Succinyl-CoA synthetase and succinyl-CoA:3-oxoacid CoA transferase

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Introduction

Succinyl-CoA synthetase (SCS) is responsible for carrying out two unrelated but vital metabolic functions. One, it catalyzes the substrate-level phosphorylation step of the citric acid cycle [1], and two, it replenishes succinyl-CoA for ketone body catabolism [2] and for porphyrin synthesis [3]. The reversible reaction catalyzed by SCS,

succinyl-SCoA + Pi + NDP ⇔ succinate + CoASH + NTP,

uses either adenosine or guanosine nucleotides. Although some species have a form of SCS that utilizes both ATP and GTP, other species have isoforms of SCS specific for each nucleotide [4]. The nucleotide-specific isoforms are believed necessary for SCS to serve its different metabolic roles. Thus, the GDP-specific SCS could maintain the high level of succinyl-CoA required for ketone body metabolism [5] (i.e., the reverse reaction above), since the mitochondrial GTP:GDP ratio is approximately 100 [6]. In contrast, since the mitochondrial ATP:ADP ratio is approximately 1, ADP-specific SCS would be suitable for the role played in the citric acid cycle [7].

E. coli SCS, in the presence of CoA, crystallizes in the tetragonal space group $P4_32$2 with unit cell dimensions a = b = 98.68 Å, c = 403.76 Å, and α = β = γ = 90˚ [8]. We have solved the structure [9] and refined it at 2.3 Å resolution using data from 23 crystals [10]. The binding site for ADP was determined using crystals soaked with ADP and Mg^{2+} ions, proving that the histidine residue phosphorylated in the reaction must swing approximately 35 Å between the binding site for CoA and the binding site for nucleotide [11]. To understand the catalytic mechanism, the binding site of succinate or the succinyl moiety must be known. Crystals of pig heart GTP-specific SCS grow in the absence of CoA [12]; we have successfully soaked them with CoA to determine the structure of the complex. The goals of our work at the Advanced Photon Source were 1) to determine the structure of *E. coli* SCS soaked with GDP and Mg^{2+} ions to prove that the guanosine nucleotide binds in the same site as the adenosine nucleotide and define the determinants of nucleotide specificity, 2) to collect a full data set from a single crystal of *E. coli* SCS at cryotemperatures, and 3) to determine the structure of pig heart GTP-specific SCS soaked with a nonhydrolyzable succinyl-CoA analogue to define the succinyl-binding site.

Succinyl-CoA:3-oxoacid CoA transferase (SCOT) is required for the catabolism of ketone bodies that are produced primarily in the liver as a source of metabolic fuel for other tissues. In the catabolism of ketone bodies, SCOT activates acetoacetate by transferring CoA from succinyl-CoA to produce acetoacetyl-CoA and succinate. The reaction proceeds with the participation of a glutamate residue of the enzyme in the formation of a thioester linkage to CoA:

E + succinyl-CoA ⇔ E-CoA + succinate
E-CoA + acetoacetate ⇔ E + acetoacetyl-CoA

We have crystallized SCOT in a monoclinic crystal form, space group $P2_1$ with unit cell dimensions a = 62.97 Å, b = 262.01 Å, c = 60.81 Å, α = γ = 90˚, and β = 112.31º, but the structure determination by multiple isomorphous replacement methods has been hampered by nonisomophism. We found a second crystal form, $P2_12_12$, with unit cell dimensions a = 101.42 Å, b = 142.94 Å, c = 69.32 Å, and α = β = γ = 90˚ that we hoped would provide a heavy-atom derivative useful for phasing.

Methods and Materials

Crystals of *E. coli* and pig heart GTP-specific SCS were grown as described in references [9] and [12]. Crystals of *E. coli* SCS were soaked with GDP and Mg^{2+} ions using the protocol worked out for ADP [11]. Crystals of pig heart GTP-specific SCS were soaked with the nonhydrolyzable succinyl-CoA analogue, S-butaryl-CoA [13]. We grew the monoclinic and the orthorhombic crystals of SCOT as well as crystals of a deletion mutant in which five residues (thought to be important in the determination of the quaternary structure of SCOT) had been removed [14]. The data were collected at the BioCARS beamline, 14-BM-C.

Results

Data from crystals of *E. coli* SCS were collected with a crystal-to-detector distance of 250 mm. The crystal soaked with GDP and Mg^{2+} ions diffracted to 2.7 Å, with an overall R_{merge}[†] of 6.2% and an R_{merge} of 36% on data in the resolution range between 2.8 and 2.7 Å

Data from a crystal of pig heart GTP-specific SCS soaked with S-butaryl CoA were collected with a crystal-to-detector distance of 200 mm. This resulted in a data set complete to 2.3 Å, but only 85.4% complete to 1.95 Å resolution. R_{merge} was 4.9% overall and 25% for data in the resolution range from 2.02 to 1.95 Å.

Two "native" data sets were collected for SCOT (one from a crystal in the monoclinic crystal form and the second from the deletion mutant in the orthorhombic crystal form). Data

[†] $R_{merge} = (\sum\sum|I_i - \langle I\rangle|)/\sum\sum I$, where I_i is the intensity of an individual measurement of a reflection and I is the mean value for all equivalent measurements of this reflection.

from the deletion mutant processed to 2.1 Å gave an overall R_{merge} of 3.7%. In the resolution range from 2.18 to 2.1 Å, R_{merge} was 8.7%. This data set was 92.3% complete overall, but only 60% complete in the resolution range between 2.2 and 2.1 Å. Surprisingly, the orthorhombic crystal of the deletion mutant was not isomorphous with the orthorhombic crystals of wild-type SCOT. A self-rotation map indicated that the noncrystallogra-phic two-fold axis is 15º from the b-axis of the crystals for the deletion mutant, while it superimposed with one of the crystallographic two-fold axes (presumably the b-axis) in crystals of wild-type SCOT. Data were also collected from six heavy atom soaks of orthorhombic crystals of SCOT. These data sets were processed to 2.5 Å. The most promising is from a crystal soaked with iridium, but the crystal displayed high mosaicity and the electron density maps calculated with phases from the derivative data do not show a clear boundary between the protein and the solvent. Work on phasing the SCOT structure is continuing.

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