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Crystallographic analysis of the proteins involved in regulation of CoA metabolism

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1 Introduction

Coenzyme A (CoA) is an important coenzyme which consists of adenosine diphosphate, pantothenate, and 2-thioaminoethane moieties. CoA forms a thioester-bond between its thiol-group and acyl-group of various compounds and acts as the acyl-carrier in the metabolic pathways, such as TCA cycle, β-oxidation, biosynthesis and degradation of fatty acids and amino acids.

In addition to the role in the metabolic pathway, recently CoA derivatives, such as acetyl-CoA and succinyl-CoA, are shown to be involved in the post-translational modification of proteins like acetylation and succinylation. The comprehensive analysis using LC-MS/MS discovered that a lot of proteins are acetylated even in bacteria. Interestingly, the proteins involved in metabolism are reported to be often acetylated, suggesting that the protein acetylation has some roles in metabolic regulation. We have identified over 200 proteins acetylated in a thermophilic bacterium, *Thermus thermophilus* HB27 [1].

In this study, we focused on the metabolic regulation in the cells based on the acyl-CoA, which are the key metabolites, and conducted the crystallographic analyses to reveal the structural basis of the regulatory mechanism. We have worked on several subjects involved in the regulatory mechanisms. Here, we describe about CoA transferase (CoAT), which catalyzes the transfer of CoA moiety of acyl-CoA to short-chain fatty acids, and acyl-CoA synthetases (ACSs), catalyzing the ATP-dependent acyl-CoA production from CoA and short-chain fatty acids, from *T. thermophilus* HB27.

2 Experiment

Purification of recombinant proteins

Since it was revealed that CoAT interacts with a regulatory protein in *T. thermophilus*, recombinant proteins of CoAT and the regulatory protein were prepared by the expression system in *Escherichia coli*. Two proteins with or without affinity tag were overexpressed in *E. coli* BL21(DE3) and the expression was induced by the addition of 1 mM of isopropyl-β-D-thiogalactopyranoside (IPTG). The cells were disrupted and the proteins were co-purified or separately purified with heat treatment, affinity-chromatography or anion-exchange chromatography, and gel-filtration chromatography.

T. thermophilus has four homologous genes encoding ACS. We prepared recombinant protein of one ACS homolog with affinity tag. ACS was overexpressed in *E. coli* BL21-codon plus-(DE3) RIL or Rosetta-gami (DE3) and the expression was induced by the addition of 0.1 mM IPTG. The tagged ACS was purified by heat treatment, affinity chromatography, and subsequent gel filtration chromatography.

Crystallization

CoAT and its regulatory protein were concentrated and used for crystallization screening at 10 mg/ml, respectively. CoAT co-purified with the regulatory protein was also used for crystallization screening. Crystallization drops were composed of each protein, its substrate (analogs), CoA and butyrate, and its cofactors. Purified protein of ACS was used at 5 mg/ml for crystallization screening and its crystallization drops were composed of each protein, CoA, ATP and acetate. Screening of crystallization condition using Crystal Screen I and II, PEG-ION screen (Hampton Research), Wizard classic I, II, III, and IV, and Wizard Precipitant synergy (Rigaku regents) was carried out with hanging drop vapor diffusion method. Optimization of the crystallization condition was also conducted by modifying the concentration of precipitant and pH of buffer used.

3 Results and Discussion

X-ray diffraction analyses of CoAT

There were several conditions in which CoAT or its regulatory protein were crystallized. Several diffraction datasets were obtained, and we could determine the structure of CoAT at 2.6 Å. Since the regulatory protein binds co-factors, we could determine the structures in apo form and in co-factor-binding form at 2.2 Å and 2.1 Å resolution, respectively, by using molecular replacement method. However, we could not obtain the crystals of complex of CoAT and its regulatory protein. Thus, further screening of the crystallization condition or using homologous protein from other species will be necessary to obtain the crystals of the complex.

X-ray diffraction analyses of ACS

There were several conditions in which ACS was crystallized with or without substrates. We could determine the structure of ACS with a reaction intermediate by molecular replacement method. However, the determined structure lacks one domain composing this protein, thus we need to find out the different crystallization conditions.

References

[1] Yoshida A., *et al. Extremophiles*. **23**, 377, (2019).

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