03-Crystallography of Biological Macromolecules

The double-headed Bowman-Birk type trypsin inhibitor(BBI), I (Mr=14,000, 123 amino acid residues) was isolated from wheat germs. To compare the tertiary structures of BBI's in cereal grains with those in leguminous plants (for example, Suzuki et al, J. Biochem.(Tokyo), 1987, **101**, 267-274), the structural study of I-2, the major component of the group I, have been carried out.
I-2 and its 1:2 complex with bovine β-trypsin have been crystallized. For I-2 two morphologically different crystal forms were obtained. Crystal form (1) is tetragonal, P4122 or P4322, a=55.45(2), c=129.1(2)A, Vm=3.55A^3/dalton, Do=1.30gcm^{-3}, Z=8 and solvent content(S)=65%. Crystal form (2) is monoclinic, space group C2. The cell parameters show significant variation even for crystals in a same batch. The median parameters are: a=83.9, b=41.5, c=45.7A, β=95.9° , Vm=2.83A^3/dalton, Do=1.33gcm^{-3}, Z=4, and S=44%. The crystals of trypsin-I-2 (2:1) complex are orthorhombic, P212121, a=73.49(2), b=120.56(3), c=70.04(2)A, Vm=2.58A^3/dalton, Do=1.28gcm^{-3}, Z=4 and S=50%. The X-ray intensity data of trypsin-I-2 complex were collected using a Weissenberg camera (Sakabe, J. Appl. Cryst, 1983, **16**, 542-547) at the BL6A2 station, Photon Factory, National Laboratory for High Energy Physics. A total of 26,565 independent reflections between 25 and 2.3A resolution were measured, giving the merging R of 0.130.
The structure of the trypsin-I-2 complex was determined by the molecular replacement method using 6~3.5A data and the structure of bovine trypsin. The structure of I-2 was traced on a 3A difference-Fourier map, and the model building was carried out using TURBO-FRODO (Cambillau, 1992). Currently 18 residues were constructed. The R-factor was 0.324 for 10,674 reflections (Fobs>5σ(F)) between 6 and 3A resolution. The partial structure of the two reactive site regions of I-2 is essentially similar to that of the 1:1 complex between trypsin and adzuki inhibitor AB-I(Tsunogae et al,. J. Biochem.(Tokyo), 1986, **100**, 1637-1646).

PS-03.07.09 STRUCTURE OF OLIGO-1,6-GLUCOSIDASE REFINED AT 2.0 Å RESOLUTION. By Y. Hata[1]*, H. Kizaki[2], K. Watanabe[2], Y. Suzuki[2] and Y. Katsube[3], [1]Institute for Chemical Research, Kyoto University, [2]Department of Agricaltural Chemistry, Kyoto Prefectural University, and [3]Institute for Protein Research, Osaka University, Japan.

Bacillus oligo-1,6-glucosidases exhibit a strong correlation between their proline content and thermostability. In order to elucidate the contribution of proline to their thermostability as well as the enzyme function, we have analyzed the structure of oligo-1,6-glucosidase (Mr=66010, 558 residues) from a mesophile *B. cereus* by X-ray method. Crystals of the enzyme grew in hanging drops (Watanabe, K., Kitamura, K., Hata, Y., Katsube, Y., & Suzuki, Y. (1991). *FEBS Lett.* 290, 221-223). The structure was solved by MIR method with three derivatives prepared in $HgCl_2$, $UO_2(NO_3)_2$ and $Sm(NO_3)_3$ solutions. The intensity data were collected using synchrotron radiation (λ=1.04 Å) at BL6A$_2$ station in KEK-PF, Tsukuba. The data were recorded on Fuji imageplates using a Weissenberg camera. The structure model was built with the FRODO on PS390 graphics by interpreting a 3.0 Å resolution electron density map ($\langle m \rangle$=0.76). The structure refinement was started at this resolution with the program XPLOR, and the resolution has been extended to 2.0 Å. The *R*-factor for the current model with 221 waters is 0.196 for 43328 reflections (92%) between 8.0 and 2.0 Å resolution. The structure of *B. cereus* oligo-1,6-glucosidase is similar to those of α-amylases and can be subdivided into three domains. The N-terminal domain has a $(\beta/\alpha)_8$-barrel with four additional α-helices. The subdomain between the third β-strand and α-helix of the N-domain is a long loop containing a small sheet of three β-strands. The C-terminal domain forms an irregular β-barrel of eight strands. The sequence homology and consequent structural similarity between the oligo-1,6-glucosidases of a thermophile and a mesophile suggest that most of the prolines responsible for increase of the thermostability should be positioned on second sites of β-turns.

PS-03.07.10 CRYSTAL STRUCTURE OF ALKALINE SERINE PROTEASE OF BACTERIAL ORIGIN. By T. Yamane[1], T. Hatanaka[1], T. Kani[1], T. Narusc[1], A. Suzuki[1], T. Ashida[1]*, T. Kobayashi[2] and S. Ito[2], [1]Department of Biotechnology, School of Engineering, Nagoya University, Nagoya 464-01, Japan, [2]Tochigi Research Laboratories, Kao Corporation, Tochigi 321-34, Japan.

A new alkaline serine protease(ALPT) was obtained from a Bacillus strain. It consists of 269 amino acids and has a molecular weight of 26,715. The sequence of ALPT is 60% homologous with subtilisin Carlsberg(SBC).
The enzyme inhibited with phenylmethylsulfonylfluoride was used for crystallization. Needle crystals (form 1) were grown in 0.05M phosphate buffer (pH 5.5), using 1.4M ammonium sulfate as precipitant. The space group is P212121 with a=57.79(2), b=75.82(2), c=54.19(1)A, Z=4 and Vm=2.15A^3/dalton. The cell parameters at first suggested that form 1 might be isomorphous to the crystal of SBC(Petsko & Tsernoglou, J. Mol. Biol., 1976, **106**, 453-456) if the a and b axes were exchanged. The other crystals (form 2) were obtained from 0.05M acetate buffer (pH 5) and belong to space group P212121 with a=47.3, b=62.5, c=75.6A, Z=4 and Vm=2.09A^3/dalton, which is isomorphous to the crystals of high-alkaline serine protease PB92 (van der Laan et al., Protein Engineering, 1992, **5**, 405-411).
The X-ray intensity data of form 1 were collected using a Weissenberg camera (Sakabe, J. Appl. Cryst, 1983, **16**, 542-547) at the BL6A2 station at the Photon Factory, National Laboratory for High Energy Physics. The needle (a*) axis was mounted parallel to the rotation axis of the camera. A total of 8,513 independent reflections up to 1.9A resolution were measured, giving the merging R of 0.119. The structure was solved by the molecular replacement method using 6~3.5A data and the structure of SBC (PDB entry number 1SBC). The crystallographic R-factor was 0.472 at this stage. The structure was refined by the X-PLOR system (Bruenger, 1990, X-PLOR Manual, Ver. 2.1, Yale Univ., New Haven, USA), with the amino acid sequence of SBC. The current R factor is 0.255 for 6404 reflections between 7 and 2.5A resolution. The structure of ALPT is essentially the same as that of SBC, though the orientation parameters from the rotation function revealed that it was not isomorphous to that of SBC.

PS-03.07.11 STRUCTURE ANALYSIS OF PHOSPHOLILASE A2 FROM VENOM OF *TRIMERESURUS FLAVOVIRIDIS*. By E. Matsueda[1], A. Suzuki[1]*, T. Yamane[1], T Ashida[1], H. Kihara[2], and M. Ohno[3], [1]Department of Biotechnology, School of Engineering, Nagoya University, Nagoya 464-01, Japan, [2]Department of Physiology, Faculty of Medicine, Kagoshima University, Kagoshima 890, Japan, [3]Department of Chemistry, Faculty of Science, Kyushu University, Higashi-ku, Fukuoka 812, Japan

Phospholipase A2 (PLA2) from the venom of *Trimeresurus flavoviridis* (habu snake) is a homo-dimeric enzyme composed of subunits with 122 residues. Crsytals belonge to space group P21 with a=44.1 Å, b=55.7 Å, c=48.8 Å, and β =92.4° . An asymmetric unit contains one dimer.
X-ray diffraction data up to 3.5Å resolution were collected on a four-circle diffractometer with CuKα radiation. High resolution data up to 2.0Å were recorded on imaging plates by using a Weissenberg camera installed at BL-6A2 station of Photon Factory. These two datasets were merged to give a total of 13822 unique reflections. The merging R was 0.054.
The structure analysis was proceeded with the molecular replacement method using the modified structure of PLA2 from the Western Diamondback rattlesnake as a search model. The search model was constructed by omitting two turn regions and side-chain atoms of residues different from the habu-snake PLA2 except C$_\beta$ atoms. Self- and cross-rotation search were performed with the Crowther fast rotation function using 6.0 to 3.5Å resolution data. Self-rotation search shows that two subunits in an asymmetric unit are related by a non-crystallographic 2-fold axis. Using this symmetry, the peaks on the cross-rotation function could be paired with one another. The pair containing the highest peak gave true orientations of two subunits in an asymmetric unit. Molecular translation along the **a** and **c** axes was determined for each subunit with the Crowther-Blow translation function using 6.0 to 3.5Å resolution data. Relative position along the **b** axis was determined by the R-search method using 6.0 to 4.0Å data.

Using the rotation and translation solutions, a PLA2 dimer was constructed from the search model, and refined with the PROLSQ program at 3.0Å resolution. R-factor reduced from 0.470 to 0.357. The resulting 2Fo-Fc map was improved by cyclic averaging of the two subunits in an asymmetric unit. Now, we are going on with model construction and over-all structure refinement.

PS-03.07.12 CRYSTALLIZATION AND PRELIMINARY CRYSTALLOGRAPHIC ANALYSIS OF TRIACYLGLYCEROL LIPASE FROM CHROMOBACTERIUM VISCOSUM

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Lipases (triacylglycerol hydrolase; EC 3.1.1.3), which are present in diverse organism, including humanes, animals, plants, fungi, and bacteria, catalyze the hydrolysis of triglycerides into free fatty acids and glycerols. Since lipases have wide versatility, considerable interest in the industrial uses of lipases has recently developed. Industrial applications of lipases include for example enzymatic fat splitting, production of cocoa butter substitutes, and use as a detergent additive. Also the enantiselectivity of certain lipases offers an attractive opportunity for the preparation of chiral intermediates for pharmaceutical syntheses.

In recent years information of crystal structures of humane, fungal and animal lipases has become available. However, no crystal structures of bacterial lipases are known.

The neutral lipase from the bacteria Chromobacterium viscosum is a single chain enzyme which contains 319 amino acid residues and one disulphide bond. The enzyme is of particular interest for industrial applications because of its sn-1,3-regioselectivity, its high temperature optimum for enzymatic activity, and its thermostability and activity over a broad pH range.

The lipase from Chromobacterium viscosum has been crystallized by vapour diffusion in sitting drops using polyethylene glycol as a precipitant. Crystals grew within one week to a final size of 0.6 x 0.45 x 0.45 mm. They diffract to at least 2.1 Å.

The crystals were investigated using a Xentronics area detector mounted on a Rigaku rotating anode X-ray source. The space group is $P2_12_12$ with a = 41.08 Å, b = 156.82 Å and c = 43.62 Å. Assuming one monomer per asymmetric unit, a V_m- value of 2.15 can be calculated (Matthews, B.W, J. Mol. Biol. 1968, 33, 491-497).

Structure solution by the MIR method is currently under way.

PS-03.07.13 Crystal Structure of a Thiol Proteinase from *Staphylococcus aureus* V-8 in the E-64 Inhibitor Complex

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Staphylococcal thiol proteinase is one of three different proteinases produced by *Staphylococcus aureus* strain V-8 [1]. The enzyme has a cleavage specificity similar to that of papain, the molecular weight is about 22000 Da and it is activated by reducing agents e.g. DTT, and strongly inhibited by heavy metal ions such as Hg^{2+}, as well as by the epoxide 1-[N-[(L-3-trans-carboxyrane-2-carbonyl)-L-leucyl]amino]-4-guanidinobutane (E-64), an irreversible inhibitor of cysteine proteinases.

Crystals of the thiol proteinase/E-64 complex were grown from 5 M ammonium acetate at pH 6.0. They belong to the space group $P6_522$ with a = b = 60.5 Å and c = 196.4 Å and diffract to about 2.0 Å resolution.

The structure was solved by multiple isomorphous replacement using data from one gold and one platinum derivative. Only the N-terminal 50 residues of the *Staphylococcal* thiol proteinase amino acid sequence are known, hence most of the polypeptide chain was built as poly-ala.

Comparison of the fifty known amino acids of *Staphylococcal* thiol proteinase with the amino acid sequence of papain show only a very low homology, nevertheless there is an obvious similarity of both three-dimensional structures, both regarding the N-terminal residues and the overall folding pattern. Particulary the design of the active site and the binding mode of the inhibitor are very similar in both structures.

[1] Arvidson, S. et al. Biochim. Biophys. Acta **302**, 135 - 148 (1973)

PS-03.07.14 STRUCTURAL ANALYSIS OF *SERRATIA* PROTEASE. By K. Hamada[1], H. Hiramatsu[1], T. Fujiwara[1], Y. Katsuya[2], Y. Hata[3], Y. Matsuura[4] and Y. Katsube[4], [1] Faculty of Science, Shimane University, [2] Hyogo Prefectural Institute of Industrial Research, [3] Institute for Chemical Research, Kyoto University, [4] Institute for Protein Research, Osaka University, Japan.

Serratia protease is a zinc-requiring protease composed of 470 amino acid residues (Miyata et al., Agri. Biol. Chem., 1970, **35**, 460-467). Recently, this enzyme has been used as a medical drug. We have carried out crystallographic studies to elucidate the three dimensional structure and the functional properties of *serratia* protease.

Serratia protease has been crystallized in the space group P212121, a = 109.2, b = 150.9, c = 42.6 Å (Katsuya et al., J. Biochem., 1985, **98**, 1139-1142). Diffraction data sets for native and Sm-derivative were collected on Weissenberg camera at Photon Factory using two kinds of wave length of 1.000 and 1.283 Å being near the absorption edge of Zn atom and on Rigaku R-AXIS IIc. The Zn position of *serratia* protease was determined by the native anomalous Patterson syntheses. Using the native data set collected at the 1.283 Å wave length as a Zn-derivative and a Sm-derivative MIRSAS starting phases were obtained up to 3.0 Å resolution. These phases were improved by Wang's solvent flattening procedure. R-factor was 0.22 and overall figure of merit being 0.87. The electron density map allowed to make a main chain tracing.

A current model of the molecule shows that the serratia protease folds into two domains. There is the active site with Zn ion in the N-terminal domain whose structure is similar to that of thermolysin. The C-terminal domain composes of several β-sheets. The improvement of the model is in progress.

PS-03.07.15 CRYSTAL STRUCTURE OF RIBONUCLEASE F1 OF *FUSARIUM MONILIFORME* IN ITS FREE FORM AND IN COMPLEX WITH 2'GMP. By D.G. Vassylyev*, K. Katayanagi, K. Ishikawa, M. Tsujimoto-Hirano, M. Danno, A. Pahler, O. Matsumoto, M. Matsushima, H. Yoshida and K. Morikawa, Protein Engineering Research Institute, Osaka, Japan.