 mm) with an Enraf-Nonius FAST Area Detector on an Enraf-Nonius FR571 rotating anode generator with copper radiation and graphite monochromator at 15°C. The refinement was carried out using the molecular dynamics program X-PLOR. Cycles of refinement were interspersed with manual model building and adjustments using the program TURBO-FRODO. The structure refined to an R-value of 0.22 for the resolution range 10 to 2.5 Å. The refined structure includes 102 solvent molecules and 2 sulfate ions.

The elbow angle for Fab Jel42 in the complex is 154°. The buried surface area at the antibody-antigen combining site, calculated by the method of Connolly using a probe radius of 1.7 Å is 665 Å^2 for the Fab and 718 Å^2 for HPr. Antibody-antigen binding is mediated by 9 hydrogen bonds and 79 van der Waals contacts ; 16 residues of HPr and 21 residues of the Fab are involved in the binding. An additional salt bridge contact involves the CDR loop (CDRL2) which previously appeared not to be involved in antigen binding. In addition, there are 18 contacts between the Fab and HPr, mediated through 11 water molecules.

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PS04.15.28 CRYSTAL STRUCTURE OF HUMAN INTERFERON-ALPHA 2. Ramaswamy Radhakrishnan[1], Leigh J. Walter[1], Paul Reichert[2], Alan Hruza[2], Tattanahalli L. Nagabhushan[2], and Mark R. Walter[1], [1]Department of Pharmacology, University of Alabama at Birmingham, Birmingham, AL 35294, USA; [2]Schering Plough Research Institute, Kenilworth, NJ 07033, USA

The human alpha interferons (hu IFN-α's) comprise a family of at least 15 closely related molecules which exhibit broad spectrum anti-viral, anti-proliferative, and immunomodulatory properties. The practical importance of these pleotropic activities may be realized by the approval of huIFN-α2 for the treatment of diseases having viral, malignant, and immune etiologies. Despite a wealth of biological and biochemical data on this multiprotein family, a representative three-dimensional structure for the huIFN-α's has not been completed. Here, we report the crystal structure of huIFN-α2.

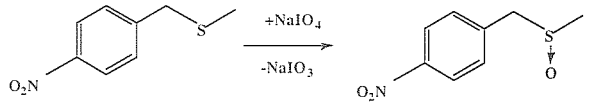
Single crystals of huIFN-α2 suitable for X-ray diffraction analysis have been grown to dimensions of 0.2 x 0.5 x 0.5 mm^3. The crystals diffract to approximately 2.9Å resolution on a R-AXIS IIC image plate detector at -170°C. Phases for the structure determination were obtained by multiple isomorphous replacement (MIR) techniques using two derivatives. The phases were subsequently improved using non-crystallographic symmetry averaging. The refined structure will be presented in light of the large amount of biochemical and structural data on related interferons and cytokines in the class 1 and class 2 cytokine receptor family.

PS04.15.29 CRYSTAL STRUCTURE OF A MURINE MONO-CLONAL ANTI-ELAM IGG1 ANTIBODY 7A9 Fab FRAGMENT Adela Rodriguez-Romero,[1] Orna Almog,[1] Maria Tordova,[1] Zafar Randawa,[2] and Gary L. Gilliland.[1] [1]Center for Advanced Research in Biotechnology of the University of Maryland Biotechnology Institute and National Insititute of Standards and Technology, 9600 Gudelsky Dr., Rockville, MD 20850. [2]Otsuka America Pharmaceutical, Inc., 9900 Medical Center Dr., Rockville, MD 20850.

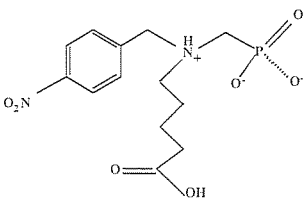
Neutrophils, involved in the early stages of many forms of acute inflammation, can cause disease by damaging normal host tissue, as in the Adult Respiratory Distress Syndrome (ARDS). In this process, cell surface receptors such as ELAM-1 (endothelial leukocyte adhesion molecule) react with a carbohydrate residue on the cell surface of the neutrophil, while the leukocyte integrins containing a CD18 antigen react with ICAM-1 (intercellular adhesion molecule-1). The 7A9 antibody effectively binds to ELAM-1 blocking neutrophil accumulation in the lungs [Mulligen et al., (1991) *J. Clin. Invest.* **88**, 1396-1406]. The three-dimensional structure of the 7A9 Fab was undertaken to further our understanding of how this antibody binds to ELAM-1. Crystals of the protein (0.2 x 0.3 x0.5 mm) were grown in vapor diffusion experiments at room temperature in the presence of 20% PEG (8K), 20 mM ammonium sulfate, 100 mM cacodylate buffer at pH 6.5. The orthorhombic crystals diffract to 2.8 and have space group $P2_12_12_1$. The unit cell parameters are a = 44.5 , b = 83.8 , and c = 132.5 . The structure was determined by the molecular replacing technique using the program XPLOR. The probe molecule, the Fab fragment of an antibody directed against the rhinovirus [Tormo et al., (1992) *Protein Sci.* **1**, 1154-1161], was selected because of its sequence homology with the 7A9 antibody. The structure has been refined using XPLOR to a current R factor of 0.18. Details of the structure determination, a description of the structure, and a comparison of the structure with those of related Fabs will be presented.

PS04.15.30 A SULFIDE OXIDATION CATALYTIC ANTIBODY. B.D. Santarsiero, L.C. Hsieh-Wilson, P.G. Schultz, & R.C. Stevens, Department of Chemistry, University of California, Berkeley, CA 94720 USA

A number of antibodies were found to catalyze the oxidation of a *p*-nitro-aromatic-sulfide to the corresponding sulfoxide with sodium periodate as a cofactor.



Earlier work on the mechanism of the oxidation lead to the description of two potential transition states, both accomodating a positive charge on the sulfur atom and an increase in the negative charge of the periodate. The hapten (below), based on these transition states, was then designed.



The antibody 28B4 was the most efficient, binding the hapten with a K_d of 52nM and catalyzing the oxidation with a k_{cat} of 8.2s^{-1}; k_{cat}/K_m=1.9x10^{5}M^{-1}s^{-1}. To better understand the catalytic mechanism of 28B4 and further examine the role of hapten binding, the structure of the Fab portion of the antibody was determined in the presence and absence of hapten. Both structures are found in space group P1. With hapten, the structure was refined to 1.7Å resolution with R=0.209 and R(free)=0.279; the cell parameters are *a*=52.7Å, *b*=58.2Å, *c*=43.2Å, α=94.3°, β=113.8°, γ=78.9°. Without hapten, the

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

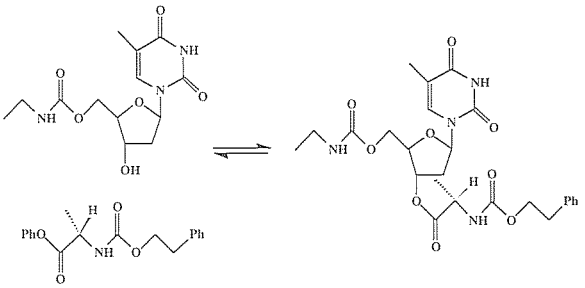
H2-M3 is a major histocompatibility complex (MHC) Ib molecule of mouse with a 10^4-fold preference for binding N-formylated peptides. H2-M3 associates with β2-microglobulin (β2m) to present a polymorphic endogenous peptide derived from the mitochondrially encoded ND1 protein to CD^{8+} cytotoxic T cells. 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