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.

