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.

This research was funded by the Medical Research Council of Canada.

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.

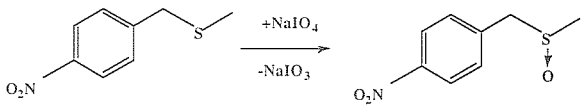
Single crystals of huIFN-α2 suitable for X-ray diffraction analysis have been grown to dimensions of 0.2 x 0.5 x 0.5 mm^3. The crystals diffract to approximately 2.9Å resolution on a R-AXIS IIC image plate detector at -170°C. Phases for the structure determination were obtained by multiple isomorphous 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.

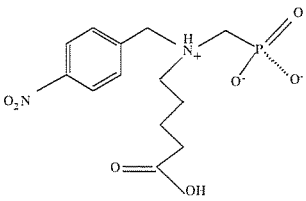
Neutrophils, involved in the early stages of many forms of acute inflammation, can cause disease by damaging normal host tissue, as in the Adult Respiratory Distress Syndrome (ARDS). In this process, cell surface receptors such as ELAM-1 (endothelial leukocyte adhesion molecule) react with a carbohydrate 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