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Crystallographic study of novel enzymes necessary for CoA biosynthesis in archaea

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1 Introduction

Coenzyme A (CoA) plays pivotal roles in a variety of metabolic pathways in all organisms. The biosynthesis pathway of CoA has been studied well in bacteria and eukaryotes. In this pathway, two enzymes which convert pantoate to 4'-phosphopantothenate are involved. These two enzymes are pantothenate synthetase (PS), which catalyzes the ATP-dependent condensation of pantoate and β-alanine to form pantothenate, and pantothenate kinase (PanK), which catalyzes the phosphorylation of pantothenate. The CoA biosynthesis pathway is regulated by feedback inhibition by CoA to PanK.

These two enzymes, PS and PanK are absent in almost all archaea. Instead, it was reported that two peculiar enzymes of archaea, pantoate kinase (PoK) and phosphopantothenate synthetase (PPS), are responsible for these conversion [1]. PoK catalyzes the ATP mediated phosphorylation of pantoate to form 4'-phosphopantoate. On the other hand, PPS catalyzes the ATP-dependent condensation of 4'-phosphopantoate and β-alanine. In the archaea PoK/PPS pathway, the order of the condensation reaction with β-alanine and the phosphorylation reaction are reversed compared to the PS/PanK reactions in bacteria/eukaryotes. In archaea, CoA biosynthesis feedback inhibition is not regulated by CoA binding to PoK or PPS, but to ketopantoate reductase (KPR) [2]. We have previously reported the crystal structure of PPS and its reaction mechanisms [3]. We report here the crystallographic studies of PoK and KPR from *Thermococcus kodakarensis*.

2 Experimental

Crystals of PoK belonging to the space group of *P*321 with the cell dimensions of *a*=94.8Å, *c*=60.0Å were obtained from PEG8,000 solutions. X-ray diffraction data were collected at the AR-NW12A beamline of the Photon Factory.

Crystals of the ternary complex of KPR from *T. kodakarensis* (Tk-KPR), CoA, and 2-oxopantoate were obtained from 2-methyl-2,4-pentanediol solution. The crystals belong to the space group $P2_12_12_1$ with the cell dimensions of a=53.5Å, b=70.8Å, and c=163.9Å. The crystallization was also performed in the presence of NADH to investigate the effect of crystal packing. Crystals belonging to the different space group, *P*1, with the cell dimensions of *a*=37.6Å, *b*=45.0Å, *c*=183.1Å, α=84.9°, β=87.8°, γ=65.2°, were obtained (P1-KPR) from PEG3,350 solutions. X-ray diffraction data were collected at the BL-1A, ARNE3A, and ARNW12A beamlines of the Photon Factory, and were processed with the HKL2000 package.

3 Results and Discussion

The crystals of PoK using glycerol as a cryoprotectant diffracted up to 2.7Å resolution. The asymmetric unit contained one PoK monomer. The crystal structure analysis is in progress.

The crystal structure of Tk-KPR was solved [4] by the molecular replacement method using the coordinates of *E. coli* KPR (2OFP.pdb) [5], and that of P1-KPR was solved using Tk-KPR coordinates [6]. The R/R_{free} values for Tk-KPR are 0.169/0.185 at 1.65Å resolution, and those of P1-KPR are 0.222/0.260 at 2.3Å resolution. In each Tk-KPR and P1-KPR crystal, protein molecules exist as a dimer (Fig. 1) in the same dimerization manner. This result suggests that the dimer structure is not an artifact of crystallization. The dimerization is also consistent with the result of size-exclusion chromatography. In Tk-KPR, $NADP^+$, which presumably derives from *E. coli* cells used as expression host, is bound to one of the two monomers (referred to as monomer 1 in Fig. 1), whereas CoA and 2-oxopantoate are bound to the other (referred to as monomer 2 in Fig. 1).

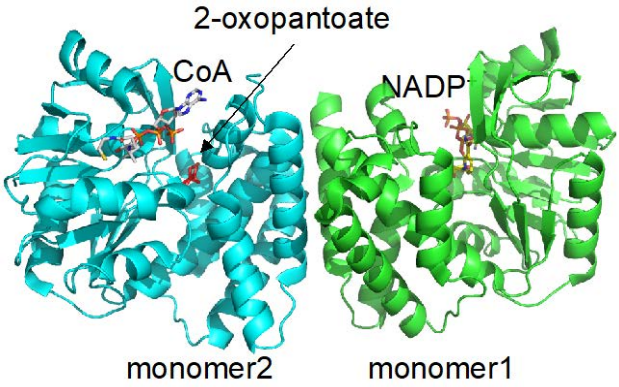

Fig. 1: Tk-KPR dimer structure.

When we superimposed two monomers in Tk-KPR crystal, the binding site of CoA is overlapped with that of $NADP^+$. This result may indicate that the activity inhibition by CoA is competitive with NADH. Notably, monomer 2 adopts a relatively closed conformation compared to monomer 1. These results suggest that CoA and 2-oxopantoate cooperatively trigger a conformational change from an open form to a closed form.

Comparison of two Tk-KPR monomers with *E. coli* KPR indicates that monomer 1 of Tk-KPR represents a

resting state as previously reported for $NADP^+$-bound *E. coli* KPR, and monomer 2 in its inhibited state is similar to products-bound *E. coli* KPR that represents an active state [5,7]. In *E. coli* KPR, the binding of pantoate is a key to adopt a closed form. Accordingly, comparisons of Tk-KPR and *E. coli* KPR also support the idea that 2-oxopantoate, located at the same position, is essential to adopt a closed form for Tk-KPR. Comparison between Tk-KPR and *E. coli* KPR also shows that the residues involved in CoA recognition in Tk-KPR are also involved in NAD(P)H recognition and highly conserved in archaea, whereas some are not conserved in *E. coli* KPR. Thus, the affinity of CoA for *E. coli* KPR would be lower than that for Tk-KPR, implying that *E. coli* KPR may not be inhibited by CoA. Significantly, Cys84, which is conserved among several *Thermococcus* strains but not among bacteria or eukaryotes, forms a disulfide bond with CoA (Fig. 2). This disulfide bond prevents dissociation of CoA from the active site, and enables strict inhibition of Tk-KPR activity upon binding of CoA.

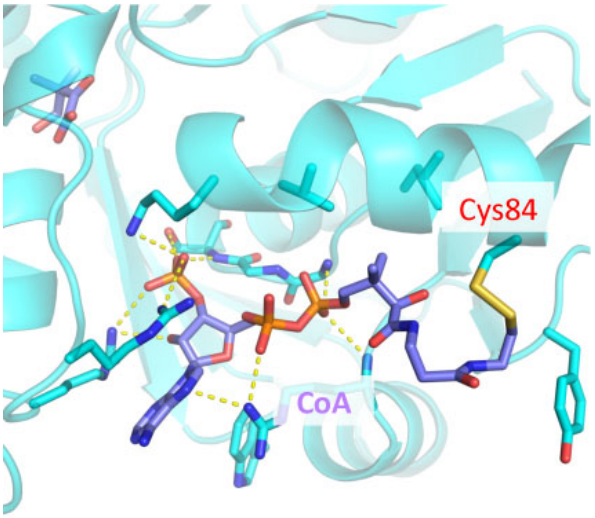

Fig. 2: Disulfide bond with CoA.

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