MS11-P6 **Identification and structure of a novel archaeal HypB for [NiFe] hydrogenase maturation.** Daisuke Sasaki,[a] Satoshi Watanabe,[a] Rie Matsumi,[b] Toshihisa Shoji,[c] Ayako Yasukochi,[b] Kenta Tagashira,[c] Wakao Fukuda,[c] Tamotsu Kanai,[b] Haruyuki Atomi,[b] Tadayuki Imanaka,[c] Kunio Miki[a] [a]*Department of Chemistry, Graduate School of Science, Kyoto University, Japan,* [b]*Department of Synthetic Chemistry and Biological Chemistry, Graduate School of Engineering, Kyoto University, Japan,* [c]*Department of Biotechnology, College of Life Sciences, Ritsumeikan University, Japan*
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[NiFe] hydrogenase catalyzes the reversible oxidation of molecular hydrogen. The large subunit of the enzyme carries a $NiFe(CN)_2(CO)$ cluster at the active site. The biosynthesis of the NiFe cluster needs maturation proteins, HypA, HypB, HypC, HypD, HypE, and HypF. After incoorporation of the $Fe(CN)_2CO$ group, HypB inserts the Ni atom together with HypA. Previously charactrized HypB protein belongs to the G3E family GTPase and GTP hydrolysis is required for the Ni insertion process and maturation. A gene encoding the G3E family HypB is not found in genome of some archaea. On the other hand, a gene showing high sequence similarity to the Mrp/MinD family ATPase is conserved adjacent to the *hypA* gene on their genome, assuming that this gene encodes a functional homologue of HypB. Here, we identify TK2007 gene product from hyperthermophilic archaeon *Thermococcus kodakarensis* KOD1 as HypB and determine its crystal structure. A severe growth defect of the ÄTK2007 strain under hydrogenase-required condition was observed and restored by addition of Ni ions, providing convincing evidence that the TK2007 gene product is a novel Mrp/MinD family HypB protein in *T. kodakarensis* (*Tk*-mmHypB). The structure of *Tk*-mmHypB was solved as homodimer related by a non-crystallographic 2-fold axis. Each monomer consists of a central seven-stranded parallel β-sheet surrounded by α-helices. Intriguingly, ADP molecules from *E. coli* expression system are tightly bound to the protein although we did not attempted to co-crystallize the purified sample with an ATP, suggesting that *Tk*-mmHypB shows high affinity with an ADP molecule. Significant structural differences between monomers indicate that the C-terminal loop-helix-loop region is related to the affinity for the ADP. Furthermore, the structure around the dimer interface reveals that the dimer formation is required for ATP hydrolysis. Comparison of the nucleotide-binding site with that of the Mrp/MinD family nitrogenase iron NifH protein suggests structural change during the hydrolysis. These structural insights imply the Ni insertion mechanism depending on nucleotide-exchange factor. Our studies on *Tk*-mmHypB shed new light on structural and functional diversity of HypB proteins in the Ni insertion process in the [NiFe] hydrogenase maturation.

Keywords: hydrogenase; maturation; HypB

MS11-P7 **The 1-Cys Prx protein from cyanobacteria is a peroxidase and a chaperone.** Sauer Uwe H.[a], Yogesh Mishra[a,b], Michael Hall[a], Patrik Storm[a] [a]*Dept. of Chemistry, Umeĺ University,* [b]*Umeĺ Plant Science Centre, Department of Plant Physiology, Umeĺ University, SE-901 87 Umeĺ, Sweden*
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Peroxiredoxins are a unique class of enzymes widespread across phylogeny. Based on the number of cysteine residue, they are broadly divided into three subfamilies: the typical 2-Cys Prxs, the atypical 2-Cys Prxs and the 1-Cys Prxs [1]. The common function of this ubiquitous and diverse group of enzymes is their ability to reduce a variety of peroxides to their respective alcohols [2,3]. The majority of the determined peroxiredoxine structures belongs to 2-Cys Prxs family, which has received much attention recently [4]. However, there is only little structural information available about the 1-Cys-Prx proteins.

We have determined the first structure of a 1-Cys Prx protein from a Cyano bacteria. Here we present the surprising results obtained from the analysis of its structure and function.

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