Acta Cryst. (1995). D**51**, 833–834

Additional crystal forms of the *E. coli* class II fructose-1,6-bisphosphate aldolase.

By YASUYUKI KITAGAWA,* GORDON A. LEONARD, STEPHEN J. HARROP, MARK R. PETERSON and WILLIAM N. HUNTER,† *Department of Chemistry, University of Manchester, Oxford Road, Manchester M13 9PL, England*, and SEEMA QAMAR and ALAN BERRY, *Department of Biochemistry and Molecular Biology, University of Leeds, Leeds LS2 9JT, England*

(*Received* 22 *November* 1994; *accepted* 28 *November* 1994)

Abstract

We have obtained two additional crystal forms of the metal-dependent class II fructose-1,6-bisphosphate aldolase from *Escherichia coli*. Crystals in the shape of elongated plates have unit-cell dimensions $a = 73.4$, $b = 120.0$, $c = 190.1$ Å, orthorhombic space group $P2_12_12_1$. Monoclinic prisms have unit-cell dimensions $a = 67.7$, $b = 104.3$, $c = 52.8$ Å, $\beta = 105°$, space group $P2_1$. Diffraction to slightly better than 3.0 Å has been observed for both forms using in-house and synchrotron facilities. These crystal forms may aid the structure solution of this enzyme by presenting additional forms for heavy-atom derivatization. These forms have multiple copies of the enzyme in the asymmetric unit and averaging methods might also be useful in the analysis.

Introduction

Aldolases are widely dispersed in nature and the fructose-1,6-bisphosphate aldolases (E.C. 4.1.2.13) which catalyse the cleavage of this bisphosphate to a ketose, dihydroxyacetone phosphate, and an aldose, glyceraldehyde 3-phosphate (Horecker, Tsolas & Lai, 1972), are amongst the most studied members of this enzyme family. They can be separated into two groups termed class I and II. The class I enzymes generally occur in eukaryotes although they have been identified in some bacteria (Baldwin & Perham, 1978; Marsh & Lebherz, 1992). They are homotetramers with a total molecular weight of approximately 160 kDa. Several crystal structures of class I enzymes have been determined. These include the enzymes isolated from rabbit muscle (Sygusch, Beaudry & Allaire, 1987) and rabbit liver (White, Allaire, Beaudry & Sygusch, 1991), human muscle (Gamblin, Davies, Grimes, Jackson, Littlechild & Watson, 1991), *Drosophila melanogaster* (Hester, Brenner-Holzach, Rossi, Smit, Struck-Donatz, Winterhalter & Piontek, 1991), the bacterial enzymes phosphogluconate aldolase (Lebioda, Hatada, Tulinsky & Mavaridis, 1982) and *N*-acetylneuraminate lyase (Izard, Lawrence, Malby, Lilley & Colman, 1994). The architecture of these enzymes is an eight-stranded α/β-barrel similar to that first observed in triosephosphate isomerase (Banner, Bloomer, Petsko, Phillips, Pogson, Wilson, Corran, Furth, Milman, Offord, Priddle & Waley, 1975) and subsequently in more than a dozen other enzymes (Brändén & Tooze, 1991).

Little is known about the class II system in comparison to the class I enzymes. In part this is attributed to difficulties in purifying the quantities necessary for structural and mechanistic studies. It is known that they are functionally active as homodimers (M_r per subunit is approximately 39 kDa) and are metal dependent. It is proposed that zinc is able to act as an electron sink during catalysis. It thus replaces the active-site lysine untilized by the class I enzymes (Mildvan, Kobes & Rutter, 1971; Littlechild & Watson, 1993). A single class II aldolase crystal structure is available, that for the L-fuculose-1-phosphate aldolase from *E. coli* (Dreyer & Schulz, 1993). However, this enzyme appears to be markedly different from other class II enzymes. For example, it is a homotetramer (M_r per subunit is approximately 24 kDa) rather than a dimer and it shows only 15–22% primary sequence identity with any other class II aldolase. This range of values is derived from sequences aligned using the program *PILEUP* (Genetics Computer Group, 1991) with a Gap weight of 3.0 and a Gap length weight of 0.1. Furthermore, it shows only about 20% identity with the class I aldolases. It is, therefore, possible that the class II aldolases may need to be further subdivided into two types of class II enzyme. The cloning and over-expression of the gene for the *E. coli* class II fructose-1,6-bisphosphate aldolase (Alefounder, Baldwin, Perham & Short, 1989) has allowed access to sufficient quantities of enzyme for structural studies. Knowledge of the structure combined with site-directed mutagenesis methods already in progress (Berry & Marshall, 1993; Packman & Berry, 1995) should allow us to delineate the structure–specificity relationship and mechanism of this enzyme.

The enzyme was isolated and purified according to Berry & Marshall (1993) to provide material for our crystallization experiments. The first crystal form we obtained, form I, is hexagonal, $P6_122$ or enantiomorph with $a = 78$, $c = 291$ Å (Naismith, Ferrara, Bailey, Marshall, Dauter, Wilson, Habash, Harrop, Berry & Hunter, 1992). In order to determine the structure of this enzyme we have adopted a strategy of heavy-atom derivatization of the hexagonal form whilst searching for additional crystal forms. The aim of further crystallization experiments is to either provide a more suitable crystal form or provide one where averaging between crystal forms might aid phase determination.

Experimental and results

Essentially only two techniques for achieving supersaturation of the enzyme were tried; (McPherson, 1982), vapour diffusion with hanging and sitting drops. Numerous conditions were tested varying such parameters as pH and type of buffer, concentration and type of precipitant, concentration of enzyme, temperature *etc.* (Ducruix & Giegé, 1992). In addition we made use of the sparse-matrix sampling screen proposed by Jancarik & Kim (1991) using reagents marketed by Hampton Research (5225 Canyon Crest Drive Suite 71-336, Riverside, CA 92507, USA). Numerous crystal morphologies have been observed in

* Permanent address: Institute for Protein Research, Osaka University, 3-2, Yamadaoka Suita, Osaka 565, Japan.
† Author for correspondence.

ISSN 0907-4449

our experiments under a wide range of conditions. However, only two of the new morphologies have produced crystals large enough to warrant exposure to X-rays. These types of crystal are designated forms II and III.

These two types of crystals grow under similar conditions. Hanging-drop vapour-diffusion methods equilibrating a 20 mg ml^{-1} protein solution in 100 m*M* Tris–HCl buffer (pH 8.0), 10 to 20%(*w*/*v*) PEG 4000, 0.02 to 0.3 *M* $MgCl_2$ against slightly higher concentration PEG solutions at room temperature. Form II presents as fragile thin elongated plates which attain a size of 1.0 × 0.3 × 0.03 mm. These grow to full size after a couple of weeks and can be obtained readily. Form III has been much more difficult to obtain. A few crystals have appeared after 10–12 months as small blocks but only two have achieved dimensions of 0.2 × 0.3 × 0.1 mm.

Crystals were mounted in thin-walled glass capillaries then sealed in the presence of mother liquor to maintain high humidity conditions. They were then exposed to X-rays (Rigaku rotating anode at 50 kV and 120 mA, utilizing a graphite monochromator) using the Rigaku R-AXIS II image-plate area detector. The unit cells were determined from three still exposures, 45° apart, using the autoindexing method of Higashi (1990*a*). Data processing was achieved using *PROCESS* (Higashi, 1990*b*). Form II is orthorhombic, space group $P2_12_12_1$ with $a = 73.4$, $b = 120.0$, $c = 190.1$ Å. Form III is monoclinic, space group $P2_1$ with $a = 67.7$, $b = 104.3$, $c = 52.8$ Å, $\beta = 105°$. The availability of some contingency time at DRAL Daresbury Laboratory allowed us to carry out resolution tests on form II. Diffraction to slightly better than 3.0 Å was observed. Form III has only been used in the home laboratory where it was observed to diffract to 2.7 Å resolution. Both crystal forms although reasonably well ordered appear to be radiation sensitive.

In the form II space group there are four equivalent positions and the volume per asymmetric unit is about 427 550 Å^3 which gives values of 2.7 Å^3 Da^{-1}, 55% solvent content (Matthews, 1968) for four subunits in the asymmetric unit. It is more likely that the functional dimer constitutes the asymmetric unit in form III with 2.3 Å^{3}Da^{-1} (48% solvent content). The range of values typically observed for protein crystals is 1.68–3.53 Å^3 Da^{-1} and 27–65% solvent volume.

In summary, we have obtained additional crystal forms of a class II fructose-1,6-bisphosphate aldolase. They present new forms with which to attempt heavy-atom derivatization and since these forms have multiple copies of the enzyme molecule in the asymmetric units, they may allow averaging methods to help determine the structures. Finally, the structure determination of the differerent forms will eventually allow us to determine any influence of crystal lattice packing effects on the molecular conformation.

We thank Sean McSweeney, Miroslav Papiz and Pierre Rizkallah for support at the synchrotron and Daresbury Laboratory for the provision of beamtime. This study is supported by the EPSRC (UK), the Wellcome Trust, and the University of Manchester. AB was a Royal Society 1983 University Research Fellow, WNH is a Nuffield Foundation Research Fellow.

References

ALEFOUNDER, P. R., BALDWIN, S. A., PERHAM, R. N. & SHORT, N. J. (1989). *Biochem. J.* **257**, 529–534.

BALDWIN, S. A. & PERHAM, R. N. (1978). *Biochem. J.* **169**, 643–652.

BANNER, D. W., BLOOMER, A. C., PETSKO, G. A., PHILLIPS, D. C., POGSON, C. I., WILSON, I. A., CORRAN, P. H., FURTH, A. I., MILMAN, J. D., OFFORD, R. E., PRIDDLE, J. D. & WALEY, S. G. (1975). *Nature (London)*, **255**, 609–614

BERRY, A. & MARSHALL, K. E. (1993). *FEBS Lett.* **318**, 11-16.

BRÄNDEŃ, C. & TOOZE, J. (1991). *Introduction to Protein Structure.* New York/London: Garland Publishing Inc.

DREYER, M. K. & SCHULZ, G. E. (1993). *J. Mol. Biol.* **238**, 549–553.

DUCRUIX, A. & GIEGÉ, R. (1992). *Crystallization of Nucleic Acids and Proteins, A Practical Approach.* Oxford: IRL Press/Oxford Univ. Press.

GAMBLIN, S. J., DAVIES, G. J., GRIMES, J. M., JACKSON, R. M., LITTLECHILD, J. A. & WATSON, H. C. (1991). *J. Mol. Biol.* **219**, 573–576.

GENETICS COMPUTER GROUP (1991). *Program Manual for the GCG Package, Version 7.* 575 Science Drive, Madison, Wisconsin, USA.

HESTER, G., BRENNER-HOLZACH, O., ROSSI, F. A., SMIT, J. D. G., STRUCK-DONTAZ, M., WINTERHALTER, K. H. & PIONTEK, K. (1991). Thirteenth European Crystallographic Meeting, Book of Abstracts. P-1.51, p. 147.

HIGASHI, T. (1990*a*). *J. Appl. Cryst.* **23**, 253–257.

HIGASHI, T. (1990*b*) *PROCESS. A Program for Indexing and Processing R-AXIS* II *Imaging Plate Data.* Rigaku Corporation, Japan.

HORECKER, B. L., TSOLAS, O. & LAI, C. Y. (1972). *The Enzymes*, ch. 6 Aldolases, edited by P. D. BOYER, 3rd ed., Vol. 7, pp. 213–258. New York: Academic Press.

IZARD, T., LAWRENCE, M. C., MALBY, R. L., LILLEY, G. G. & COLMAN, P. M. (1994). *Structure*, **2**, 361–369.

JANCARIK, J. & KIM, S.-H. (1991). *J. Appl. Cryst.* **24**, 409–411.

LEBIODA, L., HATADA, M. H., TULINSKY, A. & MAVARIDIS, I. M. (1982). *J. Mol. Biol.* **162**, 445-458.

LITTLECHILD, J. A. & WATSON, H. C. (1963). *Trends Biochem. Sci.* **18**, 36–39.

MCPHERSON, A. (1982). *The Preparation and Analysis of Protein Crystals.* New York: John Wiley.

MARSH, J. J. & LEBHERZ, H. G. (1992). *Trends Biochem. Sci.* **17**, 110–113.

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

MILDVAN, A. S., KOBES, R. D. & RUTTER, W. J. (1971). *Biochemistry*, **10**, 1191–1204.

NAISMITH, J. H., FERRARA, J. D., BAILEY, S., MARSHALL, K., DAUTER, Z., WILSON, K. S., HABASH, J., HARROP, S. J., BERRY, A. & HUNTER, W. N. (1992). *J. Mol. Biol.* **225**, 1137–1141.

PACKMAN, L. C. & BERRY, A. (1995). *Eur. J. Biochem.* Submitted.

SYGUSCH, J., BEAUDRY, D. & ALLAIRE, M. (1987). *Proc. Natl Acad Sci. USA*, **84**, 7846–7850.

WHITE, A. ALLAIRE, M., BEAUDRY, D. & SYGUSCH, J. (1991). Amer. Crystallogr. Assoc. Annual Meet. Abstract. PG20.