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

Matt Horsham,[a] Harriet Saxby,[b] James Blake,[b] Neil W. Isaacs,[b] Tim J. Mitchell[a] and Alan Riboldi-Tunnicliffe[b]*

[a]FBLS I&I, University of Glasgow, 120 University Place, Glasgow G12 8TA, Scotland, and [b]Department of Chemistry, University of Glasgow, 120 University Place, Glasgow G12 8TA, Scotland

Correspondence e-mail: alan.r-t@synchrotron.org.au

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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 overexpression, 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 His_6 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 µg ml^{-1} carbenicillin. A single colony was used to inoculate 50 ml LB supplemented with 54 µg 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 Miniprep 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 µg ml^{-1} carbenicillin. A single colony was used to inoculate 50 ml LB supplemented with 54 µg ml^{-1} carbenicillin and 34 µg 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 OD_{600} 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 5000*g* and resuspended in 50 ml buffer *A* (50 m*M* Tris–HCl pH 7.5, 500 m*M* NaCl, 20 m*M* imidazole) supplemented with 0.1% Tween. Cells were

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 6500*g* to remove cell debris and the supernatant was then centrifuged at 40 000*g* 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 m*M* Tris–HCl pH 7.5, 500 m*M* NaCl, 500 m*M* 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 program *MOSFLM* (Leslie, 1992) and scaled using the program *SCALA* (Evans, 1993).

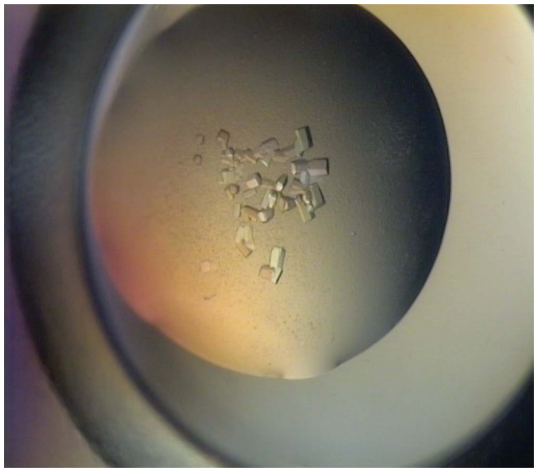

Figure 1
TktA crystals. The drop volume was 1 µl.

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 Å^3 Da^{-1}, with 39.36% of the unit cell occupied by solvent

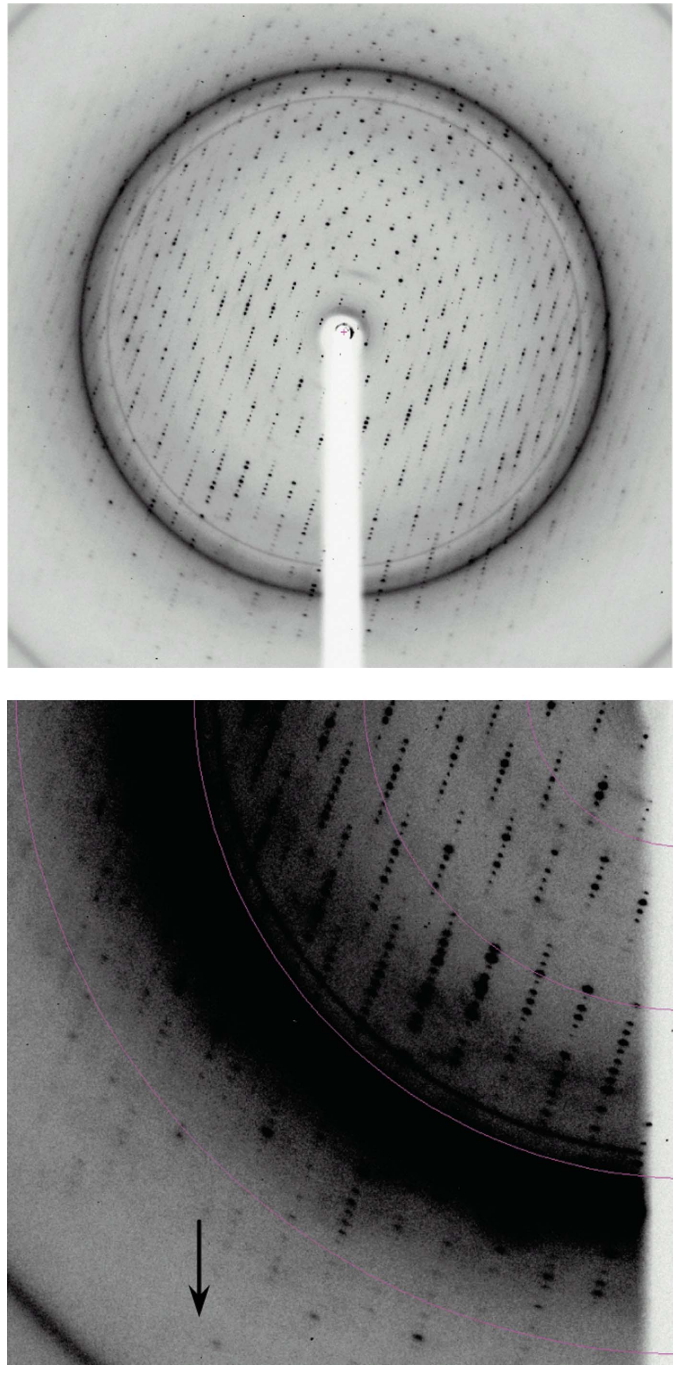

Figure 2
Diffraction image for a single crystal of TktA. The arrow indicates the limit of diffraction at 2.3 Å resolution. The outer arc shows the diffraction limit at 2.9 Å.

(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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