X-ray crystallography of 2'-aminobiphenyl-2,3-diol 1,2-dioxygenase

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2'-Aminobiphenyl-2,3-diol 1,2-dioxygenase (CarBaBb) from *Pseudomonas resinovorans* strain CA10 is an enzyme involved in the aerobic carbazole (CAR) degradation pathway, and catalyze the *meta*-cleavage of 2'-aminobiphenyl 2,3-diol in *meta* fission. CarBaBb catalyzes the *meta*-cleavage of several chlorinated trihydroxybiphenyl and chlorinated trihydroxydiphenyl ethers, that are metabolic intermediates of chlorinated dibenzofuran and chlorinated dibenzo-*p*-dioxin, respectively. Although most of *meta*-cleavage enzymes are shown to be homo multimer or monomer, CarB enzyme consists of CarBa and CarBb proteins resulting in hetero tetrameric structure ($\alpha_2\beta_2$). The determination of three-dimensional structure will help us to understand what structure of CarB is involved in the recognition of substrates, what amino acid residues are located in the active site of CarB, and how interact CarBb protein to functionally-unknown CarBa protein.

Previously, we have succeeded the purification and in obtaining crystallization of His-tagged CarB (ht-CarB). Ht-CarB crystals obtained using Index™ #54 (Hampton Research; 0.05M Calcium acetate hydrate, 0.1 M Bis-Tris buffer (pH 6.5), 30% PEG MME 550) were nice to look. To search the phase, molecular replacement method was used with the model molecule of protocatechuate 4,5-dioxygenase. However, the phase searching is not successful yet, because CarBaBb shares low homology with the model molecule. Au derivative protein crystals were prepared in order to solve the structure by multiwavelength anomalous diffraction method.

Here we report the crystallographic study of CarBaBb by using the beamline BL41XU. Diffraction data were collected using cryo loop at 100K. Crystals belonged to the space group $P2_12_12$ with unit cell parameters of a=123.4, b=144.4, c=49.3 Å. The dataset was processed using HKL2000, and observed 62250 unique reflactions with merging R-factor of 17.0% and completeness of 99.0% up to 3.0 Å resolution. Structure analysis is in progress.

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.