03-Crystallography of Biological Macromolecules

Using the initiation and translation activities, a PLAS dimer was connected from the yeast malt, and added with the Prolong program at 3.0 A resolution. R-factor reduced from 0.270 to 0.395. The resulting 2Fo-FC map was improved by cyclic averaging of the two phasens in an asymmetric unit. Now, we are going on with model construction and overall specificity refinement.

PS-03.07.12 CRYSTALLIZATION AND PRELIMINARY CRYSTALLOGRAPHIC ANALYSIS OF TRACYLGLYCOL LIPASE FROM CHROMOBACTERIUM VISCOSUM
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Lipases (tracylglycerol hydrolase; EC 3.1.1.3), which are present in diverse organisms, including humans, animals, plants, fungi, and bacteria, catalyse the hydrolysis of triglycerides into free fatty acids and glycerol. Since lipases have wide versatility and considerable interest in the industrial uses of lipases recently developed. Industrial applications of lipases include for example enzymatic fat splitting, production of cocoa butter substitutes, and as a detergent additive. Also the enantioselectivity of certain lipases offers an attractive opportunity for the preparation of chiral intermediates for pharmaceutical syntheses.

In recent years information of crystal structures of human, 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 379 amino acid residues and one disulphide bond. The enzyme is of particular interest for industrial applications because of its vast-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 0.49 x 0.45 mm. They diffract to at least 2.1 A. The crystals were investigated using a Xentaica area detector mounted on a Rigaku rotating anode X-ray source. The space group is P4_2_2_2 with a = 41.08 A, b = 156.82 A and c = 43.61 A. Assuming one monomer per asymmetric unit, a V_0 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 underway.

PS-05.07.13 Crystal Structure of a Thiol Proteinase from Staphylococcus aureus V-8 in the E-64 Inhibitor Complex
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Staphylococcal thiol proteinases 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 20000 Da and it is activated by reducing agents e.g. DTT, and strongly inhibited by heavy metal ions such as Hg²⁺, as well as by the epoxide 1-[N-(2-carboxyphenyl)-2-carboxy-L-lysyl]-3-methylimidazole (K-64), an irreversible inhibitor of cysteine proteinases.

Cystals of the title proteinase/E-64 complex were grown from 5 M ammonium acetate at pH 5.0. They belong to the space group P4_2_2_2 with a = b = 60.5 A and c = 196.4 A and diffract to about 2.0 A resolution.

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

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


PS-03.07.14 STRUCTURAL ANALYSIS OF SERRATIA PROTEASE
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Serratia protease is a zinc-requiring proteinase composed of 470 amino acid residues (Miyata et al., Agric. Biol. Chem. 1970, 34, 460-467). Recently, this enzyme has been used as a medical agent. 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 P2_1_2_1_2 with a = 109.2, b = 158.8, c = 42.6 A (Kataya et al., J. Biochem., 1985, 98, 1139-1142). Diffraction data sets for native and Srr-derivative were collected on a Weissenberg camera at Photon Factory using two kinds of wavelength of 1.000 and 1.283 A, nearly the absorption edge of Zn atom, and on Rigaku RAXIS 11C. The Zn position of serratia protease was determined by the native anomalous Patterson synthesis. Using the native data set collected at the 1.283 A wavelength as a Say-derivative and a Srr-derivative MIRAS starting phases were obtained up to 3.0 A 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 comprises of several beta-sheets. The improvement of the model is in progress.

PS-03.07.15 CRYSTAL STRUCTURE OF RIBONUCLEASE F 1 OF FUSARIUM MONOCNEME IN ITS FREE FORM AND IN COMPLEX WITH 2 GMP.
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