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,

\[\text{succinyl-SCoA} + \text{Pi} + \text{NDP} \underset{\text{E}}{\rightleftharpoons} \text{succinate} + \text{CoASH} + \text{NTP},\]

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 P4₁2₁2₁ with unit cell dimensions \(a = 98.68 \, \text{Å}, \, b = 142.94 \, \text{Å}, \, \alpha = \beta = \gamma = 90°\) [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 Mg²⁺ ions, proving that the histidine residue phosphorilated 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 Mg²⁺ 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:

\[\text{E} + \text{succinyl-CoA} \rightleftharpoons \text{E-CoA} + \text{succinate} \]
\[\text{E-CoA} + \text{acetoacetate} \rightleftharpoons \text{E} + \text{acetoacetyl-CoA}\]

We have crystallized SCOT in a monoclinic crystal form, space group \(P2_1\), with unit cell dimensions \(a = 62.97 \, \text{Å}, \, b = 262.01 \, \text{Å}, \, c = 60.81 \, \text{Å}, \, \alpha = \gamma = 90°, \, \beta = 112.31°\), but the structure determination by multiple isomorphous replacement methods has been hampered by nonisomorphism. We found a second crystal form, \(P2_12_12_1\), with unit cell dimensions \(a = 101.42 \, \text{Å}, \, b = 142.94 \, \text{Å}, \, c = 69.32 \, \text{Å}, \, \alpha = \beta = \gamma = 90°\) 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 Mg²⁺ 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 Mg²⁺ ions diffracted to 2.7 Å, with an overall \(R_{merge} = 6.2\%\) 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

\[R_{merge} = \frac{\sum \sum | I_i - \bar{I} |}{\sum \sum I_i}, \text{ where } I_i \text{ is the intensity of an individual measurement of a reflection and } \bar{I} \text{ is the mean value for all equivalent measurements of this reflection.}\]
from the deletion mutant processed to 2.1 Å gave an overall Rmerge of 3.7%. In the resolution range from 2.18 to 2.1 Å, Rmerge 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 noncrystallographical 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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References