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Crystal Structure of Phosphopantothenate Synthetase from *Thermococcus kodakarensis*

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1 Introduction

Coenzyme A (CoA) and its derivatives are essential coenzymes that play important roles in various metabolic pathways. Bacteria and eukaryotes utilize a common enzymatic pathway for CoA biosynthesis, that involves two enzymes, pantothenate synthetase (PS) and pantothenate kinase (PanK), to convert pantoate to 4'-phosphopantothenate, and this pathway has been studied for many years [1]. However, these two enzymes, PS and PanK, are absent in most of archaea. Recently, it was found that two novel enzymes, pantoate kinase (PoK) and phosphopantothenate synthetase (PPS), are responsible for this conversion in archaea [2,3].

In this study, we report the crystal structures of PPS from *Thermococcus kodakarensis* and its complexes with ATP and phosphopantoyl adenylate (PPA), a reaction intermediate [4]. Based on these structures, the mechanism of the reaction catalysed by PPS including the generation of a reaction intermediate is discussed.

2 Experiment

Expression and purification of PPS were reported previously [2,3]. Initial crystallization screenings were carried out by the sitting-drop vapor diffusion method, and the best crystals were obtained from PEG3,350 solutions containing ammonium acetate. The crystals of PPS belong to the space group of $P2_1$ with the cell dimensions of a=79.8Å, b=80.8Å, c=84.2Å, and β=110.1°. For co-crystallization with ATP and $MgCl_2$, a purified sample was incubated for several hours at 277 K in the presence of 10 mM ATP and 10 mM $MgCl_2$. For co-crystallization with ATP, $MgCl_2$, and 4-phosphopantoate (PPo, synthesized as described previously [3]), the sample was incubated at 277 K in the presence of 5 mM ATP, 5 mM $MgCl_2$, and 5 mM PPo. The former crystals (PPS/ATP/Mg) belonging to the space group of $P2_1$ with the unit cell of a=79.7Å, b=80.1Å, c=84.0Å, and β=110.4°, were obtained from PEG10,000, and the latter (PPS/PPA) crystals belonging to the space group of $P4_12_12$ with the cell of a=b=123.6Å, c=188.0Å, were obtained from tacsimate solutions, respectively.

The native PPS structure was determined as a complex with adenosine (PPS/adenosine) by the MIR method using derivatives of K_2IrCl_6, $(NH_4)_3IrCl_6 \cdot xH_2O$, $Na_3IrCl_6 \cdot xH_2O$, K_2PtCl_4, $Sm(NO_3)_3 \cdot 6H_2O$, and $OsCl_3 \cdot 3H_2O$. Data processing, phase determination, automated chain tracing, structure refinement were carried out using software programs of HKL2000 [5], SOLVE/RESOLVE [6, 7], BUCCANEER[8], and CNS [9]. The crystal structure of PPS/adenosine was determined at 2.0 Å resolution. Crystal structures PPS/ATP/Mg and PPS/PPA were refined at 2.4 Å and 2.3 Å resolution, respectively.

3 Results and Discussion

PPS forms a homodimer. The overall structure of PPS from *T. kodakarensis* monomer (261 aa, 30 kDa) consists of three domains: the N-terminal domain (residues 1-47), the core domain (residues 48-216), and the C-terminal domain (residues 217-261) (Figure 1). The N-terminal domain (forest green and blue in Figure 1) contains two α helices, and the core domain (green and cyan) contains an α/β domain containing 8 α–helices and 5 β–strands. The C-terminal domain (light green and light cyan) following the core domain contains two helices which are located at the top of the core domains. The adenosine derivatives were found at the cavity surrounded by the N-terminal and core domains at the C-terminal side of the β sheet.

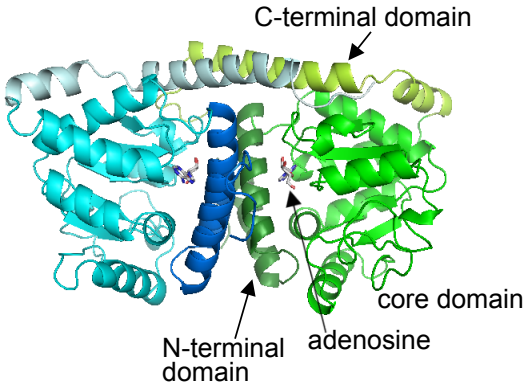

Fig. 1: Crystal structure of PPS dimer (front view).

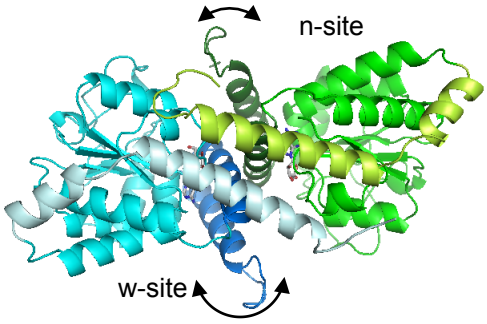

Fig. 2: Crystal structure of PPS dimer (top view).

Two monomers composing dimers are deviated from the exact 2-fold symmetry, with varied rotation degrees from 4° to 13° which is calculated with the main chains

of residues 10-250 (Figure 2). The hinge region of the PPS dimer was detected in N-terminal domain. The residues composing the hinge region and the core domain are well conserved. Therefore, the PPS molecules in other archaea may form asymmetric dimers in a manner similar to that of PPS in *T. kodakarensis*. The structure of PPS from *T. kodakarensis* is very similar to that from *Thermococcus onnurineus,* although the detail structural comparison is unable because PDB coordinates are currently unreleased [10].

Since two monomers in the PPS dimer molecule are deviated from the exact 2-fold symmetry, one cavity from the molecular surface to the nucleotide binding site is relatively wide open (we designated this cavity the "w-site"), whereas the other is narrow (the "n-site") (Figure 2). In the crystal of PPS/ATP/Mg, ATP and the Mg^{2+} ion are found in the n-site, while only ATP is found in the w-site. Both ATP molecules in the PPS active sites are accommodated in the same bent conformation, in which the γ-phosphate group locates at the N3 atom's side of the purine plane of ATP (Figure 3).

In all of the active sites of the PPS/PPA co-crystals, the electron density of PPA, which is considered to be a reaction intermediate, was observed (Figure 4). When the ATP molecule in the PPS/ATP/Mg complex and PPA were superimposed, the PPo moiety of PPA occupied a site opposite the triphosphate group of ATP. This finding indicates that the carbonyl oxygen atom of PPo makes an in-line nucleophilic attack on the α-phosphorus atom of ATP.

When two monomers in PPS/ATP/Mg dimer are superimposed, the conformation of side chains of Arg17 and Tyr45 are altered. Moreover, the relative positions of Arg17 and Tyr45 in one monomer to the n-site are different from those of Arg17' and Tyr45' in the other monomer to the w-site due to the structural asymmetry of the PPS dimer (Figure 3). As a consequence, two nucleotide binding sites (the n- and w-sites) of the PPS homodimer are not equivalent. The enzyme activity of three variant proteins, R17A, Y45A, and Y45F decreased remarkably compared to the wild-type protein. These results indicate that the residues Arg17 and Tyr45 play a crucial role in enzyme activity, and asymmetric dimer structure might be essential for PPS activity. Based on these structures, we propose that the PPS reaction mechanism which generates the reaction intermediate PPA is as follows. In the presence of ATP and the Mg^{2+} ion, both ATP and the Mg^{2+} ion bind only to one site (n-site). When the carboxyl oxygen atom of PPo makes a nucleophilic attack on the α-phosphorus atom of ATP, the reaction intermediate PPA is formed in n-site, followed by the release of the Mg^{2+} ion and diphosphate derived from ATP. At this point, one PPA is bound to one of the two active sites (n-site). Thereafter, the n- and w-sites are expected to switch their roles by structural fluctuation. In a newly formed n-site that was generated from a former w-site, ATP and the Mg^{2+} ion bind to the protein. When PPo is bound to the newly formed n-site, the second PPA is formed in the same way. Consequently, both active sites possess PPA as seen in the PPS/PPA crystal (Figure 4). Afterwards, PPA is expected to react with β-alanine to generate 4'-phosphopantothenate. Because the amino acid residues involved in binding to the PPo moiety are completely or highly conserved, the condensation reaction of ATP and PPo in archaeal PPS most likely proceeds in the same manner as in the PPS from *T. kodakarensis*.

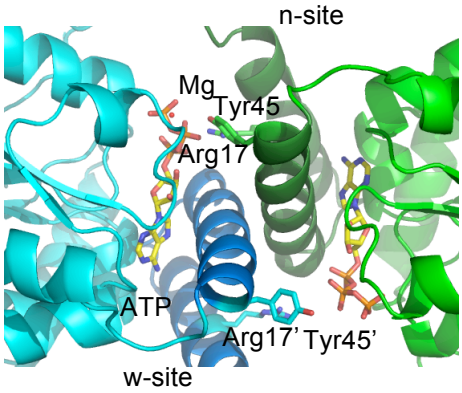

Fig. 3: Crystal structure of PPS/ATP/Mg complex. (Top view, C-terminal domains were omitted).

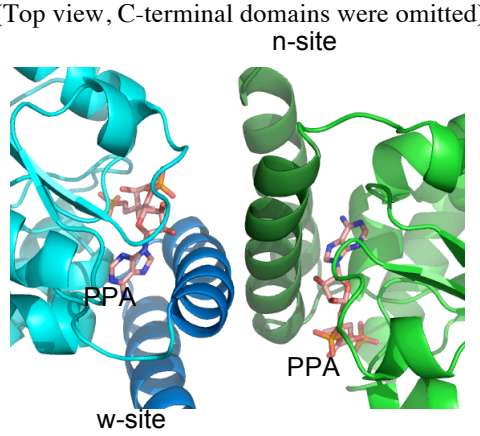

Fig. 4: Crystal structure of PPS/PPA complex (Top view, C-terminal domains were omitted).

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