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Crystallization and preliminary X-ray crystallographic data with *Escherichia coli* transketolase. By

JENNIFER LITTLECHILD* and NICHOLAS TURNER, *Department of Chemistry and Biological Sciences, University of Exeter, Stocker Road, Exeter EX4 4QD, England*, GORDON HOBBS and MALCOLM LILLY, *Advanced Centre for Biochemical Engineering, Department of Chemical and Biochemical Engineering, University College London, Torrington Place, London WC1E 7JE, England*, and AHMED RAWAS and HERMAN WATSON, *Department of Biochemistry, University of Bristol, Bristol BS8 1TD, England*

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Abstract

The *Escherichia coli* enzyme transketolase, a dimeric protein of 2 × 70 kDa (662 amino acids) has been prepared from an overexpression system in *E. coli*. The purified enzyme has been crystallized from PIPES buffer pH 6.4 and ammonium sulfate. The crystals which grow as large plates diffract to greater than 1.9 Å resolution and are of the space group $P2_12_12_1$ with unit-cell dimensions of $a = 74.6$, $b = 125.6$ and $c = 151.0$ Å ($Z = 8$ with one transketolase dimer in the asymmetric unit). The structure has been solved by molecular replacement using the yeast transketolase enzyme structure as a search model. The enzyme is being used for large-scale biotransformations using various aldehydes and hydroxypyruvate as substrates.

Introduction

The enzyme transketolase catalyses the reversible synthesis of higher carbon sugars in the pentose phosphate pathway (Racker, 1961). Specifically transketolase mediates the transfer of a two-carbon ketol unit from a ketose to an aldose. The enzyme requires both divalent magnesium ions and thiamine pyrophosphate for activity. Synthetically the reaction can be made more effective by the use of hydroxypyruvate as the ketol donor. In this way the reaction is rendered effectively irreversible owing to the release of carbon dioxide.

The substrate specificity of both the yeast enzyme (Lindqvist, Schneider, Ermler & Sundstrom, 1992) and to a lesser extent the spinach enzyme (Villafranca & Axelrod, 1971) has been investigated. These studies have revealed a relatively low specificity for the aldehyde component and recently it was shown that there is no absolute requirement for an α-hydroxyl group in the aldehyde substrate (Hobbs *et al.*, 1993). The low specificity for the aldehyde coupled with the high stereoselectivity in the C—C bond-forming step combine to make transketolase a potentially important catalyst for asymmetric C—C bond synthesis. Bearing in mind the potential of transketolase as a catalyst for organic synthesis, we have also become interested in the investigation of the underlying principles associated with operating this biocatalytic process on a large scale. The DNA sequence of the *E. coli* transketolase gene has been published recently (Sprenger, 1993) and this allows comparisons to be made between the deduced amino-acid sequence of the *E. coli* transketolase and that of yeast. The proteins appear to be highly homologous with 43% of the total amino acids conserved. The yeast enzyme structure has been solved by X-ray crystallography to 2 Å by the group of Lindqvist (Lindqvist *et al.*, 1992; Nikkola, Lindqvist & Schneider, 1994).

* Author for correspondence.

Experimental

Purification of E. coli transketolase

The initial work carried out resulted in the successful overexpression of the *E. coli* transketolase gene (Draths & Frost, 1990) in *E. coli*, JM107 and pUC18 (Hobbs *et al.*, 1993). The level of overexpression has subsequently been improved to 40% of the total cell protein (French & Ward, 1994). The overexpressed enzyme has been purified using a simple efficient purification shown in Table 1 that yielded homogenous protein. *E. coli* cells carrying 5 kb DNA fragment in the multicopy plasmid pUC18 were grown in 20–1000 l fermentation runs at University College, London. Cells were disrupted into buffer *A* [10 m*M* Tris–HCl pH 7.5, 1 m*M* EDTA, 0.006 *M* β-mercaptoethanol and the protease inhibitors 1×10^5 *M* phenylmethylsulfonyl fluoride (PMSF) and 2×10^{-5} benzamidine (BAM)] and the cell debris removed by centrifugation. The DNA was removed from the crude extract by precipitation with protamine sulfate (0.05% final concentration). The extract was centrifuged at 15 000 rev min^{-1} for 30 min. The supernatant was made up to 50% with respect to enzyme-grade ammonium sulfate, stirred for 30 min and centrifuged as above. The supernatant was made up to 75% ammonium sulfate and after stirring and centrifugation the resulting pellet containing the transketolase activity was resuspended in buffer *A*. This was dialysed against buffer *A* to remove ammonium sulfate before loading onto a Fast Flow Q Sepharose (Pharmacia) ion-exchange column. The active peak was eluted with a gradient of 0–1.0 *M* NaCl, and pooled and precipitated with 80% ammonium sulfate. After centrifugation the pellet was resuspended into buffer *A* containing 5% ammonium sulfate and fractionated by high-resolution gel filtration on a preparative Superose 12 fast protein liquid chromatography column (Pharmacia). Transketolase was assayed using the linked assay system (Heinrich, Noack & Wiss, 1972) with the linking enzymes phosphoriboisomerase, D-ribulose-5-*P*-3-epimerase, triose phosphate isomerase and α-glycerophophosphate dehydrogenase (Sigma). The active peak was pooled being careful not to include any traces of aggregated material. The enzyme was shown to be homogenous by sodium dodecyl sulfate polyacrylamide gel electrophoresis (Laemmili & Favre, 1973) and has a molecular weight of 70 000 kDa

Crystallization

The purified enzyme was concentrated to approximately 20 mg ml^{-1} using Amicon Centricon 10 microconcentrators. The sample was exchanged into 50 m*M* PIPES buffer pH 6.4 containing 20 m*M* thiamine pyrophosphate and 90 m*M* $CaCl_2$ by addition of four aliquots of 2.5 ml of buffer and repeated

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Table 1. *Purification of Escherichia coli transketolase*

Step	[TK] ($U^* ml^{-1}$)	Protein ($mg ml^{-1}$)	Activity ($U^* mg^{-1}$)	X-fold purification	Total units*	% Yield
Crude extract	167.5	19.3	8.7	1.0	10500	100
Protamine sulfate precipitation/$(NH_4)_2SO_4$ 50→75% Fractionation	112.9	4.5	25.1	2.9	3720	37
Fast flow Q Ion exchange	92.0	2.7	34.1	3.9	3312	33
FPLC preparative Superose 12 Gel Filtration	121.7	2.6	46.8	5.4	2069	21

* µmol of NADH utilized min^{-1} at 308 K, pH 7.5 in the linked assay system (Heinrich *et al.*, 1972).

concentration by centrifugation at 7000 rev min^{-1}. Material prepared in this way produced crystals grown from $(NH_4)_2SO_4$ by vapour-phase diffusion which are suitable for structural determination. The crystals grow from 46% $(NH_4)_2SO_4$, PIPES buffer pH 6.4 in approximately 1 week and can grow to 2–3 mm in their largest dimensions as shown in Fig. 1.

X-ray data collection

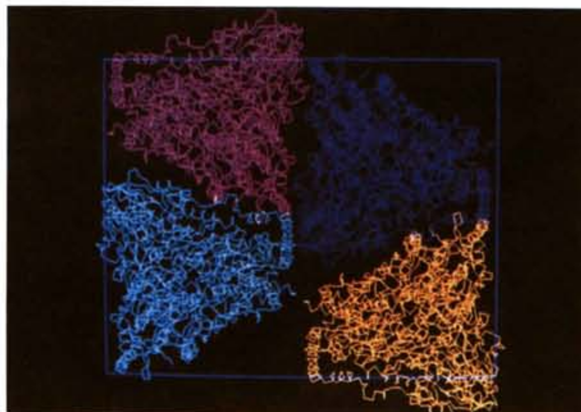
Two crystals of the *E. coli* transketolase were used to collect essentially complete medium-resolution data sets for use in molecular-replacement calculations. Data from the larger crystal was collected using a Siemens Xentronics area-detector system and exposed to a 0.3 mm collimated X-ray beam (Cu $K\alpha$, λ = 1.5418 Å) supplied by a Siemens rotating-anode generator operating at 45 kV, 80 mA. A second data set was collected from a smaller crystal using the R-AXIS image-plate system (York University Chemistry Department, Dr G. Davies).

Results and discussion

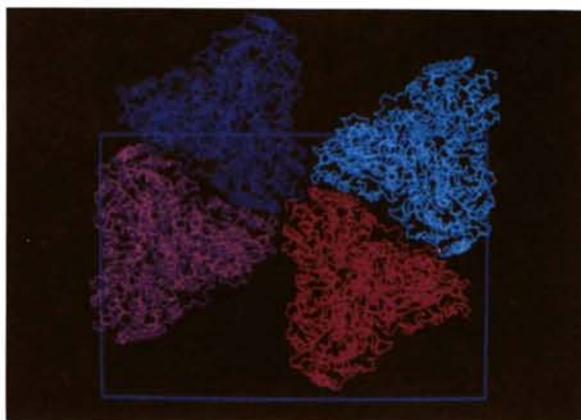
The crystals diffracted well beyond 3.0 Å resolution and 180° of data to 3.0 Å resolution were collected. The data were processed with the *XENGEN* package (Howard, 1988). These data gave R_{merge} values of 9 and 12% for intensities for the area-detector and the image-plate data, respectively. The two data sets, after scaling, gave a merging R value for all reflections of 10% and were 98% complete to a resolution of 3 Å. The crystallographic unit cell was identified using the auto-indexing program *REFINE* from the *XENGEN* package. This gave an orthorhombic crystal system and the unit-cell dimensions refined to $a = 74.6$, $b = 125.6$, $c = 151.0$ Å. The inspection of the systematic absences indicate that the space group is $P2_12_12_1$ with one transketolase dimer in the asymmetric unit. The V_m (Matthews, 1968) is 2.53 Å^3 Da^{-1} corresponding to 51.3% solvent. This is similar to the unit cell reported for the yeast enzyme ($P2_12_12_1$ $a = 76.3$, $b = 113.3$, $c = 160.9$ Å) which was found to contain one transketolase dimer in each asymmetric unit.

The structure of the *E. coli* transketolase was determined using the molecular-replacement method utilizing the data falling between 10 to 4 Å of completeness to 95%. The yeast transketolase dimer was used as search model and was placed in an artificial $P1$ unit cell with dimensions $a = b = c = 250$ Å. The refined individual atomic temperature factors of the model were used and Patterson vector of 5–45 Å, and the diffraction

Fig. 1. Crystals of *E. coli* transketolase grown from ammonium sulfate. Largest crystal in this view is 2 × 0.5 mm approximately.



(*a*)



(*b*)

Fig. 2. Crystal packing of *E. coli* transketolase. (*a*), compared with that of yeast transketolase enzyme (*b*) (Lindqvist *et al.*, 1992). The protein is represented as a Cα trace.

data falling in the 10–4 Å resolution range was used in all subsequent search operations using the *X-PLOR* software package (Brünger, 1992). Two prominent peaks were observed in the cross-rotation function, both of which were some 1.3 times higher than the next highest peak and are related by 180°. All possible solutions for the selected cross-rotation function peaks were examined using the dimer and then the monomer in the rigid-body Patterson correlation refinement procedure. The preferred solution, that corresponding to one of the principal peaks in the cross rotation function, produced a peak some four times higher than that of the general background. A translation search carried out using the preferred 'dimer' orientation produced a solution some 50% higher than the next highest peak in the translation function. The yeast dimer rotated though Eulerian angles of $\alpha = 205.9$, $\beta = 57.5$, $\gamma = 174.1°$ and placed (the dimer 'centre') at a position $x = 0.121$, $y = 0.089$ and $z = 0.380$ (fractional coordinates) from the *E. coli* cell origin, as indicated by the molecular-replacement calculations, produces a molecular-packing arrangement which is significantly different to that found for the yeast enzyme but nevertheless has few steric violations (Fig. 2). The molecular-replacement solution gave an R factor of 0.4.

High-resolution data collection from crystals grown with and without substrates is now in progress. The crystals diffract to greater than 1.9 Å resolution and the structure has been refined to an R factor of 0.13 (the details of the high-resolution structure will be published elsewhere). Comparisons will be made with the yeast transketolase enzyme. Site-directed mutants of the *E. coli* transketolase are being constructed with a view to understanding and potentially modifying substrate specificity for the industrial chemist.

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References

Brünger, A. T. (1992). *X-PLOR Manual. Version* 3.1. Yale Univ., New Haven, CT.

Draths, K. M. & Frost, J. W. (1990). *J. Am. Chem. Soc.* **112**, 1657–1659.

French, C. & Ward, J. (1995) *Biotech. Lett.* **17**, 247–252.

Heinrich, C. P., Noack, K. & Wiss, O. (1972). *Biochim. Biophys. Res. Commun.* **49**, 1427–1432.

Hobbs, G. R., Lilly, M. D., Turner, N. J., Ward, J. M., Willetts, A. J. & Woodley, J. M. (1993). *J. Chem. Soc. Perkin Trans.* I, pp. 165–166.

Howard, A. J. (1988). *A Guide to Macromolecular X-ray Reduction for the Nicolet Area Detection: the XENGEN System, Version* 1.3. Protein Engineering Department, Genex Corporation, MD, USA.

Laemmili, U. V. & Favre, M. (1973). *J. Mol. Biol.* **80**, 575–599.

Lindqvist, Y., Schneider, G., Ermler, U. & Sundstrom, M. (1992). *EMBO J.* **11**, 2373-2379

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

Nikkola, M., Lindqvist, Y. & Schneider, G. (1994). *J. Mol. Biol.* **238**, 387–404.

Racker, E, (1961). *The Enzymes*, edited by P. D. Boyer, H. Lardy & K. Myrzback. Vol. V p. 397. New York: Academic Press.

Sprenger, G. A. (1993). *Biochim. Biophys. Acta*, **1216**, 307–310.

Villafranca, J. & Axelrod, B. (1971). *J. Biol. Chem.* **246**, 31–26.