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Crystal structure analysis of NADPH-dependent acetoacetyl-CoA reductase

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1 Introduction

Nicotinamide adenine dinucleotide phosphate (NADPH)-dependent acetoacetyl-CoA (AcAcCoA) reductase (PhaB) stereoselectively reduces 3-ketone group of acetoacetyl-CoA to synthesize (*R*)-3-hydroxybutyryl(3HB)-CoA, which is known as a monomer precursor of microbial polyester polyhydroxyalkanoate (PHA). The PhaB-encoding gene was found in many bacteria including Ralstonia eutropha (also designated as Cupriavidus necator), and typically located in the phb operon together with β-ketothiolase (PhaA) and PHA synthase (PhaC). The three enzymes catalyze successive reactions synthesizing P(3HB) from acetyl-CoA. This pathway has been extensively utilized for the microbial production of P(3HB) and 3HB-based copolymers, which can be used as a biobased plastic. To use 3HB-copolymer industrially, it is important to improve the enzymatic activity. Structure based design is one of the major strategies to engineer the enzyme activity. However, the crystal structure of PhaB had not been determined, which has hampered industrial application of this enzyme.

In this study, crystal structure of PhaB from *Ralstonia eutropha* was determined in apo form and ternary complex with $NADP^+$ and acetoacetyl-CoA (AcAc-CoA) in order to understand reaction mechanism.

2 Experiment

PhaB was expressed by *E. coli* expression system, and purified by Ni-affinity chromatography and size exclusion chromatograpy. Crystals of apo-PhaB obtained from a buffer containing 0.1 M MES (pH 7.1), 1.6 M ammonium sulfate and 10% 1,4-dioxane. Crystals of PhaB complexed with $NADP^+$ and AcAc-CoA were obtained from the same buffer containing 0.9 mM $NADP^+$ and 0.9 mM AcAc-CoA. The X-ray diffraction data set was collected under cryogenic conditions (100 K). Crystals were soaked in a mother liquor containing 20% glycerol and flash-cooled in a stream of liquid nitrogen. The diffracted data were indexed, integrated and scaled using the HKL2000 program package or the XDS. The statistical data are shown in Table 1. The structure of PhaB was determined by the molecular replacement method by means of the MOLREP program using the structure of FabG from *E. coli* (PDB ID 1I01) as the search probe.

3 Results and Discussion

Four molecules of PhaB contained in an asymmetric unit formed tetramer with the point group 222. DLS analysis revealed a radius of approximately 44 Å for PhaB solution, which is in agreement with the tetrameric structure in the crystal. These observations suggested that PhaB exists as a tetramer in solution.

In the crystal structure of the ternary complex, an obvious electron density corresponding to AcAc-CoA and $NADP^+$ was observed in a large cavity of all four molecules in an asymmetric unit. The $NADP^+$ molecule was bound non-covalently in the cavity surrounded by loops between β1 - α1, β 2 - α 2, β 3 - α 3, β 4 - α 4, β 5 - α 5, and β 6 - α 7. $NADP^+$ was directly recognized by Arg40, Gly60-Asn61, Gly90-Thr92 and Pro183-Val191. An AcAc-CoA molecule was found to be adjacent to the NADP+ binding site. The nicotinamide ring of $NADP^+$ contacted with the AcAc moiety, which reasonably explains the catalytic reduction of AcAc-CoA. AcAc-CoA is recognized by Ser140, Thr92, Asp94, Gln147-Tyr153, Gly184, Tyr185 and Arg195.

Table 1: X-ray data collection statistics

	Apo form	Ternary complx
Beamline	BL1A	BL5A
Space group	$C222_1$	$C222_1$
Unit cell parameters (Å)	a = 67.7, b = 123.7, c = 260.8	a = 67.4, b = 123.4, c = 206.2
Resolution (Å)	20.6 – 1.79 (1.90 – 1.79)	50.00 – 2.14 (2.18 – 2.14)
R_{merge} (%)	10.1 (61.8)	9.1 (30.3)
Completeness (%)	97.7 (89.2)	96.9 (98.7)

Fig. 1: Crystal structure of PhaB in complex with $NADP^+$ and AcAc-CoA. (A) tetrameric structure (B) electron density of AcAc-CoA and $NADP^+$ (C) close-up view of active site

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