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Expression, crystallization and preliminary X-ray analysis of isomaltulose synthase (PalI) from *Klebsiella* sp. LX3

Nan Li,[a] Daohai Zhang,[b] Lian-Hui Zhang[b] and Kunchithapadam Swaminathan[a,c]*

[a]Laboratory of X-ray Crystallography, Institute of Molecular and Cell Biology, 30 Medical Drive, National University of Singapore, Singapore 117609, Singapore, [b]Laboratory of Biosignals and Bioengineering, Institute of Molecular and Cell Biology, 30 Medical Drive, National University of Singapore, Singapore 117609, Singapore, and [c]Department of Biological Sciences, National University of Singapore, Singapore 117543, Singapore

Correspondence e-mail: nathan@imcb.nus.edu.sg

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Isomaltulose synthase (PalI) catalyzes the hydrolysis of the α-1,2 bond between the glucose and fructose moieties of sucrose and the formation of α-1,6 and α-1,1 bonds between the two components to produce isomaltulose (α-D-glucosylpyranosyl-1,6-D-fructofranose) and trehalulose (α-D-glucosylpyranosyl-1,1-D-fructofranose), respectively. The PalI protein has been overexpressed, purified and crystallized at 295 K using the hanging-drop vapour-diffusion method. The crystals diffract to 2.2 Å resolution using synchrotron radiation and belong to the orthorhombic space group $P2_12_12_1$, with unit-cell parameters a = 59.239, b = 94.153, c = 111.294 Å.

1. Introduction

Isomaltulose synthase (PalI) catalyzes the isomerization of sucrose to produce isomaltulose (α-D-glucosylpyranosyl-1,6-D-fructofranose), trehalulose (α-D-glucosylpyranosyl-1,1-D-fructofranose) and trace amounts of glucose and fructose as byproducts (Huang *et al.*, 1998; Veronese & Perlot, 1999). Several microorganisms have been found to form isomaltulose and trehalulose from sucrose; for example, *Protaminobacter rubrum* (Weidenhagen & Lorenz, 1957), *Serratia plymuthica* (Fujii *et al.*, 1983), *Erwinia rhapontici* (Cheetham, 1984), *Klebsiella planticola* CCRC 19112 (Huang *et al.*, 1998), *Pseudomonas mesoacidophila* MX-45 (Nagai *et al.*, 1994) and *Agrobacterium radiobacter* MX-232 (Nagai-Miyata *et al.*, 1993). Recently, the gene encoding PalI from *Erwinia rhapontici* has been reported (Bornke *et al.*, 2001). To understand the basic biochemical characteristics of this isomerase, we cloned the *palI* gene (GeneBank accession No. AY040843) from the bacterial isolate *Klebsiella* sp. LX3 and the expression of fully active enzyme in *Escherichia coli* has been achieved (Zhang *et al.*, 2002). Based on amino-acid sequence similarities, PalI belongs to the glucoside hydrolase family 13 (Davies & Henrissat, 1995), including enzymes acting on starch, such as α-amylase and cyclodextrin glycosyltransferase (CGTase; Klein & Schulz, 1991), and enzymes specific for cleavage of other glucosidic linkages, such as α-1,6 and α-1,1 bonds. The three-dimensional structure of PalI is unknown, although structure prediction indicated it to contain a $(\beta/\alpha)_8$-barrel (Zhang *et al.*, 2002) similar to that of oligo-1,6-glucosidase (OGL) from *Bacillus cereus* (Kizaki *et al.*, 1993) and amylosucrase (AS) from *Neisseria polysacchrea* (Skov *et al.*, 2001). However, the biochemical mechanism of PalI is different from those of OGL and AS, as PalI is responsible for a two-step enzyme reaction, *i.e.* hydrolysis of the α-1,2 glycosidic bond of sucrose and then formation of α-1,6 and α-1,1 bonds to produce isomaltulose and trehalulose, respectively. Determination of the PalI structure is important and will contribute to a detailed understanding of the double reaction mechanism of this class of enzymes.

2. Materials and methods

2.1. PalI protein expression and purification

The *palI* gene from *Klebsiella* sp. LX3 was cloned in the vector pGEX-2T (Amersham Phamacia Biotech) to create pGEK (Zhang *et al.*, 2002) and transformed into *E. coli* BL21 (DE3). The transformed cells were grown at 310 K to an OD_{600} of 0.6 in LB medium containing 100 μg ml^{-1} ampicillin. IPTG was added to a final concentration of 0.5 m*M* to induce the GST-PalI expression at 303 K. The harvested cells were resuspended in 1× PBS buffer and lysed using a French press at 6.9 MPa. The GST fusion protein was bound to glutathione-Sepharose resin (Amersham Phamacia Biotech), washed with 1× PBS buffer and digested with thrombin protease (Amersham Pharmacia Biotech), according to the manufacturer's protocol. The released protein was loaded onto a HiPrep Sephacryl S-200 16/60 column (Amersham Phamacia Biotech) and washed with buffer (150 m*M* NaCl, 10 m*M* HEPES pH 7.5 and 1 m*M* DTT). The fractions that contained the purified protein were pooled and the protein was concentrated to 4.3 mg ml^{-1} (from spectroscopic absorption) by ultrafiltration on Centriprep and Centricon YM-10 devices (Millipore) and stored at 193 K.

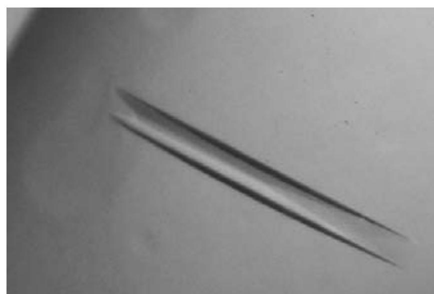

Figure 1
A crystal of PalI in a hanging drop. The longest crystal dimension is 1.2 mm.

2.2. Crystallization of PalI protein and X-ray diffraction analysis

Initial trials for crystallization conditions were set up using the hanging-drop vapour-diffusion method at 295 K with commercially available screening kits from Hampton Research (Jancarik & Kim, 1991; Cudney *et al.*, 1994). Small crystals were seen after about eight months in crystallization drops consisting of 2 µl protein solution (4.3 mg ml^{-1}) mixed with 2 µl of reservoir solution containing 0.1 *M* sodium cacodylate pH 6.5, 0.2 *M* ammonium sulfate and 30%(*w*/*v*) PEG 8000. This condition was further optimized and crystals deemed suitable for diffraction were treated with cryoprotectant (reservoir solution with a 4% increase in precipitant concentration and supplemented with 10% glycerol) for 3 min and flash-cooled in liquid nitrogen. Diffraction data were collected at SPring8, Japan (beamline BL40B2, ADSC Quantum 4 CCD detector, 100 K). The data were indexed, processed and scaled using the programs *DENZO* and *SCALEPACK* (Otwinowski & Minor, 1997).

3. Results and discussion

Overexpression of the GST-PalI fusion protein was carried out at 303 K to increase the soluble portion of the fusion protein. After purification by glutathione-Sepharose affinity and gel-filtration chromatography, the PalI protein was set up for crystallization and clusters of needle-shaped crystals appeared using several conditions. However, large crystals formed only when PEG 8000 was used as precipitant (Fig. 1). The protein concentration was optimized to minimize the initial precipitation. The precipitate gradually dissolved and vanished after several days. Crystals were seen only after about eight months and grew to their full size within a month. The crystals diffract X-rays to a resolution limit of 2.2 Å (Fig. 2). Analysis of the diffraction pattern shows that the crystal belongs to the orthorhombic space group $P2_12_12_1$, with unit-cell parameters a = 59.239, b = 94.153, c = 111.294 Å. Given the PalI molecular weight of 67.17 kDa (as deduced from the amino-acid sequence), the V_M is 2.31 Å^3 Da^{-1} (solvent content of 46.34%) and the asymmetric unit contains one enzyme molecule (Matthews, 1968). The data statistics are shown in Table 1. The overall completeness and R_{sym} are adequate for solving the structure by molecular replacement. Currently, the structure determination and model building are in progress. The structure, when completed, will explain the molecular basis of sucrose binding, hydrolysis, isomerization and the formation of different products.

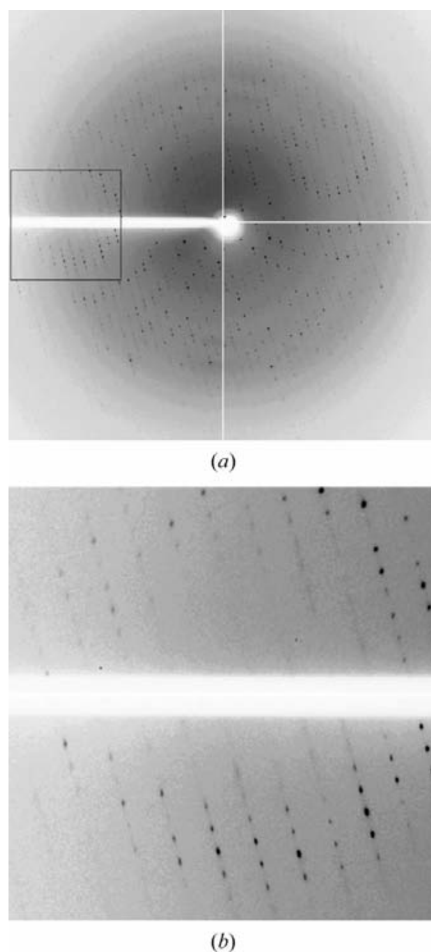

Figure 2
(*a*) X-ray diffraction pattern from a crystal of PalI. (*b*) The diffraction limit at the edge of the image is 2.2 Å.

Table 1
Diffraction data statistics for the PalI protein crystal.

Values in parentheses are for the highest resolution shell (2.28–2.20 Å).

Synchrotron-radiation source	SPring8, Japan (BL40B2)
Detector	ADSC Quantum 4 CCD
Wavelength (Å)	1.0
No. of imaging plates	180
Unit-cell parameters (Å)	a = 59.239, b = 94.153, c = 111.294
Space group	$P2_12_12_1$
Mosaicity of crystal (°)	1.0
Resolution range (Å)	20–2.2
Total No. of reflections	199659
No. of unique reflections	32456
Redundancy	6.1
Completeness (%)	99.2 (96.6)
R_{sym}† (%)	5.3 (26.9)

† $R_{sym} = \sum_j \sum_i |\langle I_i \rangle - I_i| / \sum_i I_i$.

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