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Crystal structure determination of DtsR1, a carboxyltransferase subunit of acetyl-CoA carboxylase in *Corynebadterium glutamicum*

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Introduction

Acetyl-CoA carboxylases (ACCs) catalyze the first committed step of fatty acid biosynthesis. Although ACC is an essential enzyme (complex) in every organism, the structure-function relationship of ACC remains unclear. ACC from *Corynebacterium glutamicum* is a multi-subunit complex composed of AccBC and DtsR1. As the first step for elucidating the structure-function relationship of ACC, we initiated the crystallographic analysis of DtsR1. DtsR1 is the core catalytic β-subunit of ACC from *C. glutamicum*, which catalyzes the transcarboxylation between biotin and acetyl-CoA.

DtsR1 was crystallized by the sitting-drop vapor diffusion method using PEG 6000 as a precipitant. The approximate dimensions of the obtained crystals were 0.07x0.07x0.03mm^3. Here we report the crystal structure determination of DtsR1.

Results

Data collection and processing

All the data collections were carried out at 100K. The crystals were soaked in a cryoprotectant solution for a few seconds and flash-frozen in a cold N_2 stream from a liquid-nitrogen cryostat (Rigaku). Diffraction data were collected using an ADSC Quantum CCD detector at NW-12 of PF-AR.

Data processing and scaling were performed using *HKL2000* (Table 1). The crystal belongs to the space group *R*32 and likely contains three subunits in the asymmetric unit.

Table 1: Statistics of data collection

Crystal	*I*	*II*
Cell dimensions (Å)	$a = b = 204.25$, $c = 234.00$	$a = b = 204.15$, $c = 233.72$
Matthews coefficient (Å^{3}Da^{-1})	2.62	2.61
Solvent content (%)	53.01	52.90
Resolution (Å) (outer shell)[a]	48.8-3.15 (3.26-3.15)	50.04-2.63 (2.72-2.63)
*R*merge (%)[b]	9.3 (50.1)	12.0 (87.7)
Completeness (%)	99.8 (99.8)	93.3 (58.0)
Multiplicity	7.8 (7.7)	3.4 (1.8)

a: Statistics for the highest shell are given in parentheses
b: $R\text{merge} = \Sigma_h\Sigma_i^N|I(h)_i - I(h)| / \Sigma_h(N + I(h)_i)$

Crystal structure determination

Molecular replacement was performed with the program *MOLREP* in the CCP4 suite using a 12S monomer of transcarboxylase derived from *Propionibacterium shermanii* (PDB code; 1ON3) [1] as a search model, which shows 49% amino acid sequence identity with DtsR1. The data from crystal I was used for the calculation. Because there were no significant peaks in the cross-rotation function, translation functions were calculated using each of the first 30 peaks of the rotation function. We then found three subunits in the asymmetric unit without unfavorable molecular contacts in the crystal packing and started model building with the three subunits as the initial model. The crystal packing strongly suggests that the DtsR1 is a ring-shaped homo hexamer with *32*-point group symmetry, as found in other related proteins. One subunit in the asymmetric unit forms a hexamer with its symmetry-related subunits. The other two subunits in the asymmetric unit, which make a non-crystallographic dimer, form another hexamer with their symmetry-related subunits.

Crystallographic refinement is in progress using the data of crystal *II* at 2.7Å resolution.

References

[1] P. R. Hall *et al.*, EMBO J. 22, 2334 (2003).

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