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Crystal Structure Analysis of [NiFe] Hydrogenase Maturation Proteins

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Introduction

[NiFe]-hydrogenases harbor a complex metal cofactor, $NiFe(CN)_2CO$, in their active sites. Its biosynthesis requires specific maturation machinery, in which six Hyp proteins (HypABCDEF) play key roles. Four Hyp proteins (HypCDEF) are involved in the biosynthesis and incorporation of the $Fe(CN)_2CO$ group. After Fe insertion, HypA and HypB insert the Ni ion into the hydrogenase large subunit. Finally, proteases such as HybD and HycI are involved in the cleavage of the C-terminal residues of the large subunits. We determined crystal structures of all Hyp proteins and obtained various knowledge of the maturation system. However, the transient Hyp protein complex formed in this process is not fully understood.

In this study, we have determined crystal structures of the HypA / ATPase-type HypB ($HypB_{AT}$) complex from *Thermococcus kodakarensis*, providing the structural basis of concerted actions of these proteins for Ni insertion [1]. In addition, we have also determined crystal structure of the maturation protease HybD to reveal its substrate recognition mechanism [2].

Results and Discussion

SEC analyses showed that ATPγS or AMPPCP-bound $HypB_{AT}$ can interact with HypA. The $HypAB_{AT}$ complex sample was prepared by mixing the two proteins at an equal molar concentration and ATP analogue with $NiCl_2$ for 1-2 h. Crystals of the $HypAB_{AT}$ complex were obtained by using MES-Na (pH 6.2), $MgCl_2$, and PEG3350 in addition to urea or guanidine. The X-ray diffraction data sets were collected at the BL-1A and AR-NE3A beamlines in the Photon Factory. The structure was determined at 1.63 Å resolution by the molecular replacement method, using the previously determined HypA and $HypB_{AT}$ as search models.

The $HypAB_{AT}$ complex structure consists of two HypA and two $HypB_{AT}$ molecules (Fig. 1). The Ni ions were clearly observed. Only ATP-bound $HypB_{AT}$ binds to HypA and induces drastic conformational changes of HypA, forming a Ni-binding site. These findings indicate that HypA and $HypB_{AT}$ constitute an ATP-dependent Ni acquisition cycle for [NiFe]-hydrogenase maturation.

Crystals of HybD were obtained by using Bis-Tris propane (pH 9.0) and PEG 6000. The X-ray diffraction data sets were collected at the BL-1A beamline in the Photon Factory. The structure was determined at 1.82 Å resolution by the molecular replacement method, using the *E. coli* HybD as a search model.

Comparisons of HybD with homologous proteins reveal that they share a common overall architecture with conserved Ni-binding residues, suggesting that they have similar catalytic functions. Moreover, the server-based prediction and surface electrostatic analysis provide reasonable evidence regarding the Ni binding site (Fig. 2).

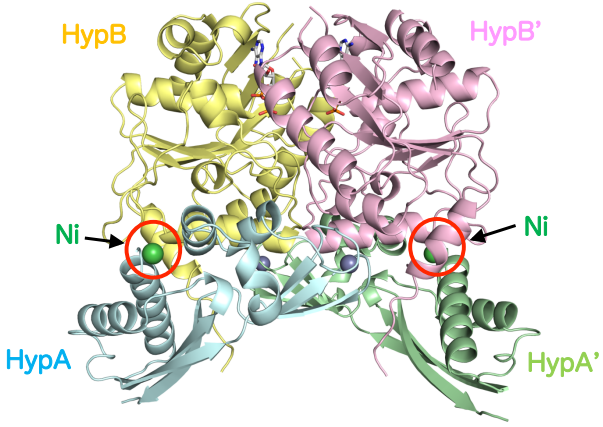

Fig. 1: Structure of the $HypAB_{AT}$ complex

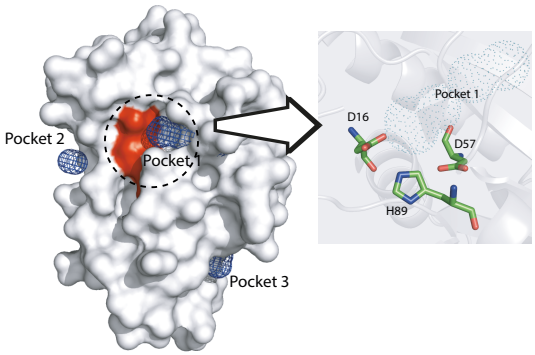

Fig. 2: Proposed Ni-binding site in HybD

References

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