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Crystal Structure Determination of Hyp Proteins for [NiFe] Hydrogenases

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Introduction

[NiFe] hydrogenases catalyze the reversible oxidation of molecular hydrogen and carry a $NiFe(CO)(CN)_2$ metal center at the active site. The biosynthesis of this metal center requires specific maturation proteins, HypA, HypB, HypC, HypD, HypE, and HypF [1]. HypA and HypB are involved in the insertion of the Ni atom into the precursor large subunit, as [NiFe] hydrogenases are composed of large and small subunits. HypE and HypF are involved in the synthesis of the cyanide ligand attached to the active site Fe atom. HypC and HypD form a complex that is presumably involved in the insertion of the Fe atom coordinated by diatomic ligands. The HypCD complex receives the cyanide ligand from HypE-thiocyanate and is assumed to insert the Fe atom to the precursor large subunit of [NiFe] hydrogenases.

The whole pathway of the maturation has been elucidated, but each maturation step is not fully understood. In order to reveal the detailed mechanism of the maturation process, we have performed crystallographic studies of Hyp proteins. Data collection in the Photon Factory was performed at the NW12 beamline.

Results and Discussion

In this study, we have determined the crystal structures of HypC, HypD, and HypE from *Thermococcus kodakaraensis* KOD1 at 1.8Å, 2.07Å, and 1.55Å resolution, respectively [1][2][3].

Crystals of HypC were obtained using PEG 4000 and NaBr as precipitants. The crystals belong to the space group $C2$ with unit cell parameters of a = 79.24 Å, b = 59.13 Å, c = 53.97 Å and β =109.0˚. The structure of HypC was determined by the MIRAS method using several heavy atoms [1][2]. The overall structure of HypC consists of an OB-fold like β-barrel domain and a C-terminal α-helix (Fig. 1A). Comparison of three HypC molecules in the asymmetric unit showed the C-terminal α helix is very flexible.

Crystals of HypE were obtained using MPD and NaCl [1][3]. The crystals of HypE belong to the space group $P2_12_12$ with unit cell parameters of a = 88.34 Å, b = 45.83 Å, and c = 75.05 Å. The structure of HypE was solved by the MIRAS using mercury and gold derivatives. The monomer structure of HypE consists of two α/β domains (domains A and B) and a C-terminal tail (Fig. 1B). HypE forms a homodimer, in which the β sheet of domain A from each monomer forms a pseudo ten-stranded β-barrel. The C-terminal tail of HypE is shown to exist in an ATP-dependent dynamic equilibrium between outward and inward conformations.

Crystals of HypD in the space group $P2_1$ grew under the condition containing PEG4000, $MgCl_2$ and ethyleneglycol. The structure of HypD was determined by the SAD method using anomalous signals of the Fe atoms in HypD [2][3]. The overall structure of HypD consists of two α/β domains and a Fe-S cluster binding domain with a [4Fe-4S] cluster (Fig. 1C). The overall architecture of HypD is not similar to any other known structures. The structure of HypD revealed that HypD has a redox cascade formed by the [4Fe-4S] cluster and two disulfide bonds, which seems to catalyze the cyanation reaction in the HypCDE complex.

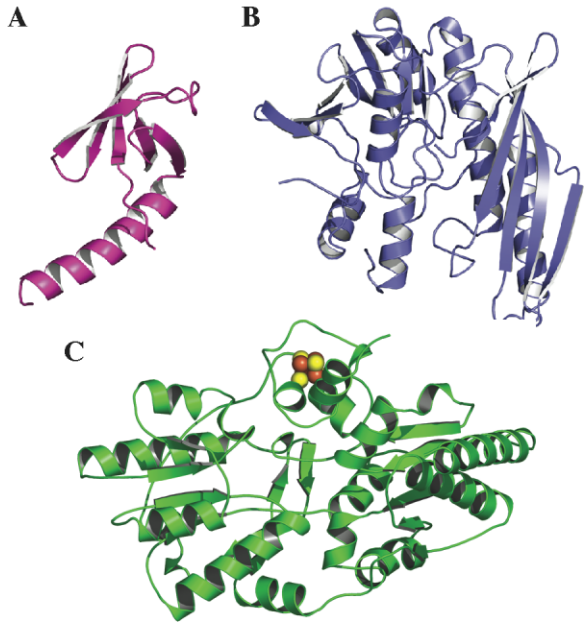

Fig 1. Overall structures of HypC (A), HypE (B) and HypD (C)

References

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