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Crystallographic analysis of a novel cupin-type phosphoglucose isomerase

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Introduction

The uniquely modified Embden-Meyerhof glycolytic pathway in certain hyperthermophilic archaea, such as *Thermococcus litoralis* and *Pyrococcus furiosus* [1], involves novel ADP-dependent glucokinases (ADPGKs), ADP-dependent phosphofructokinases (ADPPFKs), and cupin-type phosphoglucose isomerases (PGIs). ADPGKs and ADPPFKs exhibit significant sequence similarity and form a novel kinase family, which is named the PFKC family. As the first structure of the PFKC family, we have already solved the crystal structure of an ADPGK from *T. litoralis* (tlGK) complexed with ADP, and showed that it exhibits significant structural similarity with a certain ATP-dependent ribokinase family (PFKB family) [2]. Archaeal cupin-type PGIs are distantly related to PGIs in general organisms, but rather homologous to the cupin superfamily, and the iron is essential for the catalytic activity of PGI from *T. litoralis* (tlPGI) [3]. In this study, we aimed to solve the crystal structures of the unique enzymes involved in the modified Embden-Meyerhof pathway.

Results and Discussions

ADPGK

We solved the crystal structure of ADPGK from *P. furiosus* (pfGK) complexed with ADP and glucose [4]. The dataset up to 1.9 Å resolution was collected at BL6A. The structure was solved by molecular replacement method using tlGK as a search model, and refined to R = 16.8% and R_{free} = 20.5%. In comparison with the tlGK structure, the pfGK structure shows significant conformational changes in the small domain and region around the hinge, suggesting glucose-induced domain closing.

ADPPFK

We collected MIR datasets of the crystals of ADPPFK from *T. litoralis* (tlPFK) at BL18B etc [5]. Initial phase was calculated with four heavy-atom derivatives, such as K_2PtCl_4, $HAuCl_4$, PHMB, and PHMBS. Density modification and automated main-chain building were performed with the program RESOLVE, yielding a preliminary model comprising 305 amino-acid residues (Fig. 1). The overall structure of the model resembles those of ADPGKs. Characteristic β-sheets and α-helices in both domains can be seen clearly, but the main-chain trace around the hinge region remains ambiguous. The crystal structure of TLPFK seems to have open conformation, as observed for the apo structure of ADPGK from *P. horikoshii* OT3 [6]. Crystallographic refinement of tlPFK structure is currently under way.

Cupin-type archaeal PGI

The crystal structure of tlPGI was solved by Se-MAD method (Fig. 1). We collected datasets of tlPGI crystals complexed with G6P, F6P, and Fe, and refined the complex structures. However, during the course of this study, the crystal structures of another archaeal cupin-type PGI from *P. furiosus* (pfPGI) are reported [7, 8]. We are now investigating the structural differences between tlPGI and pfPGI, as well as a potential oxidative modification of the protein around the metal binding site of the active center present in tlPGI.

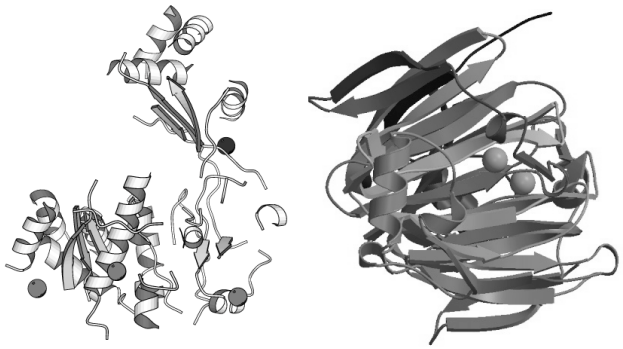

Figure 1 Ribbon diagrams of a preliminary structure of tlPFK (left) and the refined structure of tlPGI (right).

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