# THREE DIMENSIONAL STRUCTURE OF A MEMBRANE PORE

## Electron Microscopical Analysis of *Escherichia coli* Outer Membrane Matrix Porin

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Porin (the product of gene *ompF*) is an integral protein (MW 36,500) spanning the outer membrane of *Escherichia coli* and may be regarded as a paradigm for pores which allow diffusion of solutes smaller than 650d into cells (1). We have investigated its three-dimensional structure by electron microscopy to characterize its pore geometry in an attempt to relate its structure to its function. The protein has also been characterized biochemically (2) and functionally (3, 4). All structural studies (5) indicate trimers as the basic unit, consistent with functional behavior, inasmuch as pore channels are activated as multiples of three unit steps (3). For the experiments presented here we have utilized vesicles which were reconstituted in the presence of dimyristoyl phosphatidylcholine after removal of octyl-POE (octyl-oligooxyethylene), which had been used for solubilization and purification of porin (5).

Electron-microscopic and diffraction measurements were made on uranyl acetate negatively stained specimens and, more recently, on sugar-embedded preparations (resolution 20 Å and 13.7 Å,). All phase information, extracted from images obtained in tilt series (±60°) was reduced to a common origin via cross-correlation in direct space using the SEMPER image analysis system (6). Combination with electron diffraction intensity data allowed us to obtain three-dimensional images. Recent theoretical calculations have justified this approach by showing that *n*-beam dynamic pertubations are not important for diffraction intensities from thin negatively-stained membrane preparations (7).

## RESULTS

The molecular packing in the three polymorphs found in two-dimensional crystalline arrays is dependent on the lipid-to-protein ratio used for vesicle reconstitution (Table I). In these packing arrangements, the basic structural unit observed consists of the projected threefold arrangement of stain density (Fig. 1 *a*) (8). These triplet indentations are similar to those observed earlier with SDS-extracted outer membranes from *E. coli* $B^E$ (9) and with images of the more recently reconstituted vesicles made from ompC protein and lipopolysaccharide (10). This morphology might explain the activiation of pores in multiples of three unit steps (corresponding to three channels) in conductance measurements. This interpretation assumes that the stain-filled indentations indeed constitute the pores; this assumption now appears justified by three-dimensional image reconstruction. The two membranes of collapsed vesicles appear to stack in register, as revealed by the absence of twinned (hk0) diffraction peaks, and also by the observed symmetry of the electron diffraction intensities along continuous reciprocal lattice lines. The structure derived from double membranes of the small hexagonal form, belonging to trigonal space group P321 (Table I), clearly shows the stain distribution across the stacked membrane lamellae (Fig. 1 *b*). The pores in the stain-filled triplet indentations begin at the outer surface as distinct units, but apparently coalesce into a single channel before reaching the inner membrane surface. Although the resolution of this reconstruction does not allow further characterization of this region, independent evidence (4, 11) indicates a channel constriction with a likely diameter <10 Å. Also unresolved is the structural equivalent of a gating region because the three-dimensional images reveal a continuously filled pore volume for channels expected to be functionally closed at pH 7 (4). Although a higher-resolution image of the pore topology should result from a

TABLE I
CRYSTALLOGRAPHIC DATA FOR PLANAR CRYSTAL POLYMORPHS OF MATRIX PORIN (GENE PRODUCT ompF)

| Lipid-to-protein (weight) ratio | Plane Group | Cell Constants |
|---|---|---|
| 0.72 | p3 | a = 92 Å |
| 0.16 | p3 | a = 79 Å |
| 0.16 | pgg | a = 79 Å, b = 137 Å |



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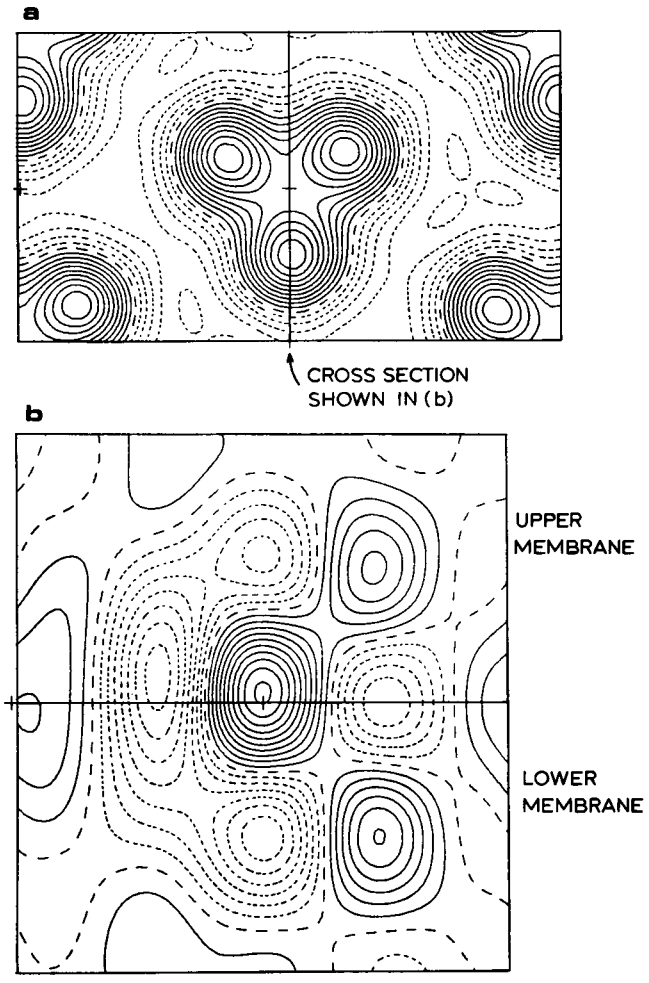


FIGURE 1 *a*, three-fold distribution of negative stain, observed as a repeating unit in all three planar crystalline polymorphs of matrix porin. Scanning transmission electron microscope determinations of mass (8) for unstained specimens give a trimer of molecular weight 100,000 ± 10,000, in agreement with earlier determinations (2, 5). *b*, section through apposed presumed pore channels in a double membrane containing regularly packed porin trimers stacked head to head. The transmembrane distribution of dense stain is apparent.

study of suitably embedded samples (with sugar or frozen-hydrated), a preliminary identification of regions positively stained by uranyl acetate can be attempted by comparing hkℓ electron diffraction intensity data of negatively stained vesicles with those obtained from glucose, sucrose, or maltose-embedded samples (12).

The high-resolution (4 Å or better) structure of this protein is expected from x-ray crystal structure analysis in progress (13) on two crystal forms at pH 7.0 and 9.8. The latter form is hexagonal (a = b = 93 Å, c = 220 Å), and strongly resembles the larger planar hexagonal form described here. With these structures as reference, further electron microscopic determinaitons will be carried out on vesicle preparations which are functionally altered—e.g., by changed phospholipid headgroups and/or headgroup conformation—to assess the effect of such perturbations on overall structure.

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