X-ray Structure Analyses of Isoamylase and Rlated Enzymes

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Isoamylase (glycogen 6-glucanohydrase EC 3.2.2.1.68) is one of the starch-debranching enzymes that catalyzes the hydrolysis of the α-1,6-glucosidic linkages specifically in α-glucans such as amylopectin or glycogen. Isoamylase belongs to the α-amylase family including various α-amylases and related enzymes such as cyclodextrin glucanotransferase (CGTase), pullulanase, neopullulanase and branching enzymes. Enzymes belonging to the α-amylase family have four highly conserved regions in their amino acids sequence. X-ray structure analyses of α-amylase family enzymes reveals that these enzymes have $(\beta/\alpha)_8$-barrel structure in their catalytic domains.

In order to understand the substrate specificity and catalytic mechanism of isoamylase, we have carried out the X-ray structure analyses of isoamylase and related enzymes such as pullulanase and maltotetraose-forming exo-amylase.

Isoamylase hydrolyzes α-1,6-glucosidic linkage of 6-O-α-maltosyl-β-cyclodextrin and the enzyme action is slightly inhibited by β-cyclodextrin. Hence, 6-O-α-glucosyl-β-cyclodextrin is expected to react as a substrate analogue. Complexed crystals with 6-O-α-glucosyl-β-cyclodextrin were prepared by soaking technique.

Diffraction data were collected for native and the complexed crystals with an imaging plate diffractometer Rigaku R-AXIS4 at the experimental hutch A of Hyogo beamline (BL24XU). The wavelength of incident X-ray was 0.834Å. The crystals were kept at 100K during the data collection. For the native crystal, 399,762 reflections were observed and 131,415 independent reflections were obtained with a merging R-value of 0.068. The diffraction data set was 90.7% complete at the resolution of 1.6Å. For the complexd crystal, 189,740 reflections were observed and 82,178 independent reflections were obtained with a merging R-value of 0.055. The diffraction data set was 79.5% complete at the resolution of 1.8Å.

The molecular replacement method was applied for each diffraction data by using the program X-PLOR. The native structure of isoamylase with 452 water oxygens was refined at 1.6Å resolution by using the program X-PLOR. The crystallographic R-factor for the model was converged to 0.217 for 130,934 unique reflections.

The positions of substrate analogue were investigated in the difference Fourier maps. Finally, we could identify three positions of glucose residues of cyclodextrin ring near the active residues. These positions of glucose residues correspond to the –1 to –3 subsites in α-amylases. The crystallographic refinement at 1.8Å resolution is now in progress.