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Crystallization and preliminary X-ray analysis of an acetone carboxylase from *Xanthobacter autotrophicus* strain Py2

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Acetone carboxylase from *Xanthobacter autotrophicus* strain Py2 catalyzes the MgATP-dependent carboxylation of acetone to acetoacetate. Interestingly, during this reaction ATP is hydrolyzed to AMP and inorganic phosphate, suggesting a novel carboxylation mechanism. Acetone carboxylase is a heterohexameric protein comprised of three different polypeptides having molecular weights of 86 342, 78 509 and 19 773 Da arranged in an $\alpha_2\beta_2\gamma_2$ quaternary structure. Here, the crystallization and preliminary X-ray data analysis of acetone carboxylase is reported. The acetone carboxylase isolated from the aerobic microorganism *X. autotrophicus* strain Py2 crystallizes in a primitive orthorhombic point group *P*222, with unit-cell parameters a = 76.2, b = 122.0, c = 264.2 Å. The Matthews coefficient calculation indicates that one $\alpha\beta\gamma$ half of the large protein complex is located in the asymmetric unit in this crystal form. Crystals have been obtained that diffract to better than 2.8 Å resolution and data have been collected to 3.2 Å resolution.

1. Introduction

Acetone is a three-carbon compound that is produced biologically by the fermentative processes of certain anaerobic bacteria or under starvation condition by mammals. Many aerobic and anaerobic bacteria grow utilizing acetone as a carbon and energy source (Sluis & Ensign, 1997). An obligatory aerobic Gram-negative bacterium *Xanthobacter autotrophicus* strain Py2 is capable of using either acetone or 2-propanol as growth substrates. The metabolism of 2-propanol is initiated by its conversion to acetone by the activity of a secondary alcohol dehydrogenase. Acetone, either as a growth substrate or formed by the activity of alcohol dehydrogenase, is converted to acetoacetate by a CO_2- and nucleotide-dependent acetone carboxylase (AC; Sluis *et al.*, 1996, 2002). Acetoacetate is then converted to the central metabolite by the activity of thiolase.

AC from *X. autothropicus* strain Py2 is a heterohexameric protein comprised of subunits having relative molecular weights of 86 342, 78 509 and 19 773 Da arranged in an $\alpha_2\beta_2\gamma_2$ quaternary structure (Sluis *et al.*, 1996; Sluis & Ensign, 1997; Wilkins *et al.*, 1998). Analysis of the metal content of AC indicates that each $\alpha_2\beta_2\gamma_2$ complex has 2 mol of Mn^{2+} ion tightly bound. The carboxylation of 1 mol of acetone catalyzed by AC is coupled to the obligate hydrolysis of 1 mol ATP to 1 mol AMP and 2 mol inorganic phosphate (Sluis *et al.*, 1996, 2002; Sluis & Ensign, 1997). Acetone carboxylase is unusual amongst the carboxylases since catalysis requires the hydrolysis of ATP to AMP and inorganic phosphate. Carboxylation of acetone is a thermodynamically unfavorable process (ΔG° = +17 kJ mol^{-1} for carboxylation of acetone with bicarbonate), but hydrolysis of ATP to ADP (ΔG° = −31 kJ mol^{-1}) could provide the energy to drive carboxylation. ATP hydrolysis in acetone carboxylase may have two functions: to allow the formation/stabilization of enolacetone and to activate bicarbonate as an electrophile (Sluis & Ensign, 1997). Since acetone carboxylation is likely to involve the attack of the carbanion of acetone on CO_2, the reaction could be facilitated by the stabilization of the enol tautomer of acetone through phosphoryl-group transfer to the O atom of the enolate. Acetone carboxylation resembles the combination of two reactions that when added together achieve pyruvate carboxylation: phosphoenolpyruvate synthetase (Cooper & Kornberg, 1965; Cooper & Kornberg, 1969; Hutchins *et al.*, 2001) and phosphoenolpyruvate carboxylase (Jane *et al.*, 1992). However, no experimental data exists so far to support such a mechanism. Since a structure of an AC has yet to be determined and amino-acid sequence searches of available databases suggest no significant sequence similarity to other proteins (Wilkins *et al.*, 1998), an imperative must be placed on determining the structure of AC. The determination of the three-dimensional structure of AC may contribute to understanding the mechanism of

carboxylation of acetone and the role of the ATP hydrolysis in the reaction.

2. Crystallization of AC

For crystallization, AC was purified to homogeneity from the soluble fraction of the cell-free extract of *X. autothropicus* strain Py2 primarily by anion-exchange and gel-filtration chromatography fractionation as described previously (Sluis & Ensign, 1997). Prior to crystallization, the protein was concentrated using an Amicon stirred ultrafiltration cell and stored in a buffer consisting of 25 m*M* MOPS pH 7.6 and 200 m*M* NaCl. The screening of conditions for crystallization has been approached by both factorial sparse-matrix screening and systematic methods of evaluating the precipitation behavior under high concentrations of precipitants *versus* different pH ranges (Garman & Mitchell, 1996; Jancarik & Kim, 1991; Cudney *et al.*, 1994). The preliminary crystallization conditions were identified using sparse-matrix crystallization screens yielding microcrystals of acetone carboxylase. For these initial screens, hanging drops were prepared by mixing 2 µl of purified AC with an equal volume of various crystallization formulations on siliconized cover slips followed by incubation in the presence of 1 ml reservoir using VDX crystallization plates. Solution No. 6 of the Hampton Cryo formulation [0.16 *M* magnesium chloride, 0.08 *M* Tris–HCl pH 8.5, 24%(*w*/*v*) PEG 4000, 20%(*w*/*v*) glycerol] yielded the best crystals in initial screens. Initial optimization of these crystallization conditions was accomplished by varying the concentration of the precipitant and pH as has been described previously (McPherson, 1990; Petsko, 1975; Weber, 1991). Crystals were grown at 291 K using the hanging-drop method by mixing 2 µl AC (45 mg ml^{-1} as determined by the method described in Chromy *et al.*, 1974) with an equal volume of reservoir solution [16%(*w*/*v*) polyethylene glycol 4000, 0.16 *M* magnesium chloride, 0.08 *M* Tris–HCl pH 9.0 and 20%(*v*/*v*) glycerol]. The colorless diamond-shaped crystals appear in 2–3 weeks and grow to a maximum dimensions of ∼0.15 × 0.05 × 0.015 mm (Fig. 1). AC crystallizes in a primitive orthorhombic space group with unit-cell parameters a = 76.2, b = 122.0, c = 264.2 Å (Fig. 1).

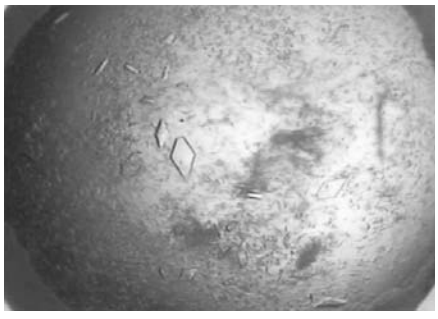

Figure 1
Crystals of acetone carboxylase (the dimensions of the largest crystals are ∼0.15 × 0.05 × 0.015 mm).

Table 1
Data statistics for primitive orthorhombic cell processed in *P*222.

Data statistics for the highest resolution shell (3.20–3.37 Å) are indicated in parentheses. Data were processed using *MOSFLM* (Leslie, 1992) and scaled using *SCALA* (Collaborative Computational Project, Number 4, 1994).

Unit-cell parameters (Å)	a = 76.24, b = 122.02, c = 264.17
Resolution (Å)	20.0–3.2
Completeness (%)	96.2 (98.6)
No. observed reflections	105648
No. unique reflections	33888
$I/\sigma(I)$	5.3 (2.0)
$R_{\rm merge}$† (%)	12.1 (23.6)

† $R_{\rm merge} = \sum_{hkl}\sum_i |I_i - \langle I\rangle|/\sum_{hkl}\sum_i |\langle I\rangle|$, where I_i is the intensity of the ith measurement of an equivalent reflection with indices *hkl*.

3. Data collection and analysis

Crystals of AC diffract to 2.8 Å resolution and to date data to 3.2 Å resolution have been collected (Fig. 2; Table 1). Diffraction data were collected at the Stanford Synchrotron Radiation Laboratory (SSRL) beamline 9-1 equipped with a Quantum-315 CCD (ADSC). For data collection, the crystals were flash-frozen in rayon loops in a liquid-nitrogen bath; during data collection, the crystals were maintained at ∼100 K. The data were processed using *MOSFLM* (Leslie, 1992) and scaled with *SCALA* from the *CCP*4 suite (Collaborative Computational Project, Number 4, 1994). As pointed out in §2, crystals belong to the orthorhombic point group *P*222. In this crystal form, the Matthews coefficient indicates that one $\alpha\beta\gamma$ half of the $\alpha_2\beta_2\gamma_2$ heterohexamer occupies the asymmetric unit in the crystal (Matthews, 1968).

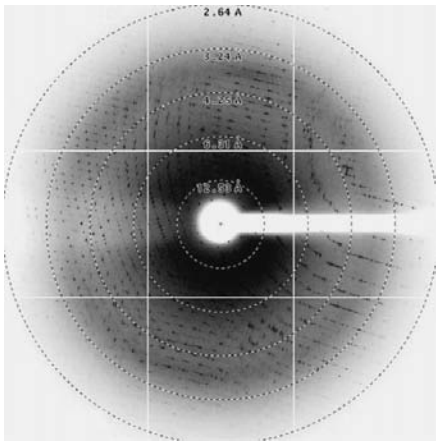

Figure 2
A 1° oscillation diffraction image of the acetone carboxylase crystals collected at SSRL beamline 9-1.

Since there are no other enzymes with significant levels of sequence identity that can be used as a search model, structure determination by the molecular-replacement (MR) method is not an option at this time. The heterologous expression of large multisubunit proteins is often difficult and as such the heterologous expression of AC has not yet been successful, therefore we are not yet able to generate selenomethionine-substituted protein for multiple-wavelength anomalous dispersion methods (MAD; Hendrickson *et al.*, 1990; Hendrickson, 1991). The intrinsic metal content is also not sufficient for the MAD technique with only one mole of Mn ions per 1661 residues in the asymmetric unit. Therefore, it will be necessary to generate heavy-atom derivatives of the protein to approach structure determination by MAD phasing using an exogenously added anomalous scatterer or the traditional method of multiple isomorphous replacement with anomalous dispersion (MIRAS).

There are a number of difficulties in determining the structure of a large protein such as AC using the MIRAS approach. The MIRAS method requires the preparation of derivatives of a protein by introducing electron-rich compounds into the crystalline lattice at a limited number of distinct locations in a manner that does not result in changes of the unit-cell parameters and symmetry. Often in the case of large proteins, the traditional heavy-atom reagents (heavy-atom salts) used for macromolecular crystallography bind the protein at too many sites, making it impossible to determine the positions of heavy-atom binding. As indicated by the Matthews coefficient, the current crystal form is likely to exist with an $\alpha\beta\gamma$ half of the $\alpha_2\beta_2\gamma_2$ heterohexamer of AC in the asymmetric unit (Matthews, 1968), which is a very favorable situation for determining the structure of this large protein by the MIRAS method. With only ∼183 kDa of the protein within the asymmetric unit, we should be able to generate heavy-atom derivatives using standard heavy-atom reagents that have a reasonable number of sites to determine their positions using Patterson methods or with the assistance of automated heavy-atom search software such as *SOLVE* (Terwilliger & Berendzen, 1999).

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