BL-5A, BL-17A, AR-NW12A, AR-NE3A /2015G546

Crystallographic analysis of the enzymes involved in regulation of CoA metabolism

Tomohiro SUZUKI, Ayako YOSHIDA, and Takeo TOMITA*

Biotechnology Research Center, The Univ. of Tokyo, Yayoi, Bunkyo-ku, Tokyo 113-8657, Japan

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 mainly two subjects; homocitrate synthase (HCS) from *Sulfolobus acidocaldarius* MW001, which catalyzes the first step of lysine biosynthesis using acetyl-CoA as a substrate, and CoA transferase (CoAT), which is acetylated in *T. thermophilus* HB27. Here, we describe the recent progress of these projects.

2 Materials and Methods

Catalytic domain of HCS

HCS from *Sulfolobus acidocaldarius* is composed of three domains: The N-terminal catalytic domain, the C-terminal regulatory domain (RAM domain), and the subdomain I, and II connecting those two domains. We designed expression system of the truncate body lacking the C-terminal regulatory domain (HCS-cat).

Purification of recombinant proteins

HCS-cat with His-tag was overexpressed in *Escherichia coli* BL21 (DE3) RIL as a host using 0.1 mM α-isopropyl-β-D-thiogalactopyranoside (IPTG) for induction. HCS was purified with Ni^{2+}-NTA affinity chromatography and the subsequent gel filtration chromatography using Superdex 200.

Crystallization

HCS-cat was concentrated and used for crystallization screening at 4 mg/ml. Crystallization drops were composed of each protein and its (possible) substrates. 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.

3 Results and Discussion

X-ray diffraction analyses of HCS-cat

There was one condition from Crystal Screen I in which HCS-cat was crystallized in the presence of 2-oxogultarate and acetyl-CoA. We obtained a diffraction data set from this crystal at 1.98 Å resolution. We are now trying to determine the crystal structure by molecular replacement method.

* uttomi@mail.ecc.u-tokyo.ac.jp