PS04.15.11 CRYSTAL STRUCTURE OF MFE-23, A CLINICALLY IMPORTANT ANTI-CEA SCFV ANTIBODY FRAGMENT, AT 0.28 NM RESOLUTION. M. K. Boehm[1], A. L. Corper[2], T. Wan[2], M. Sohi[2], B. J. Sutton[2], J. D. Thornton[1], P. A. Keep[1], R. H. J. Begent[1], S. J. Perkins[1], [1]Depts. Biochemistry Molecular Biology, and Clinical Oncology, Royal Free Hospital Sch. Med., Rowland Hill St., London NW3 2PF, UK, [2]Randall Institute, King's College London, 26-29 Drury Lane, London WC2B 5RL, UK

MFE-23 is a single-chain Fv (scFv) antibody fragment that was selected from a phage-display library for high affinity binding to carcinoembryonic antigen (CEA), and has important clinical uses in the detection, monitoring and targetting of colon cancer. CEA is a seven-domain cell adhesion molecule which is expressed at high levels on colon carcinoma cells. MFE-23 (M_r 27,150; 256 residues) consists of an N-terminal V_H domain, a 15-residue linker, a V_L domain and a 12-residue tag. MFE-23 was expressed in E. coli and purified on CEA coupled to Sepharose. Crystals were produced by the hanging-drop method. MFE-23 at 2 mg/ml was mixed 1:1 with precipitant (100 mM Tris-HCl at pH 6.5 containing 45% saturated ammonium sulfate), and a 10 µl drop of this mixture was equilibrated against 0.5 ml of precipitant at 18°C. Well-formed crystals formed within several days, and diffracted to 0.2 nm resolution. Diffraction data were collected using an R-AXIS-IIC mounted on an RU200 rotating anode X-ray source. The crystal belongs to the trigonal space group $P3_221$ and has unit cell dimensions of a = b = 6.170 nm and c = 12.794 nm. A single crystal was used for structure determination. Data to a resolution of 0.28 nm were processed using DENZO and the CCP4 program package. The structure was solved by molecular replacement using the Fv coordinates from a murine IgA Fab fragment (Brookhaven code 2FBJ). The antibody complementarity determining regions, the linker and the tag were omitted for the calculation of initial 2Fo-Fc electron density maps. Refinement of the MFE-23 structure is currently in progress, utilizing the rigid-body refinement and the positional refinement algorithms of X-PLOR and manual rebuilding of the model in O.

PS04.15.12 ARE "HOT SPOTS" [SUPER-FLEXIBLE REGIONS] ON THE SURFACE OF AN ANTIGEN ESSENTIAL FOR ANTIGEN-ANTIBODY BINDING? Gerson H Cohen, David R Davies, National Institutes of Health, NIDDK/LMB, Bethesda, MD 20892 USA

The lysozyme molecule has been studied crystallographically in complex with a number of Fv and Fab antibody fragments. In these complexes the structure of the lysozyme molecule shows significant changes at one or more of three points in its sequence. The Ca positions at residues 47, 70-71 and 101-103 of lysozyme in one complex differ from the corresponding position in a second by 1-2 Å on the average and by as much as 7 Å. The average general difference in Ca positions is 0.5-0.75 A. This main chain flexibility demonstrated by bound lysozyme is found in the published structures of native unbound lysozyme and is not merely a distortion resulting from the association with antibody fragments. In all of the complexes reported at least one of these three regions is partially buried as a result of complex formation. In an attempt to determine whether this flexibility is required for antibody binding or is merely coincidental, we examined neuraminidase and its complexes with NC41 and NC10. The neuraminidase structures do not exhibit the flexibility shown by lysozyme; the maximum difference noted in the comparison of the neuraminidases is only 1Å. Neuraminidase does not support the requirement of hot spots in the antigen-antibody interface.

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PS04.15.13 THE HIGH RESOLUTION STRUCTURE OF AN ANTI-hCG FAB. Constantina Fotinou[1], Jeremy Beauchamp[1], Annemarie deHaan[2], Ebo Bos[2] & Neil W. Isaacs[1]. [1]Dept. of Chemistry, University of Glasgow, Glasgow. G12 8QQ U.K.; [2]Dept. of Biotechnology & Biochemistry, N.V. Organon, Molenstraat 110, PO Box 20, 5340 BH, Oss, The Netherlands.

The structure of Fab3A2 - an anti-human chorionic gonadotropin (hCG) antibody - has been solved to 2.0Å resolution, with data collected to 1.4Å resolution.

hCG is a hormone essential for the maintenance of the early stages of pregnancy. It is a member of the glycoprotein hormone family which includes follicle stimulating hormone (FSH), luteinising hormone(LH) and thyroid stimulating hormone (TSH), which have a common α-subunit. The biological specificity being determined by the β-subunit. A high degree of sequence similarity exists, with hCG and LH binding to a common receptor. hCG is unique in having a C-terminal extension on the β-subunit. This C-terminal peptide of hCG has been used in WHO sponsored research to produce an anti-fertility vaccine. As some forms of cancer secrete hCG, specific antibodies against the hormone can be used as immunodiagnostics.

Antibody 3A2 is an hCG C-terminal specific antibody. The structure of the Fab fragment (Fab3A2) has been solved to 2.0Å resolution. Data were collected at Daresbury SRS under cryocooled conditions (100K). The structure was solved by molecular replacement using AMoRe(Navaza, (1994) *Acta Cryst. A*, **50**, 157-163). After some refinement, the crystallographic R-factor is 22.3%. A high resolution dataset has recently been collected to 1.4Å which is currently being used to refine the structure further. Comparison of the complementarity determining regions (CDRs) of Fab3A2 with those from the Macromolecular Structures Database reveals a mainchain conformation consistent with the canonical structure hypothesis (Chothia *et al.*, (1994) *Nature*, **342**, 877-883).

PS04.15.14 CHARACTERIZATION OF CHEMICALLY SYNTHESIZED HYBRID CXC CHEMOKINES. Tai Y. Fu, Yu Wai Chen, Yaoguang Luo, Ian Clark-Lewis and Gary D. Brayer, Department of Biochemistry and Molecular Biology, and the Protein Engineering Network of Centres of Excellence, 2146 Health Sciences Mall, University of British Columbia, Vancouver, BC, Canada V6T 1Z3

Interleukin-8 (IL-8) is a chemoattractant cytokine (chemokine) that stimulates the accumulation and activation of neutrophil leukocytes at sites of inflammation. This small protein (72 residues) has been linked to a wide variety of inflammatory events and is thought to be the causitive agent in their associated immuno-pathology. IL-8 is one of a family of related proteins termed the CXC chemokines that have four conserved cysteines, with the first two separated by one amino acid. Interferon-γ inducible peptide-10 (IP-10; 74 residues) is also a member of the CXC chemokine family and is expressed by a variety of cell types on induction with interferon-γ and lipopolysaccharide. Although IL-8 and IP-10 share 24% primary sequence identity, they appear to have very different physiological roles. IP-10 does not compete with IL-8 for binding to neutrophil receptors and has unique properties associated with T-cell mediated inflammatory reponses and host-mediated anti-tumor effects. To characterize the components of IL-8 that are responsible for its specific functions, four IL-8/IP-10 molecular hybrids were chemically synthesized in which portions of the sequence of IL-8 were replaced with the corresponding IP-10 sequence to determine the resultant functional and structural changes induced. Two of these hybrid CXC chemokines have activities comparable to wild-type IL-8, while the other two

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.

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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 $K_{ass} = 0.8x10^9$