Structural studies of [NiFe] Hydrogenase maturation proteins

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[NiFe] hydrogenases catalyze the reversible oxidation of molecular hydrogen and carry a $NiFe(CO)(CN)_2$ center at the active site. The assembly of the metal center requires specific auxiliary proteins: Hyp proteins (HypABCDFE). HypA and HypB are involved in the insertion of the Ni atom. HypE and HypF are involved in the synthesis of the cyanide ligand. HypC, HypD are required in the insertion and cyanation of the Fe atom. In order to elucidate the maturation process at an atomic resolution, we have determined the crystal structures of HypC, HypD, and HypE from *Thermococcus kodakaraensis* KOD1 [1].

The overall structure of HypC consists of an OB-fold like β-barrel domain and a C-terminal α helix. Comparison of HypC molecules in the asymmetric unit shows that the C-terminal α helix is very flexible. The structure of HypE consists of two α/β domains and is similar to other PurM superfamily proteins. The C-terminal tail of HypE shows ATP-dependent large conformational changes. The structure of HypD is composed of two α/β domains and an Fe-S cluster binding domain. Conserved regions of HypD show its probable iron-binding and active sites for cyanation. Furthermore, the [4Fe-4S] cluster environment of HypD is shown to be quite similar to that of ferredoxin:thioredoxin reductase (FTR), indicating the existence of a redox cascade similar to the FTR system. These results provide deep insights into the cyanation reaction mechanism via thiol redox signaling in the HypCDE complex.

[1] Watanabe, S., Matsumi, R., Arai, T., Atomi, H., Imanaka, T., Miki, K., Mol. Cell, 2007, **27**, 29-40