catalytic site and a specific promoter recognition residue allows the orientation of the polymerase on the template to be defined. The structure of T7 RNAP and a comparison with those of KF and RT will be discussed. Work supported by U.S. NIH Grant GM41936.

DS–03.08.03 STRUCTURE OF THE REGULATORY DOMAIN OF SCALLOP MYOSIN AT 2.8 Å RESOLUTION. By X. Xie*[1], D. Harrison[1], I. Schlichting[2], R. Sweet[3], V.N. Kalabokis[4], A.G. Szent-Gyorgyi[4] and C. Cohen[1], [1]Rosenstiel Basic Medical Sciences Research Center, [4]Department of Biology, Brandeis University, USA, [2]M.P.I. Heidelberg, Germany and [3]Brookhaven National Laboratory, USA.

The regulatory domain of scallop myosin - the protein switch that turns on this myosin motor in response to Ca^{2+} binding - can be obtained by proteolysis of scallop myosin S1 (Kwon *et al.*, PNAS, 1990, **87**, 4771-4775). This domain is a stable three chain complex (Mw ~ 45 kD) of a 10 kD heavy chain, together with two light chains. Crystals of the complex have the symmetry of the monoclinic space group $P2_1$, with unit cell dimensions a=52.5, b=87.0 and c=55.5 Å and β=114.5°. There is one molecule per asymmetric unit, with a solvent content of about 54%.

The structure has been solved to 2.8 Å resolution. The phases were determined by multiple isomorphous replacement with anomalous scattering. The light chains, as inferred from the amino acid sequences, have a "dumbbell-like" fold, similar to that of calmodulin. The "essential" light chain winds around the N-terminal portion of the heavy chain, while the "regulatory" light chain winds around the C-terminal region. The heavy chain is folded into a single long α-helix, which is stabilized by the light chain interactions. The two light chains make contact with one another over a limited region which appears to be the Ca^{2+} binding site.

DS–03.08.04 CRYSTAL STRUCTURES OF STEROID DEHYDROGENASE ENZYMES. W. L. Duax, D. Ghosh, M. Erman, W. Pangborn, Medical Foundation of Buffalo, Buffalo, NY 14203, S. Nakajin, S. Ohno and M. Shinoda, Hoshi University, Tokyo, Japan.

Short chain dehydrogenase enzymes influence mammalian reproduction, hypertension, neoplasia and digestion. We have determined the three-dimensional structures of two members of this enzyme family, bacterial 3α,20β-hydroxysteroid dehydrogenase (3α,20β-HSD) and porcine 20β-hydroxysteroid dehydrogenase (20β-HSD) and used their structures to model, 11β-hydroxysteroid dehydrogenase (11β-HSD).

3α,20β-HSD is an NAD(H)-linked enzyme of 255 amino acids that catalyzes the reversible oxidation of 3α, and 20β-hydroxyl groups of androstane and pregnane derivatives. The crystal structure of the holo form revealed a typical dinucleotide binding domain, the location of the NAD(H) cofactor and a putative steroid binding site. The quarternary association of the enzyme, the position of the cofactor and the nature of the substrate binding site are significantly different from those observed in the long chain dehydrogenases. The location of the postulated active site is compatible with the observed stereospecificity of the enzyme and the fact that it is active as a tetramer. The active site fits a cortisone molecule like a glove and is lined with residues that are highly conserved in the short chain dehydrogenase family and assumed to be involved in the mechanism of action. The structural findings eliminate one of two catalytic mechanisms previously proposed on the basis of biochemical studies. Three possible mechanisms are suggested by the observed structure. In one model an Arg side-chain between the nicotinamide ring and the steroid-binding site may facilitate hydride-transfer. Alternatively, the Arg side chain may move aside allowing the steroid to approach the cofactor for a direct hydride transfer. A proton-relay network, composed of conserved side-chains and tightly bound water molecules at the base of the catalytic cleft, has been identified. The location of the nicotinamide ring and the proton network are consistent with the stereospecific features of the reaction. In the third model both the cofactor and the substrate approach each other in the transition state, and the cofactor moves deeper into the catalytic cleft. We have also determined the crystal structure of the apo form of the enzyme in which some of the conserved side chains that have contacts with the cofactor in the holoenzyme now interact among themselves.

NADPH-dependent (20β-HSD) exhibits 85% sequence homology with human placental carbonyl reductase/$NADP^+$-dependent prostaglandin dehydrogenase. The enzyme contains a single polypeptide chain of 289 amino acids and has only 20% sequence homology with 3α,20β-HSD. While 20β-HSD will accept as substrate only steroids that have a pregnane side chain and no oxygen substituent at the C(11) position, 3α,20β-HSD accepts 11-hydroxy and 11-one substituted pregnanes as substrates. We are now in the process of preparing and interpreting a 3Å electron density map by the multiple isomorphic replacement technique. The model will be extended to 2.5Å resolution. Comparison of the substrate binding sites of the two enzymes should reveal the basis for selectivity of action, and the role played by conserved residues in binding the substrate and facilitating catalysis.

11β-HSD, controls blood pressure through tissue specific modulation of cortisol levels. 11β-HSD deficiency or inhibition of 11β-HSD by glycyrrhizic acid (from licorice) produces hypertension. We have determined that glycyrrhizic acid is also a potent inhibitor of 3α,20β-HSD. We have used the 3α,20β-HSD structure to model the structure and inhibition of 11β-HSD. Research supported in part by NIH grant No. DK26546.

DS–03.08.05 CRYSTAL STRUCTURE OF THE *AEROMONAS* TOXIN PROAEROLYSIN IN ITS WATER-SOLUBLE AND MEMBRANE-CHANNEL STATES

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Aeromonas are pathogenic Gram-negative bacteria which are most often found in water, soil, food and the gastrointestinal tract of animals. The bacterium has been shown to be cytotoxic to a large variety of cell lines *in vitro* and is known to be involved in gastrointestinal diseases and severe wound infections in humans. It has been shown that the protein toxin proaerolysin is largely responsible for the pathogenicity of *Aeromonas*. Proaerolysin is secreted as a 52kDa protoxin which can be activated by proteolytic removal of an approximately forty amino acid long C-terminal peptide. The toxin's hemolytic activity is initiated by binding to the erythrocyte transmembrane protein glycophorin followed by oligomerization and membrane insertion to produce a voltage-gated, anion-selective channel about 1nm in diameter.

We have just determined the three-dimensional structure of proaerolysin at 2.8Å resolution by X-ray crystallography. The structure has a rather bizarre elongated shape comprising four domains rich in beta-sheet with one strand longer than 90Å. We have been able to relate various structural features to toxin function (secretion, activation, oligomerization, channel formation and gating) on the basis of the behaviour of a range of single-point mutants. The protoxin contains no hydrophobic stretches of sequence long enough to span a membrane, but the dimer interface has a number of hydrophobic patches. Activation to the mature toxin would expose an additional hydrophobic region and this may account for the protein's ability to penetrate lipid bilayers. Images of an aerolysin oligomer derived from electron microscopy has enabled us to construct a model of the aerolysin channel and suggested a pathway for how the water-soluble protoxin inserts into a membrane to form a voltage-gated channel.

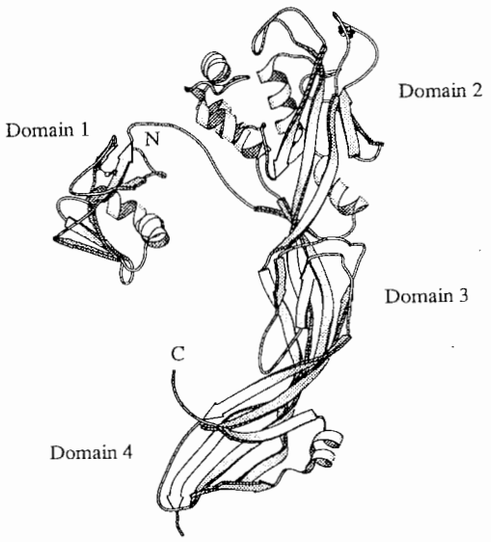

Figure: Cartoon drawing of proaerolysin as determined by X-ray crystallography. This figure was produced by the computer program MOLSCRIPT (Kraulis, J.P., J. Appl. Crystallogr. (1991) **24**, 946-950).

DS-03.08.06 CRYSTAL STRUCTURE OF THE COMPLEX BETWEEN A SINGLE CHAIN ANTIBODY AND NEURAMINIDASE: A BASIS FOR RATIONAL PROTEIN ENGINEERING. By R.L. Malby*[1], M.C. Lawrence[1], W.R. Tulip[1], V.R. Harley[2], R.G. Webster[3], P.J. Hudson[2] and P.M. Colman[1], [1]Biomolecular Research Institute and [2]CSIRO Division of Biomolecular Engineering, Parkville, Vic. 3052, Australia; [3]Department of Virology, St. Jude Children's Research Hospital, Memphis, TN 38105, U.S.A.

We have solved the structure of the complex between a recombinant single chain Fv (scFv) fragment of antibody NC10 and its antigen, neuraminidase (NA) from influenza virus. The complex crystals belong to space group $P42_12$ with cell edges a=b=141.0Å, c=217.9Å and they contain two complexes per asymmetric unit. The single chain Fv consists of the variable heavy (VH) and variable light (VL) chains of the NC10 antibody, joined by a $(GlyGlyGlyGlySer)_3$ peptide linker. Single-chain Fv molecules may be useful as therapeutic and diagnostic reagents, since they retain antigen-binding properties and are more stable under physiological conditions than double-chain Fv molecules. They are also ideal models for protein engineering studies on antibodies (Glockshuber et al., Biochemistry, 1990, 29, 1362-1367).

The scFv-NA complex structure has been solved at 3.0Å resolution using the molecular replacement routines in XPLOR, and the Fab-NA complex as a search model. Preliminary analysis of the scFv-NA structure indicates that the modes of attachment of Fab and scFv to NA are very similar. A close association between two scFv molecules related by a crystallographic twofold rotation leads to speculation that the scFvs may have crystallised in dimeric form, which has relevance to the possible utility of these molecules.

Crystallographic refinement may reveal the conformation of the peptide linker and answer the question of scFv dimerisation in this structure. Additionally, since we have recently extended the refinement of the parent NC10 Fab-NA complex structure (space group I422, a=b=171.5Å, c=160.2Å, one complex per asymmetric unit; Colman et al., Phil. Trans. R. Soc. Lond. B., 1989, 323, 511-518; Tulip, Ph.D. Thesis, University of Melbourne, 1990) to 2.2Å, refinement of the scFv-NA structure will permit comparisons to be made between the overall structures and in particular the antibody-antigen interfaces of the two NC10-NA complexes. On this basis of these two structures we intend to engineer mutations in the NC10 antibody to alter its affinity towards NA, and to design novel antibody structures.

DS-03.08.07 THE CRYSTAL STRUCTURE OF L-1 ISOZYME OF SOYBEAN LIPOXYGENASE. By Wladek Minor[1]*, Jeffrey T. Bolin[1], Janusz Steczko[2], Bernard Axelrod[2] and Zbyszek Otwinowski[3], [1]Department of Biological Sciences, [2]Department of Biochemistry, Purdue University, W. Lafayette IN 47907, [3]Department of Molecular Biophysics and Biochemistry, Yale University, New Haven, CT 06510

Lipoxygenases are non-heme, non-sulfur, iron-containing dioxygenases catalyzing the hydroperoxidation of polyunsaturated fatty acids. The iron is known to participate in the catalysis. All plant and mammalian lipoxygenases that have been so far characterized contain six conserved histidine residues. Five of these occur in a fairly well-conserved region of 40 amino acid residues which contains iron ligands.

The enzyme was crystallized in space group $P2_1$ with one 839 amino acid monomer per asymmetric unit, and cell dimensions a= 95.6Å b=94.3Å c=50.3Å and b=91.3°. Diffraction data were collected using a SDMW area detector and RAXIS-II imaging plate system to a resolution of 2.6A. The structure was solved by multiple isomorphous replacement with two heavy atom derivatives. MIR phases were calculated to 3.0Å resolution. Phase improvement and extension to 2.6Å was performed by a solvent flattening technique that incorporates maximum entropy procedures (Z. Otwinowski, to be published). The position of the active site iron atom was located in an anomalous difference electron density map. The iron atom is found in a predominantly alpha helical domain and is attached to two histidines (His 499 and His 504), both of which belong to an eight turn alpha helix. The binding of the iron atom by these two histidines is in agreement with the prediction made earlier by analysis of site specific mutations (Steczko and Axelrod Biochem. Biophysics Res. Commun. 1992, 186, 668-689). Detailed interpretation of the electron density map is in progress and will be presented together with procedures used in the structure determination

DS-03.08.08 CRYSTAL STRUCTURE OF A BACTERIAL MURAMIDASE: THE SOLUBLE LYTIC TRANSGLYCOSYLASE FROM *E. COLI* by Andy-Mark Thunnissen*, Arnoud Dijkstra[1], Ilenriette Rozeboom, Kor H.Kalk, Wolfgang Keck[1] and Bauke W.Dijkstra, BIOSON Research Institute and Lab. of Biophysical Chemistry, University of Groningen, Nijenborgh 4,9747 AG Groningen, the Netherlands, [1]F.Hoffmann-La Roche Ltd., Pharma Research Department, CH-4002, Basel, Switzerland