

Identification of the function of a putative sugar kinase from halophilic archaea

Takaaki SATO

Department of Synthetic Chemistry and Biological Chemistry, Graduate School of Engineering, Kyoto University

Research aims

According to the phylogenetic tree based on 16S rRNA sequences, organisms are classified into three domains, Eucarya, Bacteria and Archaea. Archaea possess unique metabolisms different from those of Eucarya and Bacteria. I have previously identified an archaeal pathway involved in AMP metabolism. Two novel enzymes, AMP phosphorylase and ribose-1,5-bisphosphate isomerase (R15Pi), and Rubisco, whose physiological function in Archaea has been unknown, constitute the pathway (Fig. 1)¹⁾.

Interestingly, 4 of 17 halophilic archaea whose genome analyses have been completed harbor only an R15Pi homolog (R15Pih) but not homologs of AMPpase and Rubisco. This raised the possibility that R15Pih catalyze an unknown reaction distinct from the general R15Pi reaction. Most of R15Pih form an operon with sugar kinase and uridine phosphorylase catalyzing reactions similar to those of the enzymes in the AMP metabolic pathway (Fig. 1). This suggests that these genes function cooperatively in an unknown metabolic pathway. In order to clarify the metabolic pathway composed of the three genes, I here aimed to identify the function of the sugar kinase.

Methods

Preparation of recombinant RbsK proteins

I carried out overexpression of the *rbsK* genes from halophilic archaea whose codons were changed suitable for expression in the bacterium *Escherichia coli*. The cells were then sonicated and the supernatant was subjected to an anion exchange column, Resource Q, and subsequently to a gel filtration column, Superdex 200.

Kinase activity measurement

Kinase activity was measured by determining the amounts of ADP (NDP) produced from ATP (NTP) in the kinase reaction using pyruvate kinase (PK) and lactate dehydrogenase (LDH). In this assay, the kinase, PK, and LDH reactions are coupled. As LDH consumes NADH, the decrease in absorbance at 340 nm deriving from the consumption of NADH was determined. As this method detects ADP (NDP) formation, and not the phosphorylated substrate, various sugars can be tested as substrate.

Results

Preparation of recombinant RbsK proteins

I tried preparing recombinant RbsK proteins from halophilic archaea that harbor both sugar kinase and R15Pih, *Halobacterium salinarum*, *Halorubrum lacusprofundi*, *Haloterrigena turkmenica*, and *Halopiger xanaduensis* (*Hs*-RbsK, *Hl*-RbsK, *Ht*-RbsK, and *Hx*-RbsK, respec-

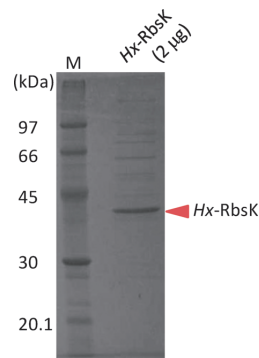


Fig. 2. Purified *Hx*-RbsK

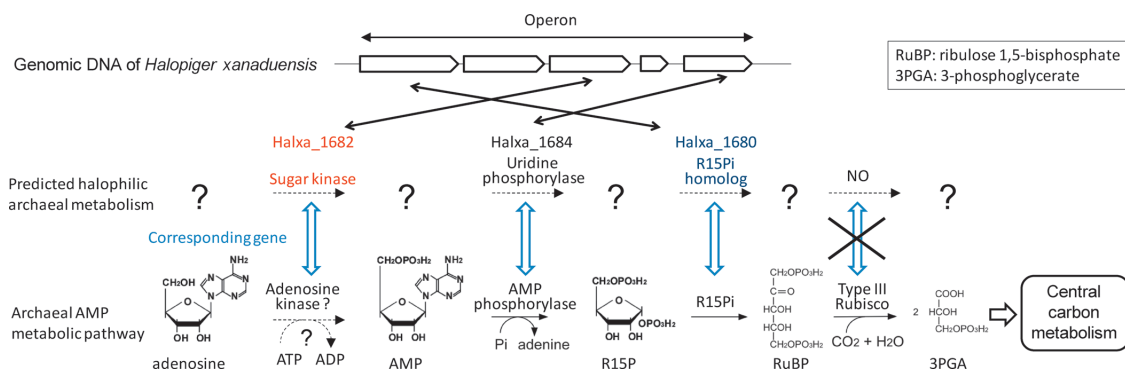


Fig. 1. Archaeal AMP metabolic pathway and predicted metabolism in haloarchaea

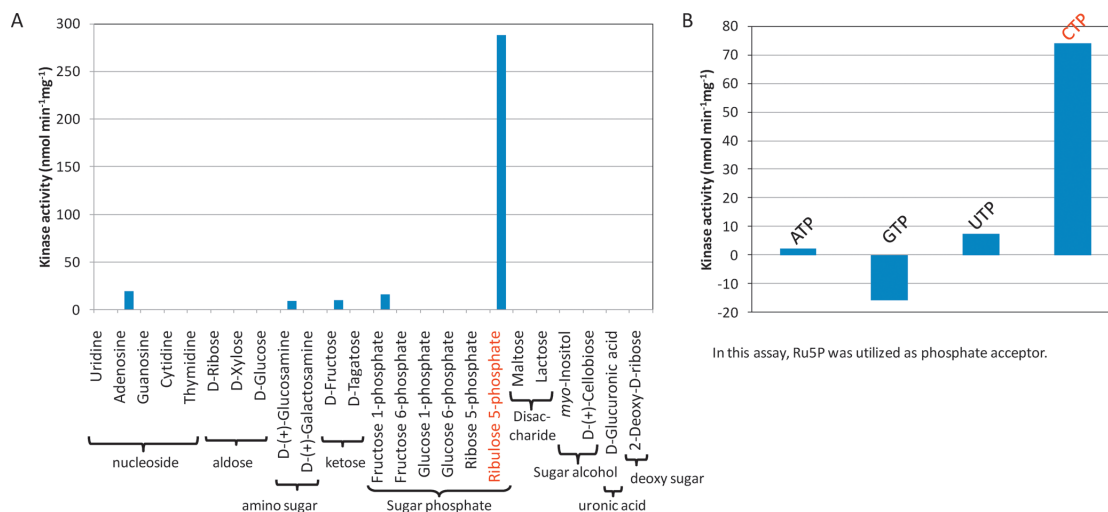


Fig. 3. Substrate specificity of *Hx*-RbsK (A). Phosphate donor specificity of *Hx*-RbsK (B)

tively).

As a result, *Hi*-RbsK and *Ht*-RbsK could not be expressed in *E. coli*. Although *Hs*-RbsK protein was detected in *E. coli*, the protein formed inclusion body. On the other hand, RbsK from *Halopiger xanaduensis* (*Hx*-RbsK) was successfully produced in *E. coli* in a soluble form after sonication of the cells. I then carried out the purification of the *Hx*-RbsK by anion exchange and gel filtration chromatography (Fig. 2).

Kinase activity measurement of *Hx*-RbsK

I investigated the substrate specificity of *Hx*-RbsK utilizing various sugars, applying monosaccharides, disaccharides, sugar phosphates, amino sugars, nucleosides as substrates. As the phosphate donor specificity of the protein was unclear, a mixture of ATP, CTP, UTP, and GTP was utilized as the phosphate donor. As a result, the protein exhibited a significant kinase activity toward ribulose 5-phos-

phate (Ru5P) (Fig. 3A). In addition, it turned out that the protein utilized only CTP as phosphate donor (Fig. 3B).

Conclusion

In this study, it is suggested that *Hx*-RbsK catalyzed phosphorylation of Ru5P. There is the possibility that product is ribulose 1,5-bisphosphate (RuBP), the substrate for Rubisco. It is interesting that halophilic archaea without Rubisco may produce the substrate for Rubisco, suggesting a novel pathway and/or enzymes. I plan to investigate whether the *Hx*-RbsK actually produces RuBP, and if so, how RuBP is metabolized.

Reference

- 1) Sato, T., Atomi, H., and Imanaka, T. (2007) Archaeal type III RuBisCOs function in a pathway for AMP metabolism. *Science* **315**: 1003–1006.