Poster Presentation

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Structural basis for the biosynthesis of the CN ligand of [NiFe] hydrogenase

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[NiFe] hydrogenases carry a $NiFe(CN)_2CO$ center at the active site, catalyzing the reversible H_2 oxidation. The complex NiFe center is biosynthesized and inserted into the enzyme by six specific maturation proteins: Hyp proteins (HypABCDEF). HypE and HypF are involved in biosynthesis of cyanide ligands, which are attached to the Fe atom in the NiFe center. First, HypF catalyzes a transfer reaction of the carbamoyl moiety of carbamoylphosphate to the C-terminal cysteine residue of HypE. Then, HypE catalyzes an ATP-dependent dehydration of the carbamoylated C-terminal cysteine of HypE to thiocyanate. Although structures of HypE proteins have been determined, there has been no structural evidence to explain how HypE dehydrates thiocarboxamide into thiocyanate. In order to elucidate the catalytic mechanism of HypE, we have determined the crystal structures of the carbamoylated and cyanated states of HypE from Thermococcus kodakarensis in complex with nucleotides at 1.53 Å and 1.64 Å resolution, respectively [1]. Carbamoylation of the C-terminal cysteine (Cys338) of HypE by chemical modification is clearly observed in the present structures. A conserved glutamate residue (Glu272) is close to the thiocarboxamide nitrogen atom of Cys338. However, the configuration of Glu272 is less favorable for proton abstraction. On the other hand, the thiocarboxamide oxygen atom of Cys338 interacts with a conserved lysine residue (Lys134) through a water molecule. Interestingly, a conserved arginine residue makes close contact with Lys134 and lowers the pKa of Lys134, suggesting that Lys134 functions as a proton acceptor. These observations suggest that the dehydration of thiocarboxamide into thiocyanate is catalyzed by a two-step deprotonation process, in which Lys134 and Glu272 function as the first and second bases, respectively.

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