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Crystallographic analysis of the enzymes involved in regulation of CoA metabolism

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1 Introduction

Coenzyme A (CoA) is a very 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 of fatty acids and amino acids, degradation of amino acids.
In addition to the role in the metabolic pathway, CoA derivatives, such as acetyl-CoA and succinyl-CoA, are shown to be involved in the acyl modification of proteins in recent years. The comprehensive analysis using LC-MS/MS discovered that a lot of proteins are acylated (for example, acetylated and succinylated) even in bacteria. Interestingly, the proteins involved in metabolism are reported to be often acylated, suggesting that the protein acylation has some roles in metabolic regulation. We have identified over 200 proteins acetylated in thermophilic bacterium, *Thermus thermophilus* HB27. One of the identified acetylated proteins, 2-isopropylmalate synthase (IPMS), which catalyzes the first step of leucine biosynthesis using acetyl-CoA as a substrate, was found to be negatively regulated by protein acetylation [1].

In this study, we focused on the metabolic regulation in the cells based on the CoA derivatives, 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 IPMS, which is regulated by protein acetylation, and CoA transferase (CoAT), which catalyzes the transfer of CoA moiety among two acyl-CoAs, from *T. thermophilus* HB27.

2 Experiment

Purification of recombinant proteins

IPMS with affinity tag was overexpressed in *Escherichia coli* BL21-codon plus-(DE3) RIL as a host. The expression was induced by the addition of 0.1 mM Isopropyl-β-D-thiogalactopyranoside (IPTG) for induction. IPMS was dissolved in buffer A (20 mM Tris-HCl pH8.0, 150 mM NaCl) and purified with affinity-chromatography and the subsequent gel filtration chromatography using Superdex 200. CoAT with or without affinity tag was also overexpressed in *E. coli* BL21-codon plus-(DE3) RIL and the expression was induced by the addition of 1 mM IPTG. The tagged CoAT was purified in the same way with IPMS. The non-tagged CoAT was purified by in combination with hydrophobic interaction chromatography, anion exchange chromatography, and gel filtration chromatography.

Purification of IPMS from T. thermophilus

IPMS was also prepared from *T. thermophilus*. We constructed over-expressing strain of strep-tagged IPMS in *T. thermophilus* by using the constitutively expressing promoter. The protein was purified with Strep-tactin column, and Superdex 200.

Crystallization

IPMS purified from *E. coli* and *T. thermophilus* were concentrated and used for crystallization screening at 5-10 mg/ml, respectively. Crystallization drops were composed of each protein and its substrates or an inhibitor, leucine. For CoAT, crystallization drops were composed of each protein, CoA, and butyrate. Screening of crystallization condition using Crystal Screen I and II, PEG-ION screen (Hampton Research), Wizard classic I, II, and III, 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 and pH.

3 Results and Discussion

X-ray diffraction analyses of IPMS

There was one condition from Crystal screen I in which IPMS was crystallized in the presence of leucine. By optimization of this condition, we succeeded in obtaining larger crystals. However, only 3 Å resolution diffraction data from this crystal was obtained and we cannot collect the diffraction data. Optimizations of the crystallization condition and cryo buffer will be necessary to obtain diffraction data sets.

X-ray diffraction analyses of CoAT

There were several conditions in which CoAT was crystallized with or without substrates. The diffraction pattern for one of the crystals showed the crystal was belonged to the space group of primitive orthorhombic. Although one data set was obtained at 2.7 Å resolution, it was estimated as twinned. Further screening of crystallization conditions will be needed.

References

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