

Crystal Structure Determination of Hyp proteins for maturation of [NiFe] hydrogenases

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Introduction

[NiFe] hydrogenases catalyze the reversible oxidation of molecular hydrogen. The active site of [NiFe] hydrogenases carries a NiFe(CO)(CN)₂ metal center. The biosynthesis of this metal center requires specific maturation proteins. HypE and HypF are involved in the synthesis of the cyanide ligand attached to the active site Fe atom. HypC and HypD receives the cyanide ligand from HypE-thiocyanate and are assumed to insert the Fe(CN)₂CO group to the precursor large subunit of [NiFe] hydrogenases. After the incorporation of the Fe(CN)₂CO moiety, HypA and HypB carry out the insertion of the Ni atom into the precursor of the large subunit. However, the detailed roles of HypA in the Ni insertion process remain unclear.

In order to reveal the detailed functions of HypA, we have performed crystallographic studies of HypA. Data collection in the Photon Factory was performed at the NW12 and BL17A beamlines.

Results and Discussion

In this study, we have determined the crystal structures of HypA from *Thermococcus kodakaraensis* in both monomeric and dimeric states.

Crystals of the HypA monomer were grown under conditions containing 0.1 M HEPES-Na, pH 7.4, 1.2 M lithium sulfate and an additive solution (1M guanidine hydrochloride, or 1M potassium chloride). These crystals belong to the space group *R*32 with two monomers (mol A and mol B) in the asymmetric unit. The monomer structure of HypA was determined by the single anomalous dispersion method using the anomalous signal of the Zn atom. The final model (residues 1-139) was refined at 2.30 Å resolution to an R_{work} value of 21.5% and an R_{free} value of 23.9%, where residues 87-107 in mol B are disordered. The structure of the HypA monomer consists of Ni- and Zn-binding domains (Fig. 1A). The relative arrangement of the two metal binding domains has been shown to be associated with local conformations of the conserved Ni-binding motif, suggesting a communication between the Ni- and Zn-binding sites.

Small crystals of the TkHypA dimer were obtained by the sitting-drop vapor-diffusion method using a precipitant solution containing 0.1M Tris-HCl pH 8.5, 24% (w/v) PEG8000, and 22% (v/v) isopropanol. Crystals of the TkHypA dimer belong to the space group *P*2₁2₁ with two dimers in the asymmetric unit. The dimer structure of TkHypA was determined at 3.3Å resolution by the molecular replacement method using each domain of the monomer structure of HypA as a search model. The HypA dimer has been shown to be stabilized by unexpected domain swapping through an archaea specific linker helix (Fig. 1B). The observed domain swapped dimer of HypA is probably formed during the folding process. These findings suggest the functional diversity of HypA proteins

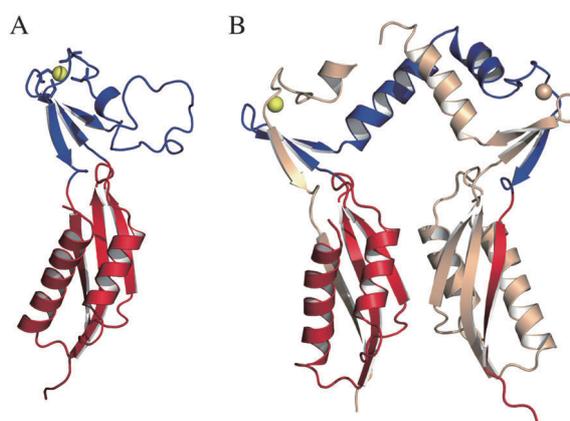


Fig 1. Overall structures of the HypA monomer and the domain swapped dimer (B).

References

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