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Crystallographic analysis of novel sugar/sulfur metabolic enzymes from a thermoacidophilic archaeon

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Introduction

Sulfolobus tokodaii strain 7 is a strictly aerobic and thermoacidophilic archaeon isolated from Beppu hot spring, Kyushu, Japan, and grows optimally at pH 2–3 and 75°C–80°C on glucose as the sole carbon source. *Sulfolobus* is thought to use an Entner-Doudoroff-like glycolysis pathway in which the activation via phosphorylation occurs at a later stage in the pathway. However, the organism has been shown to also use another route to metabolize glucose, which involves an ATP-dependent phosphorylation. Moreover, *S. tokodaii* lives in sulfur-abundant area like volcanic hot spring, solfataras and hydrothermal vents. *S. tokodaii* is known to oxidize hydrogen sulfide to sulfate intracellularly, and this organism has several ORFs related to sulfur metabolism. Therefore, sugars and sulfur are important energy source for *S. tokodaii*, but little is known about the metabolic enzymes. We have found unique enzymes involved in the sugar/sulfur metabolism of this organism, and performed structural analysis of these enzymes.

Results and Discussion

Sugar metabolic enzymes

We have purified the ATP-dependent glucose phosphorylating activity from cell extracts of *S. tokodaii* and identified the gene responsible for the activity [1]. This enzyme, *S. tokodaii* hexokinase (StHK), is a novel hexokinase that can phosphorylate broad range of sugar substrates. After our experiments at PF, we could determine the crystal structures of StHK in four different forms: (i) apo-form, (ii) binary complex with glucose, (iii) binary complex with ADP, and (iv) quaternary complex with xylose, Mg^{2+}, and ADP (Fig. 1) [2]. The four different crystal structures of the same enzyme provide "snapshots" of the conformational changes during the catalytic cycle. Sugar binding induces a large conformational change, whereas ADP binding does not. Several unique loop regions are found in the StHK structure, and they are responsible for the wide substrate specificity. A Mg^{2+} ion and coordinating water molecules are well defined in the electron density of the quaternary complex structure. This structure represents the first direct visualization of the binding mode for magnesium to hexokinase and thus allows for a better understanding of the catalytic mechanism. We have also determined other unique enzymes involved in the sugar metabolism of *S. tokodaii*. Manuscripts are currently in preparation.

Sulfur metabolic enzymes

Sulfur oxygenase reductase (SOR), which catalyzes the simultaneous oxidation and reduction of elementral sulfur (S^0) in the presence of dioxygen, is a key enzyme in the sulfur metabolism of several archaea and bacteria, e.g. *Sulfolobus* and *Acidianus*. We have determined the crystal structure of *S. tokokdaii* SOR. It has a large hollow sphere formed by 24 monomers, and encloses a positively charged nano-compartment. During the course of our structure determination of SOR from *S. tokodaii*, the crystal structure of SOR from *Acidianus ambivalens* was reported [3]. These structures are essentially same, but some differences at the catalytic center are found. We have also determined the crystal structure of flavin-containing protein from *S. tokodaii* [4]

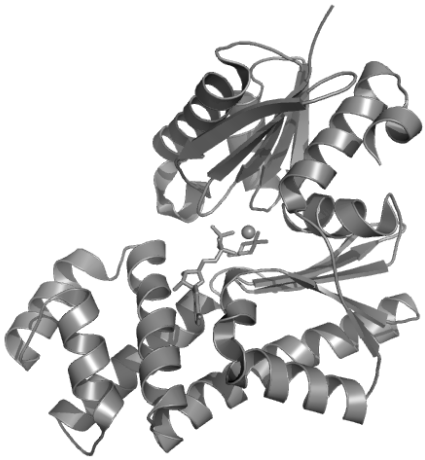

Figure 1 The crystal structure of StHK.

References

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