High Resolution X-ray Crystal Structure Analysis of Nitilic Hydratase

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Nitrile Hydratase (NtHase) catalyzes the hydration of nitrile compounds to their corresponding amides and is used for the industrial production of acrylamide. NtHase produced from Rhodococcus sp. N-771 consists of α- and β-subunits each with molecular weights of about 23kDa. The enzyme has a catalytic center of non-heme iron surrounded by a sequence of C109-X-L-C112-S-C114 in the α-subunit, which is highly conserved around NtHases already known. The iron center is associated with an endogeneous nitric oxide (NO) in the inactive form, and the dissociation of NO molecule by irradiation of visible light leads the enzyme to the active form. The crystal structure of the inactive NtHase has been analyzed at 1.7Å resolution by our group (Nature Struct. Biol. 5, 347-351 (1998)). This work clearly shows 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 C112-sulfenic acids, hydroxyl group of S113 and C114-sulfenic acid. The two of three cysteine residues in the consensus sequence of claw setting are post-translationally modified. The crystal structure of photo-activated NtHase has also been analyzed by the other group (Science 275, 591-599 (1997)). Unfortunately, however, the structure is only at a resolution of 2.6Å, which is insufficient for clarifying the claw setting structure in the photo-activated form. In order to realize much higher resolution analysis for the photo-activated NtHase, we had searched a new crystal form and determined the crystal structure determined at 1.5Å resolution. In the structure, C114-sulfenic acid was over oxidized to cysteine sulfenic acid, and the relative activity decreased to 1.7%. From above study, it is strongly suggested that C114-sulfenic acid is very important role in the enzymatic activity. So, we must determine the conformational change of C114-sulfenic acid in photo activated using higher resolution structure.

We determine the diffraction data was obtained up to 1.3Å resolution using monocromatic X-rays (0.71Å wavelength) and a high speed readable IP detector using line shaped laser and CCD (call for CCD-IP) installed in BL41XU. During data collection, the frozen crystal was maintained at 100K using a cryo stream cooler (RIGAKU Co.). The crystal to detector distance was 450mm. 344 diffraction images were obtained by an exposure time at 30 sec, and oscillation range was 2 degree per image. Data processing was carried out with the program AUTO. The merging-R value was 4.5%, completeness was 88.7%, average I/σ(I) was 29.7. Molecular replacement and structure refinement was carried out with CNS (Acta Cryst. D54, 905-921 (1998)) using the inactive NtHase coordinates. We are now refining the coordinate.