High Resolution X-ray Crystal Structure Analysis of Nitlile Hydratase

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Nitrile Hydratase (NHase) catalyzes the hydration of nitrile compounds to their corresponding amides and is used for the industrial production of acrylamide. NHase produced from *Rhodococcus* sp. N-771 consists of α- and β- subunits each with molecular weights of about 23k Da. 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 consensus around NHases 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 NHase has been analyzed at 1.7Å resolution by our group (*Nature Struc. 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-sulfinic acids, hydroxyl group of S113 and C114-sulfenic acid. The two of three cystein residues in the consensus sequence of claw setting are post-translationally modified. The crystal structure of photo-activated NHase has also been analyzed by the other group (*Structure* **5**, 691-699 (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 NHase, we had searched a new crystal form and determined the crystal structure determined at 1.5A 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 monochromatic X-rays (0.71 Å 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. 34 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 NHase coordinates. We are now refining the coordinate.

X-ray crystal structure analysis of tyrosine kinase

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The Src family kinases (SFKs) play a critical part in cell signaling by phosphorylating tyrosine residues of various cellular components. SFKs have a regulatory tyrosine residue in their carboxyl terminal (Tyr 527) that is phosphorylated by carboxyl terminal Src Kinase (Csk), which results in decrease in their kinase activity. Most of the non-receptor type tyrosine kinases, including SFKs and Csk, have homologous catalytic domain as well as regulatory peptide binding modules such as SH2 (Src Homology 2) domain and SH3 domain.

Until today structures of inhibitory form of SFKs and SH2 and kinase domains of Csk are reported. In SFKs the interaction between phosphotyrosine tail and SH2 domain is thought to have crucial role in maintaining overall conformation and other interactions between catalytic and modular domains. On the other hand, Csk does not have any regulatory tyrosine residues, and some biochemical data have suggested that domain interactions are different between SFKs and Csk.

To elucidate inter-domain interactions in Csk, we started crystallographic study. After extensive crystallization screening and its condition optimization we have finally succeeded in obtaining well diffracting crystals of full length Csk.

At first, we have planned phasing by molecular replacement method and MR analysis was almost successful. However the crystal contained 6 molecules of Csk (approximately 300kDa of protein) per asymmetric unit and the crystal structure refinement procedure might be an extremely time-consuming process.

For rapid structure determination of full length Csk, we have collected MAD data at BL41XU with one heavy atom derivative. User-oriented data collecting system at BL41XU, greatly facilitated our experimental practice. Resulting anomalous Patterson map permitted clear interpretation and we are now under phasing calculation using these datasets.

Reference

Nada,S. and Okada,M., *et al.*, ***Nature***, 351, 69-72 (1991)