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Crystal structures of the HypCD and HypCDE complexes

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Introduction

Assembly of the metal center of [NiFe] hydrogenases proceeds through a multi-step pathway, in which the six Hyp proteins (HypA, -B, -C, -D, -E and -F) function as the conserved core machinery. HypC, HypD and HypE are involved in the biosynthesis of the $Fe(CN)_2CO$ group of [NiFe] hydrogenase. A complex between HypC and HypD has been shown to receive the cyano group from the HypE-thiocyanate. However, it is unclear how HypC, HypD and HypE interact with one another and form the transient ternary complex corresponding to an intermediate for the Fe atom cyanation. To elucidate the cyanation reaction in the maturation process, we have determined the crystal structures of the HypC-HypD binary complex (the HypCD complex) and the HypC-HypD-HypE ternary complex (the HypCDE ternary complex) from *Thermococcus kodakarensis* [1].

Results and Discussion

The crystals of the HypCD complex were grown at 20˚C in sitting drops by mixing 1 µl of protein solution (23 mg/ml protein, 20 mM Tris-HCl pH 7.5, 150 mM NaCl and 1 mM TCEP) with 0.8 µl of reservoir solution (1.4 M $(NH_4)_3$ citrate/citric acid pH 4.5-4.7 and 0.7% MPD and 0.2 µl of additive solution (5% (w/v) polyvinylpyrrolidone K15). The crystals of the HypCDE complex were grown by mixing 0.7 µl of protein solution with 0.7 µl of reservoir solution (50 mM MES pH 6.4, 12-16% PEG400 and 10 mM $MgCl_2$). The X-ray diffraction data were collected on the BL17A and AR-NW12 beamlines at the Photon factory. The structures of the HypCD and HypCDE complexes were determined at 2.55 Å and 2.25 Å resolution, respectively, by the molecular replacement method using the previously determined monomer structures of HypC, HypD and HypE.

The structure of the HypCD complex reveals that the β-barrel domain of HypC is bound to the central cleft between HypD α/β domains I and II. The complex interface between HypC and HypD is formed by interactions between conserved hydrophobic residues. Closing movements of the two domains of HypD make the central cleft deeper, allowing it to better recognize and trap the β-barrel domain of HypC. On the other hand, the C-terminal α-helix of HypC undergoes a large conformational change and does not interact with any parts of HypD.

The overall structure of the HypCDE ternary complex resembles a crab with big pincers (Figure 1). The HypE dimer constitutes the body and two HypCD complexes attach to the individual sides of the HypE dimer, forming the pincers. The HypD α/β domain I and the FeS cluster binding domain are associated with the α6 helix, β11 and β12 strands and C-terminal tail in the HypE. In addition, the HypC β2-β3 loop interacts with the HypE α6 helix and its adjacent loop between the α3 and α4 helices. These observations confirm that complex formation between HypC and HypD precedes formation of the HypCDE ternary complex. The ternary complex formation induces the domain movement of HypE, which is favorable for cyanide transfer.

In the HypCDE ternary complex, the conserved motifs of HypC and HypE are located in close proximity to the conserved motifs of HypD. Cys2 of HypC is located close to Cys38 of HypD, forming an Fe binding site. The conserved C-terminal cysteine of HypE can access the thiol redox cascade of HypD. These results provide structural insights into the Fe atom cyanation in the HypCDE complex.

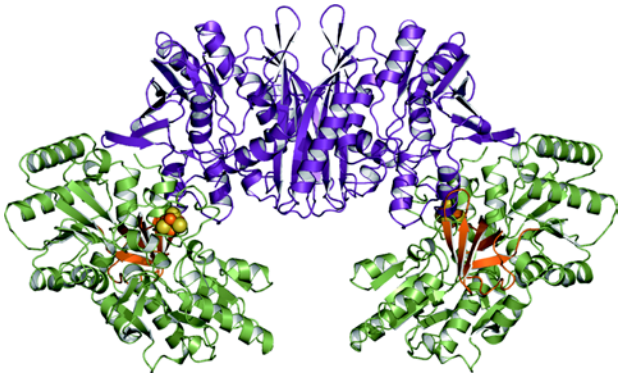

Fig. 1: Structure of the HypCDE complex. HypC, HypD and HypE are shown in orange, green, violet, respectively.

References

[1] S. Watanabe et al., *Structure*, **20**, 2124-2137 (2013).

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