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.

