MATTERS ARISING

'Lipidic particles' are intermembrane attachment sites

THE nature of lipidic 'particles' and 'pits' in freeze-fracture replicas of liposomes has been much debated. Recently, Miller¹ presented arguments that the particles and pits represent conical deformations of membranes, rather than inverted micelles sandwiched between membrane leaflets—a model repeatedly suggested by Verkleij *et al.*²⁻⁴. We have also observed lipidic particles and pits in liposomes of mixed egg phosphatidylcholine (PC)–soybean phosphatidylethanolamine (PE)⁵ and mixed PC–PE–cardiolipin, and our results essentially agreed with those of others¹⁻⁴. Moreover, we have more convincing evidence for the deformation model as presented below. The evidence has led us independently to arrive at a similar model⁶ to that proposed by Miller.

We have obtained cross-fractured profiles of the particles and pits in multilamellar vesicles. As shown in Fig. 1a, most of the remaining halves of the fractured particles and pit are seen as interlamellar contact points, although a few single particles (open arrows) are also observed. The profile of the lipidic particle is clearly conical, with the base extending to 200 nm. They are considerably larger than the mean size of proteinaceous intramembranous particles, as seen in Fig. 1b. Here, in a PC–PE vesicle containing human erythrocyte membrane protein, large conical lipidic particles are clearly seen at the fracture line as contact sites between adjacent membranes. Proteinaceous particles are hemispherical with diameters of ~10 nm. As the lipidic particles and pit are really cusp-shape distortions, they should be named as such instead. When multilamellar vesicles containing <30% PC in PE were quenched at room temperature, rows of cusps on the fractured faces were frequently observed. However, cusps are not found in large single lamellar vesicles (verified by negative staining) of the same lipid mixture. This provides independent evidence that intermembrane attachment is required for the formation, stabilization and alignment of the cusps. Our results give definite support for the cusp model¹ in preference to the inverted micelle model².

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Fig. 1 Freeze-fracture electron micrographs of multilamellar vesicles of 25% PC in PE (a), and with added crude human erythrocyte membrane protein (smaller particles) extracted with Triton X-100 (b). Lipidic particles and pits are seen as intramembrane attachment sites in cross-fractured areas (arrowhead), some of which have developed extensive contacts (solid arrows). Open arrows indicate occasional conical particles on one bilayer alone. Experimental procedures are given elsewhere^{5,6}. Scale bar, 500 nm.

VERKLEIJ AND DE KRUIJFF REPLY—We believe that 'lipidic particles' represent inverted micelles formed either within a single bilayer^{1,2} or at the nexus of intersecting bilayers during fusion of membranes³⁻⁵. We have presented^{2,3} evidence for this interpretation and

against the lipidic particles being local bilayer curvatures as inherent to the intermembrane attachment side (IMAS) model as proposed by Miller⁶ or the similar cusp model proposed by Hui and Stewart in their letter. These arguments and new facts supporting our hypothesis can be summarized as follows:

(1) The crucial point ignored by the IMAS model is that the lipidic particles have been exclusively observed in lipid systems in which at least one of the lipid species prefers the hexagonal phase II (H_{II}). In this latter phase the lipid molecules are cone shaped and oriented with their polar head group towards an aqueous channel of a small radius. Any model of the lipidic particle must take into account this favourable organization of H_{II} -type lipids, a criterion met in the inverted micelle model. This requirement, however, is not fulfilled with the IMAS model. For instance, in the cardiolipin–lecithin system⁶, cardiolipin will be present at the top of the strongly curved bilayer where it can only fit if the molecules have an 'inverted cone' shape. However, cardiolipin assumes a cone shape in the presence of Ca^{2+} .

(2) In line with this argument, a direct morphological association has been observed between the H_{II} phase and the lipidic particles^{4,7}, indicating a close relationship of these structures. Furthermore, we have found in some systems^{3,7} three-dimensional arrays of lipidic particles which probably represent an agglomerate of inverted micelles. An analogous example of structural relationships between micellar and hexagonal phase can be found in another area of lipid polymorphism in that lipids can adopt both micellar and hexagonal H_I phases in water, as, for instance, does lysophosphatidylcholine⁸.

(3) In the IMAS model, the bilayer must be strongly curved as the lipidic particles can be very small (60–130 Å in diameter)². Such a strong bilayer curvature is energetically highly unfavourable because the minimum curvature of the bilayer in the most strongly curved system (sonicated vesicles) is at least 250 Å in diameter.

(4) The IMAS model ignores the extensive ³¹P NMR studies on the lipidic particle-containing systems^{2,9}. The profound hysteresis of the isotropic peak in the ³¹P-NMR spectrum of these systems can be best explained by a model in which inverted micelles are located in rows at the fusion site of two bilayers¹⁰.

In conclusion, we feel that these arguments strongly suggest that lipidic particles represent intrabilayer inverted micelles. Also, the observation that in systems such as that described by Hui and Stewart the particles are relatively large and cone shaped can be interpreted as the