Acta Cryst. (1997). D53, 229–230

Crystallization and initial X-ray analysis of xylose isomerase from *Thermotoga neapolitana*

NAOMI E. CHAYEN,[a] ELENA CONTI[a] CLAIRE VIEILLE[b] AND J. GREGORY ZEIKUS[b,c] *at* [a]*Blackett Laboratory, Imperial College of Science, Technology and Medicine, London SW7 2BZ, England,* [b]*Department of Biochemistry, Michigan State University, East Lansing, M148824, USA, and* [c]*Michigan Biotechnology Institute, 2900 Dustin Road, Lansing M148909, USA. E-mail: n.chayen@ic.ac.uk*

(*Received 1 August 1996; accepted 5 September 1996*)

Abstract

Crystals of xylose isomerase from the hyperthermophile *Thermotoga neapolitana* have been grown using the microbatch method under oil from solutions containing Jeffamine ED 4000 as precipitant. The space group of the crystals is $C222_1$ with cell dimensions $a = 161.8$, $b = 121.9$, $c = 98.9$ Å and they contain two monomers in the asymmetric unit. The crystals diffract beyond 2.7 Å.

1. Introduction

D-xylose isomerases (E.C. 5.3.1.5) catalyse the isomerization of D-xylose into D-xylulose. As they are also able to convert D-glucose into D-fructose, they are also known as glucose isomerases. Used in the production of fructose (high fructose corn syrup), xylose isomerases represent the largest industrial use of immobilized enzymes (Crueger & Crueger, 1990). The reaction catalysed by xylose isomerases is an equilbrium reaction, and the ratio between glucose and fructose is shifted toward fructose at higher temperatures. The industrial isomerization temperature (currently 331–333 K) is limited by the marginal thermostability of the enzymes, by their pH requirements and by the unwanted side reactions (Maillard reactions) taking place at high temperatures and alkaline pHs. Isolating more thermostable and acid-tolerant xylose isomerases should allow the isomerization process to be performed at higher temperature and lower pH, thus improving the fructose yield without increasing the side reactions.

Thermotoga neapolitana xylose isomerase is the most thermophilic xylose isomerase cloned and characterized. This enzyme has been reported as a tetramer of identical 51 kDa subunits (Vieille, Hess, Kelly & Zeikus, 1995). but it can also be purified as an active dimer (V. Tchernajenko, unpublished work; M. Hess, personal communication). Highly thermostable, *Thermotoga neapolitana* xylose isomerase is optimally active at temperatures above 368 K and at pH 7.1 but retains 80% maximal activity at pH 6.1. The following traits make this enzyme a type II xylose isomerase: it is highly homologous with other type II enzymes; it contains an additional 50-residue N-terminal extension not present in type I enzymes; and, unlike type I enzymes, it is more active in the presence of Co^{2+} (with glucose as substrate) than in the presence of Mg^{2+} (Vieille, Hess, Kelly & Zeikus, 1995).

Study of the crystal structure of this hyperthermostable enzyme forms part of a joint study in our laboratories towards producing xylose isomerases with improved properties required to further stabilize this and other xylose isomerases. The conditions in which *T. neapolitana* xylose isomerase has been crystallized and the preliminary X-ray analysis of the crystals are reported below.

2. Experimental

The recombinant *T. neapolitana* xylose isomerase was purified as described by Vieille, Hess, Kelly & Zeikus (1995).

The protein was stored at 253 K in a solution containing 50 m*M* MOPS, pH 7.0, 5 m*M* $MgSO_4$, 0.5 m*M* $CoCl_2$ and 50% glycerol. Prior to crystallization the glycerol was removed by serial exchanges against 50 m*M* MOPS buffer pH 7.0, 5 m*M* $MgSO_4$ and 0.5 m*M* $CoCl_2$ and finally concentrated on a Centricon with a molecular cut-off of 30 000 M_r.

Using the microbatch under-oil method (Chayen, Shaw Stewart & Blow, 1992), indication of conditions for crystallization were obtained in 2 µl drops from a sparse-matrix screening protocol (Jancarik & Kim, 1991; A. D'Arcy, personal communication). Small crystals appeared in trials containing either polyethylene glycol (PEG) or Jeffamine ED 4000 as precipitants.

Improved crystallization conditions were obtained in further microbatch trials using the optimization mode of the IMPAX automated crystallization system (Chayen, Shaw Stewart, Maeder & Blow, 1990). The best crystals grew at 291 K in 2 µl drops under oil from the following final concentrations: 13 mg ml^{-1} protein, 14% Jeffamine ED 4000, 5 m*M* $MgSO_4$, 0.5 m*M* $CoCl_2$ and 50 m*M* MOPS pH 7.0. Crystals grown with PEG as precipitant were not as large as those obtained with Jeffamine. Single crystals appeared as small bipyramids within 2 d and reached their maximum size of 0.8 × 0.4 × 0.2 mm (Fig. 1) in approximately 10 d.

It is interesting to note that crystals of equal quality could also be grown in 1.3 µl drops and that increasing the drop volume to 4 µl did not improve crystal size or quality.

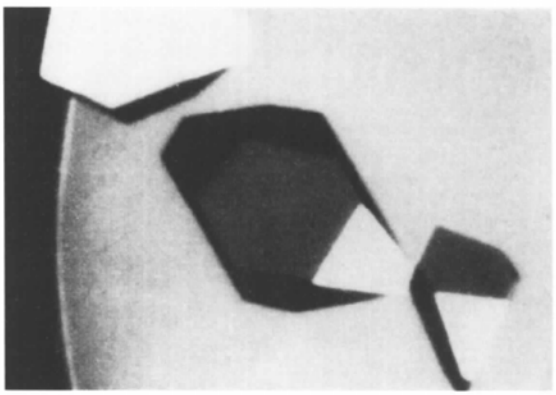

Fig. 1. Crystals of xylose isomerase from *Thermotoga neapolitana*. Actual size: 0.8 × 0.4 × 0.2 mm.

3. Crystal characterization

X-ray data were collected at room temperature on CAD-4 Enraf–Nonius diffractometer equipped with a FAST television area detector, using an Enraf–Nonius FRS71 rotating-anode X-ray generator operated at 42 kV and 99 mA.

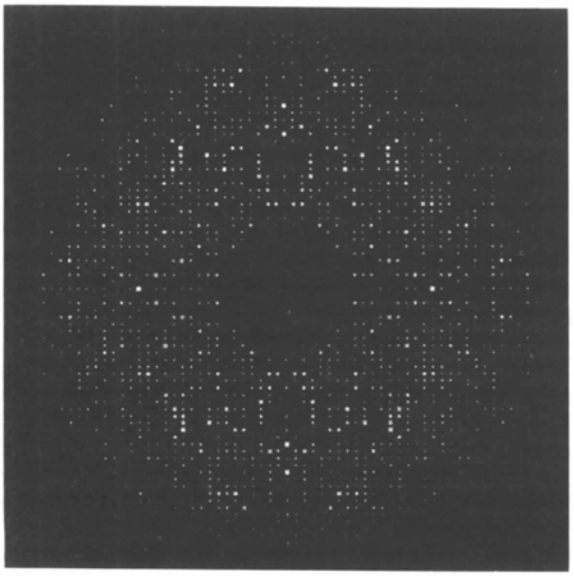

Fig. 2. Pseudo-precession image of the $h0l$ zone of the reciprocal lattice, with the h and l axes horizontal and vertical, respectively, displayed using the program *HKLVIEW* (Collaborative Computational Project, Number 4, 1994). The spots are represented as squares whose size depends on the amplitude. The outer resolution unit corresponds to 2.7 Å, where $\langle I/\sigma(I)\rangle = 4.3$.

The crystal diffraction was consistent with space group $C222_1$ with cell dimensions $a = 161.8$, $b = 121.9$ and $c = 98.9$ Å. Assuming two monomers per asymmetric unit, the specific volume $V_m = 2.4$ Å^3 Da^{-1} of protein corresponds to a solvent content of about 50%(v/v) (Matthews, 1968).

A complete data set to 2.7 Å resolution with a merging R factor of 7.7% was collected using exposure times of 90 s per 0.15° rotation frame, with the detector swung 20° from the X-ray beam axis and a detector-to-crystal distance of 100 mm. The data from the FAST area detector were evaluated on-line using the program *MADNES* (Messerschmidt & Pflugrath, 1987). A representation of diffraction in the $h0l$ zone is given in Fig. 2.

NEC wishes to thank Professor D. M. Blow FRS for support throughout the project and is grateful for funding from the BBSRC grant No. GR/H86172. Meilin Sancho is thanked for providing Fig. 1. EC acknowledges funding from an EEC grant. JGZ and CV acknowledge funding from the US Department of Agriculture, Grant 89-34189-4299.

References

Chayen, N., Shaw Stewart, P. D. & Blow, D. M. (1992). *J. Cryst. Growth*, **122**, 176–180.

Chayen, N., Shaw Stewart, P. D., Maeder, D. L. & Blow, D. M. (1990). *J. Appl. Cryst.* **23**, 297–302.

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

Crueger, A. & Crueger, W. (1990). *Microbial Enzymes and Biotechnology*, edited by W. M. Fogarty & C. T. Kelly, 2nd ed., pp. 177–226, New York: Elsevier.

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

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

Messerschmidt, A. & Pflugrath, J. W. (1987). *J. Appl. Cryst.* **20**, 306–315.

Vieille, C., Hess, J. M., Kelly, R. M. & Zeikus, J. G. (1995). *Appl. Environ. Microbiol.* **61**, 1867–1875.