CALCIUM-INDUCED PHASE SEPARATION AND FUSION IN PHOSPHOLIPID MEMBRANES

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Observations on such varied cellular activities as endocytosis, exocytosis, intracellular digestion and cell fusion have focused attention on the importance and ubiquity of membrane fusion in both cellular and subcellular systems (reviews 1–4). Despite the diversity of the various membranes that undergo fusion, and the very different types of fusion stimuli involved, a number of factors appear to be common to most, if not all, forms of membrane fusion (4). Particular attention has been given to the role of calcium ions (Ca^{2+}) in regulating the many cellular processes in which membrane fusion occurs (4, 5). It has been suggested that rapid changes in the binding of Ca^{2+} to membranes may represent a mechanism by which membranes can undergo rapid and reversible structural transitions during fusion (4) and that displacement of Ca^{2+} from membranes may constitute a common pathway by which different physiological stimuli act to induce fusion (4).

The similarities between many of the properties of phospholipid bilayers and natural membranes has prompted several groups of investigators to study fusion between lipid vesicles (6–9), and fusion between lipid bilayers (10) as potential models for the membrane fusion reaction.

In this communication, we provide a brief review of our recent studies on the effect of Ca^{2+} on molecular organization within lipid membranes and its ability to induce fusion between different lipid membranes.

It was shown several years ago that Ca^{2+} (and Mg^{2+}) can produce a drastic increase in the efflux of Na^{+} through phosphatidylserine vesicles (11) although both metals produce a considerable reduction in the area per molecule of phosphatidylserine monolayers at the air-water interface (12). It was later recognized through the use of black lipid films composed of phosphatidylserine, that the increase in conductance and instability (rupture) of these membranes was produced only when Ca^{2+} was added to one side of the membrane, while the presence of Ca^{2+} on both sides of the same membranes resulted in increased stability and lower conductance (13).

The above results were extended more recently through the use of fluorescence techniques (14) and differential scanning calorimetry (15–17). Both studies indicated that the presence of Ca^{2+} and Mg^{2+} at low concentrations (10^{-4}–10^{-3} M) produced a considerable increase on the temperature of the endothermic transition from solid to

liquid crystalline for acidic phospholipid membranes. Moreover when the concentration of Ca^{2+} was increased above a certain threshold (approximately 1 mM for phosphatidylserine membranes) the endothermic peak disappeared from within the experimental range of temperatures, and the membranes appeared to be crystalline at ambient temperature (16). These results indicate that when Ca^{2+} is added to purified acidic phospholipid membranes at temperatures at which they are "fluid," it can induce a phase transition resulting in the formation of crystalline membranes.

The ability of Ca^{2+} to reorganize phospholipid membranes is also evidenced by differential scanning calorimetry of phospholipid mixtures. When phosphatidylserine is mixed with lecithins of higher melting point, the resultant membranes suspended in aqueous NaCl exhibit broad endothermic transitions at temperatures in between those of the pure components (6, 16). Following the addition of Ca^{2+} however (but not of Mg^{2+} at similar concentrations), these membranes exhibit the endothermic transitions of the pure lecithin components, indicating a phase separation into individual phospholipid species (6, 16). Since the effect is reversible by EDTA, it can be concluded that Ca^{2+} induces a phase separation of phospholipid domains within the plane of each membrane, rather than separation into completely separate phases (particles). Similar conclusions were arrived at by observations on the effect of Ca^{2+} on the electron spin resonance spectra of spin-labeled phospholipids deposited on millipore filter membranes (18).

Several lines of evidence (6) have indicated that Ca^{2+} can also induce fusion of acidic phospholipid membranes. Thus, it has been established by changes in particle size as seen by gel-filtration and ultracentrifugation, and also by changes in melting point as seen by calorimetry, that after addition of Ca^{2+} (followed by excess EDTA) to two populations of phosphatidylserine vesicles, the resultant membranes are composed of mixed species instead of the original individual components. Although such molecular mixing from different membranes can also be the result of diffusion of individual molecules, it appears that the above observations represent actual fusion of phospholipid vesicles. Freeze fracture electron microscopy of phosphatidylserine vesicles, indicates that the addition of Ca^{2+} (1 mM) induces a remarkable morphological transformation from small (200–500 Å) single lamella vesicles to large multilamellar cylinders which seem to be spirally folded.[1]

Evidence for fusion between two populations of phosphatidylglycerol vesicles has been obtained more recently at concentrations of Ca^{2+} approximately 10 mM. As with the phosphatidylserine vesicles discussed above, this effect of Ca^{2+} occurs abruptly above a certain concentration, but only when both populations of vesicles are above their transition points (fluid) at the incubation temperature. Incubation of two populations of phosphatidylcholine vesicles under the same conditions gave no evidence for appreciable molecular mixing even after relatively long periods of time at high temperature. On the other hand, evidence of molecular mixing due to exchange diffusion

[1] Papahadjopoulos, D., W. J. Vail, K. Jacobson, and G. Poste. 1975. Cochleate lipid cylinders produced by fusion of unilamellar lipid vesicles. *Biochim. Biophys. Acta.* 394:483.

of individual molecules was obtained in two cases. One, when two populations of phosphatidylcholine vesicles were made to incorporate long chain ions of the opposite charge (6) and two, when two populations of phosphatidylglycerol vesicles were incubated in aqueous NaCl at low ionic strength (10 mM). In both these cases, exchange diffusion occurred only at temperatures when both populations of vesicles were in a "fluid" state.

Several attempts were made to fuse different populations of phosphatidylserine and also phosphatidylglycerol vesicles by addition of other multivalent cations, with negative results so far. Thus, addition of Mg^{2+} at concentrations up to twice those for Ca^{2+}, polylysine (up to 0.5 mg/ml) or cytochrome *c* (up to 5 mg/ml) had no effect in producing phase separation in mixed phospholipid membranes, or fusion of separate populations of vesicles in spite of the production of floculation and aggregation. It appears, therefore, that the effect of Ca^{2+} in such systems is not due to simple neutralization or screening of the negative charges. It is possible that a crucial step in this process is the formation of polymeric phospholipid-Ca^{2+} complexes by chelation of the metal ion by the functional groups of four vicinal phospholipid molecules (12). Whatever the precise mechanism may be, it should be noted that three independent experimental observations, i.e., sharp increase in permeability, phase separation, crystallization and fusion, all occur at the same Ca^{2+} concentrations for each system of membranes, 1 mM for phosphatidylserine (11, 16) and 10 mM for phosphatidylglycerol (20, 16). Furthermore, although Ca^{2+} induces aggregation in all the above systems, aggregation does not appear to be the principal factor, since several other systems that tend to give aggregates do not also give increased permeability or fusion.

In conclusion, it appears that Ca^{2+} can induce reorganization within phospholipid membranes resulting in both separation of domains of individual components from a mixed membrane, and also fusion of separate membranes. These effects, along with the stabilization of acidic phospholipid membranes in a crystalline state, provide new insights concerning the role of Ca^{2+} as a crucial element in the topographical organization of the components of biological membranes, and the structural changes involved in such phenomena as membrane fusion.

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