Two-dimensional lattices of porin diffract to 6 Å resolution

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Porin (the product of gene *ompF*) is an integral membrane protein (M_r 36 500) of the outer membrane of *Escherichia coli* (strain B^E). The protein has been purified to homogeneity and reconstituted in dimyristoyl-lecithin. Oriented specimen on a flat surface yielded X-ray diffraction pattern, originating from the two-dimensional protein lattice, to a resolution reaching 6 Å. Although these powder rings are broad compared to corresponding diffraction patterns from purple membranes of *Halobacterium halobium*, porin is the first reconstituted integral membrane protein which shows diffraction to this resolution.

Porin *X-ray diffraction* *2D Crystal lattice* (Escherichia coli) *Reconstitution* *Membrane protein*

1. INTRODUCTION

Bacteriorhodopsin of *Halobacterium halobium* is so far the exceptional case of an integral membrane protein which forms two-dimensional lattices of highest quality in the purple membrane. Such patches diffract electrons and X-rays (see fig. 1c for comparison) in a range of up to 3 Å resolution [1–3]. Electron microscopy of unstained preparations may give a three-dimensional high resolution picture of bacteriorhodopsin in the near future. Several other membrane proteins, which form two-dimensional lattices, have been investigated but diffract to a considerably lower resolution. We shall present here X-ray experiments on porin from the outer membrane of *Escherichia coli* which after reconstitution in dimyristoylphosphatidylcholine (DMPC) form 2D lattices and diffract to a 6 Å resolution. These experiments indicate that porin is another promising candidate for electron microscopy to extract 3D information of high resolution from 2D lattices. This would be particularly valuable for a comparison of the structure emerging from 3D crystals [4], and for the search for open and closed channel states within porin trimers.

2. MATERIALS AND METHODS

Protein reconstitution was performed as described [5]. Large hexagonal lattices of porin were used for these studies. The protein was purified to homogeneity using the nonionic detergent octyl-POE as described [4].

2.1. *X-ray diffraction experiment*

Twenty reconstituted samples containing several milligrams of porin each were investigated. From all samples a flat pellet was formed by centrifugation in a swinging bucket rotor at $350\,000 \times g$. The pellet was dried at 85% relative humidity. When equilibrium was reached, the membrane stack was first mounted perpendicular to the X-ray beam on a generator equipped with a rotating copper anode. The beam was filtered by a nickel foil and sent through an Enraf Nonius collimator 0.3 mm in diameter. X-ray diffraction was collected during a 24 h period (fig.1b). In a second experiment a smaller part of the sample, 2 mm in size, was excised and alligned such that membranes were parallel to the beam. Over another 24 h period a diffraction pattern such as that shown in fig.1a was obtained. Only 6 of 20 samples investigated were of the desired quality.

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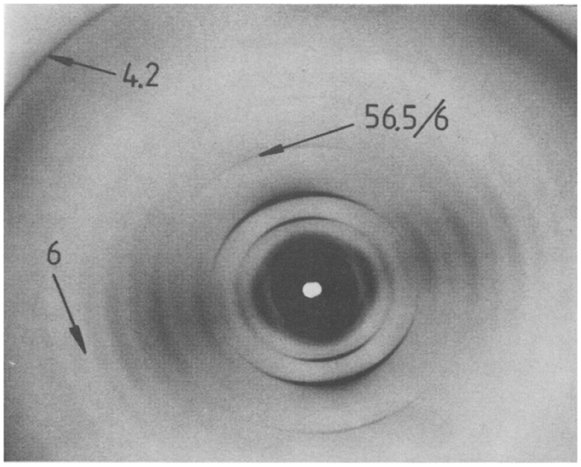
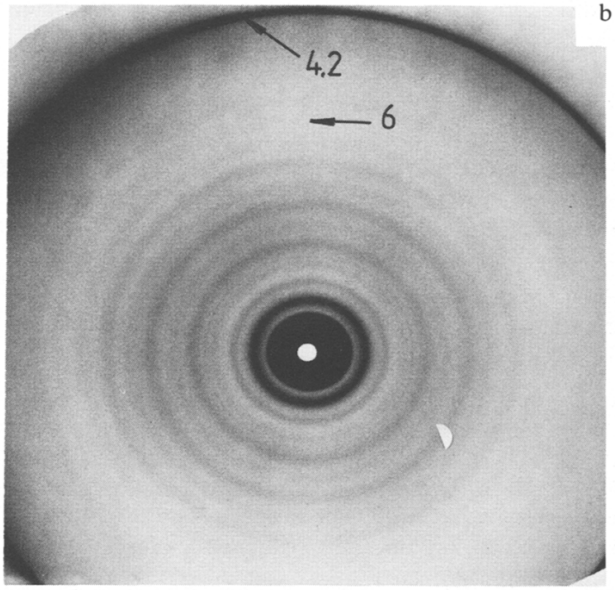
3. RESULTS AND DISCUSSION

Fig.1a shows several sharp equidistant arcs on the meridian with a spacing of 56.5 Å which we assign to multi-lamellar stacks of pure DMPC bilayers forming a separate phase. The most notable property is the sharp ring at 4.2 Å which increases in intensity towards the equator. It results from the fatty acid chain packing and indicates that these lipids are in the gel state, with the fatty acid chains in the all-*trans* conformation. The phase transition temperature between gel and fluid state of DMPC bilayers is at 23°C [6], so that domains consisting of pure lipids would, at a measuring temperature of 20°C, give results that are consistent with known results from pure lipid bilayers [6]. Lipids captured in the densely packed protein lattice are expected to have a hydrocarbon chain conformation which is determined by the uneven surface of the protein [7]. Thus, even in the large hexagonal lattice, with its more than 4-fold higher lipid content than that of the more tightly packed small hexagonal lattice [5], about 70% of the lipids appear to have direct contact with the hydrophobic surface of the protein. The observed mosaic spread of the lamellar pure lipid phase is large enough to disturb the considerably weaker arcs of the protein lattice on the equator in fig.1a. A distinction between these different kinds of reflections is possible due to the increased sharpness of the bilayer reflection. Near the beam stop, additional lamellar reflections are seen on the meridian in fig.1a which

a



b



c

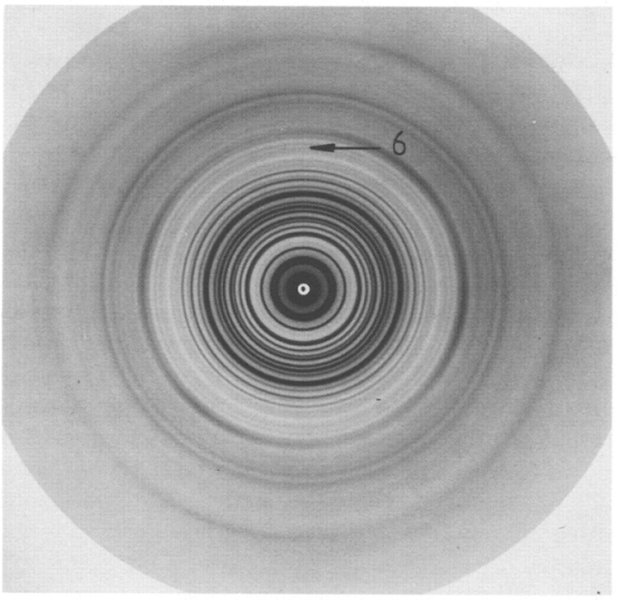


Fig.1. X-ray diffraction pattern of porin stacks aligned parallel to the beam in (a) and perpendicular to the beam in (b). (c) A diffractogram of stacks of purple membranes from *H. halobium* alligned perpendicular to the beam. The numbers in the pictures indicate the resolution in Å.

result from the stacking of bilayers containing protein lattices or from flattened vesicles.

Inspection of the protein-lattice reflections on the equator in fig.1a, or of the corresponding powder rings in fig.1b, clearly reveals the poor order in these lattices if they are compared to the extremely sharp lines resulting from purple membranes (fig.1c). It is not trivial to explain the disorder in the powder pattern of porin. It may reflect the formation of small lattice domains, or lattice disorder, or the occurrence of both. An electron microscopic analysis of single sheets, currently in progress, should answer these questions.

Indexing the protein reflections to the large hexagonal lattice (a = 91 Å) was possible on the basis of electron microscopic studies reported from equivalent specimens [5]. As the powder lines are extremely broad, it cannot be rigorously excluded that rectangular lattices of a = 79 Å and b = 139 Å could not also fit the data. A distinction can, in principle, be made from very low angle reflections. Because of the high background in this region, this has not been done so far.

The most significant aspect of the X-ray experiments reported here, is the observation of a resolution of up to 6 Å. This has not been obtained before in electron diffraction studies on reconstituted single sheets. It appears highly relevant to the prospect of electron microscopy of unstained specimens, as it indicates that low dose images, after appropriate correction for lattice distortions, may result in images in projection of 6 Å resolution. Experiments designed to obtain information about the state of the water-filled channels in porin membranes will be performed. In view of the conductance measurements that have revealed that porin channels (10 Å in diameter) exist in either open and closed states [8], electron microscopy of unstained preparations at high resolution would be the most expedient method to monitor conformational changes. This is particularly relevant since 3D crystals of porin should yield a high resolution structure in the near future. Finally, 2D lattices allow manipulations, such as the application of potentials and the observation of channel properties by conductance measurements at the same time. Electron microscopy may thus provide a significant link between the X-ray crystal structure analysis and the state of the protein in biological membranes.

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