Crystal Structure of the HypA-HypB Complex for [NiFe] Hydrogenase Maturation

Ni-metallochaperone, HypA, and GTPase/ATPase, HypB, are involved in the insertion of a Ni ion into the catalytic center of [NiFe]-hydrogenases in an unknown manner. We have determined the crystal structures of a transient complex formed between HypA and ATPase-type HypB ($HypB_{AT}$) together with Ni ions. The structure reveals that complex formation of HypA with $HypB_{AT}$ induces large conformational changes of HypA, resulting in the formation of a Ni binding site. Consequently, the Ni binding affinity of HypA is enhanced from the micromolar to nanomolar range. These results indicate that HypA and $HypB_{AT}$ perform an ATP-dependent Ni acquisition cycle for [NiFe]-hydrogenase maturation.

[NiFe]-hydrogenases catalyze reversible H_2 production with a complex cofactor, $NiFe(CN)_2CO$, in their active site [1]. Biosynthesis of the $NiFe(CN)_2CO$ cofactors is a complicated process, in which six Hyp proteins (HypABCDEF) play important roles [2]. First, four Hyp proteins (HypCDEF) catalyze the biosynthesis of CN ligand and incorporate the $Fe(CN)_2CO$ group into the large subunit of the hydrogenase. After the incorporation of $Fe(CN)_2CO$, HypA and HypB perform the insertion of the Ni ion into the hydrogenase large subunit. HypA functions as a Ni-metallochaperone, which consists of a Ni-binding domain (NiBD) and a Zn-binding domain (ZnBD). NiBD binds to a Ni ion with micromolar affinity by using a highly conserved MHE motif. HypB proteins are classified into the GTPase type and the ATPase type ($HypB_{AT}$), which share a similar overall structure, despite their low sequence similarity. HypA and HypB form a transient complex in the Ni insertion process. However, the functional relationship between HypA and HypB for the maturation process remains unclear. To elucidate the molecular details of the HypA-HypB complex, we determined the crystal structures of the HypA-$HypB_{AT}$ complex from *Thermococcus kodakarensis* at 1.63–3.10 Å resolution with and without Ni ions in the presence of ATPγS or AMPPCP [3]. Data collection was performed at BL-1A and AR-NE3A.

The structure of the $HypAB_{AT}$ complex reveals that two HypA molecules are bound to the opposite surface of the ATP-binding site of the $HypB_{AT}$ dimer (**Fig. 1a**). The structure of the complex consists of three molecular interfaces (**Fig. 1b-d**). At interface 1, N-terminal residues of $HypB_{AT}$ assume β-strand conformations, interacting with a hydrophobic patch between the α1 helix and β6 strand in the HypA-NiBD (**Fig. 1b**). At interface 2, hydrophobic interactions and several hydrogen bonds are formed between the α helices in the HypA-ZnBD and the β6-α4 and α3-β4 loops of $HypB_{AT}$ (**Fig. 1c**). At interface 3, residues surrounding the HypA zinc finger motif form hydrogen bonds with the α helices of the other monomer $HypB_{AT}$ (**Fig. 1d**).

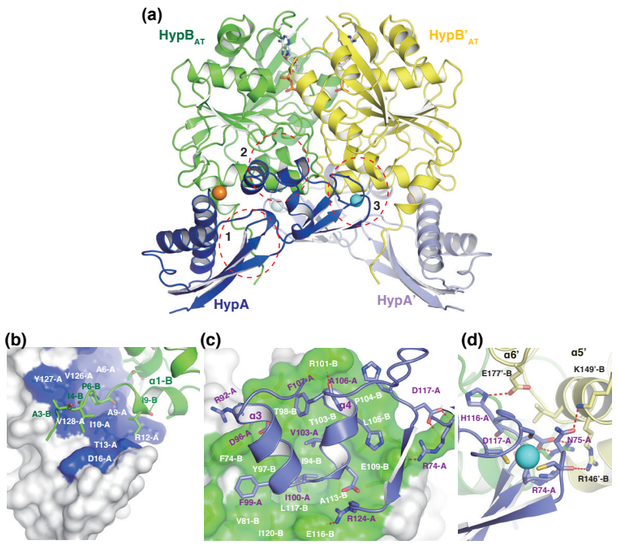

Figure 1: Structure of the HypA-$HypB_{AT}$ complex. **(a)** Overall structure of the HypA-$HypB_{AT}$ complex. Blue and light blue: HypA molecules, with Zn (cyan) and Ni (orange) atoms. Green and yellow: monomers in the $HypB_{AT}$ dimer. Three interfaces are indicated by red dashed-circles. **(b)** Close-up view of interface 1. **(c)** Close-up view of interface 2. **(d)** Close-up view of interface 3.

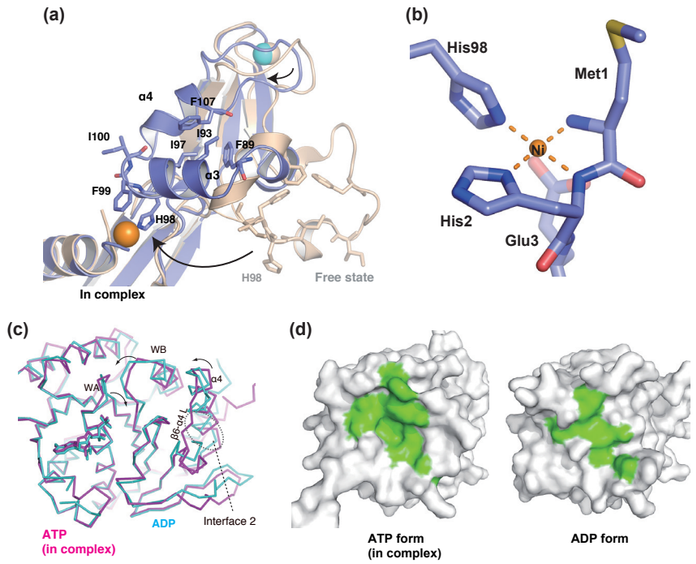

Figure 2: Conformational changes of HypA and $HypB_{AT}$. **(a)** Comparison of the ZnBD of HypA in the complex (blue) and in the isolated state (wheat). **(b)** Detail of the Ni-binding site. **(c)** Superposition of the Cα backbone of the ATP-bound (pink) and ADP-bound (cyan) states of $HypB_{AT}$. Curved arrows represent conformational changes induced by ATP hydrolysis. **(d)** Comparison of the molecular surface of the ATP-bound and ADP-bound states of $HypB_{AT}$. Residues at interface 2 are shown in green.

The complex formation induces large conformational changes of HypA (**Fig. 2a**, **b**). Upon complex formation, the ZnBD substantially rotates and several 3_{10} helices are moved toward $HypB_{AT}$ (**Fig. 2a**). This rotation disrupts a hydrophobic core in the ZnBD and results in the formation of the α3 and α4 helices. In addition, a conserved His residue (HypA-His98) is brought close to the conserved MHE motif. As a result, the amine nitrogen of Met1, the amide nitrogen and Nδ of His2, and Nε of His98 bind a Ni ion with a nearly square-planar geometry (**Fig. 2b**). The Oε of Glu3 also makes van der Waals contacts with the Ni ion. ITC experiments showed that complex formation increases the Ni-binding affinity of HypA from the micromolar to nanomolar range by the formation of the Ni-binding site in the HypA-$HypB_{AT}$ complex.

Conformational changes induced by ATP hydrolysis of $HypB_{AT}$ regulate the interaction of $HypB_{AT}$ with HypA. In the $HypAB_{AT}$ complex, the hydrophobic cleft at interface 2 traps hydrophobic residues of HypA. Upon ATP hydrolysis, the Walker A and B motifs of $HypB_{AT}$ rotate and the α4 helix and β6-α4 loop are shifted (**Fig. 2c**). As a result, the hydrophobic cleft of interface 2 in the ADP-bound $HypB_{AT}$ is changed into a flat molecular surface (**Fig. 2d**). Furthermore, the ADP-bound $HypB_{AT}$ dimer adopts a more open conformation, affecting interface 3. Therefore, conformational changes induced by ATP hydrolysis abolish interfaces 2 and 3, leading to the release of $HypB_{AT}$ from HypA. These findings indicate that there is an ATP-dependent Ni acquisition cycle for [NiFe]-hydrogenase maturation, wherein $HypB_{AT}$ regulates the Ni-binding affinity of HypA.

REFERENCES

[1] W. Lubitz, H. Ogata, O. Rudiger and E. Reijerse, *Chem. Rev.* **114**, 4081 (2014).
[2] S. Watanabe, D. Sasaki, T. Tominaga and K. Miki, *Biol. Chem.* **393**, 1089 (2012).
[3] S. Watanabe, T. Kawashima, Y. Nishitani, T. Kanai, T. Wada, K. Inaba, H. Atomi, T. Imanaka and K. Miki, *Proc. Natl. Acad. Sci. U.S.A.* **112**, 7701 (2015).

BEAMLINES

BL-1A and AR-NE3A

S. Watanabe[1, 2], Y. Nishitani[1] and K. Miki[1] ([1]Kyoto Univ., [2]Tohoku Univ.)