Time-Resolved Crystal Structure Analysis of Photoreactive Nitrile Hydratase with Large-Angle Oscillation Technique

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Nitrile Hydratase (NHase) catalyzes the hydration of nitriles to their corresponding amides and is used for the industrial production of acrylamide. NHase consists of a and b subunits each with molecular weights of ~ 23,000. The enzyme has a non-heme iron at its catalytic center. We have revealed that the iron center is associated with an endogenous nitric oxide (NO) in an inactive state and that photo-dissociation of the NO molecule causes activation of NHase. Recently, the crystal structure of the inactive NHase has been analyzed by our group (Nature Struc. Biol. 5, 347-351 (1998)). This work shows clearly the NO molecule as a sixth ligand of the non-heme iron center. The NO molecule is stabilized by an unusual claw setting composed of three oxygen atoms of Cys114-sulfenic acid, Cys112-sulfinic acids and hydroxyl group of Ser113. Furthermore, a relatively wide pocket is existing around the NO molecule, which is expected as a substrate binding site. Based on the crystal structure of the inactive NHase, we proposed a time-resolved crystal structure analysis by the large-angle oscillation technique (LOT) to reveal a soaking process of substrate into the pocket.

The Bio-Crystallography beamline (BL-41XU) was constructed for crystal structure analysis of biological macromolecules on the multiple isomorphous replacement method with optimized anomalous scattering. In order to realize this purposes, two types of imaging plate detector system were installed in the experimental hutch; an on-line system of medium active area and an off-line system of much larger size, 800 x 800 mm. The off-line system is very suitable for LOT because the large active area is indispensable to record all of independent reflections only on one frame, if possible.

We have tested abilities of LOT data collection for the inactive NHase crystal at a wavelength of 1.0 A and a specimen-detector distance of 640 mm with a large format imaging plate of 800 x 400 mm. LOT diffraction patterns are successively obtained by a 30 degree rotation of sample crystal (size; 200 mm x 150 mm x 150mm) with a 300 sec exposure. X-ray damage is not so serious for the inactive NHase crystal that the time-resolved crystal structure analysis by LOT is very much hopeful. Development of data processing software for LOT has been started by Dr. Higashi at RIGAKU Company, and it will be installed in the AUTO program system developed for BL41XU.