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Crystallization of *Escherichia coli* maltoporin in the trigonal space group *R*3

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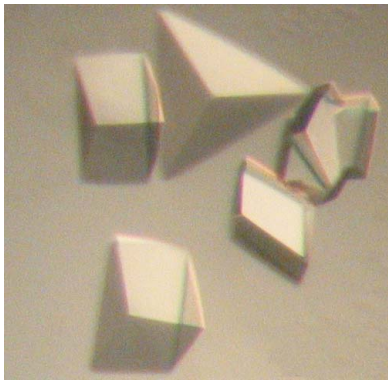
Maltoporin is an outer-membrane protein that forms a β-barrel composed of three monomers and ensures the transport of maltose and maltodextrin in Gram-negative bacteria. Previously, the crystallization of *Escherichia coli* or *Salmonella typhimurium* maltoporin has been achieved in the presence of a mixture of the detergents β-decylmaltoside and dodecyl nonaoxyethylene. These crystals all belonged to the orthorhombic space group $C222_1$ and gave rise to several structures of maltoporin in complex with different carbohydrates determined at resolutions between 3.2 and 2.4 Å. Here, the crystallization of *E. coli* maltoporin in a new crystal form is reported; the crystals belonged to the trigonal *R*3 space group and diffracted to 1.9 Å resolution. These crystals were obtained using *n*-dodecyl-β-D-maltoside as a detergent. Crystals with a lens or pyramidal morphology could be obtained using sitting or hanging drops, respectively, and despite their very different shapes they presented the same space group and very similar unit-cell parameters.

1. Introduction

In Gram-negative bacteria, transport of small metabolites of less than 1500 Da, such as ions, amino acids and carbohydrates, through the outer membrane is ensured by proteins called porins. Porins are made of transmembrane β-strands that adopt a β-meander topology in which turns and loops alternate (Wimley, 2003). The resulting architecture forms a very stable β-barrel (Nikaido & Vaara, 1985; Benz & Bauer, 1988; Jap & Walian, 1990) which is inserted in the outer membrane and forms a channel that allows the passive transport of small metabolites. Together with several other proteins of the *mal* system (Boos & Shuman, 1998), maltoporin is responsible for the catabolism and transport of maltose and maltodextrin [α(1,4)-linked polyglucosyls] in Gram-negative bacteria. In this system, maltoporin is responsible for the transport of maltose and maltooligosaccharide through the outer membrane. The first crystal structure of *Escherichia coli* maltoporin was determined by Schirmer *et al.* (1995) at 3.1 Å resolution and revealed a 144 kDa homotrimeric protein in which each monomer was composed of 18 antiparallel β-strands. The protein forms a channel that permits the passage of carbohydrates. The specificity of sugar transport by maltoporin was subsequently illustrated by several crystal structures of maltoporin bound to different carbohydrates and solved at 2.4 Å resolution (Dutzler *et al.*, 1996; Wang *et al.*, 1997). All of these structures were determined in the orthorhombic $C222_1$ space group and were crystallized in the presence of a mixture of β-decylmaltoside and dodecyl nonaoxyethylene, while other crystal forms diffracting to low resolution have been reported to be obtained using *n*-octyl polyoxyethylene, *n*-octyl β-D-glucopyranoside and *n*-octyl tetraoxyethylene (Stauffer *et al.*, 1990; Garavito *et al.*, 1984). Here, we report the crystallization of *E. coli* maltoporin in a new crystal form using *n*-dodecyl-β-D-maltoside (DDM) as a detergent. The crystals belonged to the trigonal *R*3 space group and diffracted to 1.9 Å resolution.



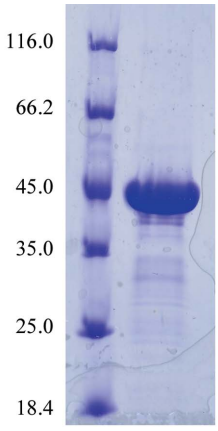
2. Material and methods

2.1. Purification of *E. coli* maltoporin

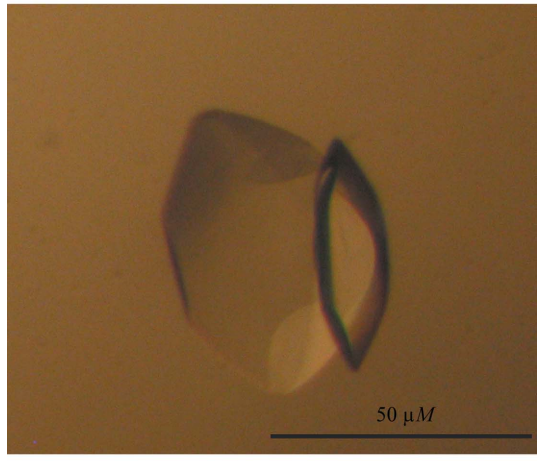
A 6 l culture of *E. coli* BL21 cells transformed with the pMAL-p4X vector (New England Biolabs) recombined with our gene of interest (not related to the maltose system) was grown to an OD of 0.8 at 310 K and induced with 0.5 m*M* IPTG overnight at 293 K. The cells were harvested, resuspended in 50 m*M* Tris–HCl buffer pH 7.6 containing 5 m*M* β-mercaptoethanol, 200 m*M* NaCl and 1 m*M* phenylmethanesulfonyl fluoride (buffer *A*) and then disrupted by sonication followed by a 1 h centrifugation at 30 000*g*. The membranes were pelleted from the resulting supernatant by a new centrifugation step at 120 000*g* for 90 min. The membranes were then resuspended in buffer *A* and homogenized, 1%(*w*/*v*) DDM (Sol-Grade, Anatrace) was added to the homogenized membranes to solubilize the proteins and after stirring the mixture for 1 h at 277 K the sample was centrifuged for 1 h at 120 000*g*. The supernatant was then loaded onto a maltose-affinity column (MBPTrap HP, 3 × 1 ml, GE Heathcare) and washed with five column volumes of buffer *A* containing 0.5 m*M* DDM. The bound proteins were eluted with ten column volumes of buffer *A* containing 0.5 m*M* DDM and 20 m*M* maltodextrin (Sigma). After this step the protein was pure, but it was submitted to an additional step of purification on a gel-filtration column (TSK-GEL GS3000SW, TosoHaas) and eluted with the following buffer: 50 m*M* Tris–HCl pH 7.6, 150 m*M* NaCl, 5 m*M* 2-mercaptoethanol, 0.5 m*M* DDM. Following this protocol, 15 mg of pure protein was obtained (Fig. 1*a*). The purified protein was identified as maltoporin by N-terminal sequencing. A culture of nontransformed *E. coli* BL21 cells otherwise grown under the same conditions gave a similar expression of maltoporin and the protein could also be purified by the procedure described above (data not shown).

2.2. Crystallization of maltoporin

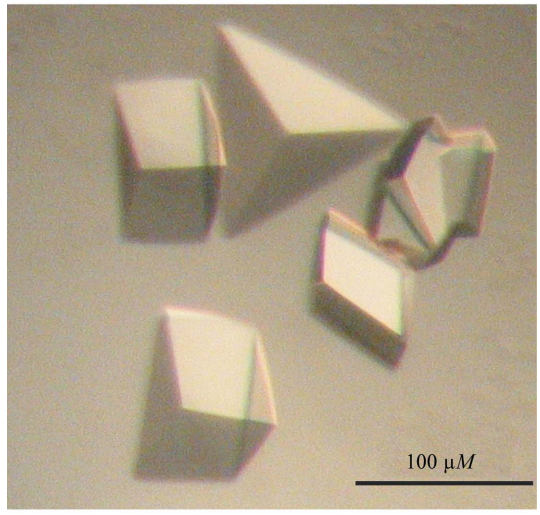
Crystallization screening was performed using the Index crystallization screen (Hampton Research), mixing 2 µl reservoir solution with 2 µl maltoporin solution at 2.8 mg ml^{-1} (monomer concentration) in sitting drops at 293 K. Many conditions gave tiny crystals; however, a condition consisting of 0.1 *M* HEPES pH 7, 30%(*v*/*v*) Jeffamine M-600 pH 7 gave biconvex lens-shaped crystals that diffracted to 3 Å resolution on a rotating-anode X-ray generator. Since the highest resolution for a maltoporin X-ray structure described so far was 2.4 Å and the crystals tested presented a new space group, we decided to optimize the crystallization conditions. The best crystals were found to grow from sitting drops made by mixing 2 µl maltoporin solution at 2.8 mg ml^{-1} and 2 µl reservoir solution consisting of 0.1 *M* HEPES pH 7, 30%(*v*/*v*) Jeffamine M-600 pH 7 and 4 m*M* $MgCl_2$. After two weeks of growth at 293 K, biconvex lens-shaped crystals with a diameter of 50 µm were flash-frozen in liquid nitrogen (Fig. 1*b*). Crystals were also optimized using the following alternative strategy. Before crystallization, the maltoporin was dialyzed against 50 m*M* Tris–HCl pH 7.6, 150 m*M* NaCl, 5 m*M* β-mercaptoethanol, 0.5 m*M* DDM and 2 m*M* maltose; this step was used to remove the maltodextrin used for protein elution on the MBPTrapHP column. After dialysis, the protein was centrifuged at 12 000*g* for 30 min. After this step, crystals were obtained using hanging drops by mixing 2 µl protein solution at 2.8 mg ml^{-1} with 2 µl reservoir solution consisting of 0.1 *M* HEPES pH 7, 32%(*v*/*v*) Jeffamine M-600 pH 7 at 293 K (Fig. 1*c*). Using this strategy, pyramidal crystals grew over two months and reached final dimensions of approximately 100 × 50 × 50 µm. These crystals were also directly flash-frozen in liquid nitrogen. It is worth mentioning that the different shape of the crystals was not a consequence of the presence of the ligand, since pyramid-shaped crystals could also be obtained under similar conditions in the presence of maltodextrin in hanging drops and, *vice versa*, lens-shaped crystals could be obtained in the presence of maltose using sitting drops.



(*a*)



(*b*)



(*c*)

Figure 1
(*a*) SDS–PAGE of maltoporin after purification. Molecular-weight markers are labelled in kDa. (*b*) Maltoporin crystals obtained in sitting drops in the presence of maltodextrin. (*c*) Maltoporin crystals obtained in hanging drops in the presence of maltose.

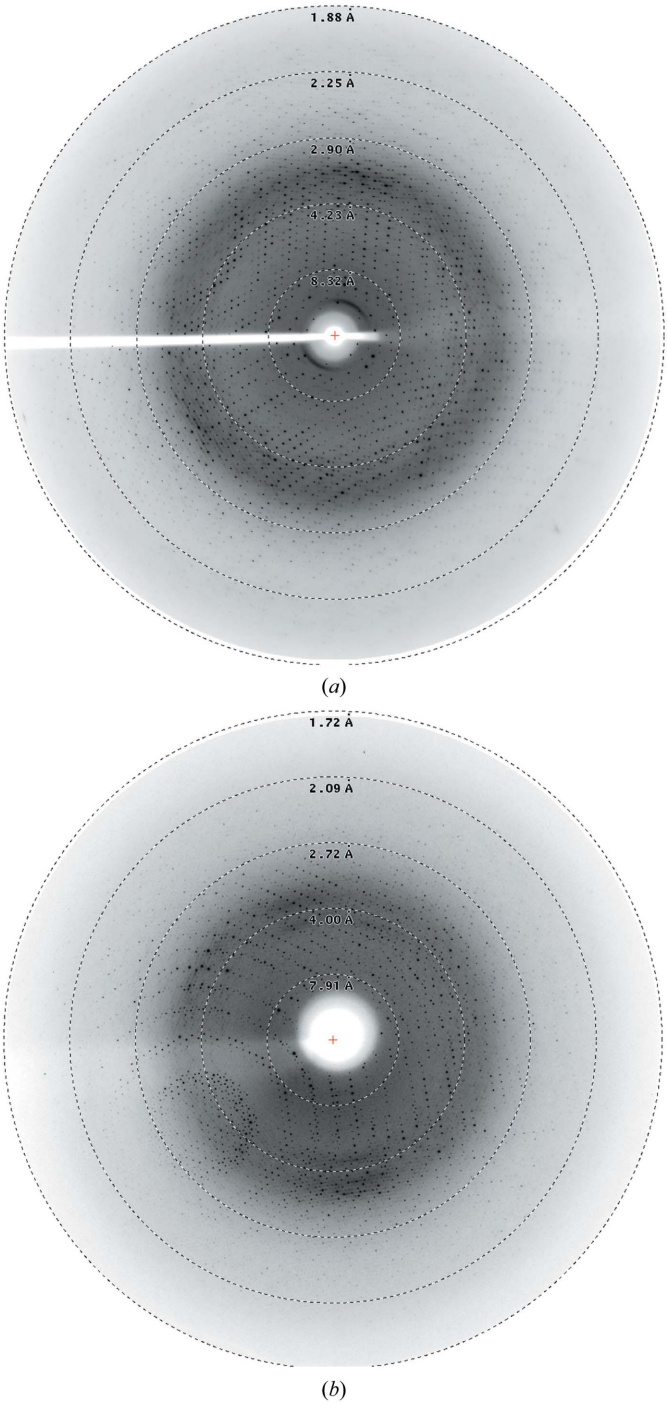


Figure 2
Diffraction images of (*a*) a maltoporin crystal obtained in a sitting drop in the presence of maltodextrin and (*b*) a crystal obtained in a hanging drop in the presence of maltose.

2.3. Data collection

A total of 224 images with a rotation range of 0.5° were collected at a wavelength of 1.037 Å from a crystal obtained in a sitting drop in the presence of maltodextrin using a crystal-to-detector distance of 130 mm on the I911-2 beamline at MAX-lab, Lund, Sweden. Data from a crystal obtained in a hanging drop in the presence of maltose were collected on the X13 beamline at EMBL/DESY, Hamburg; for this crystal, 250 images with a rotation range of 0.5° were collected at a wavelength of 0.812 Å with a crystal-to-detector distance of 158.1 mm. Reflections were processed and merged with *XDS* (Kabsch, 2010) in the trigonal space group *R*3. For both crystals diffraction spots could be observed below 1.9 Å resolution (Figs. 2*a* and 2*b*); however, a complete data set could only be processed and scaled to 1.9 Å resolution. The data-collection statistics are displayed in Table 1.

Table 1
Data-collection and refinement statistics.

Values in parentheses are for the last resolution shell.

Crystal	Sitting drop with maltodextrin	Hanging drop with maltose
Beamline	I911-2, MAX-lab, Lund	X13, DESY, Hamburg
Wavelength (Å)	1.037	0.812
Space group	*R*3	*R*3
Resolution (Å)	50–1.9 (2.0–1.9)	30–1.9 (2.0–1.9)
Unit-cell parameters (Å, °)	$a = b = 151.91$, $c = 128.83$, $\alpha = \beta = 90$, $\gamma = 120$	$a = b = 152.10$, $c = 127.77$, $\alpha = \beta = 90$, $\gamma = 120$
$R_{\rm meas}$ (%)	6.3 (54.7)	6.4 (55.9)
$R_{\rm mrgd\text{-}F}$ (%)	8.8 (51.2)	11.6 (71.0)
$\langle I/\sigma(I)\rangle$	16.22 (3.95)	16.15 (2.81)
Completeness (%)	97.2 (96.1)	99.7 (99.9)
Multiplicity	3.51 (3.31)	3.99 (3.98)
Matthews coefficient (Å^3 Da^{-1})	3.02	3.02
Solvent content (%)	59.35	59.35
Monomers per asymmetric unit	2	2

3. Results and discussion

The two crystals both present a Matthews coefficient ($V_{\rm M}$) of 3.02 Å^3 Da^{-1}, with 59.35% of the unit cell occupied by solvent, when assuming the presence of two monomers of maltoporin per asymmetric unit. Phasing of the data was achieved by molecular replacement using the *PHENIX* program suite (Adams et *al.*, 2010) with the *E. coli* maltoporin monomer as a search model (PDB entry 1af6; Wang *et al.*, 1997). In agreement with the Matthews coefficient, two monomers were found in the asymmetric unit; therefore, the asymmetric unit does not contain the biologically active homotrimer. The two structures are currently under refinement.

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