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Crystallization and preliminary X-ray analysis of AlgS, a bacterial ATP-binding cassette (ABC) protein specific to macromolecule import

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Sphingomonas sp. A1 possesses a macromolecule (alginate; average molecular size 25 700 Da) uptake system mediated by a novel pit-dependent ABC transporter. In this system, AlgS (363 amino-acid residues; 40 kDa) functions as an ATPase and provides energy for the translocation of high molecular-weight alginate across the cytoplasmic membrane. Hexahistidine-tagged AlgS of *Sphingomonas* sp. A1 was overexpressed in *Escherichia coli* and crystallized by means of the hanging-drop vapour-diffusion method with ammonium dihydrogen monophosphate as the precipitant. Preliminary X-ray analysis of the resultant crystals was performed; they belonged to the monoclinic space group $P2_1$ and had unit-cell parameters a = 57.4, b = 92.7, c = 65.8 Å, β = 102.3°. X-ray diffraction data to 3.2 Å have been collected from the native crystal.

1. Introduction

A soil bacterium, *Sphingomonas* sp. A1, incorporates high molecular-weight alginate, a polysaccharide produced by brown seaweed and some bacteria, by means of a specifically equipped molecular system which we designated a 'Super-channel' (Momma *et al.*, 1999). *Sphingomonas* sp. A1 cells are covered with many large plaits (Hisano *et al.*, 1996) and when assimilating alginate they form pits on their surface through the reconstitution and/or rearrangement of the plaits and concentrate the biopolymer in the pits. The alginate thus concentrated is delivered to a pit-dependent ABC transporter through the function of alginate-binding proteins (AlgQ1 and AlgQ2) localized in the periplasmic space (Momma *et al.*, 2001).

Similar to other bacterial ABC transporters, that of *Sphingomonas* sp. A1 is composed of a membrane-associated complex (AlgS/AlgS: AlgM1-AlgM2) which typically comprises four subunits. Two of these subunits are transmembrane proteins (AlgM1 and AlgM2) which confer specificity for substrate recognition or binding and presumably form a transporter channel. Each of the other two subunits (homodimer of AlgS) contains a nucleotide-binding site and fuels macromolecule movement across the membrane. These four subunits show significant homology among members of the ABC transporter family (Momma *et al.*, 1999, 2000).

The genes for all proteins of the pit-dependent ABC transporter system of *Sphingomonas* sp. A1 constitute an operon together with the genes for depolymerization enzymes [alginate lyases A1-I, A1-II and A1-III (Momma *et al.*, 1999) and oligoalginate lyase (OAL; Hashimoto *et al.*, 2000)] and for regulation of the transcription (*ccp*) of the operon (Momma *et al.*, 1999). The pit-dependent ABC transporter in *Sphingomonas* sp. A1 is peculiar in that it can import a macromolecule (alginate; 23 kDa) (Momma *et al.*, 2000). Such import systems are usually distinct from exporters in a number of ways. First, the ABC-protein (ATPase) is encoded separately from the membrane subunits, which are frequently composed of two different polypeptides. Secondly, the molecule to be imported normally requires a dedicated binding protein to deliver it to the membrane-transport subunits. These two general criteria are true of the pit-dependent ABC transporter of *Sphingomonas* sp. A1. Although ABC transporters for import are found in many prokaryotic microbes, almost all of them so far analyzed are those responsible for incorporation of small solutes such as maltose, histidine, peptides or ribose (Ehrmann *et al.*, 1998). Therefore, the ABC-import system for alginate found for the first time in *Sphingomonas* sp. A1 may provide a new insight into the molecular mechanism for macromolecule transport across the membrane structure by ABC transporters.

The crystal structures of energy-generating proteins (ABC-ATPases), HisP (28 kDa) of *Salmonella typhimurium* (Hung *et al.*, 1998) and MalK (42 kDa) of *E. coli* (Diederichs *et al.*, 2000), both of which are components of the ABC transporters responsible for the import of

Table 1
Data collection statistics for AlgS crystal.

Values in parentheses refer to data in the highest resolution shell (3.32–3.15 Å).

X-ray source	Cu $K\alpha$
Wavelength (Å)	1.54
Resolution (Å)	15–3.15
Space group	$P2_1$
Unit-cell parameters (Å, °)	a = 57.4, b = 92.7, c = 65.8, $\alpha = \gamma = 90$, β = 102.3
Total observations	35988
Independent reflections	11498 (1553)
Completeness (%)	98.0 (94.1)
$I/\sigma(I)$	7.02 (3.29)
R_{sym} (%)	10.4 (16.2)

histidine and maltose, respectively, have already been determined. AlgS of *Sphingomonas* sp. A1 exhibits significant identity with HisP (identity score 30%) and MalK (50%), respectively. However, AlgS (40 kDa) is approximately 100 amino acids longer than HisP and nine amino acids shorter than MalK and has the additional function of regulating pit formation (Momma *et al.*, 2000), thus indicating that the pit-dependent ABC transporter of *Sphingomonas* sp. A1 is highly specialized for the targeting and subsequent movement of a particular macromolecule, alginate. Therefore, we purified and crystallized a recombinant form of AlgS from *E. coli* cells and analyzed the preliminary statistics of the crystals.

2. Crystallization

E. coli strain BL21 (DE3) pLysS (Novagen, Inc., Madison, WI, USA) transformed with pAS4 (algS in pET14b; Novagen, Inc.) was used as a potent producer for hexahistidine-tagged AlgS (Momma *et al.*, 2000). Procedures for the cultivation of *E. coli* cells and purification of the recombinant AlgS were the same as described previously (Momma *et al.*, 2000). Since the purified AlgS was inclined to precipitate, the protein was dialyzed with a buffer comprising 20 m*M* Tris–HCl pH 7.5, 10 m*M* ATP, 0.1 m*M* EDTA, 0.5%(*w*/*v*) *n*-octyl-β-D-glucopyranoside and 20%(*v*/*v*) glycerol. Prior to crystallization, the purified AlgS was concentrated by ultrafiltration with a Centrisalts (Saltrius) to give a final concentration of 5 mg ml^{-1}. Protein concentrations were determined by the method of Bradford (1976).

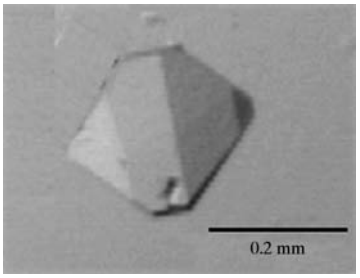

Figure 1
Crystal of AlgS of *Sphingomonas* sp. A1.

Crystallization of AlgS was achieved by the hanging-drop vapour-diffusion method on Linbro tissue-culture plates. A hanging drop (6 µl) prepared by mixing 3 µl each of the above-described protein solution and the reservoir solution was placed on a siliconized cover slip over 0.5 ml of the reservoir solution. Initial screening for crystallization conditions was performed with Screen I (Jancarik & Kim, 1991), Screen II and MembFac screening solutions (Hampton Research) at 293 K. Crystalline growth was found under ammonium dihydrogen monophosphate as precipitant and this was chosen as a starting point for further optimization. Prismatic colourless crystals were grown using 1.0 *M* ammonium dihydrogen monophosphate, 0.1 *M* trisodium citrate pH 5.6 and 20% glycerol as reservoir solution. Under these conditions, crystals grew to a maximum dimension of 0.2 mm in three months at 293 K (Fig. 1).

3. X-ray analysis

A crystal was mounted in a thin-walled glass capillary for X-ray analysis. Both ends of the capillary were filled with resorvoir solution and then sealed with wax. The diffraction data for a native crystal from 15 to 3.2 Å were collected with a Bruker Hi-Star multiwire area detector at 293 K using Cu $K\alpha$ radiation generated by a MacScience M18XHF rotating-anode generator and were processed with the *SADIE* and *SAINT* software packages (Bruker).

Preliminary characterization of the AlgS crystal indicated the monoclinic space group $P2_1$, with unit-cell parameters a = 57.4, b = 92.7, c = 65.8 Å, β = 102.3°. From the reflections (35 988) observed, 11 498 independent reflections were obtained with an R_{sym} value of 10.4%. The data exhibited a completeness of 98.0% to 3.2 Å. Data-collection statistics for the AlgS crystal are summarized in Table 1. One or two AlgS molecules per asymmetric unit give V_M values (Matthews, 1968) of 4.14 or 2.04 Å^3 Da^{-1} and a solvent content of 70 or 40%, respectively. It is probable that two molecules of AlgS are present in the asymmetric unit based on the following reasons. (i) A homodimer composed of two molecules of AlgS catalyzes the hydrolysis of ATP, as is seen in the case of HisP and MalK. (ii) Crystals of HisP and MlaK contain two molecules in the asymmetric unit. (iii) In the case of two molecules of AlgS in the asymmetric unit, the values of V_M and solvent content lie within the range usually found for protein crystals.

Data collection using a synchrotron-radiation source and a search for heavy-atom derivatives for phasing using the multiple isomorphous replacement method are now in progress.

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