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Crystallization and preliminary X-ray data of the $\alpha_2\varepsilon_2$ subcomponent of the acetyl-CoA decarbonylase/synthase multienzyme complex from *Methanosarcina thermophila*

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The $\alpha_2\varepsilon_2$ subcomponent (218.6 kDa) of the 1.99 MDa acetyl-CoA decarbonylase/synthase (ACDS) multienzyme complex is an Ni/Fe–S enzyme that catalyzes reversible CO_2/CO reduction in the context of acetyl-CoA synthesis. The ACDS complex is required for methanogenesis from acetate in methanogenic archaea. The $\alpha_2\varepsilon_2$ subcomponent from *Methanosarcina thermophila*, grown on acetate, was purified and crystallized. The crystals were mounted in a capillary and diffracted to 4.0 Å resolution at room temperature. Different flash-cooling approaches were attempted, all of which resulted in poor diffraction.

1. Introduction

Methanogens are a class of microorganisms that synthesize methane as a metabolic end-product from growth on simple carbon compounds (such as CO_2, methanol, methylamines and acetate) in a chemically reducing environment. It has been estimated that two-thirds of the roughly 10^9 tons of methane produced annually results from the growth of methanogens on acetate (Smith & Mah, 1966). This process requires the activity of the acetyl-CoA decarbonylase/synthase (ACDS) multienzyme complex (Abbanat & Ferry, 1991; Terlesky *et al.*, 1986), which catalyzes the reaction

$$\mathrm{CO_2 + H_3C\text{-}H_4SPt + CoA + 2Fd_{red} + 2H^+} \longleftrightarrow \mathrm{acetyl\text{-}CoA + H_4SPt + 2Fd_{ox}},$$

where H_3C-H_4SPt and H_4SPt are the N^5-methylated and demethylated forms of tertahydrosarcinapterin, respectively, and Fd_{red} and Fd_{ox} are the reduced and oxidized forms of ferredoxin, respectively. The ACDS complex has a $(\alpha\beta\gamma\delta\varepsilon)_8$ quaternary structure and a molecular mass of 1.99 MDa (Abbanat & Ferry, 1991; Kocsis *et al.*, 1999). Proteolysis of the ACDS complex causes dissociation into three subcomponents ($\alpha_2\varepsilon_2$, β and $\gamma\delta$), each of which retains partial activity (Grahame & DeMoll, 1996). The $\alpha_2\varepsilon_2$ subcomponent of the ACDS complex catalyzes the oxidation of the carbonyl group of acetyl-CoA in the direction of acetyl-CoA scission and CO_2 reduction in the direction of acetyl-CoA synthesis,

$$\mathrm{CO_2 + 2e^- + 2H^+} \longleftrightarrow \mathrm{CO + H_2O}.$$

The $\alpha_2\varepsilon_2$ heterodimer has a molecular mass of 218.6 kDa (Kocsis *et al.*, 1999), with a 90 kDa α-subunit and a 19 kDa ε-subunit. The $\alpha_2\varepsilon_2$ subcomponent has 14–16 Fe atoms and one Ni atom per $(\alpha\varepsilon)$ heterodimer (Balbo *et al.*, 2003). There is evidence that these are incorporated into three or four Fe–S clusters (Balbo *et al.*, 2003), including a Ni/Fe–S cluster termed the C-cluster, which is the site of CO/CO_2 redox catalysis (Anderson & Lindahl, 1994; Ensign *et al.*, 1989; Lindahl *et al.*, 1990; Ragsdale *et al.*, 1983).

Amino-acid sequence analysis has shown that the $\alpha_2\varepsilon_2$ subcomponent contains regions of sequence homology to other enzymes having CO/CO_2 oxidoreductase (also called CO dehydrogenase or CODH) activity (Kerby *et al.*, 1992; Lindahl, 2002). These enzymes are (i) the CODH component of clostridial acetyl-CoA synthase (ACS) and (ii) the CooS enzyme (CODH) from certain photosynthetic bacteria. Recently, X-ray crystal structures of two examples of CooS, those from *Rhodospirilum rubrum* (Drennan *et al.*, 2001) and *Carboxydothermus hydrogenoformans* (Dobbek *et al.*, 2001), have been published. These structures have revealed many essential features of the active site. Among these are the coordination of a histidine to a C-cluster Fe, a coordination site at the Ni, which suggests a CO-binding site, the discovery of a new Fe_4S_4 cluster (D-cluster) shared at the dimer interface and the close (10 Å) proximity of a B-cluster (an Fe_4S_4 center) to the C-cluster (an Ni/Fe–S center). Notably, the two structures differ in the coordination environment about the nickel. The structure reported by Dobbek *et al.* (2001) suggests an $NiFe_4S_5$ cluster with a distorted square-planar geometry about the Ni. The structure reported by Drennan *et al.* (2001) suggests an $NiFe_4S_4$ cluster that has a penta-coordinated Ni with an unidentified ligand (possibly CO).

The $\alpha_2\varepsilon_2$ subcomponent of ACDS from *Methanosarcina thermophila* differs in many respects from the two reported CooS structures. For example, the metal content of the

methanogen enzyme is much higher than that of its bacterial homologs, suggesting the presence of other Fe—S clusters (Balbo *et al.*, 2003). The $\alpha_2\varepsilon_2$ subcomponent is co-purified with other proteins as a multi-enzyme complex while CooS is not. Furthermore, it has been shown experimentally that the reduction of redox sites on other ACDS subunits can be coupled to CO oxidation/$\alpha_2\varepsilon_2$ reduction (Grahame, 1993; Grahame *et al.*, 1996). Finally, the methanogen enzyme functions exclusively in acetate metabolism, while the role of the *R. rubrum* and *C. hydrogenoformans* CooS is to couple the oxidation of CO to the reduction of physiological electron acceptors such as ferredoxin (Ljungdahl, 1986). Our aim is to determine the structure of the $\alpha_2\varepsilon_2$ subcomponent of ACDS in order to understand how structural properties contribute to its distinct functions relative to the two known structures of CooS. To this end, we present here the characterization of crystals of the $\alpha_2\varepsilon_2$ subcomponent of the ACDS complex from *M. thermophila*.

2. Experimental methods and results

2.1. Protein purification

All purification procedures were performed anaerobically in a Coy vinyl anaerobic chamber under an atmosphere of 95% N_2, 5% H_2; a palladium catalyst was employed to maintain O_2 levels at parts per million concentrations. The $\alpha_2\varepsilon_2$ subcomponent was isolated from the acetyl-CoA decarbonylase/synthase (ACDS) multi-enzyme complex of acetate-grown *M. thermophila* as previously described (Bhaskar *et al.*, 1998; Grahame, 1991). This procedure involves initial purification of the ACDS complex by gel-filtration chromatography, partial proteolysis of the ACDS complex with bromelain and resolution of the subcomponents by ion-exchange chromatography. After purification, the $\alpha_2\epsilon_2$ subcomponent was desalted and concentrated using a Millipore Centricon 30 ultrafiltration device. The final buffer conditions after desalting were 50 m*M* Tris–HCl pH 7.5, 0.1 *M* NaCl.

2.2. Crystallization

All crystallization experiments were set up and performed anaerobically at room temperature. The $\alpha_2\varepsilon_2$ subcomponent was stored at a concentration of 8 mg ml^{-1} as frozen pellets in liquid nitrogen. Small aliquots were thawed prior to crystallization and diluted to 6 mg ml^{-1} with 50 m*M* Tris–HCl pH 7.5. Preliminary vapour-diffusion experiments using Hampton Crystal Screen I and II yielded crystals under several conditions, all of which contained PEG 8000. These crystals appeared within 24 h and had a variable crystal morphology ranging from small triangular crystals (less that 0.1 mm in the largest dimension) to large amorphous plates which visually appeared mosaic. In order to improve the crystal size and morphology, Hampton Additive Screen was employed. The additive spermidine·4HCl allowed the growth of smaller triangular and rectangular crystals with improved morphology. The best results were obtained using the sitting-drop method. After refinement of these conditions, crystallization resulted in 20–30 crystals per well. These crystals were prepared by mixing 5 µl of the $\alpha_2\varepsilon_2$ subcomponent at 6.0 mg ml^{-1} in 10 m*M* in spermidine·4HCl with 5 µl of reservoir solution consisting of 17.5% PEG 8000, 70 m*M* magnesium acetate and 0.1 *M* sodium citrate pH 6.5. Finally, macroseeding was employed by transferring one or two crystals to a drop prepared by combining 5 µl of enzyme/spermidine (as above) with 5 µl of macroseeding solution, which consisted of 15.5% PEG 8000, 70 m*M* magnesium acetate, 0.1 *M* sodium citrate pH 6.5, using the sitting-drop method. These crystals grew in a period of 2–4 weeks to average final dimensions of 0.1 × 0.1 × 0.4 mm (Fig. 1*a*).

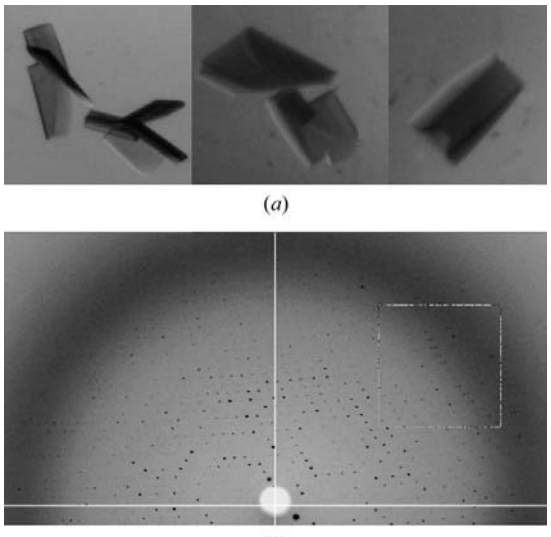

Figure 1
(*a*) Crystals of the $\alpha_2\varepsilon_2$ subcomponent of the acetyl-CoA decarbonylase/synthase from *M. thermophila*. The crystals grow as flat trapezoidal plates and required macroseeding. Crystals grew to 0.5 mm in the largest dimension. (*b*) Diffraction pattern of crystals shown in (*a*) taken at BioCARS 14-BM-C with 0.3° oscillation, a crystal-to-detector distance of 400 mm and 2 s exposure. The edge of the box shown in the figure indicates the area of highest resolution processed (4.0 Å).

Table 1
Crystallographic parameters and data-collection statistics.

Unit-cell parameters	
a (Å)	334.2
b (Å)	286.4
c (Å)	140.2
V (Å^3)	13419226.2
Space group	$P2_12_12_1$
MW, dimer (kDa)	218.2
Dimers in asymmetric unit	4–12 (V_M = 1.9 Å^3 Da^{-1} for n = 8)
Resolution (Å)	4.0
Wavelength (Å)	1.0 (BioCARS 14-BM-C)
No. exposures/No. crystals	832/48
R_{merge} (%)	19.4
Completeness (%)	64

2.3. Preliminarily diffraction analysis

X-ray diffraction experiments were performed at either the Cornell High Energy Synchrotron Source (CHESS A1 Station) or Argonne National Laboratory Advanced Photon Source (BioCARS 14-BM-C). Different data-collection strategies were tested by performing preliminary diffraction experiments on crystals that had been prepared and mounted in various ways. These included growing crystals in the presence of several cryoprotectants including glycerol, 2-methyl-2,4-pentanediol (MPD), PEG 400 and glucose. Cryocooling resulted in decreased resolution, increased mosaicity or both under all conditions tested. The best diffraction was obtained with room-temperature capillary-mounted crystals; these diffracted to a maximum resolution of about 4.0 Å (Fig. 1*b*). We used a crystal-to-detector (ADSC Quantum-4) distance varying between 300 and 400 mm depending on the size and diffraction quality of the crystals. The number of exposures (5–80 per crystal) varied according to the crystal size and quality. The optimal exposure time was determined by data scaling. We found that keeping the exposure time within 1–3 s was important in obtaining the best possible R_{merge}. Oscillation was set to 0.3°, providing a minimal overlap of reflections at the highest resolution observed.

We collected X-ray diffraction data from a total of 150 crystals that were >0.2 mm in the largest dimension. After scaling, the number of crystals in the data set reduced to 48 crystals and a total of 832 exposures. Table 1 contains the data statistics for this current

data set. The data were processed and scaled using the *HKL* suite (Otwinowski & Minor, 1997).

Our results lay out a strategy for data collection with short exposures using crystals of >0.3 mm in the largest dimension for best results. The structure determination of $\alpha_2\varepsilon_2$ will involve testing the structural homology with the related structures of the CooS enzymes of *R. rubrum* and *C. hydrogenoformans*. With our focus on improving the quality of the native data set, it is possible that we will also be able to collect diffraction data at the Fe absorption edge. This would enable us to also identify the positions of the metal clusters and to compare their arrangements relative to those observed in the *R. rubrum* and *C. hydrogenoformans* CooS structures.

Considering the unit-cell parameters and the molecular weight of $\alpha_2\varepsilon_2$, we estimate that the $\alpha_2\varepsilon_2$ crystals contain between 16 and 48 dimers in the unit cell (V_M = 3.8–1.28 Å^3 Da^{-1}), which would suggest 4–12 dimers in the asymmetric unit. The full ACDS complex has eight copies of five subunits, $(\alpha\beta\gamma\delta\varepsilon)_8$; it is therefore conceivable that $\alpha_2\varepsilon_2$ forms the core of the 1.99 MDa ACDS complex, in which case our preliminary data would suggest that there could be at least two core complexes (n = 8 dimers of $\alpha_2\varepsilon_2$) in the asymmetric unit.

One key step in the preparation of $\alpha_2\varepsilon_2$ involves the use of proteolysis to dissociate the multienzyme complex into its different subcomponents. Although this strategy has enabled the purification of the three subcomponents (Grahame & DeMoll, 1996), it might be a source of heterogeneity, which may have an impact upon the resolution of the crystals produced here. In this regard, we are working to establish a heterologous expression system and *in vitro* assembly of the different subcomponents of the ACDS complex, which may lead to higher resolution crystals.

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