Acta Crystallographica Section D
**Biological Crystallography**
ISSN 0907-4449

# Crystallization and preliminary X-ray analysis of a NADPH 2-ketopropyl-coenzyme M oxidoreductase/carboxylase

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Received 9 October 2000
Accepted 8 January 2001

NADPH 2-ketopropyl-coenzyme M (2-mercaptoethanesulfonate) oxidoreductase/carboxylase is the terminal enzyme in a metabolic pathway that results in the conversion of propylene to the central metabolite acetoacetate. This enzyme is an FAD-containing enzyme that is a member of the NADPH:disulfide oxidoreductase family of enzymes and catalyzes the cleavage and carboxylation of 2-ketopropyl-coenzyme M to form acetoacetate and coenzyme M. Crystallization trials have revealed that the highest diffraction quality crystals (better that 2.0 Å resolution) could be achieved when the substrate or product of the reaction was added to the enzyme in a stoichiometric excess.

## 1. Introduction

The aerobic microorganism *Xanthobacter* strain Py2 is one of several bacteria that have been shown to be capable of utilizing short-chain aliphatic alkenes as a sole source of carbon and energy (van Ginkel & de Bont, 1986; Habets-Crützen *et al.*, 1984; Furuhashi *et al.*, 1981). The pathways for the bacterial metabolism of propylene have been extensively studied in both *Xanthobacter* strain Py2 as well as the phototrophic *Rhodococcus rhodochrous* strain B276 (Ensign *et al.*, 1998; Small & Ensign, 1995; Allen & Ensign, 1998). The conversion of propylene to an enantiomeric excess of (*R*)-epoxypropane is catalyzed by a diiron-containing alkene monooxygenase (Small & Ensign, 1995) that has similarities to the structurally characterized methane monooxygenase from the methanotroph *Methylococcus capsulatus* Bath (Rosenzweig *et al.*, 1993). The subsequent conversion of both enantiomers of epoxypropane to acetoacetate occurs by a three-step pathway consisting of four enzyme components (Allen & Ensign, 1999). The first step results in the conjugation of coenzyme M to (*R*)- or (*S*)-epoxypropane forming the corresponding enantiomer of 2-hydroxypropyl-coenzyme M. In the next step, two stereospecific short-chain dehydrogenases catalyze the oxidation of the individual enantiomers of 2-hydroxypropyl-coenzyme M, yielding the common intermediate 2-ketopropyl-coenzyme M (Allen & Ensign, 1999; Clark *et al.*, 2000). The final step in the pathway is the conversion of 2-ketopropyl coenzyme M to acetoacetate with concomitant regeneration of free coenzyme M. This reaction is catalyzed by NADPH:2-ketopropyl-coenzyme M oxidoreductase/carboxylase (2-KPCC), a unique member of the FAD-containing NAD(P)H disulfide oxidoreductase family of enzymes by primary sequence similarities (Swaving *et al.*, 1996). Several members of this enzyme family have been structurally characterized, including glutathione reductase, dihydrolipoamide dehydrogenase and thioredoxin reductase. The activity of 2-KPCC is distinct from these examples, catalyzing not only the reductive cleavage but also the carboxylation reaction resulting in the formation of acetoacetate. There is significant interest in the details of the mechanism by which bacteria degrade epoxides such as epoxypropane owing to their mutagenic and carcinogenic nature. The pathway described above results in the chemical conversion of the potentially harmful epoxide into a central metabolite, acetoacetate. Significant information can be obtained on the manner in which the carboxylation reaction and the oxidoreductase activity are coordinated and the relationship between 2-KPCC and the other members of the NAD(P)H oxidoreductase family of enzymes through the structural characterization of 2-KPCC in the presence of its reaction substrate and product.

## 2. Crystallization of 2-KPCC

For crystallization, purified 2-KPCC was obtained as described previously (Clark *et al.*, 2000). Using a sparse-matrix crystallization screen (Jancarik & Kim, 1991; Garman & Mitchell, 1996), conditions have been identified that yield diffraction-quality crystals of 2-KPCC. Crystals could be obtained by the hanging-drop or sitting-drop vapor-diffusion method using a 1:1 ratio of precipitating solution to protein ($\sim$20 mg ml$^{-1}$) with 0.8 ml of reservoir solution in standard Linbro plates. The substrate and product for 2-KPCC were

added in molar excess (final concentration 10 m*M*) directly to the protein solution prior to crystallization. Crystallization of 2-KPCC alone or in the presence of 2-ketopropyl coenzyme M occurred using a precipitating solution consisted of 0.17 *M* ammonium acetate, 0.085 *M* trisodium citrate dihydrate pH 5.6, 25.5%(*w*/*v*) polyethylene glycol 4000 and 15%(*v*/*v*) glycerol. In the presence of coenzyme M, crystallization of 2-KPCC occurred using a precipitating solution consisting of 0.17 *M* sodium acetate, 0.085 *M* Tris–HCl pH 8.5, 25.5%(*w*/*v*) polyethylene glycol 4000 and 15%(*v*/*v*) glycerol. Under these conditions in either the presence or absence of substrate or product, crystals appear after ~1–2 weeks incubation at 294 K. Crystals of 2-KPCC grown in the presence of substrate, 2-ketopropyl coenzyme M, or product, coenzyme M, differed in gross morphology when compared with crystals of 2-KPCC grown without the addition of substrates or products.

The crystals grown in the presence of either 2-ketopropyl coenzyme M or coenzyme M are identical in appearance, with overall dimensions of <0.1 × 0.2–0.4 × 0.5–1.0 mm, are bright yellow in color and belong to the monoclinic space group $P2_1$. The crystals grow as extended plates or lathes in either single crystals or clusters (Fig. 1*a*). The thin dimension of the crystals results in the crystals being quite fragile and difficult to handle. Although at this point it is impossible to know whether these crystals represent the substrate- and product-bound states, the requirement for either 2-ketopropyl coenzyme M or coenzyme M for crystallization of 2-KPCC is highly suggestive that the resulting structure represents substrate- and product-bound states of the enzyme.

In contrast to these crystals are the crystals of 2-KPCC grown in the absence of substrates or products, which have an overall morphology that is markedly different. They are more three-dimensional with very nice edges (Figs. 1*b* and 1*c*) and although they sometimes grow in clusters, they are most often found as single crystals with overall dimensions of 0.3–0.5 × 0.8–1.0 × 0.8–1.0 mm.

## 3. Data collection and analysis

The difference in gross morphology observed in the crystals of 2-KPCC with and without substrate or product are consistent with observed differences in cell symmetry. Although the unit-cell parameters are similar, implying some degree of relatedness, the crystals of 2-KPCC in the presence of substrate or product occur in the monoclinic space group $P2_1$, whereas the data from the crystals grown in the absence of substrate or product can only be reduced in the triclinic space group *P*1 (Table 1). Differences are also observed in the diffraction quality of the two crystal forms. Contrary to their appearance, the crystals of the substrate or product diffract well and data can be collected routinely to 2.5 Å resolution on our home Cu $K\alpha$ X-ray source and, as is commonly the case with small crystals, the diffraction limit is dramatically improved at a synchrotron source such that data to greater than 1.7 Å resolution can be collected. In contrast, the crystals grown in the absence of substrates or products diffract to 2.8 Å resolution on our home source and there is little or no improvement in the diffraction limit when data is collected at a synchrotron source.

We have been able to collect several data sets from these crystals and the resulting data statistics are shown in Table 1. The data for the triclinic crystal form of 2-KPCC grown in the absence of substrates or products is a representative data set collected on a Cu $K\alpha$ source. For data collection, the crystals were flash-cooled in the aforementioned mother-liquor solution on rayon loops in a liquid-nitrogen bath and during data collection the crystals were maintained at near-liquid-nitrogen temperature (~93 K) with a stream of liquid nitrogen over the crystals at all times. Diffraction data were collected using a Rigaku 3HR (Rigaku, Tokyo, Japan) X-ray generator with a Cu-anode source and a R-AXIS IIc imaging-plate area detector (Molecular Structure Corporation, The Woodlands, Texas, USA). The data were processed and scaled using the programs *DENZO* and *SCALEPACK* (Otwinowski & Minor, 1997). The diffraction data for the monoclinic crystal form were collected at the Stanford Synchrotron Radiation Laboratories beamline 9-1 equipped with a MAR345 imaging-plate detector (MAR USA, Inc., Illinois, USA). The data were processed using *MOSFLM* (Leslie, 1992) and scaled using *SCALA* from the *CCP*4 suite of programs (Collaborative Computational Project, Number 4, 1994).

**Table 1**
Data statistics.

Numbers in parentheses indicate values for the highest resolution bin.

| Crystals | 2-KPCC | 2-KPCC + 2-KCoM | 2-KPCC + CoM |
|---|---|---|---|
| Space group | *P*1 | $P2_1$ | $P2_1$ |
| Unit-cell parameters | | | |
| *a* (Å) | 65.59 | 87.98 | 87.79 |
| *b* (Å) | 87.54 | 60.12 | 60.05 |
| *c* (Å) | 100.71 | 105.60 | 105.63 |
| $\alpha$ (°) | 72.06 | 90.00 | 90.00 |
| $\beta$ (°) | 73.37 | 102.50 | 99.91 |
| $\gamma$ (°) | 69.77 | 90.00 | 90.00 |
| Resolution (Å) | 20.0–2.8 | 20.0–1.64 | 20.0–2.20 |
| Completeness (%) | 92.1 (90.2) | 98.2 (99.1) | 97.8 (99.5) |
| Observed reflections | 213523 | 504165 | 227087 |
| Unique reflections | 48234 | 164972 | 58516 |
| $I/\sigma(I)$ | 10.9 (2.8) | 7.8 (2.4) | 7.6 (2.7) |
| $R_{\rm merge}$† (%) | 9.8 (18.9) | 6.7 (30.9) | 7.9 (25.7) |

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

The similarities in unit-cell parameters but differences in cell symmetry and diffraction quality may suggest that the crystal packing is similar but that addition of substrates or products results in a conformationally distinct form of the enzyme when compared with the substrate or product-free form of the enzyme. From previous biochemical studies, we anticipate that 2-KPCC will exist as a dimer with a monomer molecular weight of ~57 000 Da (Allen & Ensign, 1997). Both unit cells have a similar volume of ~550 000 Å$^3$ which would result in a Matthews coefficient (Matthews, 1968) of ~2.4 Å$^3$ Da$^{-1}$ for both crystal forms, with the triclinic form having two dimers in the asymmetric unit and the monoclinic form having one dimer in the

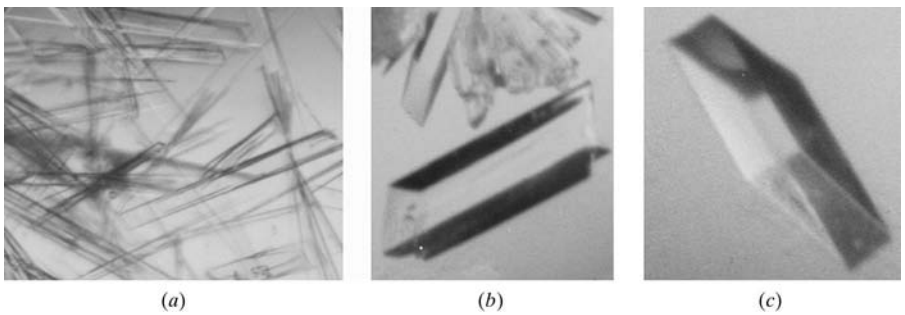


**Figure 1**
Crystal forms of 2-KPCC generated either (*a*) in the presence of substrate 2-ketopropyl-coenzyme M (2-KCoM) or coenzyme M (CoM) or (*b*) and (*c*) in its absence.

asymmetric unit. The determination of the structure of 2-KPCC in the presence of bound substrate and product at the current resolution of the data will contribute significantly to the understanding of the mechanism of the enzyme, especially in terms of how this unique member of the NAD(P)H disulfide oxidoreductase class is able to coordinate the reductive cleavage of 2-ketopropyl coenzyme M with carboxylation.

The data collection facility at SSRL is funded by the US Department of Energy, Office of Basic Energy Sciences and by the NIH Biomedical Research Technology Program, Division of Research Resources. This work was supported in part by National Institutes of Health Grant GM51805 to SAE.

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