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Structure Biology of [NiFe] Hydrogenase Maturation Proteins

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1 Introduction

[NiFe] Hydrogenases catalyse the oxidation of hydrogen molecules (H_2) and the reduction of hydrogen ions (H^+). They consist of two (large and small) subunits, and the large subunit contains a Ni-Fe cluster at the active site. The formation of the metal cluster does not occur spontaneously and requires a maturation process involving specific accessory proteins such as Hyp proteins (HypA, HypB, HypC, HypD, HypE, and HypF). After the metal insertion, a maturation endopeptidase is involved in the cleavage of the C-terminal residues of the large subunit. It is also known that a Ni ion is necessary for the substrate recognition by endopeptidase.

A Ni chaperone HypA is responsible, together with HypB, for Ni insertion into the active site. The structures of HypA and the HypA-HypB complex have been determined, but details of the interaction mechanism between an immature large subunit (HyhL) and HypA remain obscure. Structures of HyhL in complex with Ni delivery proteins are indispensable for understanding how HypA interacts with HyhL for the Ni insertion and how it affects HyhL in the maturation process. We have determined crystal structures of HypA in complex with HyhL from a hyperthermophilic archaeon, *Thermococcus kodakarensis* [1].

2 Experimental

The HyhL–HypA complex was prepared by mixing HyhL and HypA at a 1:1.4 molar ratio, and the mixture was purified using SEC. Crystals of the HyhL–HypA complex were obtained by using PEG 3350 as a precipitant in two forms with space groups *C*222 and *P*23 at 20 °C and 4 °C, respectively. The structure was solved by the molecular replacement method and refined at 3.30 Å and 3.24 Å resolution for orthorhombic and cubic crystals.

3 Results and Discussion

Figure 1 shows the crystal structure of the HyhL-HypA complex, which revealed two major binding modes of HyhL and HypA: hydrophobic interactions and β-sheet formation. It was also observed that the N-terminal tail of HyhL interacts with the C-terminal β-strand of HypA, forming an extended β-sheet with the three β-strands of HypA. These interactions bring the metal binding sites of HypA and HyhL close to each other and the distance between Cys65 of HyhL and His2 of HypA is 12.2 Å, which is likely enough to deliver Ni into the active site with adequate movement. Comparison of the structures of immature HyhL provides structural insights into the conformational changes after the metal insertion steps. Remarkably, the C-terminal extension of immature HyhL, which is cleaved in the mature form, adopts a β-strand adjacent to its N-terminal β-strands. The position of the C-terminal extension corresponds to that of the N-terminal of the mature large subunit, preventing the access of endopepdidase to the cleavage site of HyhL. These findings suggest that the Ni insertion into the active site induces spatial rearrangement of both N- and C-terminals of HyhL, which is an important step for incorporation of the Ni-Fe cluster in the maturation process of [NiFe] Hydrogenases.

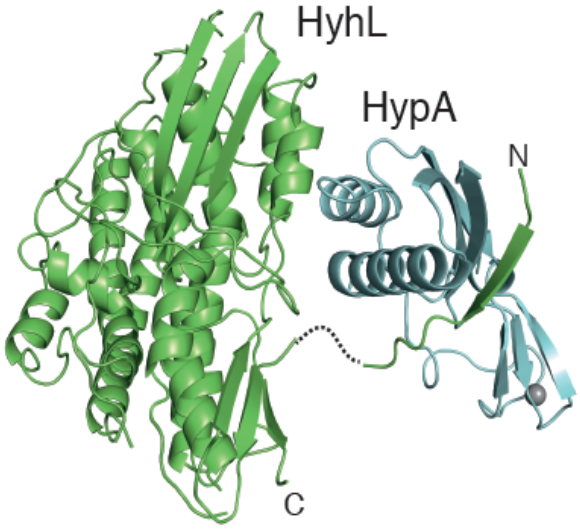

Fig. 1: Structure of the immature large subunit, HyhL and HypA from *Thermococcus kodakarensis*

Reference

[1] S. Kwon, S. Watanabe, Y. Nishitani, T. Kawashima, T. Kanai, H. Atomi, and K. Miki, *Proc. Natl. Acad. Sci. USA*, **115**, 7045-7050 (2018).

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