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Expression, purification, crystallization and preliminary X-ray crystallographic data from TktA, a transketolase from the lactic acid bacterium *Lactobacillus salivarius*

The enzyme transketolase from the lactic acid bacterium *Lactobacillus salivarius* (subsp. *salivarius* UCC118) has been recombinantly expressed and purified using an *Escherichia coli* expression system. Purified transketolase from *L. salivarius* has been crystallized using the vapour-diffusion technique. The crystals belonged to the trigonal space group $P3_221$, with unit-cell parameters $a = b = 75.43$, $c = 184.11$ Å, and showed diffraction to 2.3 Å resolution.

1. Introduction

The transketolase enzyme was first identified in *Saccharomyces cerevisiae* (Turner, 2000). The enzyme acts as a link between glycolysis and the pentose-phosphate pathway and relies on divalent magnesium ions and thiamine pyrophosphate for activity. Transketolase catalyses the reversible synthesis of higher carbon sugars in the pentose-phosphate pathway, mediating the transfer of a two-carbon ketol unit from a ketose to an aldose (specifically from D-xylulose 5-phosphate to D-ribose 5-phosphate, generating D-sedulose 7-phosphate and D-glyceraldehyde 3-phosphate in the process; Turner, 2000). Here, we present work on the protein LSL_1946 from the lactic acid bacterium *L. salivarius*, which has been identified as the transketolase enzyme TktA. Here, we present data on the over-expression, purification, crystallization and preliminary X-ray diffraction data analysis of LSL_1946.

2. Materials and methods

2.1. Protein expression and purification

The PCR product coding for the transketolase gene LSL_1946 was cloned into the expression vector pOPINF using In-Fusion cloning technology (Clontech) in-frame with an N-terminal His₆ tag separated by a HRV-C3 protease cleavage site. The vector was transformed into competent *Escherichia coli* DH5- α cells and grown at 310 K on LB agar plates supplemented with 54 $\mu\text{g ml}^{-1}$ carbenicillin. A single colony was used to inoculate 50 ml LB supplemented with 54 $\mu\text{g ml}^{-1}$ carbenicillin and the culture was grown overnight in a shaking incubator operated at 310 K and 170 rev min⁻¹. Plasmids harvested from the overnight culture using a Qiagen Plasmid Mini-prep kit (Qiagen) were transformed into the protein-expression strain *E. coli* 2(DE3)pLysS and grown at 310 K on LB agar plates supplemented with 54 $\mu\text{g ml}^{-1}$ carbenicillin. A single colony was used to inoculate 50 ml LB supplemented with 54 $\mu\text{g ml}^{-1}$ carbenicillin and 34 $\mu\text{g ml}^{-1}$ chloramphenicol and the culture was grown overnight at 310 K and 170 rev min⁻¹. Two 500 ml cultures of LB medium were each inoculated with 5 ml of this starter culture and incubated at 310 K and 170 rev min⁻¹ until an OD₆₀₀ of 0.6 was reached. The cultures were then induced with 0.5 ml 1 M isopropyl β -D-1-thiogalactopyranoside (IPTG) and incubated at 295 K for the remainder of the 24 h period. Cells were harvested by centrifugation at 5000g and resuspended in 50 ml buffer A (50 mM Tris-HCl pH 7.5, 500 mM NaCl, 20 mM imidazole) supplemented with 0.1% Tween. Cells were

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Table 1

Data-collection statistics for TktA.

Values in parentheses are for the outer shell.

Source	ID14-1, ESRF, Grenoble
Wavelength (Å)	0.934
Resolution (Å)	36.94–2.30 (2.42–2.30)
Space group	$P3_221$
Unit-cell parameters (Å, °)	$a = b = 75.43$, $c = 184.11$, $\alpha = \beta = 90.0$, $\gamma = 120.0$
R_{merge}	0.126 (0.352)
Total No. of reflections	55793
No. of unique reflections	25397
Average redundancy	2.2
Completeness (%)	92.8 (93.6)

lysed by five passes through a French pressure cell set at 6.5 MPa. The cell lysate was centrifuged at 6500g to remove cell debris and the supernatant was then centrifuged at 40 000g for a further 30 min. The cleared lysate was applied onto a 1 ml HisTrap HP nickel-affinity column (GE Healthcare) and the protein was eluted from the column with a linear gradient to buffer *B* (50 mM Tris–HCl pH 7.5, 500 mM NaCl, 500 mM imidazole) on an ÄKTAprime plus (GE Healthcare). The purified protein was dialysed overnight into buffer *A* to remove excess imidazole.

2.2. Crystallization and data collection

The protein was concentrated to 13.2 mg ml^{−1} and crystallized using the sitting-drop vapour-diffusion technique, mixing equal volumes (500 µl) of protein solution and reservoir solution to form the drop (using a HoneyBee 8+1 robot). Crystallization trials included Cryo Screens 1 and 2, Crystal Screens 1 and 2 and PEG/Ion Screens 1 and 2. Crystals grew over a period of 3 d at 293 K in many conditions. The best-quality crystals were observed in condition 6 of PEG/Ion Screen 1 (Hampton Research), which consisted of 0.2 *M* sodium chloride, 20% (w/v) PEG 3350. The crystals appeared as hexagonal rods of approximately 200 × 40 × 40 µm in size (Fig. 1). The crystals were flash-cooled in a stream of nitrogen gas maintained at 110 K, using dried paraffin oil as the cryoprotectant (Riboldi-Tunnicliffe & Hilgenfeld, 1999).

Diffraction data were collected from a single crystal on beamline ID14-1 at the ESRF using an ADSC Q210 CCD detector with a crystal rotation of 1° per frame. A summary of the data-collection statistics is given in Table 1. The data were processed using the

Figure 1
TktA crystals. The drop volume was 1 µl.

program *MOSFLM* (Leslie, 1992) and scaled using the program *SCALA* (Evans, 1993).

3. Results and discussion

The crystal belonged to the trigonal space group $P3_221$, with unit-cell parameters $a = b = 75.43$, $c = 184.11$ Å, and diffracted to 2.3 Å resolution (Figs. 2*a* and 2*b*). The calculated Matthews coefficient (V_M) for one molecule of the protein in the asymmetric unit is 2.03 Å³ Da^{−1}, with 39.36% of the unit cell occupied by solvent

Figure 2
Diffraction image for a single crystal of TktA. The arrow indicates the limit of diffraction at 2.3 Å resolution. The outer arc shows the diffraction limit at 2.9 Å.

(Matthews, 1968); the biologically relevant dimer is created through a symmetry operation. The structure has been solved by the molecular-replacement method using the *PHENIX* suite of crystallographic programs (Adams *et al.*, 2002) and is currently undergoing refinement.

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References

- Adams, P. D., Grosse-Kunstleve, R. W., Hung, L.-W., Ioerger, T. R., McCoy, A. J., Moriarty, N. W., Read, R. J., Sacchettini, J. C., Sauter, N. K. & Terwilliger, T. C. (2002). *Acta Cryst.* **D58**, 1948–1954.
- Evans, P. R. (1993). *Proceedings of the CCP4 Study Weekend. Data Collection and Processing*, edited by L. Sawyer, N. Isaacs & S. Bailey, pp. 114–122. Warrington: Daresbury Laboratory.
- Leslie, A. G. W. (1992). *Jnt CCP4/ESF-EACBM Newsl. Protein Crystallogr.* **26**.
- Matthews, B. W. (1968). *J. Mol. Biol.* **33**, 491–497.
- Riboldi-Tunnicliffe, A. & Hilgenfeld, R. (1999). *J. Appl. Cryst.* **32**, 1003–1005.
- Turner, N. J. (2000). *Curr. Opin. Biotechnol.* **11**, 527–531.