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Crystallization and preliminary X-ray analysis of the bacterial ATP-binding-cassette (ABC) protein MalK

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The ATP-binding protein, MalK, of the bacterial ABC (ATP-binding-cassette) transport complex $MalFGK_2$ provides the energy for the translocation of maltose and maltodextrins across the cytoplasmic membrane. The MalK protein from *Salmonella typhimurium* was overexpressed in *Escherichia coli* and crystallized by the hanging-drop method using $(NH_4)_2SO_4$ as a precipitant. The crystals belong to space group $P6_x22$ (most probably $x = 1$ or 5) with cell dimensions $a = 181.8$ and $c = 182.5$ Å, corresponding to three or four molecules per asymmetric unit. They diffract to a resolution of about 3 Å on a synchrotron X-ray source and are suitable for structure determination.

1. Introduction

The rapidly growing family of ABC (ATP-binding-cassette) transport systems comprise an extremely diverse class of membrane-transport proteins that couple the energy of ATP hydrolysis to the translocation of solutes across biological membranes. ABC transporters not only accomplish the uptake of nutrients but are also involved in a large variety of processes, such as signal transduction, protein secretion, drug and antibiotic resistance, antigen presentation, bacterial pathogenesis and sporulation systems (Higgins, 1992).

Typically, an ABC transporter is composed of four parts: two membrane-integral domains, each of which spans the membrane six times, and two ATP-hydrolyzing domains. In eukaryotic systems, these modules are mostly fused to yield a single polypeptide chain, while bacterial ABC transporters are made up of individual subunits. The ATP-hydolyzing domains are characterized by a set of Walker A and B motifs that constitute a nucleotide-binding fold (Walker *et al.*, 1982) and by a highly conserved sequence motif ('linker peptide') (Ames & Lecar, 1992), immediately preceding the Walker B site. The Walker A and B motifs are linked by a moderately hydrophobic, less conserved peptide fragment that is predicted to essentially form an α-helical structure ('helical domain') (Hyde *et al.*, 1990; Mimura *et al.*, 1991). This fragment, for which evidence has been presented that it might be involved in the interaction with the membrane-integral components (Wilken *et al.*, 1996; Mourez *et al.*, 1997), is variable in length between different proteins. Both the linker peptide and the putative helical domain represent unique features of ABC-transport systems (reviewed in Schneider & Hunke, 1998) that are absent in other nucleotide-binding and nucleotide-hydrolyzing proteins whose structures have been solved (Abrahams *et al.*, 1994; Story & Steitz, 1992; Diederichs & Schulz, 1990; Pai *et al.*, 1990).

Bacterial binding-protein-dependent transport systems that accomplish the uptake of a large variety of nutrients represent the best-characterized subclass of the family (Boos & Lucht, 1996). Prominent eukaryotic members include several medicinally important mammalian proteins such as P-glycoprotein that, when amplified, enables certain cancer cells to extrude chemotherapeutic drugs (Gottesman & Pastan, 1993), and the cystic fibrosis transmembrane regulator protein (CFTR) which is mutated in patients affected by the common hereditary disease cystic fibrosis (Collins, 1992).

The mechanism, at the molecular level, by which ABC-transport systems operate is only poorly understood. This is mainly due to the lack of structural data for any ABC transporter to date. Since the crystallization of membrane proteins is still a rather difficult task to achieve, the availability of the crystal structure of an ATP-hydrolyzing domain would be a major step towards understanding their role in the transport process. Here we report on the crystallization of MalK, the ATP-hydrolyzing component of the binding-protein-dependent transport system ($MalFGK_2$) for maltose and maltodextrins from *S. typhimurium*. MalK has a molecular weight of 41 kDa and displays a spontaneous ATPase activity when purified in the absence of the membrane-integral components MalF and MalG (Walter *et al.*,

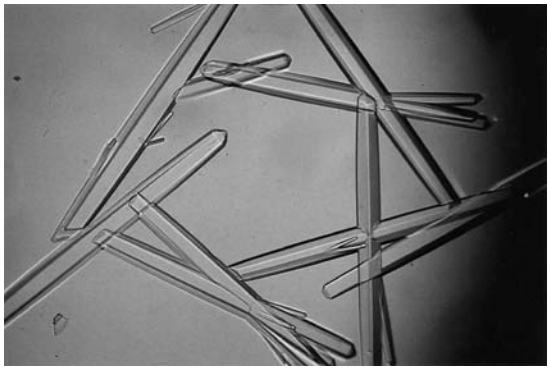

Figure 1
Crystals of the MalK protein from *S. typhimurium*. The hexagonal-shaped needles have a size of about 0.03 × 0.03 × 0.4 mm.

1992; Morbach *et al.*, 1993). Binding of MgATP is accompanied by a specific conformational change that affects the helical domain (Schneider *et al.*, 1994). The enzymatic properties of the protein not only compare favourably with those measured with the reconstituted transport complex of *E. coli* (Davidson *et al.*, 1992) but also with data reported on the mammalian P-glycoprotein (Urbatsch *et al.*, 1994). In contrast to most other ATP-hydrolyzing subunits, MalK has a C-terminal extension that is crucial for regulatory activities of the protein within the maltose regulon (Kühnau *et al.*, 1991; Schneider & Walter, 1991).

2. Material and methods

MalK from *S. typhimurium* was purified from the cytoplasmic fraction of the overproducing *E. coli* strain BL21 (DE3) (pLysS, pES67) (expression vector pRSET5d) in milligram quantities by a two-step chromatographic procedure as described elsewhere (Schneider *et al.*, 1995). The protein, dissolved in a buffer containing 50 m*M* Tris–HCl pH 7.5, 1 *M* NaCl, 10 m*M* ATP, 5 m*M* $MgCl_2$, 5 m*M* dithiothreitol, 0.1 m*M* EDTA, 0.1 m*M* phenylmethylsulfonyl fluoride, and 20%(*v*/*v*) glycerol, was concentrated by ultrafiltration and centrifugation to a final concentration of about 0.4 mg ml^{-1}. After addition of 10 m*M* *n*-octyl-β-D-glucopyranoside or 10 m*M* *n*-dodecyl-D-maltoside, MalK was further concentrated to about 10 mg ml^{-1} with a Centrisart (cutoff 20000) centrifugal microconcentrator (Sartorius, Göttingen, Germany). Protein concentrations were determined by the method of Bradford (1976).

Crystallization trials were performed with the hanging-drop vapour-diffusion method immediately after concentrating the protein. An initial crystallization screening at 277 K with the commercial Hampton Research crystallization kit I (Jancarik & Kim, 1991) revealed tiny needles with $(NH_4)_2SO_4$ or Li_2SO_4 as precipitants. Optimal crystallization conditions were found at a temperature of 293 K by mixing 2 µl of enzyme solution and 2 µl reservoir solution containing 2 *M* $(NH_4)_2SO_4$, 50 m*M* HEPES pH 7.0 and 10% DMSO in the drop. Without the presence of a detergent, MalK was merely soluble to a concentration of about 3 mg ml^{-1} and no crystals could be produced under any conditions. In order to avoid radiation damage, crystals were loop mounted in a cryoprotectant, consisting of 2 *M* $(NH_4)_2SO_4$, 50 m*M* HEPES pH 7, 10% DMSO, 10 m*M* detergent and 25%(*v*/*v*) glycerol.

Preliminary X-ray experiments were performed in-house using a Rigaku RU-200 X-ray generator with Cu $K\alpha$ radiation and an MAR Research imaging-plate detector. Measured reflection intensities were processed with *DENZO* and scaled with *SCALEPACK* (Otwinowski, 1993).

3. Results and discussion

Thin hexagonal-shaped crystals of MalK appeared overnight and reached a maximum size of 0.04 × 0.05 × 0.4 mm (Fig. 1). These crystals grew in space group $P6_x22$ (most probably $P6_122$ or $P6_522$) with cell dimensions a = 181.8 and c = 182.5 Å determined by autoindexing oscillation photographs using *DENZO* (Otwinowski, 1993). Assuming three or four molecules in the asymmetric unit, the crystal volume per protein mass V_m (Matthews, 1968) was 3.6 and 2.8 Å^3 Da^{-1}, respectively, both of which fall in the normal range found for protein crystals. The corresponding solvent contents were determined to be 66 and 56%, respectively. The resolution limit of the crystals was about 3.0 Å using a synchrotron X-ray source. The obtained crystals, which decay within one week, were highly radiation-sensitive and cryo-cooling was therefore essential. Because of their small size, synchrotron radiation was required for data collection. The crystals only diffract to about 4.5 Å resolution with the in-house X-ray source.

Native reflection intensities were measured at the beamline BM14 at the European Synchrotron Radiation Facility (ESRF) using a wavelength of 1.18 Å and an oscillation range of 0.5°. The combined data collected from three crystals had 69903 observations which were reduced to 30474 unique reflections. The data had a completeness of 85% and an $R_{\rm sym}$ value of 8.4% in the resolution range 3.1–10.0 Å. We are currently applying the multiple isomorphous replacement method to solve the crystal structure.

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