Supplementary materials

Crystal structure of the carboxyltransferase domain
of acetyl coenzyme A carboxylase

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Materials and Methods

Protein expression and purification. Residues 1429-2233 of *S. cerevisiae* ACC was sub-cloned into the pET24d vector (Novagen) and over-expressed in *E. coli* at 20 °C. The soluble protein was purified by nickel-agarose affinity and anion exchange chromatography. The protein was concentrated to 10 mg/ml in a buffer containing 20 mM Tris (pH 7.0), 100 mM NaCl, 5% (v/v) glycerol, and 10 mM DTT. The sample was flash-frozen in liquid nitrogen and stored at –80°C. The C-terminal His-tag was not removed for crystallization.

For the production of selenomethionyl proteins, the expression construct was transformed into DL41(DE3) cells. The bacterial growth was carried out in defined LeMaster media, and the protein was purified using the same protocol as for the wild-type protein.

Protein Crystallization. Crystals of the protein were obtained at 4°C by the vapor diffusion method. The reservoir solution contained 100 mM NaCitrate (pH 5.5), 10% (w/v) PEG8000, and 5% (v/v) glycerol. The protein was at 7 mg/ml in a solution that also contained 1 mM acetyl-CoA. Micro-seeding was used to obtain crystals of sufficient size for data collection. Three different crystal forms were observed under this condition, and our structural analyses showed only very weak electron density for the acetyl-CoA. A fourth crystal form was obtained using the crystallization condition 100 mM Tris (pH 7.0), 13% (w/v) PEG8000 and 10% (v/v) glycerol. The protein was pre-incubated with 2 mM acetyl-CoA, and the binding of this compound to the enzyme was observed in the electron density map. The crystals were cryo-protected with the introduction of 25% (v/v) ethylene glycol and flash frozen in liquid propane.

Data Collection. X-ray diffraction data were collected on an ADSC CCD at the X4A beamline of Brookhaven National Laboratory. For initial structure determination, a selenomethionyl single-wavelength anomalous diffraction (SAD) data set to 2.7 Å
resolution was collected at 100K on a crystal grown at pH 5.5. Significant decay in the
crystal diffraction quality precluded data collection at other wavelengths. The diffraction
images were processed and scaled with the HKL package (1). This free enzyme crystal
belongs to the space group \( C2 \), with cell dimensions of \( a=246.0 \text{ Å}, b=123.9 \text{ Å}, c=145.1 \text{ Å}, \) and \( \beta=94.1 \degree \). There are three molecules in the asymmetric unit, giving a \( V_m \) of 4.0
\( \text{Å}^3/\text{Dalton} \). Two of the molecules form a non-crystallographic dimer, whereas the third
molecule is part of a crystallographic dimer. To determine the binding mode for acetyl-
CoA, a native data set to 2.7 Å resolution was collected on a crystal grown at pH 7. It
belongs to space group \( P2_1 \), with cell dimensions of \( a=92.9 \text{ Å}, b=138.1 \text{ Å}, c=101.4 \text{ Å}, \) and \( \beta=114.4 \degree \). There are two molecules in the asymmetric unit, giving a \( V_m \) of 3.2
\( \text{Å}^3/\text{Dalton} \). The data processing statistics are summarized in Table 1.

**Structure determination and refinement.** The locations of Se atoms were determined
with the program SnB (2) and further confirmed with SHELXS (3) based on the
anomalous differences in the SAD data set. Reflection phases to 2.7 Å resolution were
calculated and improved with the program SOLVE (4). The atomic model was built into
the electron density with the program O (5). The structure of the CoA complex was
determined by the molecular replacement method with the program COMO (6). The
structure refinement was carried out with the program CNS (7). The statistics on the
structure refinement are summarized in Table 1.

**Enzyme kinetic assays.** The CT domain can catalyze the decarboxylation of malonyl-
CoA to produce acetyl-CoA in the presence of biotin methyl ester. The kinetic assays
monitored the production of acetyl-CoA by coupling it to citrate synthase and malate
dehydrogenase, which ultimately lead to the reduction of \( \text{NAD}^+ \) (8). Mutations in the
active site were designed based on the structural information. The mutants were made
with the QuikChange kit (Stratagene) and sequenced for confirmation. They were
purified and assayed kinetically under the same condition as the wild-type protein. The
kinetic parameters for each enzyme were obtained by non-linear least-squares fitting to the initial velocity data.
**Table 1**

Kinetic parameters of wild-type and mutant CT

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>$V_{\text{max}}$ (^1) (x 10^{-5} \text{ AU/s})</th>
<th>$K_m$ (µM) (for malonyl-CoA)</th>
<th>$V_{\text{max}}/K_m$</th>
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<tbody>
<tr>
<td>Wild-type</td>
<td>148 ± 3</td>
<td>67 ± 5</td>
<td>2.2 ± 0.1</td>
</tr>
<tr>
<td>L1705I</td>
<td>12 ± 0.3</td>
<td>1342 ± 180</td>
<td>0.009 ± 0.001</td>
</tr>
<tr>
<td>R1731S</td>
<td>385 ± 10</td>
<td>909 ± 72</td>
<td>0.42 ± 0.02</td>
</tr>
<tr>
<td>Y1738F</td>
<td>152 ± 3</td>
<td>53 ± 6</td>
<td>2.9 ± 0.3</td>
</tr>
<tr>
<td>R1954S</td>
<td>39 ± 6</td>
<td>5100 ± 1200</td>
<td>0.0076 ± 0.0008</td>
</tr>
<tr>
<td>E1994Q</td>
<td>272 ± 3</td>
<td>109 ± 5</td>
<td>2.5 ± 0.1</td>
</tr>
<tr>
<td>E2026Q</td>
<td>144 ± 6</td>
<td>218 ± 24</td>
<td>0.66 ± 0.05</td>
</tr>
<tr>
<td>R2036E</td>
<td>133 ± 3</td>
<td>41 ± 4</td>
<td>3.3 ± 0.3</td>
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</table>

1. All reactions contain 2.5 µM of the enzyme. AU: absorbance unit.
Figure Legends

**Fig. 1.** Alignment of the CT domain sequences. The secondary structure elements (S.S.) are shown and labeled. The residue numbers shown are for yeast ACC. Residues in the core of the monomer structure are colored green. Residues in the dimer interface are colored purple. The symbol = represents a residue that is strictly conserved among 30 CT domain sequences. A ‘-’ indicates a residue that is identical to that in yeast CT, and a dot represents a deletion.

**Fig. 2.** Experimental electron density map. Omit electron density map for residues 2121-2125, contoured at 3σ. Produced with Ribbons (9).

**Fig. 3.** Binding modes of CoA to CT and crotonase. Overlay of the binding mode of CoA in complex with CT (gray for carbon atoms) and octanoyl-CoA in complex with crotonase (green for carbon atoms). Produced with Grasp (10).

**Fig. 4.** Inhibition of yeast CT by the herbicide haloxyfop. (a). Chemical structure of haloxyfop. (b). Double reciprocal plot showing the competitive inhibition of wild-type yeast CT by haloxyfop.
References:

SFig. 2. Zhang, Yang, Shen & Tong
SFig. 3. Zhang, Yang, Shen & Tong
SFig. 4. Zhang, Yang, Shen & Tong