Acta Cryst. (1998). D**54**, 1399–1400

Crystallization of OmpC osmoporin from *Escherichia coli*

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(*Received 29 January 1998; accepted 24 March 1998*)

Abstract

OmpC porin, one of the major outer-membrane proteins of Gram-negative bacteria, participates in bacterial osmoregulation by counteracting OmpF porin. Although these two osmoporins from *Escherichia coli* share high sequence homology, their crystallization behavior was found to be very different. OmpC could be crystallized under a variety of conditions by either microdialysis or hanging-drop methods using PEG 4000 as precipitant. The crystals belong to space group $P2_1$ with unit-cell constants a = 117.6, b = 110, c = 298.4 Å, $\beta = 97^\circ$. They diffract beyond 4 Å with a rotating anode and show intense non-Bragg scattering.

1. Introduction

Osmoporins belong to a subclass of a porin protein family found in the outer membrane of Gram-negative bacteria (for a review, see Jap & Walian, 1991). A generic functional osmoporin consists of three identical subunits associated by strong interactions, although the existence of a functional dimer (Rocque & McGroarty, 1989) and of heterotrimers of different subunits (Gehring & Nikaido, 1989) has been suggested. Two distinct types of osmoporins, OmpF and OmpC, have been identified as participating in the osmoregulation of *E. coli*. Depending on the environmental osmolarity, the relative ratio of the two osmoporins changes in the outer membrane while the overall amount of porin proteins remains constant.

The expression of osmoporins is regulated mainly at the level of transcription (Csonka & Hanson, 1991). The regulatory mechanism is a conventional two-component signal-transduction system, in which EnvZ and OmpR are the osmosensor and the regulator, respectively (Mizuno & Mizushima, 1990). OmpR is a bi-functional transcriptional regulator so that OmpC expression is promoted and OmpF synthesis is repressed at high osmolarity, but OmpF is favorably expressed over OmpC at low osmolarity. Compared to the well characterized mechanism of transcriptional regulation, post-translational processes such as export and oligomerization are largely unknown. However, oligomerization seems to involve several stages of conformational transition, from water-soluble monomer to membrane monomer, to intermediary dimer and finally to mature trimer (Reid *et al.*, 1988).

The regulation of osmoporins at the molecular level suggests the presence of differential osmopore activity in the two osmoporins. Some biochemical studies suggest that there is a slight difference in the effective pore size between the two osmoporins (Nikaido & Rosenberg, 1983; Benz *et al.*, 1985). Moreover, it has been thought that they have a complicated regulatory mechanism in order to achieve a tight control of intracellular osmolarity. However, the structural basis of osmoporin-mediated osmoregulation could not be elucidated because of the lack of an OmpC structure, despite recent successes in the structure determination of bacterial porins, including three *E. coli* porins, OmpF, PhoE (Cowan *et al.*, 1992, 1995) and LamB (Schirmer *et al.*, 1995), and porins of other bacteria, *Rhodobacter capsulatus* (Weiss & Schulz, 1992), *Rhodopseudomonas blastica* (Kreusch *et al.*, 1994), *Salmonella typhimurium* (Meyer *et al.*, 1997) and *Paracoccus denitrificans* (Hirsch *et al.*, 1997).

2. Methods and results

The OmpC protein was purified from *E. coli* strain ECB ($OmpC^+$, $OmpF^-$, $OmpA^-$; a generous gift from Dr E. J. McGroarty of Michigan State University) by adapting the previously reported purification procedure for OmpF porin (Garavito & Rosenbusch, 1986). Material extracted from porins using a 3–5% solution of the detergent C_8POE (octylpolyoxyethylene) was highly associated with heterogeneous lipopolysaccharide (LPS) molecules, as manifested by the occurrence of multiple bands in sodium dodecyl sulfate polyacrylamide gel electrophoreses (SDS–PAGE). Therefore, the main purification effort was focused on efficient removal of the bound LPS without altering the crystallizability of the proteins. The loosely bound LPS could be removed by incubating proteins in the presence of 40 m*M* EDTA and 20 m*M* NaCl, followed by molecular-sieve chromatography [Ultrogel AcA in 20 m*M* 4-(2-hydroxyethyl)-1-piperazine ethanesulfonic acid (HEPES) pH 7.6 containing 0.5% C_8POE]. In contrast, the tightly bound LPS appeared to be removed only by repeated cycles of DEAE–cellulose (20 m*M* HEPES pH 7.6 containing 0.5% C_8POE) and hydroxylapatite (10 m*M* sodium phosphate pH 7.0 containing 0.5% C_8POE) chromatography. Use of either SDS or low-pH buffer (for example, sodium succinate pH 4.0) was found to enhance the removal of LPS, but seemed to cause a deterioration in the quality of the crystals.

The hanging-drop vapor-diffusion technique was used for an initial screening of crystallization conditions. The protein concentration was maintained at about 10 mg ml^{-1} in the standard crystallization buffer [20 m*M* HEPES pH 7.6 containing 1.6% octyl-β-glucoside (β-OG) and 0.1% NaN_3]. This buffer could be exchanged by multiple rounds of microdialysis prior to crystallization trials.

It was initially expected that the crystallization behavior of OmpC would be similar to that of OmpF, considering the high sequence homology shared by the two osmoporins (64% identity). However, OmpC precipitated heavily under well established crystallization conditions for both tetragonal (Garavito & Rosenbusch, 1986) and trigonal (Pauptit *et al.*, 1991) crystal forms of OmpF. Instead, OmpC was crystallized from a variety of buffer conditions [sodium phosphate pH 6.5; HEPES pH 7.6; Tris–HCl pH 8.5; triethanolamine (TEA) pH 9.5 and cyclohexylaminoethanesulfonic acid (CHES) pH 10.5], salts (NaCl, LiCl, $MgCl_2$ and $CaCl_2$) and detergents (β-OG,

nonyl-β-D-glucoside, decyl-β-D-maltoside and cyclohexyl-propyl-β-D-maltoside). The different crystallization niches of the two osmoporins could be explained by the fact that the sequence homology is least in the loop regions, which are known from the structural analysis to be involved in crystal contacts.

With the hanging-drop method, plate-like crystals appeared in a week, but usually had a maximum dimension of less than 0.3 mm. The crystal size, particularly the smallest dimension of the plate, could be significantly improved using microdialysis (Garavito & Rosenbusch, 1986). The optimized crystallization conditions in microdialysis were 20 m*M* TEA pH 9.5, 10–12% PEG 4000, 230–300 m*M* $MgCl_2$ and 0.9% β-OG, with an initial PEG concentration of 7.5%. In this case, the crystals grew slowly to a size of 0.9 × 0.8 × 0.3 mm (Fig. 1).

The preliminary characterization of the OmpC crystals was performed with an Enraf–Nonius precession camera mounted on a Rigaku RU200 rotating-anode generator operated at 40 kV and 100 mA. The crystals diffract beyond 4 Å with this X-ray source. The space group of the crystals was determined to be monoclinic ($P2_1$) with unit-cell constants of a = 117.6, b = 110, c = 298.4 Å, β = 97°. This space group is consistent with analysis of the partial X-ray data set collected on a FAST area detector and processed by *MADNES* (Messerschmidt & Pflugrath, 1987). The voume of an asymmetric unit is about 2×10^5 Å^3. An asymmetric unit contains about five trimers, assuming a molecular weight of 170 kDa for the OmpC–detergent complex and a crystal density of 2.5 Da Å^{-3}.

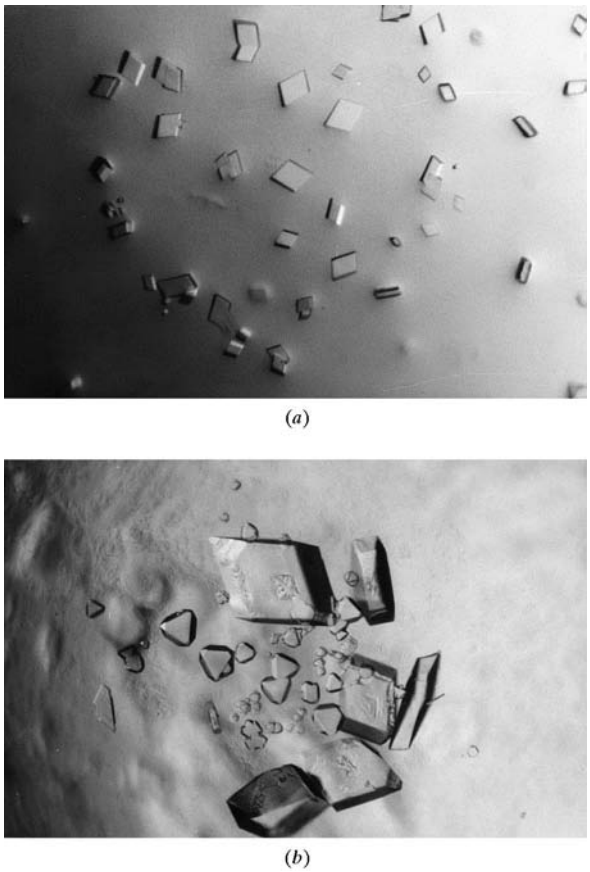

Fig. 1. (*a*) OmpC crystals obtained by the hanging-drop method. The largest dimension of the crystal is about 0.25 mm. (*b*) OmpC crystals obtained by the microdialysis method. The largest dimension of the crystal is about 0.8 mm.

The diffraction image of the crystals showed intense non-Bragg diffuse scattering, which is probably due to the presence of disordered molecules in the crystals. This type of diffuse scattering was also observed in the tetragonal form of OmpF, in which trimers are heavily surrounded by disordered detergent molecules (Kim & Garavito, unpublished work; Pebay-Peyroula *et al.*, 1995). The tetragonal form of OmpF was composed of two interleaved lattices of porin trimers displaying only detergent-mediated interlattice contacts. This is distinct from the situation in the trigonal crystal form of OmpF, in which bound detergents do not prevent micelle fusion during the crystallization process and allow the establishment of more direct protein–protein contacts. In either case, however, the distribution pattern of the detergent molecules is similar to that found for membrane proteins with lipids in biological membranes.

The author wishes to thank Dr R. Michael Garavito (now at Michigan State University) for his help and advice during the entire research project (conducted in the Department of Biochemistry and Molecular Biology at the University of Chicago), and Dr E. J. McGroarty for providing *E. coli* strains. This research was supported by the National Institutes of Health under grant No. R01 GM39550.

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