

Crystallographic study on the initial stage in the photo-reactivation of nitrile hydratase

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Nitrile hydratase (NHase; EC. 4.2.1.84) catalyzes the hydration of nitriles to their corresponding amides and consists of α and β subunits with M_s of about 23k. The enzyme having a non-heme iron atom in the active center is called Fe-type NHase. Some Fe-type NHases show an extraordinary characteristic: they completely lose the catalytic activity after aerobic incubation of microorganisms in the dark (inactive state), but immediately recover it upon light irradiation (active state). In the inactive state, an endogenous nitric oxide (NO) molecule persistently binds to the non-heme iron center and dissociates upon light-irradiation. The photo-dissociation of the NO molecule causes the activation of NHase. The crystal structure analysis on Fe-type NHase from *Rhodococcus* sp. N-771 revealed that the active center is composed of, so-called, 'claw setting' structure formed by one serine and two cystein residues post-translationally modified into cystein-sulfinic (Cys-SO₂H) and -sulfenic (Cys-SOH) acids.

Until now, the crystal structures of 'native' NHases have been reported in the inactive the active state. However, the detailed structural dynamics in the primary process of the photo-reactivation still remain to be elucidated. In the present X-ray diffraction experiment, we have tried to collect the diffraction data toward the structural analysis for the photo reactivation process.

The crystals used were obtained in the

presence of ammonium sulfate and dioxan and belonged to the space group $P2_12_12$ with lattice constants of $a = 117.4$, $b = 145.6$, $c = 52.1$ Å. In the cooling process, the lattice constant c sometimes became double-sized. The diffraction intensity data were collected at BL41XU with MarCCD-165 detector. The X-ray wavelength was set at 0.7100 Å, and the diffraction patterns were recorded through a series of oscillation of 0.6 ° in an exposure time of 2 sec. For one crystal, two data sets were collected under the dark and the light-irradiation. In addition, to examine the temperature dependence of the photo-reactivation process, several data sets were collected both at 90 K and 165 K. The diffraction data collected up to resolutions of 1.7 - 1.8 Å were processed using the programs DENZO and SCALEPACK and had good statistics indicated by R_{merge} of less than 0.06.

The crystallographic structure refinement revealed that there were little structural changes between the dark and light conditions at the two temperatures. This finding indicates two possibilities in the photo reactivation dynamics. (1) The endogenous NO molecule still resides at the active-center in the temperature range examined. (2) The primary process of the photo-dissociation of the NO molecule may be trapped in temperature range far below as well as the photo-dissociation of CO molecule from the heme of myoglobin.

Infrared Spectral Analysis of Coals

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Coal is the type of the sample with uneven and complicated chemistry structure. In infrared spectrum measurement, there are some problems such as multiplex dispersion of light and mixing of the specular light by the influence of a high refractive index powder samples.

That is, these cause of a spectrum distortion are annoying problems, which degrade spectrum accuracy.

This time, by using the powerful light source of Spring8, we made the trial production of the optical accessories which mitigate these influences.

First, instruction by Moriwaki and Kimura was done about the handling of a total main part of BL43IR, and the method of dealing with it about the vacuum system of a beam line was mastered in general using first two shifts.

Next, MCT detector was moved from the original position, and the light which carried out incidence to the sample room received reflection by the sample, and dispersion, and once, it performed the positioning so that it might be enough condensed by the detector.

However, measurement of the sample which that the focal position of a condensing mirror was a little inaccurate and the fixed method of a sample position were planning from it having been unripe in this measurement -- only the part of sample coals has been measured very much. Moreover, instruction was received about the usage of a workshop so that work might be myself possible.

When the following measurement opportunity was obtained, prior preparations were made scrupulously and it keenly realized using a beam time effectively and that it was important to take time, such as experiment arrangement, enough before and after a beam time.