X-ray Structure Analysis of Hydrogenase at High Resolution

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Introduction

Hydrogenase is an enzyme which catalyzes the oxidation and reduction of molecular hydrogen. Three types of hydrogenases, iron only, nickel iron and nickel-iron-selenium hydrogenases have been found in sulfate-reducing bacteria, *Desulfovibrio*. Two crystal structures of the nickel-iron hydrogenases from *Desulfovibrio gigas* and *Desulfovibrio vulgaris* Miyazaki F were analyzed at 2.85 Å[1] and 1.8 Å[2], respectively. They showed the location, coordination, and geometry of the metal centers in the molecule[1, 2]. The novel features of the histidine-ligated Fe_4S_4 cluster, Fe_3S_4 cluster and an iron atom near the nickel site were also reported. Furthermore an additional Mg site has been also found in the Miyazaki structure[2]. The active Ni-Fe metal center has the most unusual ligand structure. The Ni atom is coordinated by four sulfur atoms of protein cysteine residues. Two of them also coordinate the iron atom making the bridge between the Ni and the Fe atoms. The four out of six coordination positions of the iron atom are occupied by four non-protein ligands. Three of four non-protein ligands have been assigned as SO, CO and CN diatomic molecules, and the remainder has been considered as a sulfur atom from the nature of the electron density and the refined atomic parameters[2]. The assignments of these non-protein ligands has been controversial between structural biologists in this field. The high resolution up to 1.5Å resolution x-ray diffraction study of the enzyme has been done in order to get the information about the plausible assignments for the non-protein ligands to the Fe atom in active center.

Material and Methods

Hydrogenase from *D. v.* Miyazaki F was isolated and purified from the membrane fractions of the bacterial sonicates and crystallized by siting-drop vapor diffusion method using 2-methyl-2,4-pentane-diol as a precipitating agent. The small brown crystals in the typical size of 0.02 X 0.02 X 0.1 mm were used for diffraction study. The data collection was done at 100K using a Rigaku R-axis IV system which was installed at the beam line of BL41XU at SPring-8. Experimental conditions in detail are listed in Table 1. The diffraction patterns were processed by the programs of DENZO and SCALEPACK[3].

Table 1. Condition of data collection

Rotation axis		*c*
No. of oscillation		15
Rotation angle / film	(°)	1.0
Exposure time / film	(sec)	30
Scan speed	(°/sec)	0.5
Wavelength of x-ray	(Å)	0.7

Results and Discussions

Total of 97 diffraction patterns were successfully collected on Fuji Imaging Plates for about 10 hours. Though the diffraction spots in each film were very fine and isotropic with small mosaicity (<0.3), many diffraction spots have been rejected in the integration process because of the strong diffraction patterns by ice which were due to the high humidity of the experimental hutch. The data completeness of the final intensity data at 1.5Å is only 51% with 15% of the outer shell (1.7-1.5Å). Reprocessing of the diffraction data by manual indexing is now in progress.

References

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