Acta Cryst. (1997). D**53**, 352–353

Characterization of two crystal forms of 3-carboxy-*cis,cis*-muconate lactonizing enzyme from *Pseudomonas putida*

MIRIAM S. HASSON,[a]† ILME SCHLICHTING,[a]‡ MARGARET M. MCGOWEN,[b] ELISA M. WOOLRIDGE,[b]§ JOHN W. KOZARICH,[b,c] GREGORY A. PETSKO[a] AND DAGMAR RINGE[a] *at* [a]*Departments of Chemistry and Biochemistry and the Rosenstiel Basic Medical Sciences Research Center, Brandeis University, Waltham, MA 02254-9110, USA,* [b]*Department of Chemistry and Biochemistry, University of Maryland, College Park, MD 20742, USA, and* [c]*Merck Research Laboratories, PO Box 2000, R80M-101, Rahway, NJ 07065-0900, USA. E-mail: ringe@binah.cc.brandeis.edu*

(*Received 23 July 1996; accepted 17 December 1996*)

Abstract

Two crystal forms of 3-carboxy-*cis,cis*-muconate lactonizing enzyme from *Pseudomonas putida* have been characterized. Form *A* is in space group *P*6, with unit-cell dimensions $a = b = 232$, $c = 79$ Å, $\alpha = \beta = 90$, $\gamma = 120°$. Form *B* is orthorhombic, with cell dimensions $a = 163$, $b = 139$, $c = 90$ Å, $\alpha = \beta = \gamma = 90°$.

1. Introduction

The β-ketoadipate pathway of *Pseudomonas putida* is composed of two branches, each producing the end product β-ketoadipate from different starting compounds, catechol and protocatechuate (Ornston & Stanier, 1966). The second enzyme of the protocatechuate branch of the pathway, 3-carboxy-*cis,cis*-muconate lactonizing enzyme (CMLE; E.C. 5.5.1.2), catalyzes the conversion of 3-carboxy-*cis,cis*-muconate to 4-carboxymuconolactone (Ornston, 1966*a*). The second enzyme of the catechol branch, muconate lactonizing enzyme (MLE; E.C. 5.5.1.1) catalyzes the identical reaction on a substrate that differs by only one carboxylate group; it converts *cis,cis*-muconate to muconolactone (Ornston, 1966*b*). The similarities in function and metabolic context of the two enzymes led to the belief that they were homologous proteins (Patel, Meagher & Ornston, 1973). However, further studies showed that the two are quite different.

The primary sequences of *P. putida* CMLE and MLE are unrelated. CMLE is a member of the class II fumarase family (Williams *et al.*, 1992), while MLE is a member of a group of enzymes, including mandelate racemase, that catalyze diverse reactions involving the abstraction of a proton from a Cα to a carboxylate group (Babbitt *et al.*, 1995). The structure of MLE has been determined (Hasson *et al.*, 1997; Helin, Kahn, Guha, Mallows & Goldman, 1995), as have the structures of two members of the class II fumarase family, δ-crystallin (Simpson *et al.*, 1994) and fumarase C (Weaver, Levitt, Donnelly, Stevens & Banaszak, 1995). The folds of proteins in the two families are completely different.

The reactions catalyzed by *P. putida* CMLE and MLE are carried out with the opposite stereochemical course (Chari, Whitman, Kozarich, Ngai & Ornston, 1987); CMLE catalyzes an *anti*-cycloisomerization, while MLE catalyzes a *syn* addition. A rationalization of the difference in strategy of the two enzymes, based on the difference of a carboxyl group, has been adduced (Benner, Glasfeld & Piccirilli, 1988).

Recently diffraction-quality crystals of CMLE from *Neurospora crassa* have been obtained (Glumoff, Helin, Mazur, Kozarich & Goldman, 1996); this enzyme has no sequence similarity with either CMLE or MLE from bacteria (Mazur, Henzel, Mattoo & Kozarich, 1994). The stereochemical course of the reaction it catalyzes is identical to that catalyzed by MLE and opposite to that catalyzed by *P. putida* CMLE (Mazur *et al.*, 1994). Thus, *N. crassa* CMLE seems to define yet another class of lactonizing enzymes whose structure may soon be available.

Comparison of the structures of the active sites of CMLE from *P. putida* and *N. crassa* and of MLE would allow a better understanding of the contrast between three strategies for performing similar reactions. To this end, we have begun the determination of the crystal structure of CMLE from *P. putida*. We have obtained two crystal forms suitable for this type of structural analysis.

2. Methods

2.1. *Crystallization*

CMLE was expressed in *Escherichia coli* and purified as described previously (Williams *et al.*, 1992). Crystals of form *A* were grown at room temperature by hanging-drop vapor diffusion against a well solution of 13% polyethylene glycol 8000, 0.1 *M* potassium phosphate (pH 7). Drops contained equal volumes (2 µl) of well solution and protein solution consisting of 50 mg ml^{-1} CMLE in 25 m*M* ethylenediamine chloride, pH 7.3, 5 m*M* β-mercaptoethanol and 1 m*M* EDTA.

Crystals of form *B* were grown at room temperature by hanging-drop vapor diffusion against a well solution of 0.75 *M* sodium citrate, 0.1 *M* HEPES pH 7.5 or 0.1 *M* Tris–HCl pH 8.5, 0.6% 2-methyl-2,4-pentanediol. Drops contained equal volumes of well solution and a protein solution consisting of 10–20 mg ml^{-1} CMLE in 50 m*M* Tris–HCl (pH 7.3), 0.01 m*M* $MnCl_2$, 0.04% sodium azide. The 2-methyl-2,4-pentanediol prevented a phase separation and allowed the growth of single crystals.

2.2. *Data collection*

Crystals were mounted in quartz capillaries (Charles Supper Co.) for diffraction analysis. Data from crystals of form *A* were collected to 3 Å resolution on a Siemens X-100A multiwire proportional X-ray area detector. X-rays were generated by an Elliot GX-6 rotating anode (30 kV, 30 mA) equipped with a

† Present address: Purdue University, Department of Biological Sciences, West Lafayette, IN 47907-1392, USA.

‡ Present address: Max Planck Institut für Molekulare Physiologie, Abteilung Physikalische Biochemie, Rheinland Damm 201, D-44139 Dortmund, Germany.

§ Present address: International Paper Corporate Research Center, Long Meadow Road, Tuxedo, NY 10987, USA.

ISSN 0907-4449

nickel filter, a 0.5 mm focusing cup and a 0.3 mm collimator. Crystals were kept at a temperature of 277 K during X-ray experiments with a stream of chilled air. The set of X-ray diffraction images was reduced to integrated indexed intensities and processed with *XDS* (Kabsch, 1993) to determine the unit-cell dimensions, Laue symmetry and space group. A partial data set from a crystal of form *B* was collected at the X12C beamline at Brookhaven National Laboratory.

3. Results and discussion

Large crystals of form *A*, up to 1–2 mm in each dimension, grow in several days. The crystals appear hexagonal and uniform at one end and are often somewhat frayed at the other; they diffract to better than 3 Å resolution. The space group of the crystals, determined by the data-reduction program *XDS*, was found to be hexagonal, with cell dimensions $a = b = 232$, $c = 79$ Å, $\alpha = \beta = 90$, $\gamma = 120°$. A graphical representation of the *hk*0 zone of the data reduced in space group *P*6 is shown in Fig. 1. The data show that the crystals do not have the symmetry of space group *P*622, as mirror symmetry is not apparent in this level. The intensities of the reflections collected on the area detector show no systematic absences, indicating that no screw axis is present. Thus, the space group of the crystals is tentatively assigned as *P*6.

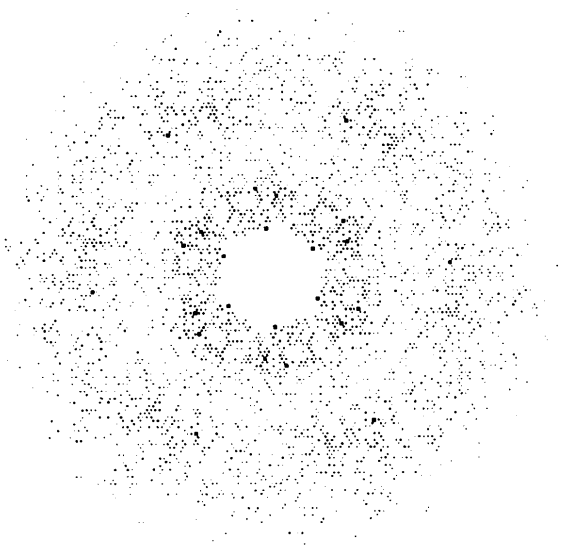

Fig. 1. A graphical representation of the *hk*0 zone of the data from a crystal of form *A*. The data were plotted by the program *HKLVIEW*, a part of the *CCP*4 software package (Collaborative Computational Project, Number 4, 1994).

The assumption that crystals of form *A* have a typical volume-to-mass ratio (V_m) of 2.4 Å^3 Da^{-1} (Matthews, 1968) leads to the conclusion that the asymmetric unit contains six CMLE monomers of 42 kDa each. Given the fact that CMLE seems to form a tetramer in solution, six monomers can be accommodated in the asymmetric unit if one tetramer is on the crystallographic twofold axis present in *P*6, contributing two subunits to the asymmetric unit, and another tetramer is built up entirely of non-crystallographic symmetry elements and contributes four subunits to the asymmetric unit. We are currently attempting to collect data on heavy-atom derivatives of this crystal form.

Crystal form *B* is orthorhombic, with cell dimensions $a = 163$, $b = 139$, $c = 90$ Å, $\alpha = \beta = \gamma = 90°$. The crystals have a rod-like morphology. Assuming this crystal form has a typical solvent content ($V_m = 3.0$ Å^3 Da^{-1}) the asymmetric unit contains a tetramer. We are now attempting to collect data on frozen crystals of form *B*, as they are extremely radiation sensitive at 277 K.

References

Babbitt, P. C., Mrachko, G. T., Hasson, M. S., Huisman, G. W., Kolter, R., Ringe, D., Petsko, G. A., Kenyon, G. L. & Gerlt, J. A. (1995). *Science*, **267**, 1159–1161.

Benner, S. A., Glasfeld, S. & Piccirilli, J. A. (1988). *Topics in Stereochemistry*, Vol. 19, edited by E. L. Eliel & S. H. Wilen, pp. 127–207. New York: John Wiley.

Chari, R. V. J., Whitman, C. P., Kozarich, J. W., Ngai, K.-L. & Ornston, L. N. (1987). *J. Am. Chem. Soc.* **109**, 5514–5519.

Collaborative Computational Project, Number 4. (1994). *Acta Cryst.* D**50**, 760–763.

Glumoff, T., Helin, S., Mazur, P., Kozarich, J. W. & Goldman, A. (1996). *Acta Cryst.* D**52**, 221–223.

Hasson, M. S., Schlichting, I., Moulai, J., Barrett, B., Gerlt, J., Babbitt, P., Kenyon, G., Petsko, G. & Ringe, D. (1997). Submitted.

Helin, S., Kahn, P. C., Guha, B. L., Mallows, D. G. & Goldman, A. (1995). *J. Mol. Biol.* **254**, 918–941.

Kabsch, W. (1993). *J. Appl. Cryst.* **26**, 795–800.

Matthews, B. W. (1968). *J. Mol. Biol.* **33**, 491–497.

Mazur, P., Henzel, W. J., Mattoo, S. & Kozarich, J. W. (1994). *J. Bacteriol.* **176**, 1718–1728.

Ornston, L. N. (1966*a*). *J. Biol. Chem.* **241**, 3787–3794.

Ornston, L. N. (1966*b*). *J. Biol. Chem.* **241**, 3795–3799.

Ornston, L. N. & Stanier, R. Y. (1966). *J. Biol. Chem.* **241**, 3776–3786.

Patel, R. N., Meagher, R. B. & Ornston, L. N. (1973). *Biochemistry*, **12**, 3531–3537.

Simpson, A., Bateman, O., Driessen, H., Lindley, P., Moss, D., Mylvaganan, S., Narebor, E. & Slingsby, C. (1994). *Nature Struct. Biol.* **1**, 724–733.

Weaver, T. M., Levitt, D. G., Donnelly, M. I., Stevens, P. P. & Banaszak, L. J. (1995). *Nature Struct. Biol.* **2**, 654–662.

Williams, S. E., Woolridge, E. M., Ransom, S. C., Landro, J. A., Babbitt, P. C. & Kozarich, J. W. (1992). *Biochemistry*, **31**, 9768–9776.