BL*1A, BL*5A, BL*17A, NW12A/2012G177

Crystal structures of a novel archaeal HypB and intermediate states of HypE

Satoshi Watanabe[1], Daisuke Sasaki[1], Taiga Tominaga[1], Rie Matsumi[2], Tamotsu Kanai[2], Haruyuki Atomi[2], Tadayuki Imanaka[3] and Kunio Miki[1*]
[1]Graduate School of Science, Kyoto University, Sakyo-ku, Kyoto, 606-8052, Japan
[2]Graduate School of Engineering, Kyoto University, Katsura, Nishikyo-ku, Kyoto 615-8510, Japan
[3]College of Life Sciences, Ritsumeikan University, Kusatsu 525-8577, Japan

Introduction

Biosynthesis 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) are involved. HypC, HypD, HypE and HypF are involved in the biosynthesis of the $Fe(CN)_2CO$ group of [NiFe] hydrogenase, while HypA and HypB perform Ni insertion. However, the GTPase HypB homolog proteins are not conserved in some archaeal species including *Thermococcales*. In this study, we have identified a novel archaeal HypB from *Thermococcus kodakarensis* %TkHypB&and have determined its crystal structure [1]. In addition, we have also determined structures of intermediate states of HypE for the biosynthesis of cyanide ligands [2].

Results and Discussion

The crystals of TkHypB were obtained by using sodium citrate tribasic dihydrate (pH 5.6) and ammonium phosphate monobasic (Condition I), or sodium phosphate monobasic monohydrate and potassium phosphate dibasic (pH 5.0). Both of crystals belong to the space group *I*222. The X-ray diffraction data sets were collected at the BL*17A beamline in the Photon Factory. The structure was determined at 2.1 Å resolution by the single-wavelength anomalous dispersion method using a Pt-derivative crystal.

The overall structure of TkHypB consists of a central eight-stranded parallel β-sheet surrounded by several α- and 3_{10}-helices, and is similar to those of the Mrp/MinD family ATPases (Fig. 1). TkHypB forms a stable homodimer and the ADP molecules are sandwiched in parallel by both subunits at the dimer interface. Structural comparisons suggest the ATP-binding dependent conformational changes of the TkHypB dimer.

Carbamoylation of the conserved cysteine residue of HypE was performed by chemical modification using KOCN before crystallization. Crystals of the modified HypE were obtained using reservoir solution containing ammonium sulfate, polyethylene glycol 400 and glycerol. Diffraction data sets were collected at BL*1A, BL*5A and AR-NW12A. The crystal structures of the carbamoylated and cyanated forms of HypE in complex with nucleotides have been determined at 1.53- and 1.64-Å resolution, respectively. These structures reveal the detailed interactions around the carbamoylated cysteine residue (Fig. 2), providing structural basis for the dehydration of thiocarboxamide into thiocyanate.

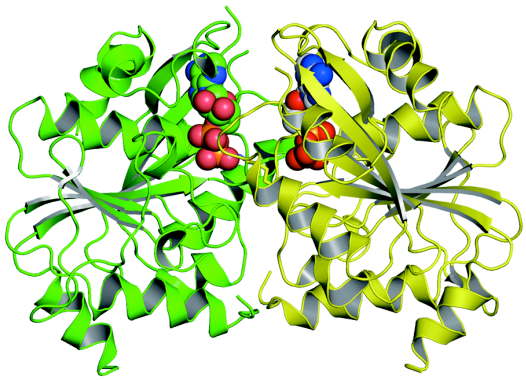

Fig.1: Structure of the TkHypB dimer+

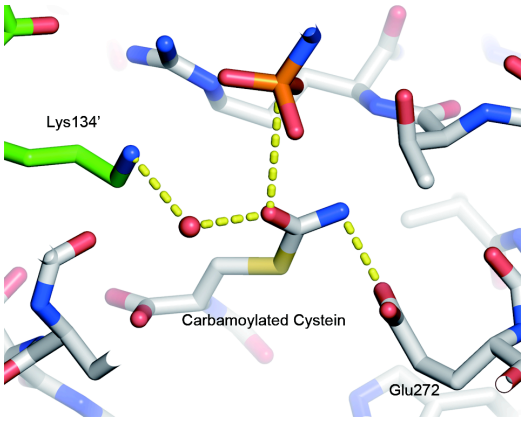

Fig. 2: Interactions around the carbamoylated cysteine.

References

[1] D. Sasaki, S. Watanabe, R. Matsumi, T. Shoji, A. Yasukochi, K. Tagashira, W. Fukuda, T. Kanai, H. Atomi, T. Imanaka, and K. Miki, *J. Mol. Biol.*, **425**, 1627 (2013).
[2] T. Tominaga, S. Watanabe, R. Matsumi, H. Atomi, T. Imanaka, and K. Miki, *Proc. Natl. Acad. Sci. USA*, **110**, 20485 (2013).

*miki@kuchem.kyoto-u.ac.jp