Crystal Structures of [NiFe] Hydrogenase Maturation Proteins, HypC, HypD, and HypE

[NiFe] hydrogenases catalyze the reversible formation of molecular hydrogen in a variety of microorganisms. The core enzyme of [NiFe] hydrogenases carries a $NiFe(CO)(CN)_2$ metal center at the active site. The synthesis of this metal center is a complex process, in which specific maturation proteins, six Hyp proteins (HypABCDEF), are required [1]. HypA and HypB are involved in the insertion of the Ni atom into the precursor large subunit. HypE and HypF are involved in the synthesis of the cyanide ligand attached to the active site Fe atom. HypF catalyzes the transfer of the carbamoyl group from a carbamoylphosphate to the C-terminal cysteine residue of HypE coupled with the conversion of ATP to AMP and PPi. An ATP-dependent dehydration by HypE converts HypE-carboxamide to HypE-thiocyanate. 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 into the precursor large subunit of [NiFe] hydrogenases. However, the mechanism of the CN transfer to the iron atom in the ternary complex remains unclear. In order to gain insight into the mechanism of the cyanation reaction, we have determined crystal structures of HypC, HypD, and HypE from a hyperthermophilic archaeon, *Thermococcus kodakaraensis* KOD1 at 1.8 Å, 2.07 Å, and 1.55 Å resolution, respectively, using beamlines **BL41XU** and **BL44B2** [2].

The overall structure of HypC consists of a β-barrel (oligonucleotide/oligosaccharide binding (OB)-fold) domain and a C-terminal α helix (Fig. 1(a)). N-terminal residues adopt an extended conformation, which allows the essential cysteine residue (Cys2) to make contact with the solvent. Comparison of three HypC monomers in the asymmetric unit has shown that the arrangement of the OB-fold domain and the C-terminal helix differs between the monomers (Fig. 1(b)), suggesting that the C-terminal α helix is very flexible.

The overall structure of HypE consists of two α/β domains (domains A and B) and a C-terminal tail (Fig. 2(a)). This structure is similar to that of other PurM proteins, whose members are characterized by a common motif for ATP hydrolysis. In the crystal, HypE forms a homodimer with a symmetry-related molecule (Fig. 2(b)). The structure of the HypE dimer also resembles that of the PurM dimer. In the HypE dimer, four conserved motifs are assembled at the active site, suggesting that the HypE dimer is a functional unit for ATP-dependent dehydration.

The crystal structure prepared by co-crystallization with ATP shows that the C-terminal tail of HypE exists in an ATP-dependent dynamic equilibrium between outward and inward conformations (Fig. 2(c)). In the

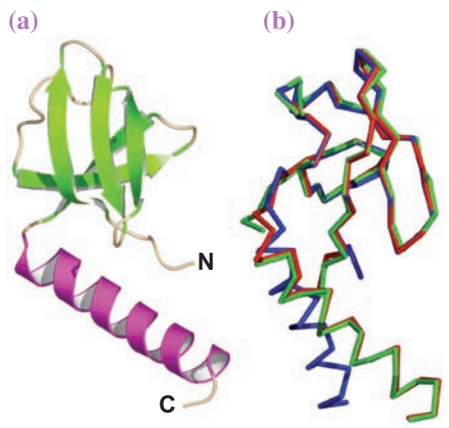

Fig. 1. Crystal structure of HypC. **(a)** Overall structure of HypC in a ribbon representation. The OB fold domain is shown in green; the C-terminal α helix is shown in pink. **(b)** A superposition of the Cα backbone of the three HypC molecules in the asymmetric unit.

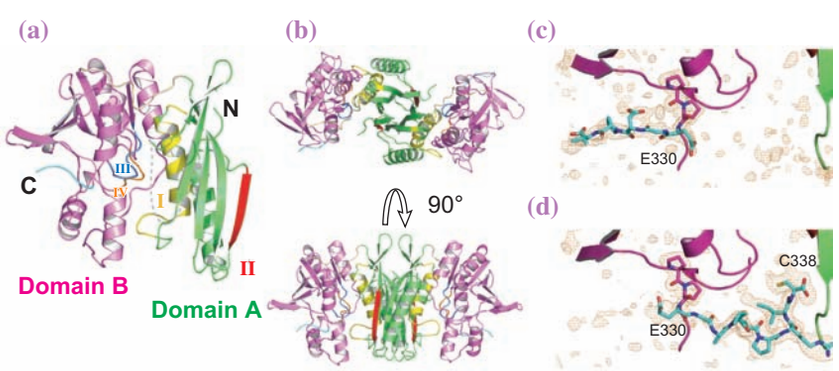

Fig. 2. Overall structure of HypE. **(a)** Ribbon representation of the overall structure of HypE. The two α/β domains A and B, and the C-terminal tail are shown in green, pink, and cyan, respectively. The conserved motifs I, II, III, and IV are colored yellow, red, blue, and orange, respectively. **(b)** Top (upper panel) and side (lower) views of the HypE dimer. **(c** and **d)** Electron density of a simulated annealing omit map (omitting residues 328-338) around the C-terminal tail in the absence of ATP **(c)** or in the presence of ATP **(d)** is shown at 2.5σ (blue).

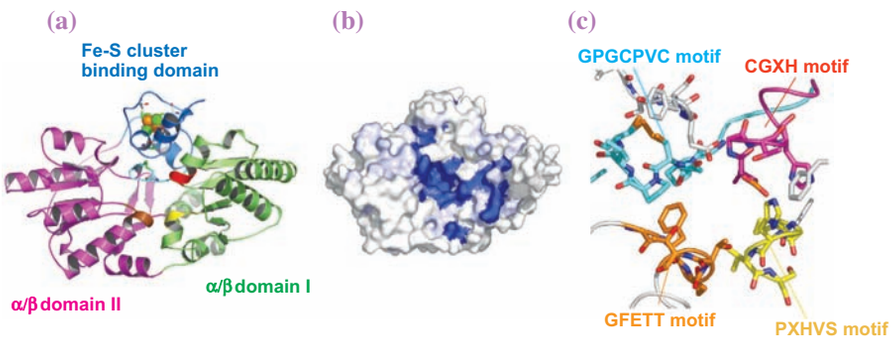

Fig. 3. Overall structure of HypD from *T. kodakaraensis*. **(a)** Ribbon representation of the overall structure of HypD. The two α/β domains I and II, and the Fe-S cluster binding domain are shown in green, magenta, and blue, respectively. Cysteine residues and the [4Fe-4S] cluster are represented in a stick-and-sphere model. **(b)** Surface representation of HypD. Identical and conserved residues among HypD proteins are colored in blue and light blue, respectively. The orientation is identical to that in Fig. 3**(a)**. **(c)** Close view of the assembly of the conserved motifs in a stick representation. The conserved motifs are shown in different colors: CGXH motif, magenta; GPGCPVC motif, cyan; GFETT motif, orange; and PXHVS motif, yellow, respectively.

ATP-unbound state, the C-terminal tail of HypE takes the outward conformation, which is appropriate for receiving carboxamide from a carbamoylphosphate by HypF or for transferring thiocyanate to the HypCD complex. Binding of ATP to HypE will shift the equilibrium toward the formation of the inward conformation, in which HypE dehydrates the S-carboxamide moiety to yield thiocyanate.

The overall structure of HypD consists of three domains: an α/β domain I, an α/β domain II, and an Fe-S cluster binding domain (Fig. 3(a)). The Fe-S cluster-binding domain carries a [4Fe-4S] cluster. The overall architecture of HypD is not similar to any other known structures. These three domains form a cleft at the center of the molecule. Mapping of conserved residues on the molecular surface has shown that residues around the center cleft are highly conserved (Fig. 3(b)). In particular, the four conserved motifs are assembled at the bottom of the cleft (Fig. 3(c)). Therefore, these observations strongly suggest that the active site of HypD is constructed by the four conserved motifs.

The crystal structure of HypD reveals that the disulfide bond SS2 (Cys325-Cys354) is located very close to the [4Fe-4S] cluster (Fig. 4). The sulfur atom of Cys325 in the reduced form makes a close contact with the iron atom. Furthermore, the disulfide bond SS1 (Cys66-Cys69) is located close to SS2. Unexpectedly, the [4Fe-4S] cluster environment of HypD is quite similar to that of ferredoxin:thioredoxin reductase (FTR), suggesting the existence of a redox cascade between the 4Fe-4S cluster and two disulfide bonds.

The present structures of HypC, HypD and HypE provide deep insights into the cyanation reaction. In particular, the assembly of conserved residues of HypD around the center cleft suggests that the coordination of the cyanide ligand to the Fe atom takes place at the conserved motifs (Fig. 3(c)). The interface between HypD and other Hyp proteins is probably the conserved region around the center cleft (Fig. 3(b)). An extended conformation of each conserved motif of HypC and HypE allows the essential cysteine residues of both proteins to interact with the active site of HypD. Revealed FTR-like redox cascade implies that the cyanation reaction is catalyzed by unique thiol redox signaling in the HypCDE complex.

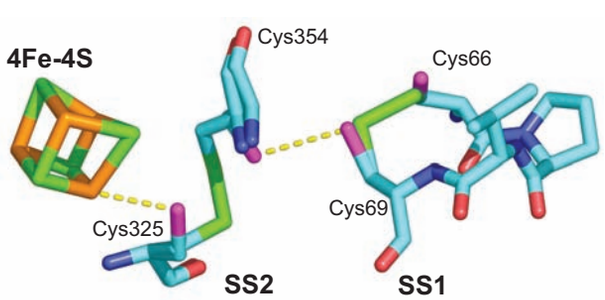

Fig. 4. Redox cascade between the [4Fe-4S] cluster and two disulfide bonds of HypD. Sulfur atoms in the dithiol form of cysteine residues are shown in magenta.

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References

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