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FUSION OF PHOSPHOLIPID VESICLES IN ASSOCIATION WITH THE APPEARANCE OF LIPIDIC PARTICLES AS VISUALIZED BY FREEZE FRACTURING

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Summary

The addition of Ca^{2+} to small unilamellar vesicles of an equimolar mixture of egg phosphatidylcholine and cardiolipin induces fusion of these vesicles in association with the appearance of lipidic particles on the fusion sites.

Membrane fusion clearly requires that participating lipids adopt transitory non-bilayer configurations during the intermediate stages. The nature of the possible intermediate structures (such as micellar [1] or inverted micellar [2, 3]) and their relation to the physical properties of membrane lipids remain, however, a matter of some speculation. Recently, it has been argued that endogenous lipids which preferentially adopt the hexagonal (H_{II}) phase under certain conditions may be directly involved in fusion events [4]. In this regard it has been established that the presence of Ca^{2+} is vital to the fusion process [5] and that the addition of Ca^{2+} can trigger formation of the hexagonal (H_{II}) phase in model membrane systems consisting of certain pure [6] or mixed [7] species of naturally occurring phospholipid. It is therefore of interest to first establish that Ca^{2+} can also induce fusion between model systems partly comprised of such phospholipids, and secondly to establish that such fusion events proceed via the non-bilayer structures engendered by the presence of Ca^{2+}. In this work we present results obtained employing model systems comprised of an equimolar mixture of bovine heart cardiolipin and egg yolk phosphatidylcholine in conjunction with

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freeze-fracture techniques. It is shown that the addition of Ca^{2+} does induce fusion, and that intermediary inverted micellar lipid structures may be observed at the fusion interface.

The model membrane system employed consisted of an equimolar amount of egg yolk phosphatidylcholine and bovine heart cardiolipin (Sigma Chem. Co, Ltd., St. Louis) which was initially dispersed in a Triton X-100 (0.5%), 10 mM Tris/acetate pH 7.4, 150 mM NaCl solution to give a lipid concentration of 2.5 mM. Subsequently the mixture was incubated in the presence of Bio-Beads SM2 (0.3 g/ml) overnight in order to remove the detergent [8]. This material was then centrifuged (30 min at 30 000×g) to remove larger structures and the clear supernatant diluted to arrive at a 2 mM lipid concentration. These vesicles were incubated for 30 min in the presence of varying amounts of Ca^{2+} and were then concentrated for freeze-fracture studies by centrifugation at 150 000×g. Glycerol was added to 30% (vol./vol.) to prevent freeze damage. Replicas were obtained according to well-established techniques and were observed employing a Philips 301 electron microscope.

The morphological features observed as the Ca^{2+} concentration is varied between 0 and 10 mM are illustrated in Fig. 1a–j. In the absence of Ca^{2+} (Fig. 1a) a population of unilamellar vesicles with diameters ranging between 250 Å and 1000 Å is observed. Both the convex and the concave fracture faces are smooth. The presence of 2 mM $CaCl_2$, which caused the vesicle suspension to become translucent, is seen to result in a population of larger vesicles, demonstrating that vesicle fusion has occurred. Higher Ca^{2+} contents which result in an increasingly milky appearance of the vesicle suspension, are observed to result in corresponding increases in the average vesicle size (Fig. 1b–j). Even vesicles with diameters larger than 5000 Å are apparent (Fig. 1h–j).

Two characteristics of these results are that no multilamellar structures are observed and, more importantly, that the fusion process is associated with the appearance of particles and pits on the concave and convex fracture faces respectively. The average diameter of these particles is approx. 100 Å, although larger ones can be detected. The complementary pits are approx. 30 Å smaller than the particles due to the replication technique. It should be noted that particles and pits are predominantly located on vesicles which have an unusual dimpled appearance (Fig. 1b–j). Such features are thought to reflect vesicles in intermediate stages of fusion. The most interesting observation however is that the particles and pits appear to be preferentially located in regions which correspond to the edges of fusing vesicles. This is particularly apparent in the micrographs of Fig. 1d and 1e.

Freeze-fracture micrographs of the precipitate obtained at high (> 10 mM) Ca^{2+} concentrations have a granular texture (Fig. 1k and 1l) which may be attributed to densely packed particles and pits of approx. 100 Å and 70 Å diameter, respectively. In addition, some regions of hexagonal (H_{II}) phase lipid may be observed (Fig. 1m).

We consider the interpretation of the results presented in this communication to be relatively straightforward. In previous freeze-fracture work on cardiolipin-egg yolk phosphatidylcholine dispersions we have interpreted the

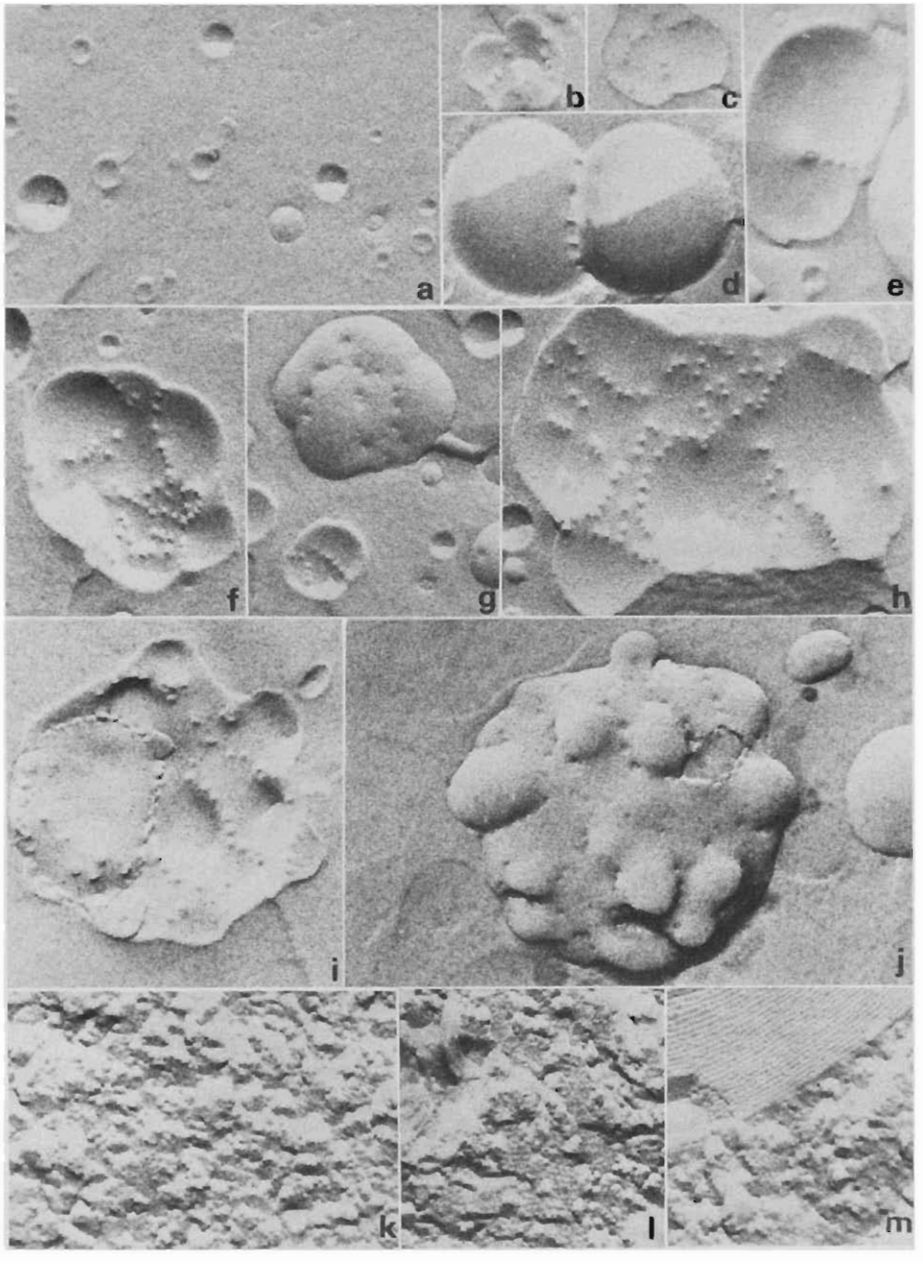

Fig. 1. Freeze fracture micrographs of an equimolar mixture of egg-yolk phosphatidylcholine and bovine heart cardiolipin prepared by the Triton X-100/SM-2 beads procedure: a, without $CaCl_2$; b—j, representative for $CaCl_2$ concentrations between 2 and 10 mM $CaCl_2$; and k—m at Ca^{2+} concentrations larger than 10 mM $CaCl_2$. Magnification is about 100 000 X. Shadowing direction is from bottom to top for all micrographs.

particles observed as inverted micelles sandwiched between two lipid monolayers [9, 10]. Such structures appear to occur as intermediaries in the bilayer to hexagonal (H_{II}) phase transitions observed for cardiolipin on addition of Ca^{2+} [6, 11]. We therefore consider that the fusion of vesicular model membrane systems composed of cardiolipin and egg yolk phosphatidylcholine arises from the ability of the endogeneous cardiolipin to facilitate formation of non-bilayer lipid structures in the presence of Ca^{2+}. In particular, we conclude that the fusion event proceeds via formation of intermediary inverted micellar structures at the fusion interface.

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