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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. CoA can constitute the thioester-bond between its thiol-group and acyl-group of various compounds to be used in the metabolic pathways, such as TCA cycle, β-oxidation, biosynthesis of fatty acids and amino acids, degradation of amino acids, and so on.

In addition to the role in the metabolic pathway, it is revealed that CoA derivatives, such as acetyl-CoA and succinyl-CoA, are involved in the acyl modification of proteins. 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 role in metabolic control via regulation of metabolic enzymes. Therefore, the importance of CoA metabolism is increasing recently.

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 mainly two subjects; 2-isopropylmalate synthase (IPMS) from *Thermus thermophilus* HB27, which catalyzes the first step of leucine biosynthesis using acetyl-CoA as a substrate, and CoA transferase (CoAT), which is acetylated in *T. thermophilus* HB27.

2 Materials and Methods

Purification of recombinant proteins

We constructed the over-expression system of IPMS with Strep-tag in *T. thermophilus* HB27 and IPMS was purified with Strep-tactin affinity chromatography and the subsequent gel filtration chromatography using Superdex 200.

CoAT was overexpressed in a His-tagged form using *Escherichia coli* BL21-Codon-Plus (DE3) RIL as a host. The expression was induced by the addition of 0.1 mM isopropyl-β-D-1-thiogalactopyranoside (IPTG). Since CoAT was found to compose the protein complex with a putative amino acid dehydrogenase (ADH), ADH was also overexpressed in the Strep-tagged form in *E. coli* BL21-Codon-Plus (DE3) RIL. Each protein was purified with Ni^{2+}-NTA column or strep-tactin column followed by the gel filtration chromatography using Superdex200. To obtain the protein complex of CoAT and ADH, the cell lysates from each *E. coli* cell were mixed and incubated at 70 ºC for 30 min to promote the complex formation. The supernatant separated by centrifugation was then applied to Strep-tactin column and Superdex 200.

In addition, protein acetyltransferase (PAT) which can acetylate CoAT was also overexpressed and purified in His-tagged form in the same way.

Crystallization

Each purified protein is concentrated and used for crystallization screening at 5-10 mg/ml. Crystallization drops were composed of each protein and its (possible) substrates or inhibitors. Screening of crystallization condition using Crystal Screen I and II, PEG-ION screen (Hampton Research), and Wizard classic I, II, and III (Rigaku regents) was carried out with hanging drop vapor diffusion method. Optimization of the crystallization condition was also conducted by modifying the concentration of each solution and pH.

3 Results and Discussion

Crystallization of IPMS

There was one condition from Crystal Screen I in which IPMS was crystallized in the presence of leucine, the feedback-inhibitor of IPMS. Although the optimization of this condition let the crystals bigger, it diffracted at only 3.0 Å resolution. Because of the poor quality of the diffraction, we have not collected the diffraction data so far. Further examination of the crystallization condition will be necessary to obtain diffraction data.

X-ray diffraction analyses of PAT and CoAT·ADH complex

We found several crystallization conditions for PAT in the presence of CoA by the screening, but we could not collect the diffraction data because of the poor diffraction (upto 7.0 Å).

Several conditions in which CoAT·ADH complex was crystallized were found. We could obtain three diffraction data from different crystals at 2.35-2.6 Å resolution. One of the data was turned out to be a “perfect twinned”. The cell content analyses for the other two data revealed that one crystal contains only one molecule (CoAT or ADH) and the other has two molecules. The molecular replacement using homologous structures as search models was failed. Now further screening and optimization of crystallization condition is performed.

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