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Crystallization and preliminary X-ray crystallographic analysis of water channel AQP1

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Aquaporin-1 (AQP1), a water channel from bovine red blood cells has been deglycosylated, purified to homogeneity and crystallized in a form suitable for X-ray crystallographic study. Crystals are grown using polyethylene glycol as precipitant and belong to the tetragonal space group $I422$, with unit-cell parameters $a = b = 93.4$, $c = 180.4$ Å. The crystals diffract beyond 2.2 Å resolution.

1. Introduction

Aquaporins (AQPs) are a family of water-channel proteins involved in water transport across cell membranes. There are currently ten AQP water-channel subfamilies: AQP0–AQP9 (Kuwahara *et al.*, 1999). Over 150 AQP water channels have been identified to date. A wealth of information about the structure and function of water channels has been obtained from biophysical, biochemical and genetic studies. This includes the overall architecture of APQ1, derived from electron crystallography.

AQP1 (MW = 28 kDa) in erythrocyte membranes was the first identified water channel (Preston *et al.*, 1992; van Hoek & Verkman, 1992) and other members were subsequently found to exist in a range of cell types from bacteria to those found in man; in mammals, AQP water channels are distributed in a variety of tissues from organs such as kidney, gall bladder, spleen, lung, intestine, salivary glands and eyes (Hasegawa *et al.*, 1993; Nielsen *et al.*, 1993; Agre *et al.*, 1993; Gresz *et al.*, 1999). AQP water channels are believed to be osmotically regulated and to transport water across a number of epithelial and endothelial cell layers during fluid absorption and secretion. Biochemical and biophysical studies have indicated that AQP1 forms tetramers in lipid bilayers (Smith & Agre, 1991; Verbavatz *et al.*, 1993) and that each monomer functions as a single channel (Zhang *et al.*, 1993; Preston *et al.*, 1992; van Hoek *et al.*, 1991). These results have been confirmed by several electron-crystallographic structural studies (Jap & Li, 1995; Li *et al.*, 1997; Walz *et al.*, 1997; Cheng *et al.*, 1997); these studies also revealed the general α-helical organization of the AQP1 water-channel structure within lipid bilayers at a resolution of about 6 Å. An AQP1 tetramer is believed to contain two glycosylated and two non-glycosylated monomers; the functional significance of such a mixture is not clear. Deglycosylation of the protein does not, however, affect its channel activity (van Hoek *et al.*, 1995).

Although substantial structural information on AQP1 water channels has already been obtained, a detailed understanding of their functional mechanisms, such as the specificity of water transport through the channels, requires the availability of the structure at atomic resolution. The ability to produce the high-quality crystals described here is an important step in obtaining the atomic model of AQP1 water channel.

2. Experimental procedure

2.1. Deglycosylation and purification

Native AQP1 exists as a tetramer consisting of both glycosylated and non-glycosylated forms, as shown by SDS–PAGE (Fig. 1). It is commonly accepted that glycosylation can

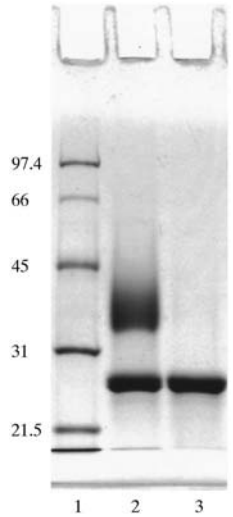

Figure 1
SDS–PAGE of purified AQP1 stained with Coomassie blue. Lane 1, molecular-weight markers; lane 2, purified AQP1 before deglycosylation; lane 3, purified AQP1 after deglycosylation.

greatly affect the quality of crystals. Deglycosylation of AQP1 from bovine red blood cells was therefore performed with the goal of obtaining high-quality crystals. The deglycosylation of purified AQP1 has been described previously (van Hoek *et al.*, 1995), involving a lengthy incubation of purified protein in the presence of a high level of detergent at an elevated temperature; this incubation process could greatly affect the functional integrity of the protein. In an effort to maintain protein integrity, we have performed the deglycosylation of AQP1 while in the cell membrane and before detergent solubilization as described below.

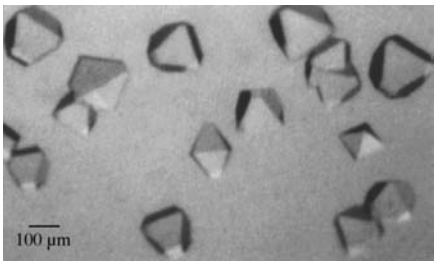

Figure 2
Bipyramidal crystals of AQP1 water channel. Crystals grew to maximum size in 3–4 d.

The purification of AQP1 from bovine erythrocytes follows the basic method described previously by Denker *et al.* (1988) and van Hoek *et al.* (1993) with some modifications. In short, stripped bovine red blood cell ghosts were washed three times with 3% *N*-laurylsarcosine in 10 m*M* sodium phosphate buffer pH 7.4 containing 1 m*M* dithiothreitol, 0.2 m*M* EDTA and 3 m*M* NaN_3; this negative purification step extracts and removes a major portion of other membrane proteins. The membranes were then washed twice with phosphate buffer to remove the *N*-laurylsarcosine detergent. The washed membrane was then treated with *N*-glycosidase F (Boehringer-Mannheim) and incubated at 295 K for 2 d, followed by solubilization with *n*-nonyl-β-D-glucoside (NG) for 1 h at room temperature. After removal of the remaining unsolubilized membranes by centrifugation, the solubilized proteins were applied to a DEAE Sephacel column which had been equilibrated with a solution containing 20 m*M* Tris–HCl pH 7.5 and 13 m*M* NG and then eluted with a sodium chloride gradient. The peak fractions containing deglycosylated AQP1 were collected and concentrated; the concentrated protein was then applied to a Sephacryl S200-HR column to remove any minor contaminants and protein aggregates. The peak fractions containing deglycosylated homogenous AQP1 (Fig. 1, lane 3) was concentrated to about 20 mg ml^{-1} for subsequent crystallization.

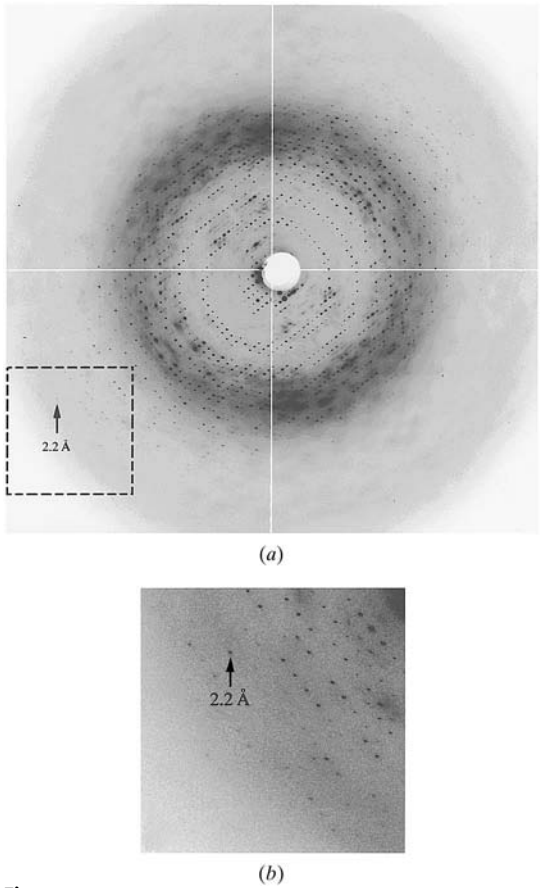

Figure 3
(*a*) An X-ray diffraction pattern image from an AQP1 crystal. This diffraction pattern was recorded with a 1° oscillation at ALS beamline 5.0.2. (*b*) An enlarged image of the indicated area in (*a*) shows diffraction spots extending to a resolution better than 2.2 Å.

2.2. Crystallization

Several polyethylene glycols (PEGs) were screened to determine conditions useful for crystallization of AQP1. This screening provided clues that low-molecular weight PEGs would be worthwhile investigating in further crystallization trials. A variety of such PEGs were screened in depth; this included the use of additives, various ionic strengths and PEG types. After extensive crystallization experiments, AQP1 crystals were obtained with the use of PEG 400, PEG 600 and PEG monomethyl ether 550 which produced the highest quality crystals.

Crystallization trials of AQP1 were performed using the sitting-drop technique. The protein was concentrated to about 20 mg ml^{-1} in 20 m*M* Tris–HCl buffer pH 7.5 and mixed with crystallization solution containing 20% PEG monomethyl ether 550 and 10 m*M* Tris–HCl pH 7.5 in a 1:1 volume ratio. The mixture was then placed in a 277 K environment to equilibrate with the crystallization solution in the reservoir. Crystals reached dimensions of up to 0.2 mm in about 3–4 d. Well diffracting crystals showed a bipyramidal morphology, as shown in Fig. 2.

2.3. X-ray crystallographic studies

Prior to flash-freezing, crystals were transferred in steps to cryoprotectant solutions containing twice the critical micelle concentration of detergent and PEG monomethyl ether 550 at concentrations of 20, 23 and 25% before being mounted on cryo-loops and plunged into liquid nitrogen. The flash-frozen crystals were then placed on the goniometer head and maintained below 123 K during crystal evaluation and data collection. Crystals were screened for their diffraction quality in terms of resolution and mosaicity, using in-house X-ray equipment. Synchrotron data collection was conducted at the Advanced Light Source (ALS), Lawrence Berkeley National Laboratory. The diffraction data were processed using *DENZO*/*SCALEPACK* (Otwinowski & Minor, 1997).

3. Results and discussion

AQP1 from bovine red blood cells has been deglycosylated and purified to homogeneity. Deglycosylation of AQP1 was performed on cell membranes prior to detergent solubilization in order to reduce the amount of time that the protein is in detergent; a prolonged incubation of protein in detergent can affect the conformational homogeneity of the AQP1 protein which is critical for obtaining high-quality crystals. High-quality AQP1 crystals have been obtained using vapor-diffusion techniques and 20% PEG monomethyl ether 550 as precipitant. The diffraction data collected at the synchrotron source under cryo-conditions extended anisotropically (Fig. 3*a*), showing visible diffraction spots to a resolution of 2.7 Å in the poorest direction and beyond 2.2 Å in the other direction (Fig. 3*b*). A complete data set to 2.5 Å has been collected and processed with an overall $R_{\rm merge}$ of 5%; the

R_{merge} of the last resolution shell, with a width of 0.1 Å, is below 26%. The processed data show that the crystals belong to the tetragonal space group $I422$, with unit-cell parameters $a = b = 93.4$, $c = 180.4$ Å, $\alpha = \beta = \gamma = 90^\circ$.

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