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Structure analysis of novel enzymes in CoA biosynthesis pathway in archaea

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1 Introduction

Coenzyme A (CoA) is an essential metabolic cofactor in cells from all three domains of life, bacteria, eukaryotes, and archaea. The CoA biosynthesis pathway in bacteria or eukaryotes includes 8 well characterized enzymes [1-3]. In contrast, in archaea, many enzymes of the CoA biosynthesis pathway remained unknown until recently. We have previously characterized the enzymes of the CoA biosynthesis pathway in the hyperthermophilic archaeon, *Thermococcus kodakarensis* (*T. kodakarensis*) [4-8].

Dephospho-CoA kinase (DPCK), which is utilized in all three domains of life and catalyzes the final reaction of CoA biosynthesis, converts dephospho-CoA to CoA. An archaeal DPCK could not be readily identified from the genomic sequence information and remained unknown. Recently, the protein encoded by TK1697 gene in *T. kodakarensis*, was subsequently characterized and identified as the DPCK in *T. kodakarensis* [9]. The *T. kodakarensis* DPCK (*Thermococcus kodakarensis* dephospho-CoA kinase, TkDPCK) consists of 177 amino acid residues and its calculated molecular weight is about 20,000 Da. From the result of gel filtration chromatography, it was suggested that TkDPCK is a monomer in solution [9]. Orthologs of TK1697 are present in most archaea, suggesting that the structural features and catalytic motifs are conserved throughout the archaeal domain of life.

Here we report the crystal structure of TkDPCK and its complex with GTP and a magnesium ion (TkDPCK-GTP-Mg) [10].

2 Experiment

Purified TkDPCK was concentrated to 10–12 mg/mL for the crystallization experiments. Crystals of TkDPCK with a bipyramidal shape were appeared from a mixture of protein solution and polyethylene glycol (PEG) solutions. The crystallization was also carried out for TkDPCK in the presence of GTP and magnesium (TkDPCK-GTP-Mg). The crystals of TkDPCK-GTP-Mg were obtained from the PEG solutions. All diffraction data were collected using synchrotron radiation and processed with the program XDS [11]. The crystal structure of TkDPCK-GTP-Mg structure was determined by the single anomalous dispersion (SAD) method using Au derivative data, with the protocol from the program SHARP/autoSHARP [12]. The initial structure was further rebuilt manually and refined using the programs COOT [13] and Refmac5 [14], with the TkDPCK-GTP-Mg data at 2.4 Å. The TkDPCK structure was solved by molecular replacement method at 2.15 Å resolution with the program Molrep [15] using the coordinates of TkDPCK-GTP-Mg.

3 Results and Discussion

The crystal structure of the TkDPCK monomer consists of continuous amino acid residues 3 to 174 and contains 7 α-helices (H1-H7) and 10 β-strands (S1-S10) (Fig. 1(a)). TkDPCK molecule adopts a Rossmann-like, 3-layer, α/β/α sandwich fold. In contrast to TkDPCK, the crystal structure of the TkDPCK-GTP-Mg monomer contained three large disordered regions at N-terminal region, C-terminal region, and the middle region (Fig. 1(b)). There is a large cleft on the surface of TkDPCK-GTP-Mg that is constructed by the helices 2, 3, and 6, the strands 4, 8, and 9, and residues in their close vicinity (Fig. 1(c)). In this cleft, GTP is located in an extended form.

The guanine ring of GTP in the TkDPCK-GTP-Mg crystal structure is held by four hydrogen bonds, those between amino groups at the N1 and N2 positions of the guanine ring and protein main chain carbonyl oxygen of Gly25, amino group of N2 position and protein main chain carbonyl oxygen of Asp147, and nitrogen at position 7 and hydroxyl oxygen atom of the side chain of residue Tyr31 (Fig. 2). A reason why ATP is not utilized as the phosphate donor by TkDPCK is considered that the absence of two interactions, GTP/N2-Gly25/CO and GTP/N2-Asp147/CO (red dashed lines in Fig. 2), in adenine might render ATP binding unstable, at least if bound in the same location as GTP.

Among the highly conserved residues in archaea DPCKs, both Asp48 and Asp67 side chains are located at suitable positions to activate GTP for nucleophilic attack on the γ-phosphate by deprotonation of the hydroxyl group of the substrate (Fig. 3). Each mutant in which these residues were replaced by alanine rendered *T. kodakarensis* auxotrophic for CoA [9]. Therefore, it is considered that one of these residues is likely to play a critical role in deprotonation, and the other or both would be involved in maintaining the phosphate moiety of GTP and the substrate in their proper positions. We consider that Asp48 might be the catalytic residue because the specific activity was completely lost in the D48A mutant protein whereas a low level of activity was still remained in the D67A mutant protein [10].

The possible modes of dephospho-CoA binding by TkDPCK can be considered. The adenine ring of dephospho-CoA could be accommodated in the cleft of TkDPCK-GTP-Mg, through π-π stacking interactions between the adenine ring and the side chain of Tyr143 (the ellipse in Fig. 3). Tyr143 is highly conserved in archaeal

DPCKs, and the Y143A mutant showed a decrease in activity [10]. The elucidation of the binding mode of dephospho-CoA is essential for understanding the TkDPCK reaction mechanism.

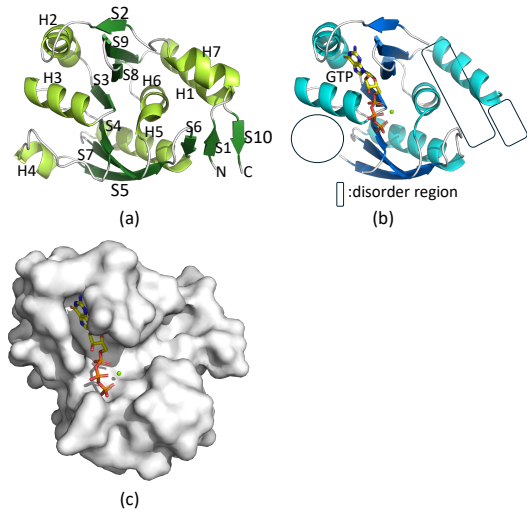

Fig. 1: Overall structure of TkDPCK.
(a) TkDPCK, (b) TkDPCK-GTP-Mg,
(c) molecular surface of TkDPCK-GTP-Mg.

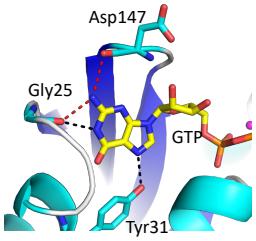

Fig. 2: Interaction between guanin ring of GTP and TkDPCK protein.

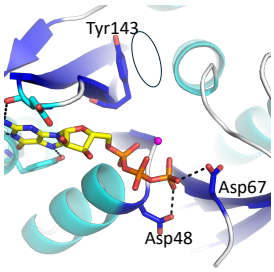

Fig. 3: Two important aspartic acids and the phosphate moiety of GTP.
The residue Tyr143 is also drawn (see text).

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