03-Crystallography of Biological Macromolecules

PS–03.07.31 THREE-DIMENSIONAL STRUCTURE ANALYSIS OF *ACHROMOBACTER* PROTEASE I AT 1.2 Å RESOLUTION. By Y.Kitagawa, Y.Katsube, K.Sasi, Y.Matsuura, S.Norioka and F.Sakiyama, Institute for Protein Research, Osaka University, Suita, Osaka 565, JAPAN

Achromobacter protease I (API) is one of serine proteases and can split the lysyl bond specifically. API consists of 268 amino acid residues with three disulfide bridges in the molecule. The crystal of API was obtained by hanging drop vapour diffusion method using polyethelene glycol 6000. The crystal belonged to a space group $P1$ with unit cell dimensions a=39.54 Å, b=40.36 Å, c=43.93 Å, α=114.80°, β=113.73°, γ=74.00°. The asymmetric unit contained one molecule. The structure was determined by multiple isomorphous replacement method using three heavy atom derivatives ($K_2Pt(NO_2)_4$, $Hg(CH_3COO)_2$, and $Sm_2(SO_4)_3$). The average figure of merit at 2.8 Å resolution was 0.91. The atomic model was then built using "FRODO" and the structure refinement was performed using "PROLSQ" at 1.2 Å resolution. The R-factor was 15.0 % after the 476 cycles of the refinement. The basic structure of the API molecule is almost similar to other serine proteases. API mainly composes of several β-strands and some short helix regions. The structure of active site is also similar to that of trypsin except for the shape of hydrophobic pocket. The carboxyl group of Asp189 located in the hydrophobic pocket in trypsin can interact the side chain of the substrate basic residues. In the case of API, the position is occupied by a valine residue. Instead of this replacement, API has an alternative acidic residue on the other side of the hydrophobic pocket; that is Asp225. It is considered that Asp225 in API located at the bottom of the pocket takes the same role as Asp189 in trypsin. Moreover, it is interesting that the distance between the active Oγ atom of Ser190 and the carboxyl oxygen atom of Asp225 in API is shorter than that in the case of trypsin. So, an arginine side chain is too big to enter the hydrophobic pocket in the case of API.

PS–03.07.32 The Ability of Time-Resolved Laue Crystallography to Discriminate Small Changes in Structure: Appearance of a Hydrolytic Water Molecule in Trypsin Catalysis. By P.T. Singer, Argonne Nat'l Lab., USA; A. Smalås, University of Tromsö, Norway; R.P. Carty, State Univ. of N. Y. Health Sci. Ctr. at Brooklyn, USA; and W.F. Mangel and R.M. Sweet*, Brookhaven Nat'l Lab., USA;

Crystals of bovine trypsin were acylated at the reactive residue, serine 195, to form the transiently stable p-guanidinobenzoate. Hydrolysis of this species was triggered in the crystals by a jump in pH. The course of the hydrolysis was monitored by three-dimensional Laue crystallography. Three x-ray diffraction structures resulted, all from the same crystal and each representing approximately 5 seconds of x-ray exposure. The structures were determined at a nominal resolution of 1.8 Å, and were of sufficient quality to reproduce subtle features in the electron density maps for all of the structures. Comparison of the structures before and after the pH jump reveals that a water molecule has positioned itself to attack the acyl group in the initial step of hydrolysis of this transient intermediate. [Singer, *et al.* Phil. Trans. R. Soc. Lond. A. (1992) **340**, 285-300; Singer, *et al.* Science (1993) **259**, 669-673.] Comparison of these structures to others of serine proteases with peptides bound leads to general conclusions about the role of water in hydrolysis of the acyl intermediate in trypsin catalysis.

03.08 – Hot Structures (New Macromolecular Structures)

DS–03.08.01 HIGH RESOLUTION CRYSTAL STRUCTURE OF INTACT ELONGATION FACTOR TU IN ITS ACTIVE FORM REVEALS MAJOR DOMAIN REARRANGEMENTS

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Elongation factor Tu (EF–Tu) is a guanine nucleotide–binding protein essential for bacterial protein biosynthesis. It transfers aminoacyl–tRNA in the form of a ternary complex with GTP to the ribosome and controls the fidelity of translation. During the elongation cycle EF–Tu binds successively to different molecular entities such as GDP, GTP, aminoacyl–tRNA, the ribosome, and elongation factor Ts, thereby undergoing a series of conformational changes.

Using the structures of individual domains of trypsinized EF–Tu from *E. coli* in its inactive GDP form (KJELDGAARD, M. & NYBORG, J. . (1992) *J. molec. Biol.* **223**, 721–742) as separate search models for molecular replacement, we have determined the crystal structure of intact EF–Tu from *Thermus thermophilus* and refined it using data extending to 1.45 Å. The protein, complexed to the slowly hydrolysing GTP analogue, guanosine–5'–(β,γ–imido)triphosphate (GppNHp), is in its active form which enables it to bind both aminoacyl–tRNA and the ribosome, binding sites for which are evident in the three–dimensional structure. Molecular mechanisms will be proposed for the transduction and amplification of the signal induced by GTP binding as well as for the intrinsic and effector–enhanced GTPase activity of EF–Tu. Comparison of the structure with that of EF–Tu•GDP reveals major mutual rearrangements of the three domains of the molecule.

DS–03.08.02 STRUCTURE OF BACTERIOPHAGE T7 RNA POLYMERASE AT 3.3 Å RESOLUTION. By Rui Sousa, Yong Je Chung, John P. Rose and Bi-Cheng Wang*, Departments of Crystallography and Biological Sciences, University of Pittsburgh, Pittsburgh, PA 15260 USA

The crystal structure of T7 RNA polymerase (T7 RNAP) has been determined at 3.3 Å resolution revealing a molecule organized around a cleft which can accommodate a double-stranded DNA template. The molecule has an overall size of approximately 75 x 75 x 65 Å. It consists of 32 α–helices and at least 9 strands of β-sheets. There are three molecules of 99 kD each in an asymmetric unit. Isomorphous replacement methods, together with three-fold non-crystallographic symmetry averaging and solvent flattening, were used in solving the structure. A portion (~60%) of the T7 RNAP displays extensive structural homology to the Klenow fragment (KF) polymerase domain and more limited homology to HIV-1 reverse transcriptase (RT). A comparison of the structures and sequences of these polymerases identifies structural elements which may be responsible for discriminating between rNTP and dNTP substrates, and RNA and DNA templates. The relative locations of the

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.