Crystallographic Studies of *Tapes japonica* Lysozyme

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Lytic enzymes, which belong to the lysozyme family, have been classified on the basis of organism, activity, and structure. Recently, it has been recognized that the lysozyme from the marine bivalve Tapes japonica belongs to i-type lysozyme. But little is known about structure and function of i-type lysozymes.

Lysozyme from the marine bivalve *Tapes japonica* (*Tapes japonica* lysozyme) is composed of 123 amino acids (13.8 kDa). The lytic activity of *Tapes japonica* lysozyme against *Micrococcus luteus* is 425%, the chitinase activity is 85%, and the binding ability to $(NAG)_3$ is 22%, compared to hen egg lysozyme. A primary sequence of *Tapes japonica* lysozyme indicates 46% homology identity to the destabilase from medicinal leech. The destabilase from medicinal leech is an enzyme that hydrolyses ε-(γ-Glu)-Lys cross linkage between Glu and Lys in stabilized fibrin. Destabilase has been studied on the base of thrombosis. Based on this homology, we confirmed hydrolysis activity of *Tapes japonica* lysozyme against three substrates: L-γ-Glu-pNA, D-γ-Glu-pNA, and ε- (γ-Glu)-L-Lys. The optimal pH of chitinase and isopeptidase activity is 5.0 and 7.0, respectively. The isopeptidase activity is inhibited with serine protease inhibitor, but the lytic and chitinase activities are not. Moreover, only isopeptidase activity is decreased by lyophilization, but lytic and chitinase activities are not. We conclude that *Tapes japonica* lysozyme expresses isopeptidase and chitinase activity at different active sites. Therefore, we attempt to analyze of detail bi functionally enzymatic function by X-ray.

X-ray diffraction measurements were carried out under cryogenic condition (100K) using flash-cooling technique. X-ray diffraction data were collected up to 2.8 Å resolution using CCD detector. The data were processed using the program HKL2000 for integration and scaling.

X-ray Crystallographic Studies of the Multi-Hemoglobin System

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Pogonophorans are deep sea animals without mouth and gut. Their nutrition is provided by symbiotic bacteria living inside the worm. Recent genetic and embryological studies suggest that pogonophorans, vestimentiferans, and annelids are closely related. *Oligobrachia mashikoi* (pogonophora) inhabits the seabed in Tsukumo Bay, and it has the 400 kDa multi-hemoglobin system. This giant hemoglobin is thought to transport O_2 to a host and H_2S to symbionts simultaneously. Little is known about pogonophoran's hemoglobin, and no three-dimensional structure is available.

Crystals of the giant hemoglobin from *O. mashikoi* were obtained under slightly modified condition from that previously reported in the 2003B experiments. Well-shaped rhombohedral crystals with dimensions of 0.1 × 0.1 × 0.3 mm^3 were obtained at 293 K from a protein solution of 30 mg/ml, 13 % PEG 10,000 in 100 mM Tris-HCl buffer at pH 8.0. The crystals belong to the space group *R*32 with cell dimensions of a = 111.5 Å and c = 276.8 Å. These crystals were stabilized in the cryo-protectant buffer containing 20 % (v/v) glycerol.

X-ray diffraction experiments were performed at the BL41XU beamline using a marCCD165 X-ray CCD detector. The wavelength, camera length, oscillation range, and exposure time were 1.0000 Å, 200 mm, 0.5 degree, and 20 sec, respectively. A total of 250 frames were obtained. All the data collections have been executed at 90 K with the nitrogen gas stream cooling system.

The crystal diffracted X-rays up to 3.2 Å resolution, and the data were processed using the program HKL2000. The resolution limit of diffractions from the crystals was very much improved compared with that in the previous 2003B experiments. It was found that the present crystals enable us to perform the conventional structure determination of this large molecular assembly. A total of 84,440 diffractions were collected with an overall R_{merge}, completeness, and redundancy were 11.0 %, 98.5 %, and 7.5 respectively. Structure determination using these data is under progress.