論文の内容の要旨

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論文題目 Phosphoserine phosphatases and serine related metabolism of *Hydrogenobacter thermophilus* TK-6

(*Hydrogenobacter thermophilus* TK-6 の Phosphoserine phosphatases とセリン関連代謝)

Introduction

Metal-independent phosphoserine phosphatases (iPSPs: iPSP1 and iPSP2)) were firstly identified from *H.thermophilus* TK-6, assimilating carbon dioxide as the sole source for carbon via RTCA cycle. Although the presence of disulfide bond was suggested by the crystal structure of iPSP1(PspA-PspB), there was still much to be resolved. At first, the presence of disulfide bond *in vivo*. This was because disulfide bonds are rarely found in intracellular proteins due to the reducing environment of the cytosol. Second, if there are disulfide bond within iPSPs what kinds of the role does the disulfide bonds have? Third, catabolic pathway of serine or glycine was that since glycine cleavage system was not detected from *H.thermophilus.* Lastly, function of thiol in TK-6 was also of interest. This was because that thiol stands out for making disulfide bond and response for the intra cellular redox state. To solve these questions, biochemistry analysis and gene manipulation were performed.

Chapter 1. Disulfide bond

1.1 Detection of intermolecular disulfide bonds

This chapter clarified the presence of disulfide bond in *vitro* and *in vivo*. Even if intracellular environment was expected to be strong reduced condition, iPSPs and other intracellular proteins had lots of intra- or inter-molecular disulfide bond within *H.thermophilus.* In addition, reversible disulfide bond was also detected under redox condition. Therefore, taking into *H.thermophilus* genome information consideration, the formation and cleavage of intracellular disulfide bond was proposed by the role of protein disulfide bond isomerase and thioredoxin, which showed significant fold change in transcriptome analysis.

1.2 Thermostability

The function of intermolecular disulfide bond made from thiols of iPSPs was investigated. Interestingly, enhanced thermostability by intermolecular disulfide bond was detected not only from homodimeric iPSP1(PspA-PspA) but also from heterodimeric iPSP2 (PspA-PspB). As far as I know, thermostability of heterodimer by intermolecular disulfide bond was

firstly identified from this study. Furthermore, thermostability by proteins-proteins association based on intermolecular disulfide bond was also firstly identified in this study. Therefore, it was revealed that intermolecular disulfide bonds were deeply related with protein thermostability.

1.3 Thermal characteristics between iPSP1 and iPSP2

Although Intermolecular disulfide bond enhanced the thermostability of both iPSP1 and iPSP2, there were different thermo stability between them. Therefore, thermal characteristics of both PspA and PspB subunits were investigated. I found iPSP1 had other several thermostable characteristics related with amino acid composition, compact conformation, and protein secondary structure.

Chapter 2. Physiology of iPSPs

With an aim to identify the physiology of iPSPs, gene manipulation and transcriptome analysis were performed.

2.1 pspA gene deletion serine auxotroph

The results of *pspA* gene deletion firstly identified that obligate chemolithoautotrophy, *H.thermophilus*, could be changed into heterotrophy, suggesting that this newly discovered method will open new avenue for autotrophy. Also for the first time, *psp*A gene deletion serine auxotroph from this study clarified that Liv system could transport external serine after the speculation of 40 years ago. With regard to metabolic property of *pspA* in *vivo, pspA* gene deletion serine auxotroph and absence of glycine cleavage system identified the one carbon metabolism not by glycine but by serine, suggesting that serine was significantly important for cell proliferation. Therefore, it can be suspected that proper control for *pspA* might give clue for controlling unwanted cell.

2.2 Physiology of PspA

Transcriptome and environmental stress analysis were performed to find the physiology of *pspA in vivo*. *pspA* mutants couldn't grow under 30% O_2 condition, suggesting thiols of *pspA* may have direct or indirect role as a ROS scavenger or as regulator for intracellular oxygen level, which was consistent with the gene expression results of ROS detoxifying genes. In addition, transcriptome analysis also revealed that *H.thermophilu* had Liv system for L-serine uptake and One carbon metabolism by only serine.

2.3 Physiology of PspB

Gene expression levels, relative gene quantification, and environmental stress were also estimated by using *pspB* gene deletion *H.thermophilus.* Interestingly, *pspB* expected as a client protein to make intermolecular disulfide bond revealed that *pspB* gene influenced on intracellular oxygen level. In addition, the *pspB* deleted *H.thermophilus* showed markedly up-regulation of cobalt and nickel transport genes, indicating that *pspB* deleted *H.thermophilus* could accumulate lots of Nickel and Cobalt. Therefore, it was suspected that this finding will also open new avenue for controlling intracellular environment.