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# The essential role of specific *Halobacterium halobium* polar lipids in 2D-array formation of bacteriorhodopsin

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The mechanism whereby bacteriorhodopsin (BR), the light driven proton pump from the purple membrane of *Halobacterium halobium*, arranges in a 2D-hexagonal array, has been studied in bilayers containing the protein, 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC) and various fractions of *H. halobium* membrane lipids, by freeze fracture electron microscopy and examination of optical diffractograms of the micrographs obtained. Electron micrographs of BR/DMPC complexes containing the entire polar lipid component of *H. halobium* cell membranes or the total lipid component of the purple membrane, with a protein-to-total lipid molar ratio of less than 1:50 and to which 4 M NaCl had been added, revealed that trimers of BR formed into an hexagonal 2D-array similar to that found in the native purple membrane, suggesting that one or more types of the purple membrane polar lipids are required for array formation. To support this suggestion, bacteriorhodopsin was purified free of endogenous purple membrane lipids and reconstituted into lipid bilayer complexes by detergent dialysis. The lipids used to form these complexes are 1,2-dimyristoyl-*sn*-glycerol-phosphocholine (DMPC) as the major lipid and, separately, each of the individual lipid types from the *H. halobium* cell membranes, namely 2,3-di-*O*-phytanyl-*sn*-glycero-1-phosphoryl-3′-*sn*-glycerol 1′-phosphate (DPhPGP), 2,3-di-*O*-phytanyl-*sn*-glycero-1-phosphoryl-3′-*sn*-glycerol 1′-sulphate (DPhPGS), 2,3-di-*O*-phytanyl-*sn*-glycero-1-phosphoryl-3′-*sn*-glycerol (DPhPG) and 2,3-di-*O*-phytanyl-1-*O*-[β-D-Gal *p*-3-sulphate-(1 → 6)-α-D-Man *p*-(1 → 2)-α-D-Glc *p*]-*sn*-glycerol (DPhGLS). When examined by freeze-fracture electron microscopy, only the complexes containing 2,3-di-*O*-phytanyl-*sn*-glycero-1-phosphoryl-3′-*sn*-glycerol-1′-phosphate or 2,3-di-*O*-phytanyl-*sn*-glycero-1-phosphoryl-3′-*sn*-glycerol-1′-sulphate, at high protein density (less than 1:50, bacteriorhodopsin/phospholipid, molar ratio) and to which 4 M NaCl had been added, showed well defined 2D hexagonal arrays of bacteriorhodopsin trimers similar to those observed in the purple membrane of *H. halobium*.

## Introduction

Bacteriorhodopsin, the light-driven proton pump produced in the purple membrane (PM) of *Halobacterium halobium*, when grown under low aeration conditions, packs in an hexagonal 2D-array in the membrane [1]. This fortuitous arrangement has enabled electron microscopic and diffraction methods to be applied successfully to sheets of the membrane to resolve structural details of BR to a resolution of 3.5 Å [2,3].

The reasons why BR, and other integral membrane proteins, arrange into 2D-arrays in the bilayer are unclear. Bacteriorhodopsin in PM sheets treated with non-solubilizing amounts of detergent, arrange into other orthogonal arrays [4]. This suggests that if a component (possibly a lipid) is removed from the PM by detergent, the resulting loss of hexagonal protein packing would be due to the removal of this component [4]. To support this suggestion, BR, purified entirely free of PM lipids and reconstituted into 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC) bilayers, does not arrange into 2D-arrays [5]. Furthermore, since the protein is in high density (approximately 10 lipids per protein) in the purple membrane, the protein packing density may play some role in array formation.

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Abbreviations: BR, bacteriorhodopsin; HHPL, total polar lipids extracted from *H. halobium* cells; PML, lipids of the purple membrane; DMPC, 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine; DPhPGP, 2,3-di-*O*-phytanyl-*sn*-glycero-1-phosphoryl-3′-*sn*-glycerol 1′-phosphate; DPhPGS, 2,3-di-*O*-phytanyl-*sn*-glycero-1-phosphoryl-3′-*sn*-glycerol 1′-sulphate; DPhPG, 2,3-di-*O*-phytanyl-*sn*-glycero-1-phosphoryl-3′-*sn*-glycerol; DPhGLS, 2,3-di-*O*-phytanyl-1-*O*-[β-D-Gal *p*-3-sulphate-(1 → 6)-α-D-Man *p*-(1 → 2)-α-D-Glc *p*]-*sn*-glycerol; DSC, differential scanning calorimetry; TLC, thin-layer chromatography; $T_m$, endothermic transition temperature; nSTF, non-specific lipid transfer protein; PF, protoplasmic fracture face; EF, endoplasmic fracture face.

Here, bilayer complexes of BR and DMPC, together with either the total lipid or the polar lipids of the purple membrane, have been produced and examined by freeze-fracture electron microscopy to monitor the involvement of lipids in BR-array formation. We have also fractionated the different lipid types of the *H. halobium* cell membrane and added these individually to BR-DMPC complexes formed with BR which had been isolated and purified entirely free of purple membrane phospholipids and does not form an hexagonal array in complexes with only DMPC at any protein/lipid ratio [5], to identify specifically the lipid type responsible for array formation.

## Materials and Methods

*Purification of BR. Halobacterium halobium* cells were grown and harvested and purple membrane (PM) isolated and purified as described previously [6]. Delipidation of the BR was performed as detailed previously [5,7].

*Lipids.* DMPC, which was pure by TLC analysis, was obtained from Sigma Chemical and used without purification.

*Isolation of lipid fractions from H. halobium cell membranes. H. halobium* polar lipids (HHPL), 5–10 mg per g of bacteria (wet weight), were extracted from whole cells as described previously [8–10]. HHPL contain mostly diphytanylphosphatidylglycerol phosphate and diphytanylglycolipid sulphate with traces of diphytanylphosphatidylglycerol and diphytanylphosphatidylglycerol sulphate. The purple membrane of *H. halobium* has a similar lipid composition to the cell membranes, although the cell membranes do not contain DPhPGS which is exclusive to the purple membrane [10,11]. The individual lipid types were separated by two-dimensional thin-layer chromatography (TLC) [12] adapted from earlier methods [13].

The separated, purified lipids were first dissolved in chloroform. The lipid mixture of DMPC and required lipid fraction from HHPL was then dried under a stream of nitrogen and the remaining traces of solvent removed under high vacuum for several hours. A small volume (< 1 ml) of cholate elution buffer (see below) was then added to the lipid mixture for solubilization by agitation at room temperature until optically clear.

After concentration of the delipidated BR-cholate micelles to 5–10 ml by ultrafiltration, the required amount of protein was mixed with the solubilized lipids and the solution dialysed at room temperature in the dark against 1 litre of buffer (10 mM Tris, 2 mM EDTA, 150 mM KCl, 0.02% $NaN_3$, pH 8.0) containing 1.2 g of sodium cholate. The buffer was renewed once or twice a day for about 5 days until a slight cloudiness could be observed. Once vesicles were formed, the sample was dialysed against the same buffer, but without cholate, and adding washed SM-2 Bio-Beads (10 ml slurry) in order to complete the detergent removal for about 5 more days. The BR-containing complexes were purified on a sucrose gradient (10–45%, w/v), washed three times (150 mM KCl; 10 mM Tris-HCl. pH 8.0) and the phospholipid:protein mole ratios determined.

*BR-DMPC complex formation.* Complexes of BR-DMPC with different protein/lipid ratios, and containing all the lipid of the purple membrane (PML), were produced by inserting DMPC into PM fragments, mediated by a non-specific phospholipid transfer protein from bovine liver as previously described [8]. Three complexes were produced with BR/DMPC/PML molar ratios of 1:14:9, respectively.

Other complexes of BR-DMPC containing the polar lipids (HHPL) extracted from the *H. halobium* cell membranes or their individual lipid fractions, were prepared by detergent dialysis. Protein, entirely free of endogenous PM lipids, was produced and characterized as previously reported [5,7]. Detergent solubilized protein and lipids were recombined to include predetermined amounts of the total polar lipid fraction extracted from the *H. halobium* cell membranes (HHPL) and DMPC by shaking over-night at room temperatures in 150 mM KCl, 10 mM Tris-HCl, 0.12% cholate at pH 8. The solution was dialysed for 5 days in 150 mM KCl, 10 mM Tris-HCl (pH 8.0), which initially contained 0.12% cholate and which was successively reduced, and then without cholate but with Bio-Rad SM-2 Bio-Beads (washed). The residual detergent, doped with [$^{14}$C]cholate, was typically reduced to a level of 1 cholate per 700 lipid molecules.

All complexes were subjected to sucrose density gradient (10–45%, w/v) centrifugation (100 000 × *g*; 4°C, 17 h) and only the single major band, after washing three times (150 mM KCl, 10 mM Tris-HCl, pH 8.0), was used for further studies by $^{31}$P-NMR to monitor phospholipid content [5], analysis using a modified Lowry method [14], phosphate assay [15] and then for electron microscopy.

The absorption spectrum of BR, with $\lambda_{max}$ at 560 nm, in all complexes was very similar to that for BR in the purple membrane.

*Freeze-fracture electron microscopy.* The BR-containing complexes were studied by freeze-fracture electron microscopy after quenching from a range of temperatures from 4 to 55°C, initially without added salt. Subsequently, NaCl was added to an aliquot of the same complex to a concentration of 4 M and the samples again studied by electron microscopy. Some samples were re-examined by microscopy after NaCl had been removed by dialysis against 150 mM KCl, 10 mM Tris-HCl (pH 8.0). Samples were quenched using liquid propane and the sandwich technique, in which the samples were mounted between two plain-faced copper holders to give a quenching rate of faster than

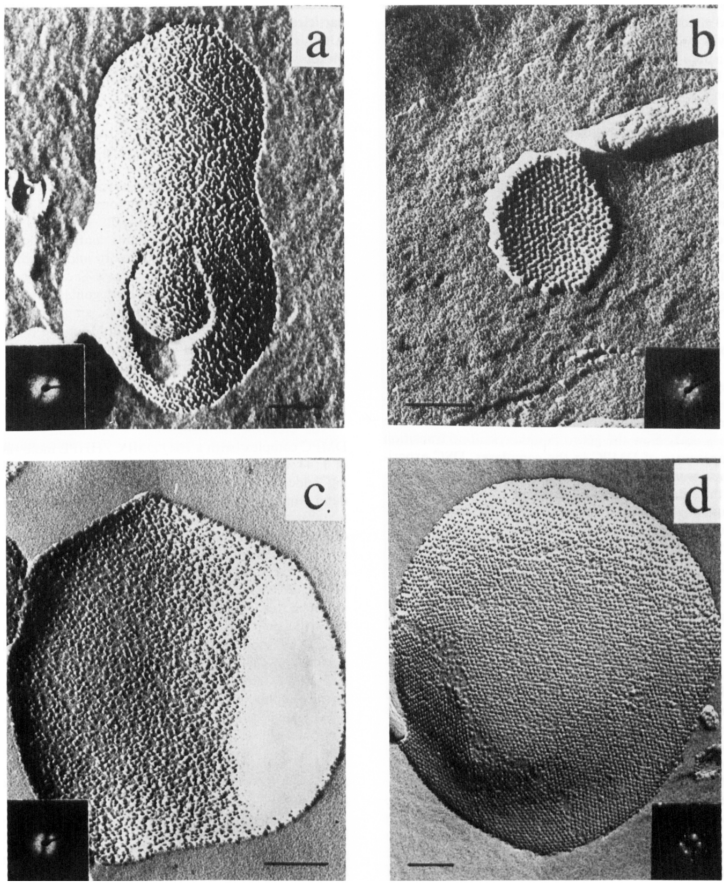


Fig. 1. Freeze-fracture electron micrographs of PM fragments into which DMPC has been inserted using the bovine non-specific lipid transfer protein (as described in the text) to give molar ratios of BR/DMPC/PML of 1:14:9 (a) and (d) and 1:46:9 (b) and (c). Complexes were quenched for freeze-fracturing from either 4°C (a) and (b) or 55°C (c) and (d), and either in the absence (a) and (c) or presence (b) and (d) of 4 M NaCl. Bar represents 100 nm. Shadowing direction is from bottom to top of the micrographs. The insets show optical diffractograms from selected areas of the micrographs.

$10^4$ K $s^{-1}$ which has been shown to be f... enough to prevent reorganization of protein particles in the bilayers during quenching [5]. The samples were fractured and shadowed in a Balzers BAF-400D freeze-fracture device at −120°C. The cleaned replicas were examined in a Jeol-100-B electron microscope.

Light optical diffractograms produced from the electron micrograph negatives were used to judge the quality of the order of the protein particles in the micrographs on areas showing reasonable order.

*Differential scanning calorimetry (DSC).* Heat capacity measurements were performed on a computer controlled Perkin-Elmer DSC-7 scanning calorimeter. Pellets of BR-containing phospholipid vesicles in 4 M NaCl (25 μl, ~ 1 mg BR) and were sealed in stainless steel pans. The reference cell was an identical pan containing air. The scanning rate used was 5 C°/min. Following the first scan of each sample, a reheat scan was recorded and subtracted from the first scan to correct the base line for reversible transitions.

## Results

All complexes formed from DMPC and BR were examined by freeze-fracture electron microscopy by quenching the complexes for replica formation from above and below the gel to liquid crystalline transition temperature of DMPC in the complexes. DSC experiments were performed to determine the temperature of the bilayer transition (data not shown) which was found to be centred at about 23°C but considerably broadened by BR content (as shown previously) [16]) and presence of other phospholipids when compared to protein-free DMPC bilayers.

### *BR-DMPC complexes formed from PM fragments*

Electron microscopic examination of Pt-C replicas of freeze-fractured complexes showed that they were all large (100–2000 nm) bilayer and closed, vesicular structures.

Electron micrographs from the two complexes formed from PM fragments into which DMPC has been inserted, mediated by nsTP, with BR/lipid mole ratios of 1:14:9 and 1:46:9, (BR/DMPC/PML), are shown in Fig. 1.

No residual PM fragments were observed in these complexes. Also, in these complexes, particles were observed on the protoplasmic fracture face (PF) and the endoplasmic fracture face (EF) and they were large enough (diameter ~ 9.2 ± 0.2 nm) to represent BR trimers (Fig. 1), and thus there was no indication of particle asymmetry on either the PF or EF as seen in the PM in which BR is only observed on the PF [16]. No complex, quenched from either 55°C or 4°C in buffer but in the absence of 4 M salt (Figs. 1a and 1c), showed regular packing of the protein units. However, in the presence of added 4 M NaCl (Figs. 1b and 1d), the same complexes displayed regular hexagonal, and additionally in some cases orthogonal arrays of protein particles, with some degree of long range disorder. Although protein-free areas are visible in the complexes with higher protein density (Figs. 1a, 1b and 1d), no regular arrays were observed for the BR-DMPC-PML complex containing less protein (micrograph not shown), with a mole ratio of 1:93:9 (BR/DMPC/PML). Removal of the NaCl by dialysis against 150 mM KCl, 10 mM Tris-HCl (pH 8.0) and examination by EM, showed that the regular protein arrays were now not present, thereby demonstrating that the presence of NaCl is essential to array formation.

The optical diffractograms (insets in Fig. 1) from selected regions of the micrographs confirm a hexagonal array of protein-particles (Figs. 1b and 1d) when 4 M NaCl was added to the complexes. The spacing for these protein particles in the hexagonal array was determined to be 9.2 ± 0.2 nm which is approximately 30% more than that observed (5.9 nm) for the PM [2], as confirmed by correlation averaging of the micrographs to give surface relief reconstruction profile [17].

### *BR-DMPC complexes containing H. halobium polar lipids*

The electron micrographs of a representative BR-DMPC complex (with a BR/DMPC/HHPL mole ratio of 1:44:8), made from phospholipid-free BR, containing added *H. halobium* polar lipids and formed by detergent dialysis, are shown in Fig. 2 and show similar features to those of Fig. 1. The BR/HHPL mole ratios chosen for these complexes were similar to that found in the PM, at approximately 1:9 although they also contain excess DMPC. Complexes with BR/DMPC/HHPL mole ratios of 1:44:8 and 1:43:3 were formed and seen to be large (100–200 nm) and closed vesicular bilayer structures. Irrespective of the BR/DMPC ratio, 2D-hexagonal arrays were formed only the in the presence of 4 M NaCl when quenched from both 55°C and 4°C, as confirmed by examination of the optical diffractograms (insets Fig. 2), which show the array to have a unit cell of 9.4 ± 0.2 nm using the methods described above.

### *BR-DMPC complexes containing individual H. halobium polar lipids*

Four bacteriorhodopsin-lipid complexes were formed in which DMPC was the major bilayer phospholipid, at mole ratios of 1:15:5, BR/DMPC/DPhPGP; 1:16:9, BR/DMPC/DPhPGS; 1:11:5, BR/DMPC/DPhPG and 1:13:6, BR/DMPC/DPhGLS. In addition, complexes were made from BR with DPhPGP and DPhGLS in molar ratios of about 1:40 for both complexes. These complexes were examined by freeze-fracture EM in buffer (150 mM KCl, 10

mM Tris-HCl, pH 8.0) with added 4 M NaCl or 10% glucose. Some complexes were re-examined after removal of 4 M NaCl by dialysis against buffer.

Electron microscopic examination of the Pt/C replicas of freeze-fractured complexes showed that they all were closed vesicular structures. In the complexes con-

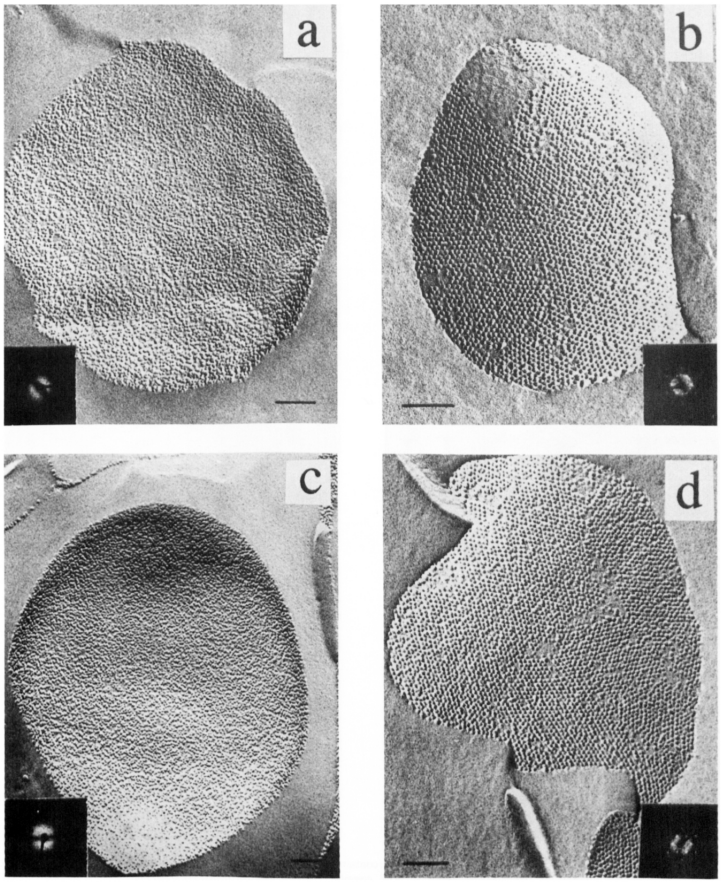


Fig. 2. Freeze-fracture electron micrographs of a bilayer complex containing BR, DMPC and the polar lipids from *H. halobium* cell membranes (HHPL) by detergent dialysis formed using BR, initially entirely free of PM lipids (see text) with mole ratios of BR/DMPC/HHPL of 1:44:8 quenched from 4°C [(a) and (b)], or 55°C [(c) and (d)] in the presence [(b) and (d)] and absence [(a) and (c)] of 4 M NaCl. Bar represents 100 nm. Shadowing is from bottom to top of micrograph. The insets are optical diffractograms from selected regions of the micrographs.

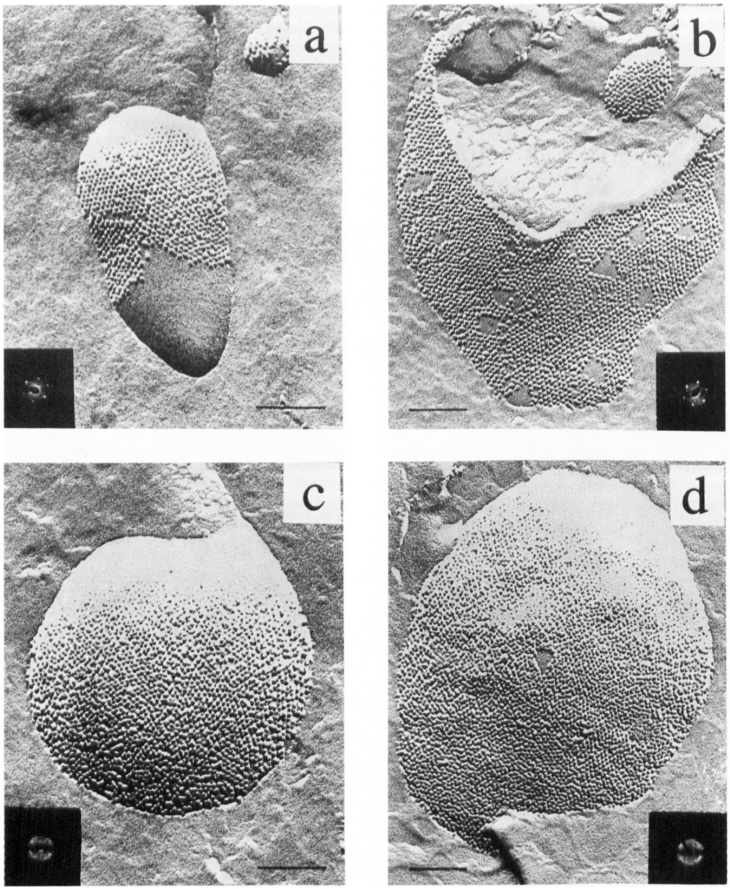


Fig. 3. Freeze-fracture electron micrographs of bilayer complexes containing BR, DMPC and the individual lipids from *H. halobium* cell membranes by detergent dialysis as described in the text. The complexes contained BR/DMPC/DPhPGP, 1:15:5, mole ratios (a), BR/DMPC/DPhPGS, 1:16:9, mole ratio (b), BR/DMPC/DPhPG, 1:11:5, mole ratios (c) and BR/DMPC/DPhGLS, 1:13:6, mole ratios (d). All complexes were in 4 M NaCl and quenced from 4°C which is below the bilayer gel to liquid crystalline transition temperature. Bar represents 100 nm. Shadowing direction is from bottom to top of micrographs. Optical diffractograms (insets) were made from selected areas of the negative.

taining the individual phospholipid fractions, however, the vesicles were generally smaller than those of Figs. 1 and 2 (below 100 nm in diameter), and are shown in Figs. 3a, b and 4a, b, whereas the complexes containing the glycolipid fraction (Figs. 3d and 4d) showed much larger vesicles (of several 100 nm in diameter). Additionally there was always a disproportionate number of protein particles visible in the vesicles depending upon

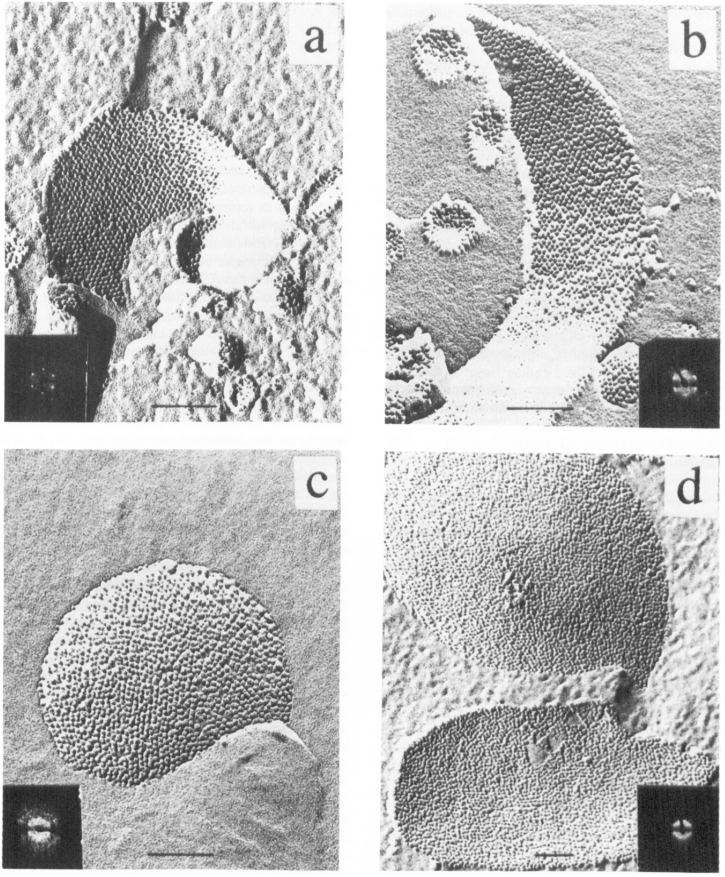


Fig. 4. As for Fig. 3 but all samples were quenched from 55°C which is well above the bilayer gel to liquid crystalline phase transition temperature, as shown by DSC.

their size with smaller vesicles being fully packed and larger vesicles showing no or few protein particles.

Freeze-fracture electron micrographs from complexes containing the various lipid fractions isolated from *H. halobium* cell membranes and quenched from 4°C are shown in Fig. 3. Only the complexes containing DPhPGP or DPhPGS and to which 4 M NaCl had been added showed hexagonal arrays of protein particles, with a unit cell dimension of 8.5 ± 0.2 nm. Complexes of BR/DMPC containing DPhPG or DPhGLS (Figs. 3c and 3d) at similar BR/lipid ratios as those in Figs. 3a and 3b, or with any added type of HHPL but without 4 M NaCl, do not reveal any regular protein packing (micrographs not shown). Also, complexes which did show hexagonal protein arrays in 4 M NaCl, showed no such arrays when the NaCl was subsequently removed by dialysis.

The temperature from which the complexes were quenched appears not to be important in determining array formation since quenching from 55°C produced micrographs which were very similar to those shown in Fig. 3. Indeed, the array is seen to be even better ordered in the complex containing DPhPGP quenched from 55°C rather than 4°C, with higher order reflections visible in the optical diffractogram to second (inset Fig. 4b) or even third orders (inset in Fig. 4a). The unit cell for the BR arrays was determined to be 9.5 nm which is about one third larger than the lattice constant of the purple membrane.

## Discussion

The mechanism whereby bacteriorhodopsin, or other integral membrane proteins, arrange into 2D-arrays in bilayer membranes is not well-defined. Such mechanisms may be specific and different for each protein. However, the similarity of the gross structural features now being recognized for families of proteins, for example the 7-helix bundle receptors [18], may imply that some similar driving forces could promote integral proteins to form arrays in a close packing condition for a homogeneous protein content in a bilayer of suitable composition and under appropriate conditions. On the other hand, bacteriorhodopsin may be a special case although this needs to be demonstrated.

Freeze-fracture electron microscopy is a good method for examining 2D-array formation of membrane spanning proteins, such as bacteriorhodopsin which group of lipid types, and under what conditions, are required to re-establish BR trimers into a two-dimensional bilayer array similar to that found in the purple membrane. Previously we showed that BR, free of endogenous PM lipids, does not form into 2D-arrays in DMPC bilayers over a wide range (1440:1 to 67:1, DMPC/BR mole ratios) of protein/lipid ratios [5]. In the present study, it has been shown that it is the DPhPGP and the DPhPGS components of the PM, which are capable of re-establishing BR trimers into an hexagonal array in DMPC containing complexes when 4 M NaCl is present.

Here we have shown that hexagonal 2D-arrays of BR can be re-established in DMPC bilayers and by salt and the presence of one or more components of the PM-derived phospholipids, either DPhPGP or DPhPGS, both having highly charged polar headgroups. Previously it was shown that the protein-protein contacts alone in complexes of DMPC with no other lipid are insufficient for array formation [5]. In addition, even though the complexes were formed by very different procedures, with one type containing all PM lipids and the other only the *H. halobium* cell membrane phospholipids, together with DMPC in both types, the final result in terms of array formation is the same.

Since DPhPGS is the phospholipid that is found only in the PM, and not the *H. halobium* cell membrane [11], this lipid is the most favoured candidate for this function. Indeed, it is the complexes containing the polar lipid of either DPhPGS or DPhPGP that show the well ordered hexagonal BR arrangement, as seen in Figs. 3a, 3b, 4a, 4b, with even second-order (inset in Figs. 4a, 4b) and third-order reflections produced in the optical diffraction pattern (inset in Fig. 4a).

It is not thought that salt is important in determining the oligomeric state of BR, since little difference in the protein particle size is observed without (Figs. 1a and 1c, 2a and 2c) or with salt (Figs. 1b and 1d, 2a and 2c). In addition, salt is necessary to form stable polymorphic complexes of purple membrane lipids, as shown by calorimetric measurements [19,20]. The role of salt is unclear in protein trimer array formation although its presence does preclude any simple electrostatic involvement in the generation of arrays. The well ordered BR-pattern generated within bilayers of these complexes under the influence of high salt, disappeared when the salt was dialyzed below a level of 2 M. PM fragments used for electron diffraction studies to deduce structural information [3], are frequently prepared in glycerol as a cryoprotectant which, like salt, can reduce the overall water content of the bilayer. In order to obtain extensive dehydration, glucose (1 and 10% by weight) and trehalose (1 and 10% by weight) were added to the samples instead of high salt. The electron micrographs (not shown) did not reveal any hexagonal BR arrangement in samples containing BR/DMPC/HHPL. Therefore, limited dehydration by salt and relatively high protein density may be amongst the requirements for good ordering of this protein in 2D-arrays.

However, the results presented here do suggest that the lipid headgroups have a major effect in BR array formation, since all the bacterial lipids have the same acyl chain composition with saturated phytanyl groups

ether linked to the glycerol backbone [21]. The complexes containing the lipids with the more charged headgroups show the well ordered BR arrays, and therefore it appears that array formation is not mediated by an acyl chain effect alone.

The degree of order obtained for BR trimers in the complexes formed and shown in Figs. 1b and 1d, 2b and 2d, 3a and 3b, and 4a and 4b, is rather high and indeed higher than obtained by us using the same procedure for the PM (unpublished data not shown). In the optical diffractograms, single order reflections were obtained with PM and recombined complexes quenched from lower temperatures, 4°C (Fig. 1b and 2b, Fig. 3), but two or three orders of diffraction are routinely observed from micrographs of recombined complexes quenched from higher temperatures, 55°C (Figs. 1d, 2d, 4a, 4b).

The particles forming the arrays are thought to be trimers, both from their size (9.2 nm from trimer to trimer centre) and since protein-free areas are observed at high protein density (BR/total lipid; from 1 : 16 upwards) even though, in the complexes shown in Figs. 1a and 1d, Figs. 3a and 3b, Figs. 4a and 4b there is sufficient lipid to form only one annulus of 25 lipids around a monomer of BR, calculated on geometric grounds from the electron density profile of the protein [2,22]. The size of the particles observed here are somewhat larger than reported for the purple membrane itself, being $9.6 \pm 0.2$ nm and 6.2 nm [23], respectively. It has been shown that shadow angle and technique (freeze-fracture or negative stain) can influence the size of particles observed in replicas, which are assigned to BR trimers here, observed in electron microscopic examinations. In addition, since DMPC is added to the complexes here with protein, the trimers in these complexes may occlude more phospholipid than is occluded in the PM.

The long range (7–20 particle diameters) order of an hexagonal, as well as some orthogonal, array of particles is seen to be not perfect. Some discontinuity in particle order is observed after more than 5–7 particles although the same direction of particle alignment is maintained over much greater distances (> 50 particle diameters) in the best examples (Fig. 1d). This differential packing can be seen from the discontinuity in the protein arrangement at lower temperatures (Figs. 3a, 3b) due to the competition between gel phase lipid long-range ordering competing with protein ordering in complexes quenched from below $T_m$ where gaps in the lattice domains are seen (Figs. 3a, 3b) and for which second order reflections are depressed. The coherent lattice domains here are small and sometimes angled with respect to each other so that the broad reflections in the optical diffractograms may coalesce to a Debye-Scherer-Ring thereby reducing their intensity [17].

The tendency of DPhPGP to form non-bilayer lipid phases [21] may be the reason for the smaller size of many of the liposomes containing higher amounts of DPhPGP, these liposomes being more highly curved and high in BR content. In order to find the minimal number of constitutents of BR-lipid complexes which are able to form an hexagonal BR arrangement, complexes made from BR and the individual PM lipids fractions, but without DMPC, were produced by the same methods described here. BR-DPhPGP complexes yielded small vesicles only (micrographs not shown) and pattern recognition was not possible on such small bilayer areas. When BR was reconstituted in DPhPGS alone, a lipid which is known to be a good layer forming lipid [21], and examined by the same methods, large liposomes were formed but again no hexagonal BR arrangement was observed (micrographs not shown) as was also true for BR-DPhGLS complexes both with and without DMPC.

Although no generalizations can be extended to other integral proteins at present, the use of such lipids and reconstitution methods may be useful as appropriate promoters for 2D-crystallization into sheets of other membrane proteins.

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