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The Membrane Geometry of the Prolamellar Body

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Summary

When etioplasts are exposed to light, the branched tubular lattice of the prolamellar body becomes the large flat discs of the primary lamellae. We show that in spite of the very different apparent morphology of these two membranous structures, their membranes have similar average curvature and inside-outside surface areas. This implies that the packing or molecular organization of the lipids and proteins can be similar in the two structures, and that consequently the transformation does not require much high energy "flip-flop" of membrane components from one side to the other. We also discuss the relative physiological stability of the two structures.

Keywords: Avena sativa; Etioplasts; Membrane geometry; Phototransformation; Prolamellar bodies; Thylakoids.

1. Introduction

The branched tubular structure in an etioplast which transforms into the lamellar membrane structure of a chloroplast is called the prolamellar body (PLB) (see Fig. 1; Gunning and Steer 1975, Kirk and Tilney-Bassett 1978). Exposure to light changes the apparently rigid lattice of the PLB into the large flat discs of the thylakoid lamellae. It was previously believed that the intricate membrane structure of the PLB was governed by proteins and ribosomes. However recent data (Ruppel *et al.* 1978, Kesselmeier and Budzikiewicz 1979, see also Wellburn *et al.* 1977) have shown that the extracted PLB lipids alone and steroidal saponins in particular reaggregate into branched tubular structures similar to natural PLBs, and that proteins are not essential building units in this process.

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It is still not clear what physical mechanisms govern the geometry of the PLB and the lamellae, nor what intervening shapes are involved in the transition, though the two structures often appear to be contiguous and the transformation reversible (Gunning and Steer 1975, Kirk and Tilney-Bassett 1978, Ch. 27). It is possible that lipids (and proteins) may be transferred from one side of the membrane bilayer to the other during the transition—a process which would almost certainly require a very high activation energy. But this appears unlikely in view of the apparent ease with which the two structures interchange. The conclusion that emerges from the calculations presented here is that such an energetically expensive transfer is not necessary. It may be shown that the average membrane curvature of the two structures is similar despite the striking morphological differences between them: they can therefore be formed from bilayers whose inner and outer surfaces differ in area by a similar amount. This further implies that the lipid packing in the membranes of these two structures may also be very similar.

The concept of molecular packing has recently been used to explain the observed properties, such as the elasticity, sizes and asymmetries, of bilayers and bilayer vesicles (Israelachvili *et al.* 1977, Carnie *et al.* 1979). This theory ascribes to each species of lipid some intrinsic geometric or packing characteristics—the surface area per molecule at the aqueous interface and the maximum extended length of the hydrocarbon tails. These packing properties constrain the molecules to aggregate only into a limited number of structures (*e.g.*, micelles, bilayer vesicles, planar bilayers, etc.) in which the packing stresses—and hence the interaction free energies—are minimized. Israelachvili (1977, 1978) and Petrov *et al.* (1978) have extended the concept of molecular packing to lipid-protein membranes and the fluid mosaic model, and have used it to examine qualitatively such processes as lipid-protein clustering, lipid pore formation and membrane shape changes.

One corollary of the molecular packing theory is this: In a given section of bilayer membrane, since "flip-flop" (or exchange of molecules between the two monolayers) is extremely slow (Bloj and Zilversmit 1976) then during changes in membrane shape the areas of the two sides of the membrane are unchanged (see also Evans and Hochmuth 1978). Thus, if a membrane becomes bent or warped it can only change its shape in such a way as to conserve any original difference that existed between the two areas. This is equivalent to conserving the overall curvature of the membrane (Evans 1974). We show below that this situation obtains, or nearly so, in the structures of the PLB and the lamellar membrane [2].

[2] In some cases the area per molecule may be changed asymmetrically; thus for example a change in ion concentration on one side only of a planar bilayer membrane might produce wrinkling caused by an asymmetric change in area (Papahadjopoulos 1976).

2. The Geometry of the Prolamellar Body

The basic units of construction of the PLBs are illustrated in Figs. 1 A and *B*.

From a detailed analysis of electron micrographs of the plastids of *Avena sativa* GUNNING and JAGOE (1967) proposed that one of the structures of the prolamellar body is as shown in Fig. 1 *A*. This structure may be considered

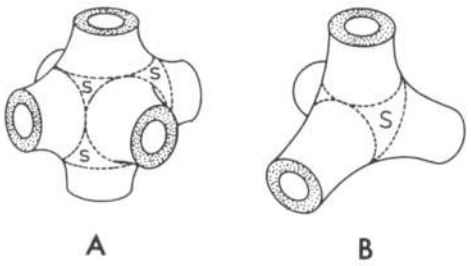

Fig. 1. The six-armed (*A*) and four-armed (*B*) branched tubular "nodal units" of the cubic and tetrahedral prolamellar body structures (after GUNNING and STEER 1975). These units can join together to form a continuous three-dimensional membrane lattice which at almost any point is convex along one axis and concave along the axis at right angles to the first

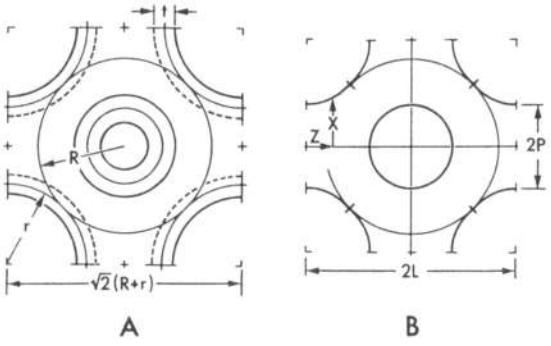

Fig. 2. (*A*) A "nodal unit" similar in shape to that suggested by GUNNING and JAGOE (1967). The arms are toroidal sections which abut a sphere of radius R. The toroid minor radius is r and the membrane thickness t. (*B*) Here the toroidal sections are replaced by axially symmetric figures whose radius X is given by $X = P\cosh(Z/P)$

to comprise six-armed "nodal units" linked together in a continuous cubic lattice. By contrast the membrane structure in the thylakoid lamellae is not very different from a stack of planar membranes with highly curved regions only at the edges.

In the "nodal unit" in Fig. 1 *A*, the arms are symmetric about their axes. If the eight approximately triangular segments s were portions of a spherical surface they would match smoothly and continuously onto the arms (dotted

circles), and so these triangular segments cannot be very different in shape from spherical. If this spherical surface has radius R then the area of the eight nearly triangular segments can be shown by simple geometry to be $A_s = 0.49\,\pi\,R^2$. The difference between the areas of triangles with radii $(R + {}^1/_2\,t)$ and $(R - {}^1/_2\,t)$ is therefore $0.49\,\pi\,[(R + {}^1/_2\,t)^2 - (R - {}^1/_2\,t)^2] = 2\,(0.49)\,\pi\,Rt$. If the six tubular arms are toroidal in shape (Fig. 2 *A*), the total area of these toroidal sections can be shown to be

$$6\,A_{tor} = 6 \times 2\pi r \left\{ \frac{\pi}{4}(R + r) - r \right\} \sin\frac{\pi}{4}.$$

The difference between the areas of toroidal sections displaced ${}^1/_2\,t$ inside and ${}^1/_2\,t$ outside this surface is therefore

$$6\,\Delta A_{tor} = 6 \times 2\pi t \left\{ 2r - \frac{\pi}{4}(R + r) \right\} \sin\frac{\pi}{4}.$$

Thus the total membrane area is

$$A = A_s + 6\,A_{tor} = 2\pi\,[0.24\,R^2 + 3.33\,Rr - 0.91\,r^2]$$

and the difference between inner and outer surface areas is

$$\Delta A = \Delta A_s + 6\,\Delta A_{tor} = 2\pi t\,[5.15\,r - 2.85\,R].$$

From the measurements of Gunning and Jagoe: $(2\,r + t) = 38$ nm, and $\sqrt{2}\,(R + r) = 59$ nm. This yields, using their measured membrane thickness of $t = 6$ nm: $A = 7 \times 10^{-3}\ \mu m^2$, $\Delta A = 2 \times 10^{-3}\ \mu m^2$, and so $\Delta A/A = 0.30$. (In comparison, a sphere which has the same ratio of $\Delta A/A$ has a diameter of 80 nm. This is substantially larger than the size of the prolamellar body repeat unit.) This result is easy to see qualitatively: since the two principal radii of curvature of the membranes in the toroidal arms are of opposite sign (see also GUNNING and JAGOE 1967) the overall curvature of these sections, defined conventionally by $(1/R_1 + 1/R_2)$, is small. The spherical "triangular" sections are small and do not have a large curvature; elsewhere convexities in one direction are counterbalanced by concavities. The average curvature of the whole unit is therefore small and not as different from a flat lamella as might be expected. This conclusion suggests an alternative analysis:

Suppose the structure is not, as the sketch of Gunning and Jagoe suggests, composed of toroidal and spherical sections, but is such a shape that its curvature *is* everywhere small or zero, which implies that $\Delta A/A \approx 0$. Such a structure, shown in Fig. 2 *B*, is practically indistinguishable from that in Fig. 2 *A*. This structure requires that the "arms" have a cross-sectional radius which is a cosh function of the distance along their axes, *i.e.*, $X = P \cosh (Z/P)$. In order for the arms to fit together smoothly, we further require that at $Z = L - R\cos\frac{\pi}{4}$, $X = R\sin\frac{\pi}{4}$, and $\frac{dX}{dZ} = \sinh (Z/P) = 1$. Thus we find

that P = R/2 and L = 1.15 R, and therefore the ratio L/P is determined and equal to L/P = 2.3. The measurements of Gunning and Jagoe give L/P = 59/(21 — 6) = 3.9. The assumptions of this analysis are crude, and depend sensitively on the exact thickness and location of the membrane surfaces. Nevertheless, the "theoretical" and experimental ratios are not too dissimilar and, as shown above, the difference corresponds to an area difference of only 30%. The membrane in the PLB is evidently organized in such a manner that the difference between its inner and outer membrane areas is non-zero, but not as large as might be expected for such an ornate structure.

Prolamellar bodies are more commonly observed in tetrahedral (4-armed) lattices, shown in Fig. 1 *B*, whose shape also suggests toroidal sections (or perhaps cosh functions of revolution) abutting on spheres. Indeed, a straightforward extension of the above analysis readily shows that there exist at least three types of tubular lattices in which the membranes can have small or zero curvature throughout. These are composed of 4-armed, 6-armed and 12-armed units. Further, if θ is the angle between any two adjacent tubular arms (radiating out from a nodal centre) then the condition for zero or near zero membrane curvature and surface area difference is attained when

$$\frac{\text{length of tubule}}{\text{width of tubule}} = \frac{L}{P} = \frac{\cos(\theta/2)}{\sin^2(\theta/2)} + \sinh^{-1}(1/\tan(\theta/2)).$$

For a 6-armed unit, $\theta = 90^\circ$, the above ratio is 2.3 as already noted. For a 4-armed unit, θ equals the normal tetrahedral angle of 110° (Ikeda 1968) and the above equation now gives a ratio of 1.5. Kirk and Tilney-Bassett (1978, Ch. 11), and Simpson (1978) quote values of about 50 nm and 20–22 nm for the lengths and widths respectively of the tetrahedral tubules in both bean and barley PLBs [though Wehrmeyer (1965) measured a smaller tubule length of about 35 nm, and Ikeda (1968) obtained 21 nm and 9 nm for the length and width of bean PLB tubules using $KMnO_4$ fixations]. It appears therefore that the measured ratio of (tubule length)/(tubule width) is about 500/(200 — 60) = 3.6, which is close to the value of 38/9 = 4.2 obtained by Gunning and Steer, Fig. 36 e (1975). These ratios are higher than the "theoretical" ratio of 1.5 expected for a membrane of zero curvature, but nevertheless corresponds to a difference in membrane surface area $\Delta A/A$ of only 40–50%. Thus the membranes of tetrahedral PLBs have a structure qualitatively similar to the cubic PLBs, both characterized by a low $\Delta A/A$.

As already mentioned the above analysis suggests that a 12-armed tubular lattice can also satisfy the condition of a small or zero net curvature, with $\theta = 60^\circ$ so that the length/width ratio equals about 5, though such structures have not been observed.

3. Conclusion and Discussion

Whatever the intermediate transition states may be, the above analysis shows that the membrane of the prolamellar body can transform to the mainly flat thylakoid lamellae without requiring much "flip-flop" of lipids or proteins from the outer to inner membrane monolayers. More importantly, the transition does not require a large change in local membrane curvature and there is therefore only a small molecular packing energy difference between the two apparently very different membrane structures. These conclusions are of some relevance in view of the observations that PLB membranes transform to thylakoid membranes by a simple structural rearrangement without the production of new membranes, and that the crude chemical composition of PLB membranes is not grossly different from that of thylakoid membranes (Kirk and Tilney-Bassett 1978, Ch. 27). This analysis, however, does not yet clarify the conditions which give rise to such a regular structure with such ornate geometry nor the possible survival advantages conferred by the ability of the plastids to make the transition from PLB to thylakoid.

The thylakoid lamellae have an obviously convenient shape not only because a larger area than the PLB may be required for photon capture, but also because they have a convenient stacking and unstacking response to osmotic changes probably induced by changes in the local ionic concentration (Duniec *et al.* 1979). Of what use then is the shape of the prolamellar body? Gunning and Jagoe (1967) argue that it efficiently stores a large amount of membrane in a small space. Further advantages may be that PLB-type membraneous structures are unique in possessing continuous inner and outer regions, while maintaining a more or less continuous low curvature throughout.

Indeed, for a paracrystalline membrane whose lowest energy configuration is a curved surface the PLB structure appears to be the only one which allows for a three-dimensional network of constant curvature at all points of the membrane. This is in contrast to the lamellar structure which has highly curved (and possible highly stressed) regions at the edges. This may confer on the PLB certain advantages over the discontinuous and less rigid thylakoid membrane structure. However, so long as the curvature or $\Delta A/A$ remain fixed, the inner and outer volume ratio of the prolamellar body must also remain fixed—it cannot swell, shrink or deform much in response to osmotic (photo-induced) stresses, as can the thylakoid lamellae and the rod outer segment disc membranes (Norisuye and Yu 1977). Perhaps an osmotic effect occasioned by exposure to light is the simple cause of the transformation from the prolamellar body to the thylakoid lamellae.

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