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Molecular Mechanism of Strategy for Minimization of the Intracellular Formaldehyde Level in Microorganisms and Its Application

Hiroya YURIMOTO

Division of Applied Life Sciences, Graduate School of Agriculture, Kyoto University

Research aims

Formaldehyde, which is a cytotoxic compound, is a central metabolic intermediate in the metabolic pathway for assimilation and dissimilation of one-carbon (C1) compounds, such as methane, methanol, and formaldehyde, in methylotrophs. To achieve efficient industrial use of C1 compounds by microorganisms, it is important to elucidate the molecular basis of how methylotrophs cope with the toxicity of formaldehyde. Recently, several biochemical and genetic approaches have established the molecular mechanisms by which the intracellular level of formaldehyde is regulated during microbial growth on C1 compounds[1,2]. In this study, I described the physiological significance of the ribulose monophosphate (RuMP) pathway, which are widely distributed not only in methylotrophic bacteria but also in non-methylotrophic bacteria and archaea, in a hyperthermophilic archaeon and the molecular mechanism of gene regulation of this pathway in *Bacillus subtilis*. I also described the characterization of a transcriptional factor involved in C1 compounds-induced gene expression in methylotrophic yeast.

Results and Discussion

1) Physiological role of the ribulose monophosphate pathway enzymes in archaea

The RuMP pathway, involving 3-hexulose-6-phosphate synthase (HPS) and 6-phospho-3-hexuloisomerase (PHI), is now recognized as a widespread prokaryotic pathway for formaldehyde assimilation and detoxification. Interestingly, HPS and PHI orthologs are also found in archaeal strains, and recent biochemical and genome analyses have proposed that the reverse reaction of formaldehyde fixation, i.e. ribulose 5-phosphate (Ru5P) synthesis from fructose 6-phosphate, may possibly function in the biosynthesis of Ru5P in several archaeal strains whose pentose phosphate pathways are incomplete. In this study, we have taken a genetic approach to address this proposal using the hyperthermophilic archaeon *Thermococcus kodakaraensis* KOD1 in which gene disruption system has been developed and HPS and PHI are encoded in a single open reading frame (TK0475).

The recombinant HPS-PHI fused enzyme exhibited the expected HPS and PHI activities in both directions (formaldehyde-fixing and Ru5P-synthesizing). The TK0475-deletion mutant did not exhibit any growth in minimal medium, while growth of the mutant strain could be recovered by the addition of nucleosides to the medium. This auxotrophic phenotype together with the catalytic properties of the HPS-PHI-fused enzyme reveal that HPS and PHI are essential for the biosynthesis of Ru5P, the precursor of nucleotides, showing that the RuMP pathway is the sole pathway for Ru5P biosynthesis which substitutes for the conventional pentose phosphate pathway[3].

2) Analysis of the novel DNA-binding protein that acts as a positive regulator of the formaldehyde-inducible RuMP operon in Bacillus subtilis

In *B. subtilis*, *hxlA* and *hxlB*, coding for HPS and PHI, respectively, make up the *hxlAB* operon and expression of this operon is induced by the presence of formaldehyde. Upstream of the *hxlAB* operon, the open reading frame of *hxlR* is oriented in the opposite direction relative to *hxlAB*. The HxlR protein belongs to the DUF24 protein family of unknown function which contains DNA-binding helix-turn-helix motif. The *hxlR* gene was essential for the formaldehyde-induced expression of HPS and PHI because the *hxlR* disruptant strain did not exhibit HPS or PHI activity after exposure to formaldehyde.

Purified recombinant HxlR showed specific binding to the upstream region of the *hxlAB* operon. Using gel-retardation and DNase I-foot printing assays, two 25-bp binding regions for HxlR within the upstream DNA were identified. Surface plasmon resonance analyses suggested that two HxlR dimers sequentially bound to these regions. Finally, we showed that each of the two binding regions for HxlR was necessary for formaldehyde-induced expression of the *hxlAB* operon[4]. Thus, I have shown that HxlR is a novel DNA-binding protein that activates the formaldehyde-induced expression of *hxlAB*. HxlR is now designated as a transcriptional activator of the "helix-turn-helix, HxlR type" (http://www.ebi.ac.uk/interpro/IEntry?ac=IPR002577).

3) Trm1 is a transcriptional activator involved in regulation of methanol-inducible gene expression in yeast

Methylotrophic yeasts have been widely used as a host for the heterologous gene expression system because the promoters of the genes encoding methanol-metabolizing enzymes are highly inducible by C1 compounds[5]. However, the molecular mechanism of C1 compounds-inducible gene expression is still unclear. We have screened for the mutants that transcriptional activity of C1 compounds-in-

ducible promoters was reduced using *Candida boidinii* as a host strain and acid phosphatase from Saccharomyces cerevisiae as a reporter. As a result, the *TRM1* gene was found and its properties were analyzed.

Trm1 belongs to Zinc finger protein family of a $Zn(II)_2 Cys_6$ type restricted to eucaryotic microbe. The *TRM1* gene disrupted strain was not able to grow on methanol, but showed normal growth on glucose or other non-fermentative carbon sources. In *TRM1* gene disrupted strain, the transcriptional activity of several methanol-inducible promoters was examined. As a result, the drastic reduction of transcriptional activity was observed compared with wild-type strain when methanol or formaldehyde is used for induction substrate. In addition, Trm1 was localized in nucleus. From these results, it was suggested that Trm1 was a main transcriptional factor of C1 compounds-inducible gene expression.

Conclusion

Molecular basis of formaldehyde metabolism in microorganisms was investigated in this study. First, it was clearly indicated that the reverse reaction of formaldehyde fixation catalyzed by HPS and PHI play an indispensable role in pentose phosphate biosynthesis in archaea. The fact that the forward and reverse reactions have different physiological roles in bacteria and archaea is interesting from the viewpoint of evolution of metabolic pathways. Second, as for the molecular mechanism of regulation of gene expression in response to C1 compounds, I have obtained and characterized two DNA-binding proteins which activate C1-compunds-inducible gene expression from *B. subtilis* and *C. boidinii*, respectively. Further analyses are necessary to understand the molecular mechanism such as recognition of C1 substrates and signal transduction.

References

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