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.

03-Crystallography of Biological Macromolecules

RNase F1, a guanine-specific ribonuclease from *Fusarium monitiforme* was crystallized in two different forms, in the absence of an inhibitor and in the presence of 2'GMP. Both crystal structures were solved by molecular replacement method. The crystal structure of RNase F1 free form was refined to a final R-factor 18.7% at 1.3Å resolution. The crystal structure of the complex was refined to a final R-factor 16.8% at 2Å resolution. The two crystal structures of RNase F1 free form and the complex with 2'GMP are very similar to each other (r.m.s.d. 0.43Å for all C^{α} atoms). The main differences between the two structures are associated with binding of 2'GMP in the substrate binding site. A structural comparision between RNase F1 and RNase T1 shows a substantial similarity between all C^{α} atoms (r.m.s.d. 1.4Å). The loop from residues 32 to 38 was strikingly different between these two enzymes. The side chain of a catalytically active residue, His92, is shifted away from the catalytic site in RNase F1 by 1.3Å and 0.85Å with respect to the corresponding positions in RNase T1 free form and in the RNase T1 complex with 2'GMP, respectively. In the RNase F1 complex, the guanine base of 2'GMP has a *syn* conformation about the glycosyl bond, and the furanose ring assumes a 3'-*exo* pucker, which is different from that found in the complex with RNase T1. In the catlytic site of the RNase F1 complex with 2'GMP, one water molewas observed, which bridges the phosphate oxygen atoms of 2'GMP and the side-chains of the catalytically important residues, His92 and Arg77, through hydrogen bonds. A water molecule occupying the same position was found in the RNase F1 free form. This water molecule may play an important role during the catalytic reaction.

D.G. Vassylyev, K. Katayanagi, K. Ishikawa, M. Tsujimoto-Hirano, M. Danno, A. Pahler, O. Matsumoto, M. Matsushima, H. Yoshida and K. Morikawa (1993). *J. Mol. Biol.* 230, in press.

PS-03.07.16

CRYSTALLIZATION AND PRELIMINARY STUDY OF ENDOGLUCANASE (EGIII) FROM *Trichoderma reesei*

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The endoglucanase EGIII is one component of the enzyme mixture, called "cellulases" which contains multiple cellobiohydrolases (exoglucanases) and endoglucanases. All these cellulases act synergistically to degrade both crystallin and amorphous cellulose. EGIII from fungus *Trichoderma reesei*, has been crystallized. It is a low molecular weight (Mr=25,000 dalton) unglycosylated enzyme with pI=7.4. The EGIII gene has been cloned and the amino acid sequence has been deduced from genomic DNA. The crystals diffract at least to 2.8Å resolution. The crystals are monoclinic, space group $P2_1$ with cell dimensions of a=75.5Å, b=96.6Å, c=67.4Å, β=107.2°. The solvent content is 49% with V_m=2.4 if there are four molecules per assymetric unit. Self rotation function revealed the presence of a non-crystallographic dyad axis located at Φ=42°, Ψ=90°, suggesting two dimers per asymmetric unit. Two potential heavy atom derivatives have been identified so far, with K_2PtCl_4 and $KAuCl_4$.

PS-03.07.17 CRYSTAL STRUCTURE OF LYSOZYME FROM *STREPTOMYCES ERYTHRAEUS* AND CRYSTALLIZATION OF LYSOZYME FROM *STREPTOMYCES GLOBISPORUS*
By S. Harada*, C. Uematsu, Y. Kai, and N. Kasai Department of Applied Chemistry, Faculty of Engineering, Osaka University, Suita, Osaka 565, Japan.

Lysozyme is the enzyme which causes lysis of cell walls of bacteria by hydrolyzing the β-(1,4)-glycosidic bonds of the polysaccharide backbone of the peptidoglycan. On the basis of the homology of amino acid sequence, lysozyme is classified into four distinct types: (1) chicken, (2) phage, (3) goose and (4) bacteria. The crystal structure of a bacterial lysozyme produced by *Streptomyces erythraeus* (SEL) has been determined by X-ray diffraction analysis using the isomorphous replacement method. SEL consists of 202 amino acid residues and its amino acid sequence is totally different from other lysozymes whose crystal structures are known. The three-dimensional model of SEL shows that there are eight β-strands, six α-helices in the molecule. Six β-strands forms a parallel β- sheet. The parallel β-sheet, in which adjacent strands are connected by helices, is a barrel-like shape. Thus the folding pattern of SEL is topologically different from other lysozymes. A deep cleft which is identified as the active site exists on the C-terminal ends of the parallel β-sheet. The refinement of the structure is in progress. *Streptomyces globisporus* produces two kinds of lysozymes (M-1 and M-2 lysozymes) and secretes them in the cultural broth. The molecular weights are about 20,000 and 11,000 for M-1 and M-2 lysozymes, respectively. Their amino acid sequences are not known. M-1 lysozyme has been crystallized in two crystal forms ($P4_12_12$ ($P4_32_12$), $a=b=63.09$, $c=121.44$Å and $P6122$ ($P6_522$), $a=b=128.9$, $c=144.0$Å). Since the amino acid sequence of M-1 lysozyme is supposed to be similar to that of SEL, the structure of M-1 lysozyme is intended to be determined by the molecular replacement method using the refined structure of SEL.

PS-03.07.18 CRYSTAL STRUCTURE OF TURKEY LYSOZYME COMPLEXES WITH OLIGOSACCHARIDES. By K. Harata, National Institute of Bioscience and Human-Technology, Tsukuba, Ibaraki 305, Japan

Crystal structure of monoclinic turkey lysozyme and its complexes with N-acetylglucosamine(NAG) di-N-acetylchitobiose(NAG2), and tri-N-acetylchitotriose(NAG3) has been determined by the X-ray analysis. The NAG3 molecule occupies A,B, and C subsites and is bound in the manner that assumed in the catalytic reaction. The NAG molecule is found near the B subsite with the orientation different from that found in the NAG3 complex. One sugar residue of NAG2 with the α-anomeric form is bound near the D subsite while the other residue protrudes outside from the active site cleft. Therefore, the mode of binding differs according to not only the number of NAG residues but also the anomeric form of the terminal residue.