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

