show only low activity. To fully understand the underlying structural principles behind functional activity shifts we have crystallized and solved the structures of all four hybrid proteins. Our results show that the overall polypeptide chain fold for these four hybrid proteins is similar to that of IL-8. However, also apparent are differential side chain interactions that appear to be correlated to observed functional differences. This has lead to a hypothesis as to which portions of the surface of IL-8 are essential for the expression of its activity.

PS04.15.15 STRUCTURAL STUDIES OF CATALYTIC ANTIBODIES. A. Heine, E. A. Stura, K. D. Janda, C. F. Barbas III, R. A. Lerner, I. A. Wilson, The Scripps Research Institute, Department of Molecular Biology, 10666 North Torrey Pines Road, La Jolla, CA 92037.

The diversity of the immune system can be exploited to generate catalytic antibodies, which not only catalyze a wide variety of reactions similiar to enzymes, but also otherwise disfavored or difficult reactions. The structures of catalytic antibodies and their corresponding complexes with transition state analoges or substrates are essential to understand the antibody-antigen interaction and the mechanism involved in catalysis. A comparison of such mechanisms with those of corresponding enzymes might give indications for different rate enhancements. By knowing which residues in the active site are involved in catalysis, site directed mutagenesis can be done to improve the catalytic rate.

The aldolase antibody 38C2 mimics the natural class I aldolase enzyme. A similiar mechanism for catalysis is proposed. The ε-amino group of a lysine residue in the active site forms an enamine with a ketone substrate, activating it as an aldol donor. Crystals diffract to at least 3.2 Å and a dataset was collected to that resolution. Subsequently studied antibodies include the anti-metallocene antibody 13G5, which catalyzes a Diels-Alder reaction. A ferrocene derivative was used as the haptenic group. Data were collected to 2.7 Å for the Fab and to 2.8 Å for the isomorphous complex. A syn elimination of an acyclic substrate is catalyzed by antibody 1D4. In the absence of the catalytic antibody this disfavored reaction does not occur. Crystallization conditions, structure solution and progress in refinement will be reported.

PS04.15.16 STRUCTURE OF THE VARIABLE DOMAIN OF HUMAN IMMUNOGLOBULIN κ_{IV} LIGHT CHAIN LEN. D.-B. Huang, C. Ainsworth, C.-H. Chang, F. J. Stevens, M. Schiffer, Center for Mechanistic Biology and Biotechnology, Argonne National Laboratory, Argonne, IL 60439-4833

The structure of κ_{IV} protein LEN was determined using molecular replacement with the κ_I protein REI as a search structure. The R factor is 15% for data extending to 1.8 Å. The protein was crystallized from 1.5 M ammonium sulfate in space group $C222_1$: the unit cell dimensions are a=43.1, b=83.5, and c=54.5 Å. There is one V domain in the asymmetric unit. The crystallographic twofold axis parallel to the b axis forms the twofold of the dimer observed in other crystal structures of light chains. Although the geometry of this dimer is similar to that observed in human κ_I type protein REI and mouse protein McPC603, the crystal contacts are different. The LEN dimers form a very tightly packed crystal, with head to tail contacts. All other kappa V domain dimers form a helix related by the sixfold screw axis of the unit cell; the contacts between neighboring dimers are through residues 9-12 forming a ß-pleated sheet utilizing a local or crystallographic twofold axis. We speculate that this contact in the LEN crystal is not energetically favorable, since Ser residues 9 and 12 are replaced by Asp and Ala, respectively. Protein LEN has six additional residues in its CDR1 segment compared with REI. The LEN CDR1 segment has the same length as murine κ light chain McPC603; its amino acid sequence differs in 5 out of 17 residues. The conformations of these segments are also homologous. Proteins with closely related sequences to LEN form various deposits in patients, while LEN does not; therefore it can serve as a standard to identify pathogenic features of light chains.

This work was supported by the U.S. Department of Energy, Office of Health and Environmental Research, under Contract No. W-31-109-ENG-38 and by U. S. Public Health Service Grant DK43757.

PS04.15.17 CRYSTAL STRUCTURE OF EXTRACELLULAR DOMAIN OF TISSUE FACTOR COMPLEXES WITH AN INHIBITORY FAB. Mingdong Huang, Rashid J. Syed, Enrico A. Stura, Wolfram Ruf, Thomas S. Edgington, and Ian A. Wilson. Department of Molecular Biology The Scripps Research Institute, La Jolla, CA 92037.

The macromolecular assembly of Tissue factor (TF) with factor VIIa plays the central role in the cellular activation of the blood coagulation cascades. An Fab (5G9) was found to be an effective immediate anticoagulant in plasma, which binds strongly to TF and displaces VIIa from the preformed TF-VIIa complex. We report here the crystal structure of the complex between Fab 5G9 and the extracellular domain of TF. The interdomain angle of TF in the complex is basically the same as for the free TF, demonstrating the strong interdomain interaction in TF. The current model of the complex is consistent with the TF mutagenesis data. For example, K169 and N171 interact with L1, L2, L3, and H3 CDR loops of Fab 5G9 with large contact area (138Å^2), which explains when these residues are mutated to alanines both the binding of TF to Fab 5G9 and TF's function are greatly reduced. The TF-Fab complex is the first protein structure in P2 space group (based on current pdb release). The L shape of the complex molecule may explain the adoption of this rare space group for protein and the weak diffraction of the crystal. The crystal used for data collection diffracted to 3Å with an overall I/σ (I) of 7.5 and an Rsymm of 13%. The structure is currently refined to an R value of 24.0% and an R-free of 28.5% with strict NCS applied to the two NCS-related molecules in the asymmetric unit.

PS04.15.18 PRELIMINARY STRUCTURAL FEATURES OF HUMAN HEPARIN BINDING PROTEIN. Lars F. Iversen, Jette S. Kastrup and Ingrid Kjøller Larsen. Dept. of Medicinal Chemistry, Royal Danish School of Pharmacy, Universitetsparken 2, DK-2100 Copenhagen.

The highly glycosylated protein, human heparin binding protein (HBP) also known as CAP37 or Azurocidin has been crystallised in the primitive orthorhombic space group $P2_12_12_1$. A full data set has been collected to 2.8 Å and diffraction was observed to at least 2.2 Å. A molecular replacement solution using human neutrophil elastase as a search model was obtained, showing one monomer per asymmetric unit. The correlation coefficient and *R*-factor were 50.4 and 44.8%, respectively, after 10 cycles of rigid body refinement.

HBP is an inactive serine protease homologue [1]. The inactivity is caused by selective mutations in the serine 195 and histidine 57 in the active site triade. The human HBP exhibits 47% sequence identity with human neturophil elastase [1]. HBP consist of 225 amino acids, is highly glycosylated and possess three putative N-glycosylation sites. Human HBP has been expressed in the baculo virus system as a 28 kDa protein, resulting in a glycosylation degree of 13-14%.

HBP is believed to be involved in host defence during infections and inflammations [2]. Monocyte chemotaxis, survival and differentiation are activated by HBP [1,3]. In addition, it has been shown that HBP binds strongly to endotoxin with $K_{ass} = 0.8x10^9$

M^{-1} and possess antibacterial properties.

The multifunctionality of HBP makes it highly relevant for structural investigations. The structural information contributing to the elucidation of HBP's multifunctionality and the role of the heavy glycosylation.

1. Flodgaard H. et al., Eur. J. Biochem, 197, 535-547 (1991).
2. Østergaard E. and Flodgaard H., J. Leukocyte Biol., 51, 316-323 (1992).
3. Østergaard E. et al., APMIS, 100, 1073-1080 (1992).

PS04.15.19 CRYSTALLIZATION AND PRELIMINARY X-RAY STUDY OF THE VISCUM ALBUM ML1 TOXIN IN ITS NATIVE STATE. N.V.Konareva, A.A.Mikhailov, Institute of Crystallography, Moscow, Russia A.G.Tonevitskii, I.I.Agapov, D.E.Temjakov SRIIMGS, Moscow, Russia A.N.Popov, H.D.Bartunik GBF/MPG, DESY, Hamburg, Germany

The Viscum album ML1 toxin belongs to a group of phytotoxins, such as abrin, ricin, or modeccin, which inhibit the protein synthesis of eukaryotic cells. The toxins of this group are glycoproteins with molecular weights of about 60 kDa. They consist of two subunits, which have their own functions. One of the subunits is lectin with two sites of galactose binding, and the other subunit is a highly specific N-glycosidase - an enzyme, which modifies the 28S RNA-60S ribosomal subunits. The catalitic A (active) and the binding B (binding) subunits are linked into a dimer by a disulfide bond. A handing-drop version of vapor diffusion was used for crystal growth. A precipitating countersolution consisted of ammonium sulfate (in a concentration of 30-35% to a saturated at 20 C solution) and 0.14 M NaCl in a 0.1 M acetate or 0.1M phosphate buffer in a pH range from 4 to 5. The crystallization solution contained equel volumes of a solution of protein (10-13 mg/ml) in one of the above-mentioned buffers and a countersolution. To reduce the number of crystal nuclei, dioxane was added in an amount of 0.01 to 0.02 the volume of the crystallization solution. The X-ray difraction data were collected using the BW6 synchrotron station with a DORIS storage ring (DESY,GBF/MPG, Hamburg, Germany). Intensities were registered using a MARresearch Imaging Plate Scanner. Crystal data are: sp.gr.P6122 (P6522); a=b=110.4 Å, c=309.6 Å. Intensities of 20,683 unique reflections with I>s were measured in the resolution region from20 to 3 Å resolution. The completeness of the data set is 89% and R(I)merge=5.5%.

PS04.15.20 STRUCTURAL BASIS OF HIV-1 PROTEASE INHIBITION BY A MONOCLONAL ANTIBODY. Julien Lescar[1], Renata Stouracova[1,2], MarieMadeleine Riottot[1], Véronique Chitarra[1], Jiri Brynda[1,2], Juraj Sedlacek[2], Graham A. Bentley[1], [1]Unité d'Immunologie Structurale, Institut Pasteur, Paris, [2]Department of Gene Manipulation, Institute of Molecular Genetics, Academy of Sciences of the Czech Republic, Prague

The crystal structure of the Fab fragment of the anti-HIV-1 protease monoclonal antibody, F11.2.32, has been solved both in the unligated state and as two different complexes with cross-reacting peptide fragments from the viral enzyme corresponding to residues 36-46 and 36-57. This antibody inhibits HIV-1 protease activity with an inhibition constant in the micromolar range. Diffraction measurements made at cryogenic temperatures gave Bragg intensities to 3.0 Å resolution for the free Fab, 2.1 Å resolution for the Fab complexed to peptide 36-46 and 2.6 Å resolution for the Fab complexed to peptide 36-57. Initial models for the three crystal structures were obtained by molecular replacement and refined by simulated anealing.

The structures reveal the peptide to be bound at the centre of the antigen-binding site between residues 38 to 42, which adopt a type II β turn conformation. There is only partial correspondence between the structure of this region of the peptide and that of the native antigen.

PS04.15.21 CRYSTAL STRUCTURE OF OUTER SURFACE PROTEIN A (OspA) OF *BORRELIA BURGDORFERI* COMPLEXED WITH A MURINE MONOCLONAL Fab. Hong Li[1], John Dunn[1], Benjamin J.Luft[2], & Catherine L. Lawson[1], [1]Biology Department, Brookhaven National Laboratory, Upton, N.Y. 11973, [2]Division of Infectious Diseases, School of Medicine, State University of Stony Brook, Stony Brook, N.Y. 11794-8153

OspA is an abundant outer surface protein of *Borrelia burgdorferi* that is currently being tested in several forms as a prophylactic vaccine against Lyme disease. The crystal structure of recombinant OspA (with amino-terminal lipidated cysteine replaced by non-lipidated alanine) was solved in a complex with the Fab fragment of an agglutinating monoclonal antibody. The structure of OspA will be useful for mapping sequence variability and for defining antigenic epitopes to aid in vaccine design.

OspA consists almost entirely of antiparallel ß-strands with short turns or small loops. Nine strands (5-13) form a central ß-sheet with right-handed twist. Strands 1 to 4 pack against the central sheet in an orthogonal sandwich motif. Strands 14 to 17 and 18 to 21 form two sheets that pack against the sole a-helix terminating at lysine 273, forming a $\beta/\beta/\alpha$ barrel. A homology search of the protein structure data base (DALI) suggests that OspA does not share structure homology with any known proteins. The Fab184.1 binds to loops in the region of the N-terminal sandwich and may stabilize an otherwise flexible N-terminal region. Sequence variability maps to two C-terminal loops on one edge of the central sheet, plus one loop of the C-terminal barrel.

The structure was solved by a combination of multiple isomorphous replacement and molecular replacement methods. The refined model consists of residues 22 to 273 of OspA (28 kD), all residues of Fab184.1 (50 kD), and 324 waters (current R-factor is 22.9 % and R free is 29.4 at 1.95 Å).

PS04.15.22 CRYSTAL STRUCTURE OF A CDR3 MUTANT OF A T CELL ANTIGEN RECEPTOR Va DOMAIN. Hongmin Li[1], Marina I. Lebedeva[1], E. Sally Ward[2], and Roy A. Mariuzza[1]. [1]Center for Advanced Research in Biotechnology, University of Maryland Biotechnology Institute, 9600 Gudelsky Drive, Rockville, MD 20850; [2]University of Texas Southwestern Medical Center, 5323 Harry Hines Boulevard, Dallas, TX 75235-8576

The crystal structure of an engineered T cell receptor Va domain containing a grafted CDR3 was determined at 2.3Å resolution by molecular replacement using the wild type Va domain (1) as a search model. The final R factor is 0.156 with R.M.S. deviations from standard bond lengths and bond angles of 0.010Å and 1.936°, respectively. Like the wild type Va, the mutant Va crystallized as a homodimer very similar to antibody V_LV_H dimers However, the relative orientation of the two chains in the mutant Va homodimer differs from that in the wild type by a rotation of 14°. The buried surface area in the dimer interface of the mutant is 24.1Å^2 less than that in the wild type. While the residues forming the interface are essentially the same in the two structures, there are only five pairs of interface hydrogen bonds in the case of mutant compared with 17 for the wild type. This implies that the mutant Va homodimer is stabilized mainly by hydrophobic interactions. The difference in relative orientation of the Va domains in the two dimers will be discussed in the context of the variability in domain orientation reported for V_LV_L and V_LV_H dimers.

1. Fields et al, Science 270, 1821-1824 (1995).