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

Satoshi WATANABE1, Rie MATSUMI2, Takayuki ARAI1, Haruyuki ATOMI2, Tadayuki IMANAKA1 and Kunio MIKI*1

1Department of Chemistry, Graduate School of Science, Kyoto University, Sakyo-ku, Kyoto 606-8502, Japan
2Department of Synthetic Chemistry and Biological Chemistry, Graduate School of Engineering, Kyoto University, Katsura, Nishikyo-ku, Kyoto 615-8510, Japan
3Department of Biotechnology, College of Life Sciences, Ritsumeikan University, Kusatsu 525-8577, Japan

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. 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 P212121 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


*miki@kuchem.kyoto-u.ac.jp