Crystallographic analysis of the proteins involved in the 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. It is well-known that acetyl-CoA synthetase (ACS), which produce acetyl-CoA in an ATP-dependent manner, is regulated by acetylation in a feedback manner. We have identified over 200 proteins acetylated in a thermophilic bacterium, Thermus thermophilus HB27, and revealed that an enzyme involved in the leucine biosynthesis is regulated by the protein acetylation [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 the regulation of ACS and CoA transferase (CoAT), which was found as highly acetylated protein in T. thermophilus. Here, we describe about CoAT, catalyzing the transfer of CoA moiety of acyl-CoA to 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.

In addition, to maintain the native status of the protein modification in CoAT, we prepared the complex of CoAT and the regulatory protein using T. thermophilus strain overproducing these proteins.

Crystallization

CoAT co-purified with the regulatory protein and CoAT itself were used for crystallization screening. Crystallization drops were composed of each protein, its substrate (analogs), CoA and butyrate, and its cofactors. 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

So far, we could determine the crystal structures of CoAT at 2.6 Å but it does not contain any substrates. Therefore, we tried co-crystallization with its substrates and crystal soaking before flash freezing with liquid N2. However, we could not obtain the good diffraction from the crystals. We suppose that it is necessary to find other crystallization conditions or to crystallize homologous proteins from other species.

X-ray diffraction analyses of the complex of CoAT and the regulatory protein

We have determined the structure of the regulatory protein in the presence/absence of its ligand, but the crystals from the screening using the mixture of CoAT and the regulatory protein always contains only the regulatory protein. Since the size of the complex is over 400 kDa, we now try to determine the structure by CryoEM. Preliminary analysis shows that those prepared as the complex of two proteins contain a mixture of different ratios of CoAT and the regulatory protein, which we believe made crystallization difficult.

References


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