The Paracrystalline Nature of the Purple Membrane of *Halobacterium halobium* I. Lattice Distortions within the Membrane

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Purple Membrane of *Halobacterium halobium*, X-Ray Diffraction, Paracrystalline Distortions, Crystal Size

X-Ray diffraction patterns from oriented purple membrane of *Halobacterium halobium* show that the packing of proteins within the membrane has a paracrystalline structure. The paracrystalline mean distance fluctuations, g, were found to be 1.5 ($\pm$ 0.1)% for the (100) packing planes of the proteins. The corresponding paracrystallite sizes are of $\sim$ 850 Å. It is suggested that paracrystalline lattice distortions within the membrane may be explained by conformational singularities of the proteins or by an irregular packing of adjacent proteins and lipids.

Introduction

Much attention has been given lately to the study of the structure of the purple membrane of *H. halobium* [1–3]. The interpretation of X-ray and electron diffraction patterns has certainly provided a valuable information about the arrangement of protein and lipid components within the membrane. According to Blaurock and Stoeckenius [4] the membrane consists of a two-dimensional mosaic of proteins and lipids packed side by side with a stacking period of 49 Å. Such a molecular packing results in a $\sim$62 Å hexagonal unit cell containing 3–4 protein molecules and approximately 40 lipids. More recently Henderson [5] concluded from diffraction studies on oriented specimens that the protein components are packed across the membrane into clusters of 3 components with a 3 fold axis at the centre of each cluster. In addition, a broad equatorial diffraction halo at 10 Å and axial maxima at 5 and 1.5 Å indicate that the protein molecules which are contributing to $\sim$75% of the mass of the membrane are made up of α-helices. The lipids are less exactly positioned in the lattice than the protein. Model calculations [1] suggest that they are arranged within the membrane as in a bilayer though exhibiting a higher distribution of lipids on one side than on the other side of the membrane.

This paper presents a further experimental investigation of the X-ray diffraction pattern from oriented intact purple membrane concerning the size of the coherently diffracting domains extending parallel to the plane of the membrane and the type of disorder within the protein lattice. We set out to establish whether the disorder present within the membrane is the result of structural distortions which preserve long range order in the crystallites (microstrains or distortions of the first kind) or of distortions in which long range order is not preserved (distortions of the second kind). The latter type of disorder, also known as paracrystallinity, has been thoroughly discussed by Hosemann and coworkers and has been shown to exist not only in synthetic polymers [6], catalytic ammonia crystals [7], ferrites [8], molten metals [9] but also on biopolymers [10].

To distinguish between the two types of distortions by X-ray line broadening, it is required to analyzed at least three orders of a given hkl reflection. In the case of the purple membrane this condition is only met for the 100 reflections. According to Hosemann *et al.* [11] distinction can be made between the presence of distortions of the first kind (microstrains) and distortions of the second kind (paracrystallinity) by plotting the X-ray diffraction integral width, $\delta\beta$, as a function of the order, m, of the hkl reflection. If the plot of $\delta\beta$ against m turns out linear microstrains are present. If, on the contrary, the plot of $\delta\beta$ against m^2 is linear the structure is admittedly paracrystalline.

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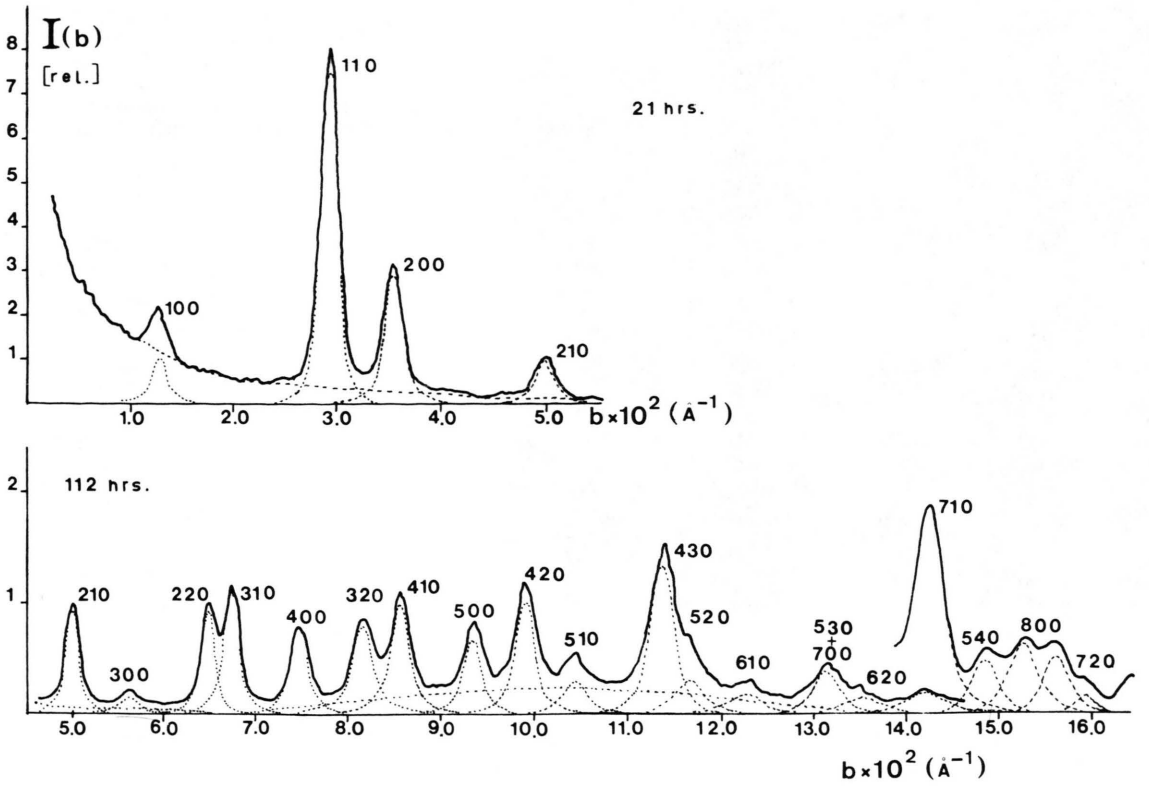

Fig. 1. X-ray diffraction resolved profiles for the equatorial reflections of oriented purple membrane of *Halobacterium halobium*. Exposure times of 21 and 112 h respectively.

Experimental

The *H. halobium* bacterium – kindly supplied by Dr. J. Gonzalez – was cultured and the purple membrane isolated according to the procedure of Oesterhelt and Stoeckenius [12]. The X-ray diffraction reflections were recorded using a Kiessig point-collimation camera with Ni filtered copper K_α radiation from a Rigaku rotating anode generator working at 8 Kw. Collimators of 0.3/0.5/0.3/0.2 mm were used. The incident beam was ~0.2 mm at the specimen. The specimen-film distance used was 100 mm. Exposure times of 21 and 112 h were required for the, second and third reflections, and for the higher orders respectively (Fig. 1). The collimation errors were corrected by deconvolution of the experimental (Lorentz squared) profile with that of the primary beam assuming a Lorentz squared profile. The intensities and spacings of the reflections were measured from densitometer traces that were recorded on a Joyce-Loebl microdensitometer. The reflections were subsequently resolved with a Dupont 310 curve analyzer. The diffraction experiments were carried out at room temperature. The lattice parameters of the proteins within the purple membrane were measured from the positions of the reflections on traces as shown in Fig. 1.

Results and Discussion

The X-ray diffraction pattern from an oriented pellet of purple membrane with the X-ray beam incident parallel to the plane of the membranes stack shows the presence of a series of equatorial sharp reflections beyond 3.5 Å (Fig. 2). This pattern confirms the existence of the two dimensional hexagonal lattice first observed by Blaurock [1] and Henderson [5]. In addition to the peaks previously reported by Henderson [5] three new reflections have been indexed in the present work. These are the 540, 800 and 720, respectively corresponding to lattice spacings of (6.815 ± 0.005) Å, (6.651 ± 0.005) Å and (6.508 ± 0.005) Å. Two diffuse halos centered at

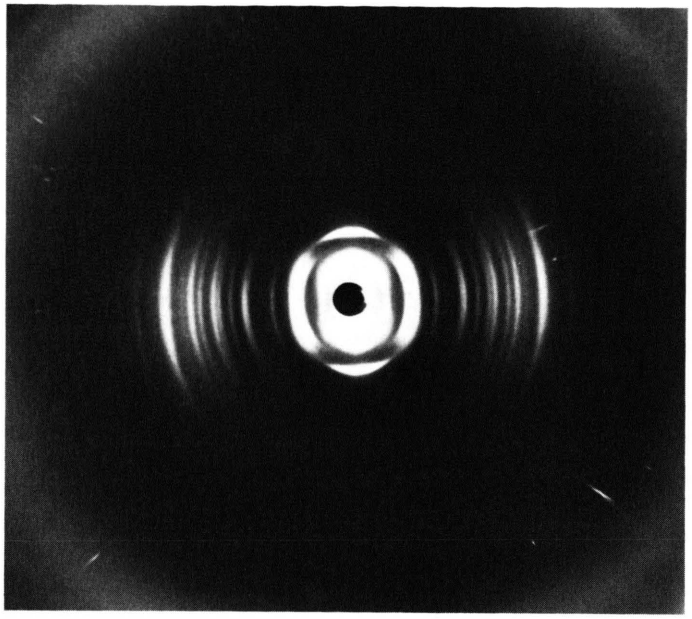

Fig. 2. X-ray diffraction photograph of sedimented pellet of purple membrane at room temperature. X-ray beam parallel to the membrane stacking plane which in the present mounting corresponds to the horizontal. Specimen film distance: 100 mm. Exposure time: 21 h.

~10 Å and 4.6 Å have also been observed. The former presumably arises from the irregular side-to-side packing of the α-helixes [2] and the latter has been associated to the liquid-like arrangement of lipid chains perpendicularly to the plane of the membrane [5]. The percent paracrystalline distortion (g) and the size of the coherently diffracting domains (D) parallel to the plane of the membrane were derived according to the theory of paracrystals [13] from the equation:

$$\delta\beta_{hkl}=1/D_{hkl}+(\pi g m)^2/\bar{d}_{hkl}$$

where $\delta\beta$ is the integral width, m the order of each reflection, $\bar{d}_{hkl}$ the average netplane separation and $g=\Delta d_{hkl}/\bar{d}_{hkl}$, where Δd represents the mean distance fluctuation between net-planes. From the $\delta\beta$ vs m^2 plot values of g and D were obtained for the packing planes of the proteins. Fig. 3 illustrates the conspicuous linear increase of $\delta\beta$ as a function of b^2 ($b=2\sin\Theta/\lambda$) in the case of the seven orders occurring for the family of (100) lattice planes. The apparent deviation of the 700 reflection from the least square straight line is probably due to the contribution of the 530 reflection which appears at the same spacing. It is interesting to note that this is the first time that such a large number of higher order reflections with increasing $\delta\beta$ are reported for a paracrystal. The plot of $\delta\beta$ against m shows, on the other hand, a parabolic increase suggesting that the contribution of defects of the first kind is negligible.

The g and D-values for the (100), (110), (210) and (310) planes are shown in Table I. The set of reflections investigated shows the existence of anisotropy in D. The smallest coherently diffracting domains are obtained in the direction of closest packing of protein arrays. The paracrystalline character of model membranes (cerebrosides) has been previously reported [14–15]. In this case, however, the

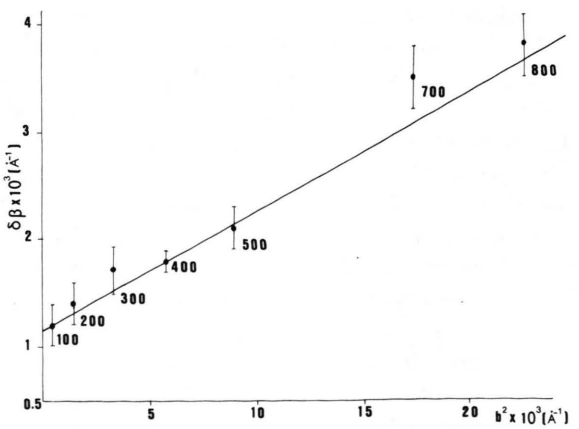

Fig. 3. Corrected integral width of the (h 00) X-ray diffraction peaks against the square of reciprocal vector b for the hexagonal structure of the purple membrane.

Table I. Lattice spacing, d_{hkl}, percent paracrystalline distortion, g_{hkl}, coherent mean size of diffracting domains parallel to membrane plane, D_{hkl}, for various planes of the hexagonal lattice of the purple membrane.

Lattice planes	d [Å]	g [%]	D [Å]
(100)	53.2 ± 0.6	1.5 ± 0.1	850 ± 100
(110)	30.7 ± 0.3	1.0 ± 0.5	920 ± 140
(210)	20.1 ± 0.1	2.2 ± 0.6	940 ± 250
(310)	14.8 ± 0.1	3.1 ± 0.8	1150 ± 400

coherent mean size and paracrystalline distortions measured was referred to dimensions normal to the lamellar stacking, offering the number of bimolecular lamellae which are diffracting coherently. The basic netplane fluctuation $g_{100} \sim 1.5\%$ for the purple membrane corresponds to irregularities in the packing of proteins. This value is of the same order of magnitude than the fluctuations found in synthetic polymers [16]. The g_{210} and g_{310} values are slightly larger because the net-planes involved are smaller than those corresponding to the d_{100} packing of the protein clusters. Paracrystallinity in the case of the purple membrane implies a limiting degree of order restricted to a few intermolecular distances ($n \sim 13-18$). The lattice disorder becomes increasingly larger over greater intermolecular distances being proportional to $\sqrt{n}$ [17], where n is the limiting number of intermolecular vectors within the paracrystal. Thus the domain size D refers to the extent of the structure over which the total fluctuation reaches the size of the mean separation of the netplanes. Having more disordered intermolecular arrangements than exist in the crystalline state paracrystalline structures are presumably more reactive so-that with such a structure the purple membrane would more efficiently be able to fullfil its biological role. This should be confirmed directly when experiments with wet membranes, showing possible changes of g with varying degree of water content, are completed. We have previously shown, in fact, that colloidal structures can only prevail if paracrystalline distortions do occur [18]. These distortions could be caused in the purple membrane case by three dimensional incorporation of conformational distortions and/or maybe through an irregular side-by-side packing distribution of adjacent proteins and lipids.

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