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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 is consisted 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 role in metabolic control via regulation of metabolic enzymes. Therefore, the importance of CoA metabolism is increasing.

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. Of which, here, we describe about homocitrate synthase (HCS), which catalyzes the first step of lysine biosynthesis. This reaction is aldol-type condensation of 2-oxoglutarate (2-OG) with acetyl-CoA to yield homocitrate. Previously analyzed bacterial HCS consists of TIM barrel domain, subdomain I, and subdomain II and is inhibited by lysine binding at 2-OG binding site. On the other hand, archaeal HCS has a regulatory domain in addition to these three domains. We hypothesized the regulatory domain was essential to regulate the activity of HCS and performed crystallization of HCS. Here, we describe the recent progress of this project.

2 Materials and Methods

Purification of recombinant proteins

HCS 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 α-isopropyl-β-D-thiogalactopyranoside (IPTG) for induction. HCS was dissolved in buffer A (20 mM Tris-HCl pH8.0, 150 mM NaCl) and purified with chromatography and the subsequent gel filtration chromatography using Superdex 200.

Crystallization

HCS was concentrated and used for crystallization screening at 5-10 mg/ml. Crystallization drops were composed of each protein and its (possible) substrates or an inhibitor, lysine. 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 of and pH.

3 Results and Discussion

X-ray diffraction analyses of HCS

There was one condition from Precipitant synergy in which HCS was crystallized in the presence of 2-OG and acetyl-CoA. By optimization of this condition we succeeded in obtaining better shape of crystals. However, only 6 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.

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