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Crystallization of the hydantoin transporter Mhp1 from *Microbacterium liquefaciens*

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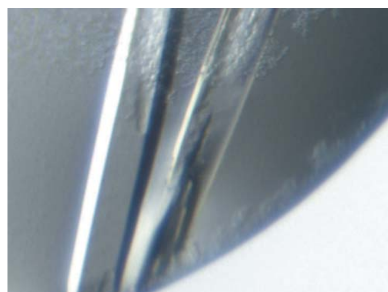

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The integral membrane protein Mhp1 from *Microbacterium liquefaciens* transports hydantoins and belongs to the nucleobase:cation symporter 1 family. Mhp1 was successfully purified and crystallized. Initial crystals were obtained using the hanging-drop vapour-diffusion method but diffracted poorly. Optimization of the crystallization conditions resulted in the generation of orthorhombic crystals (space group $P2_12_12_1$, unit-cell parameters a = 79.7, b = 101.1, c = 113.8 Å). A complete data set has been collected from a single crystal to a resolution of 2.85 Å with 64 741 independent observations (94% complete) and an $R_{\rm merge}$ of 0.12. Further experimental phasing methods are under way.

1. Introduction

Transporters mediate the uptake and efflux of extremely diverse solutes including cations, anions, drugs, sugars, amino acids, neurotransmitters and metabolic end products (Pao *et al.*, 1998). The nucleobase:cation symporter 1 (NCS-1) family is a family of electrochemical potential-driven transporters (Busch & Saier, 2002). The NCS-1 family includes several hundred proteins found in bacteria, archaea, yeast, fungi and plants. The members of this family are typically composed of 400–650 amino acids and possess 12 putative transmembrane α-helices. These proteins transport small molecules such as thiamine, uracil, cytosine, purines, allantoin and nicotinamide riboside. Some of the NCS-1 family members function in uptake by substrate:H^+ symport. At present, no structures of NCS-1 family members have been reported.

Recently, the membrane protein Mhp1, encoded by the *hyuP* gene from *Microbacterium liquefaciens*, has been cloned and expressed in *Escherichia coli* (Suzuki & Henderson, 2006). Mhp1 is composed of 489 amino acids with a molecular weight of 54.6 kDa and is predicted to have 12 transmembrane helices. Sequence analysis suggested that Mhp1 belongs to the NCS-1 family. Mhp1 transports 5-substituted hydantoin compounds, which are converted to amino acids as part of a metabolic salvage pathway. The transporter activity is proposed to be proton-dependent (Suzuki & Henderson, 2006) because the uptake of a hydantoin compound was not accelerated by the addition of NaCl but was affected by pH. However, the details of the transporter mechanism of Mhp1 are unclear owing to a lack of structural information.

Here, we report the crystallization of Mhp1 from *M. liquefaciens*. The crystals diffracted to 2.85 Å resolution. Determination of the three-dimensional structure of Mhp1 will provide new insights into its function and more generally into the structure and function of NCS-1-family proteins.

2. Material and methods

2.1. Expression and purification

E. coli culture conditions and protein expression have been described previously (Suzuki & Henderson, 2006). Cell pellets were resuspended in 50 m*M* Tris–HCl pH 8.0 with protease inhibitors (Roche) and sonicated. Membranes were then collected by ultra-

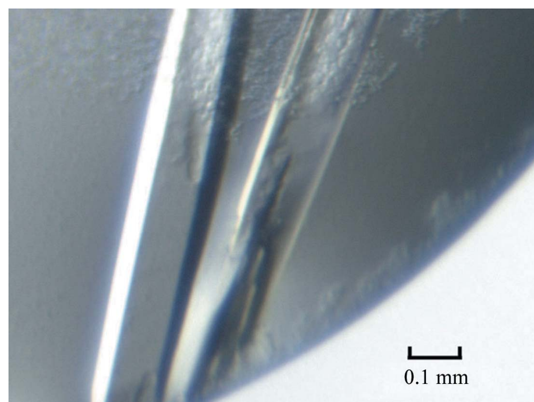

Figure 1
Crystal of Mhp1 from *M. liquefaciens*.

centrifugation using an SW28 rotor at 28 000 rev min^{-1} for 60 min. Membranes were solubilized in 20 m*M* Tris–HCl pH 8.0, 20 m*M* imidazole pH 8.0, 300 m*M* NaCl, 1% *n*-dodecyl-β-D-maltopyranoside (DDM), 20% glycerol and protease inhibitors (Roche). The solubilized membranes were mixed with Ni^{2+}–NTA beads (Qiagen) and incubated for ~16 h at 277 K. The Ni^{2+}–NTA beads were transferred into a column and washed with 15 column volumes of wash buffer *A* [20 m*M* Tris–HCl pH 8.0, 20 m*M* imidazole pH 8.0, 10% glycerol, 0.05% DDM and protease inhibitors (Roche)]. The Mhp1 protein was eluted with an elution buffer consisting of 200 m*M* imidazole pH 8.0, 5% glycerol and 0.05% DDM. The purified protein was concentrated using a Vivaspin system (100 kDa cutoff; Vivascience) and the buffer was exchanged to 10 m*M* Tris–HCl pH 8.0, 2.5% glycerol and 0.05% DDM. The protein was finally concentrated to ~20 mg ml^{-1} and used for crystallization trials.

After *n*-nonyl-β-D-maltopyranoside (NM) was found to be effective in improving the crystal size (described in §3), we modified the final steps of the purification procedure as outlined below. After the Ni^{2+}–NTA beads had been washed with buffer *A*, a second wash step was applied consisting of three consecutive column volumes of wash buffer *B* (20 m*M* Tris–HCl pH 8.0, 20 m*M* imidazole pH 8.0, 5% glycerol, 0.7% NM). Mhp1 was then eluted using an elution buffer consisting of 200 m*M* imidazole pH 8.0, 5% glycerol and 0.7% NM.

Table 1
Data statistics.

Values in parentheses are for the last shell.

Space group	$P2_12_12_1$
Resolution (Å)	30–2.85 (2.95–2.85)†
Unit-cell parameters (Å)	*a* = 79.7, *b* = 101.1, *c* = 113.8
Total observations	64741
Unique observations	22414
Completeness (%)	94 (81)
Redundancy	2.9 (2.3)
R_{merge} (%)	12.2 (77.3)
Average $I/\sigma(I)$	7.2 (1.0)
Wilson *B* factor (Å^2)	60.9

† 3.07 Å when a 2.06 $I/\sigma(I)$ cutoff criteria was applied.

The eluted protein was concentrated and the buffer was exchanged to 10 m*M* Tris–HCl pH 8.0, 2.5% glycerol and 0.5% NM.

2.2. Crystallization and optimization

Crystallization conditions were screened at 277 and 293 K by the hanging-drop vapour-diffusion method using Linbro plates (Hampton Research, USA). A 0.5 µl droplet containing ~20 mg ml^{-1} Mhp1 dissolved in 10 m*M* Tris–HCl pH 8.0, 0.05% DDM was mixed with an equal volume of reservoir solution and the droplet was allowed to equilibrate against 300 µl reservoir solution. In the initial screening experiment, Crystal Screen, Crystal Screen II and MembFac (Hampton Research) as well as handmade screening solutions were used as reservoir solutions.

To optimize the crystallization conditions, we varied the pH, PEG type and PEG concentration in the initial conditions. Additive and Detergent Screens 1, 2 and 3 (Hampton Research) were also used for optimization; 1 µl of each additive was mixed with 9 µl reservoir solution and used to prepare hanging drops as described above.

2.3. X-ray data collection

Because of its high PEG 300 content, the mother liquor for the crystals could be used as a cryoprotectant and crystals were directly flash-cooled in liquid nitrogen prior to data collection. Data collection from frozen crystals was performed at 100 K. Diffraction data were collected to 2.85 Å resolution on beamline X06A of the Swiss Light Source using a MAR CCD detector. The oscillation range,

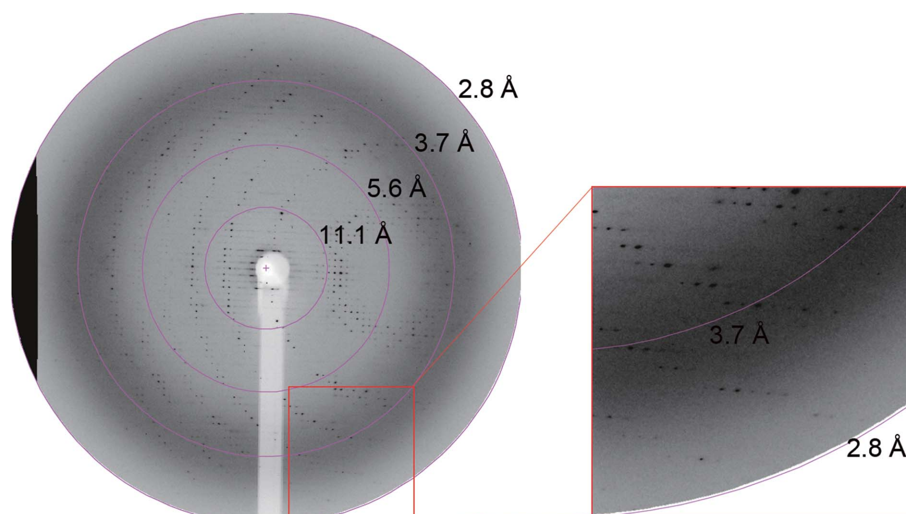

Figure 2
X-ray diffraction image from a native Mhp1 crystal.

exposure time and crystal-to-detector distance were 0.5°, 2 s per frame and 220 mm, respectively. Image data were processed using the *DENZO* and *SCALEPACK* programs (Otwinowski & Minor, 1997). All data better than $-3.0\sigma(I)$ were used for scaling.

3. Results and discussion

In the initial screening of crystallization conditions, needle-shaped or rod-shaped crystals were obtained within a week at 277 and 293 K using reagents containing PEGs (MW 400–20 000) at pH 6.5–9.0. These crystals were very thin or small and diffracted poorly. Therefore, we further optimized the crystallization conditions by varying the pH, PEG type, PEG concentration, detergents and additives. Notably, addition of NM improved the crystal size, whereas additions of longer detergents (*n*-decyl-*β*-D-maltopyranoside and *n*-undecyl-*β*-D-maltopyranoside) did not improve the crystal quality. Based on these observations, we changed the detergent used in the elution buffer from 0.05% DDM to 0.7% NM. This modification dramatically improved the thickness of the crystals. Addition of NaCl at an appropriate concentration also improved the crystal size.

After iterative optimization cycles, the final optimized crystallization conditions were 0.1 *M* sodium phosphate pH 7.0, 0.1 *M* NaCl, 27–35% PEG 300. Crystals appeared within a week at 293 K (Fig. 1). The best crystal diffracted to 2.85 Å resolution (Fig. 2). Analysis of the symmetry and systematic absences in the diffraction patterns indicated that the optimized crystals of Mhp1 from *M. liquefaciens* belonged to space group $P2_12_12_1$, with unit-cell parameters a = 79.7, b = 101.1, c = 113.8 Å. We obtained a data set from a single crystal (Fig. 1). The data were integrated and scaled to 2.85 Å resolution (Table 1). The data set consisted of 64 741 total and 22 414 independent observations in the resolution range 30–2.85 Å with an overall completeness of 94% (81% for the last shell) and an $R_{\rm merge}$ of 12.1%. The overall $I/\sigma(I)$ is 7.2. Assuming the presence of one Mhp1 molecule in the asymmetric unit, the Matthews coefficient $V_{\rm M}$ (Matthews, 1968) of the crystals is 4.2 Å^3 Da^{-1} and the solvent content is 71%. These values are reasonable for crystals of a membrane protein. A heavy-atom derivative search is currently in progress in order to solve the crystal structure of Mhp1.

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