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SELECTIVE DECORATION OF HYDROPHILIC MOIETIES OF MEMBRANE MOLECULES IN FREEZE FRACTURE

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Summary

Selective decoration of the hydrophilic moieties of phospholipid molecules on freeze fractured bilayer faces was made using residue water vapor in an oil-free vacuum unit. The preferential decoration technique was applied to label structural faults of bilayers, such as domain boundaries and other regions of molecular dislocation which are not visible by conventional morphological observations.

The usefulness of freeze fracture electron microscopy in membrane structural studies would be greatly improved if various molecules on the fractured surfaces could be labelled. Several ingenious methods have been used to chemically identify the features seen on freeze fractured faces [1,2]. In this article we report the use of specific decorations with water vapor to label the hydrophilic moieties of freeze fractured membrane faces.

It has been known for some time that when a heterogeneous surface is exposed to condensing vapor, condensation may occur preferentially on some parts of the surface [3]. The phenomenon is commonly known as decoration in freeze fracture terminology. The selective decoration on the freeze fracture faces of the plasmalemma of Baker's yeast by water vapor condensation was described by Gross et al. [4]. They attribute the mechanism of preferential condensation of water vapor to electrostatic interactions with the polar moiety of the membrane molecules. When membranes are freeze fractured, most exposed faces represent the hydrophobic interior of the lipid bilayer [5]. Hydrophilic portions of the membrane molecules are exposed only in areas such as those along cross fractured edges; along structural faults such as solid domain boundaries [6] or around the pits where transmembrane structures have

been pulled out [4]. The frozen, aqueous buffer surface surrounding the specimen is of course hydrophilic.

Two examples of selective decoration of the hydrophilic portion of freeze fractured membranes are shown in Figs. 1 and 2. The membrane vesicles in

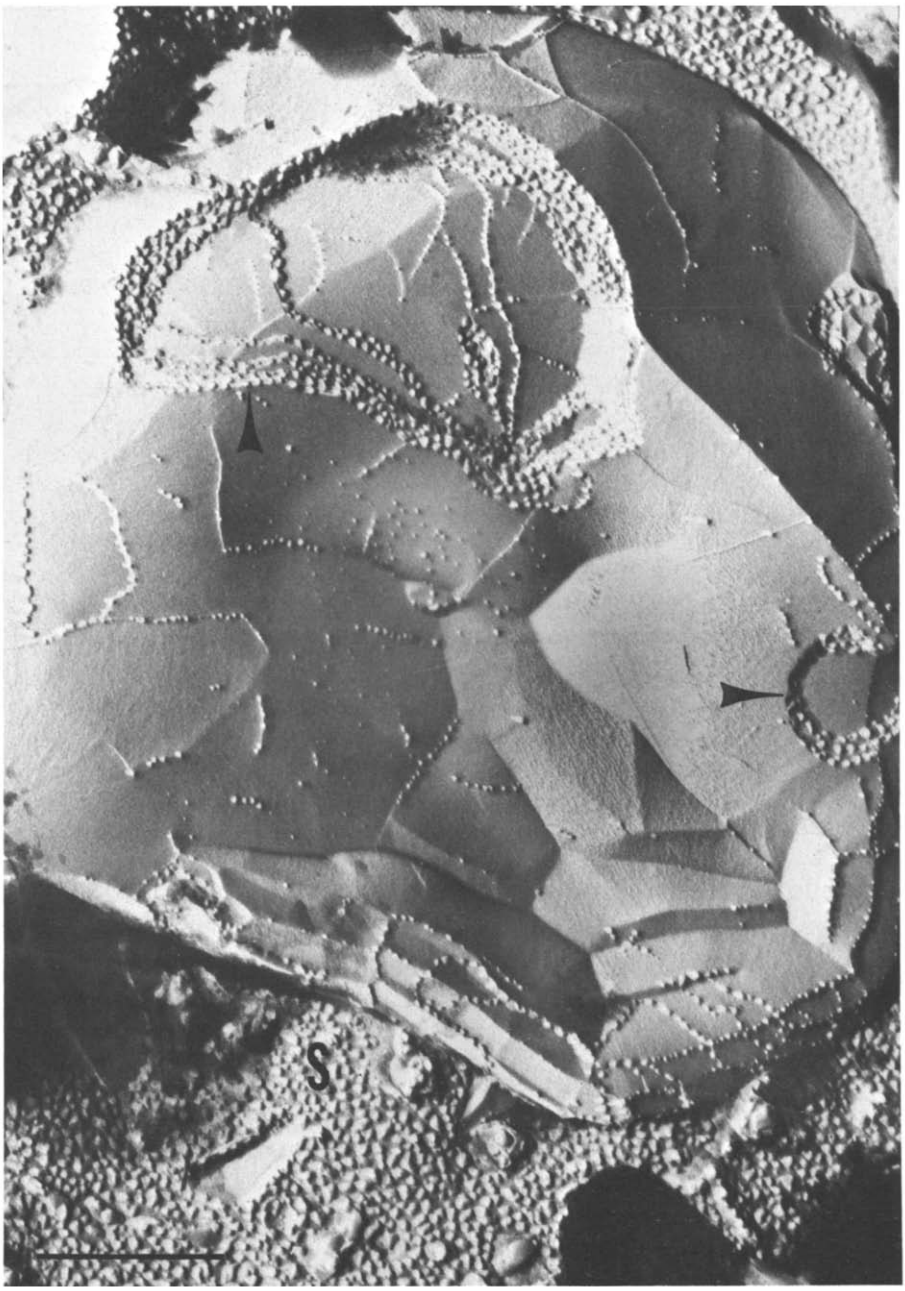

Fig. 1. Decorated freeze fractured face of a multilamellar vesicle of mixed 16,16-diacylphosphatidylcholine and bovine brain phosphatidylserine at a molar ratio 8:2, and quenched from 4° C. Decorations are concentrated on the cross lamellar fractured edges (arrowed) and on the frozen aqueous buffer surface (S). Bar = 500 nm.

these micrographs are multilamellar vesicles of mixed 16:0 / 16:0 - phosphatidylcholine and a natural phosphatidylserine extracted from bovine brain, in a molar ratio of 9:1 or 8:2. The vesicles were made by suspending vacuum dried, mixed lipids in a buffer solution containing 100 mM NaCl, 2 mM histidine, 2 mM TES and 20 mM $CaCl_2$. The detailed sample preparation and freeze fracture procedures were described previously [7]. The samples were quenched from 4°C into melting Freon 22. The fracture and replication were performed in a Polaron E7500 freeze fracture module pumped by a Ultek TNB-X ion pump and titanium sublimation booster unit. The system is entirely oil free. In order to deliberately induce decoration, the specimens were fractured at a temperature between —115 to —120°C, with a liquid-nitrogen-cooled knife blade. After the initial fracture, a trace of the aqueous specimen was deposited on the blade. The same knife blade was then used to refracture the specimen, so that a minute amount of water vapor was produced around the specimen at the impact of the second fracture, which momentarily warmed up the aqueous deposit. Samples fractured once were not decorated, even at temperatures as low as —150°C. Alternatively, decoration could also be induced by closing off the freeze fracture chamber from the ion pump and allowing the vacuum to deteriorate to 10^{-4} Torr. After exposing the sample to 10^{-4} vacuum for one minute the sample was again covered with the shroud and the system was pumped to 10^{-7} Torr within one minute, at which time it was replicated. These two methods of decoration produced similar results.

Electron micrographs of decorated liposome suspensions show that the condensing particles concentrated on the frozen buffer background. Only when the background ice surface is covered do they appear on the membrane fracture face. In vesicles showing cross fractured lamellae, the decorations were concentrated on the cross fractured, open edge of the bilayer lamellae (Fig.1). It is here where the polar head groups of the phospholipids are exposed. On vesicles which were fractured entirely along the interior of a single lamella decoration is seen along sharply angulated ridges, or along lines whose planar patterns are similar to those of domain boundaries [7—9]. At 4°C, these mixtures of phospholipids are in the L_{β}' phase (unpublished result) where no banded pattern is observable. The structural faults outlined by decoration shows straight edges and turning angles of approximately 120°, similar to those seen at higher temperatures where domain boundaries may be discerned by the banded patterns of the P_{β}' phase [7]. Apparently, at 4°C, staggered molecular packing at the domain boundary and other structural faults expose sufficient portions of the polar head groups of the phospholipid to be labelled (Fig.2).

The most likely source of the decorating particles is water vapor desorbed from the knife blade and elsewhere in the vacuum system. Deliberately leaking-in water vapor produced similar results [4]. Since the decorations preferentially concentrate on the frozen buffer background, cross fractured lamellae and faults in the bilayer where the hydrophilic portions of the lipid molecules are exposed, it is likely that the decorations are a result of electrostatic interactions as proposed by Gross et al. [4]. The condensation does not nucleate on areas of topographic roughness, which is also in agreement with Gross et al. [4]. The hydrophilic decorations produced here and in other oil-free vacuum systems [4] is different from those seen preferentially on the hydrophobic

fracture face of lipids [5]. The latter is often caused by hydrocarbon contaminations from diffusion pump oil, used in most freeze fracture vacuum systems.

The geometry of domain boundaries in lipid bilayers is difficult to observe, because each domain differs from its neighbor only by molecular packing. In

Fig. 2. Decorations on a vesicle (molar ratio 9:1) under similar conditions as in Fig. 1. When cross lamellar fracture is absent, the decorating particles are seen mostly along molecular dislocations. Bar = 500 nm.

some cases, neighboring domains may be differentiated by their differently oriented surface patterns, such as those in P phases [7—9]. Domain boundaries may also be visualized by diffraction contrast electron microscopy [10]. Decoration techniques have been applied at the light microscopic level to visualize mesomorphic phase domains of liquid crystals [11] and in lipid multilayers [12]. The application at the electron microscopic scale has potential in labelling structural faults in membranes at the molecular scale. Since surface banding (P phase) exists only under particular temperatures and hydration ranges in a limited number of phospholipids, the decoration technique is a more universal way to delineate domain boundaries in lipid bilayers. It is also useful in pinpointing the parts of the fracture face where transmembranous components are pulled out of the bilayer, thereby exposing the outer surface of the membrane [4]. Possible hydrophilic channels in the membrane interior may also be detected in this manner.

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