Time Resolved Crystal Structure Analysis of Photoreactive Nitrile Hydratase with Laue Diffraction 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 α and β subunits each with molecular weights of ~ 23k Da. 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. The crystal structure of 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 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. In order to clarify the dynamic structure change of NHase at the photo-activation reaction, we have proposed a time-resolved crystal structure analysis of inactive NHase by the white Laue technique at BL44B2.

The crystal structure of active NHase has already been analyzed by other group (Structure **5**, 691-699 (1997)), which will be very useful for our project as the final structure of the photo-activation reaction. Unfortunately, however, the structure is analyzed only at a resolution of 2.6Å, which is insufficient for clarifying the claw setting structure in an active state. Recently, we have succeeded to crystallize the active NHase in a different form with that previously reported. The new crystals diffract X-rays well up to 1.5Å resolution. Since the high resolution structure of active NHase is very important for our project, we have started to analyze the new crystal before the time-resolved analysis by the Laue technique.

The diffraction intensity data were collected with the RIGAKU RaxisIV detector (300 x 300 mm) at a specimen-detector distance of 160 mm using X-rays of 1.0Å wavelength. The collimator size was 0.1 mm. The diffraction patterns were obtained by a 3 degree rotation of sample crystal (0.1 x 0.1 x 0.5 mm) with a 5 min exposure per film. Total rotation range was 190 degree and total exposure time was 5 hours.

The diffraction intensities were measured by the AUTO program system newly developed by Dr. Higashi at RIGAKU Company. A total of 197,697 reflections was observed and 70,866 independent reflections were obtained with a merging R value of 0.072. The diffraction data set was 99.7% complete at the resolution of 1.5Å. The X-ray structure determination of active NHase is in progress by molecular replacement technique using the active NHase structure deposited in PDB.