Effect of electrostatic repulsion on the morphology and thermotropic transitions of anionic phospholipids

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Samples of dimyristoylphosphatidylglycerol and dimyristoylphosphatidylserine which exhibit highly cooperative phase transitions in suspension with 0.5 M NaCl, 10 mM sodium phosphate, pH 7.0, demonstrate a marked broadening of the phase transition when suspended in distilled water at pH 7. The cooperativity of the thermal transition of dimyristoylphosphatidic acid at pH 7.0, in contrast, was little affected by the presence or absence of 0.5 M NaCl. The most dramatic changes in phase transition properties were observed with phosphatidylglycerol. The morphology of phosphatidylglycerol was also altered by NaCl. At high salt concentrations, typical multilamellar structures were observed by freeze-fracture electron microscopy. However, in distilled water the large multilamellar structures are disrupted, with opened shells and smaller particles of 20–30 nm diameter being observed, in addition to a few larger vesicles. Structures of differing morphology could be partially separated from aqueous 'solutions' of phosphatidylglycerol by ultracentrifugation. The supernate contained only small particles. The results are discussed in terms of the large effective head group volume resulting from strong electrostatic repulsion at low ionic strengths. This allows the anionic lipids, particularly phosphatidylglycerol, to form hydrophilic surfaces with high curvature so as to seal the bilayer edges of discs or shells. Thus, certain anionic lipids in distilled water destabilize bilayers by a mechanism which is opposite to that found with lipids which destabilize bilayers by forming the hexagonal phase.

Phospholipid *Phase transition* *Freeze-fracture electron microscopy* *Differential scanning calorimetry* *Phosphatidylglycerol* *Phosphatidylserine*

1. INTRODUCTION

There has been considerable interest in recent years in the bilayer-destabilizing action of 'cone-shaped' phospholipids. Relative to bilayer-forming lipids, these lipids have hydrophobic acyl chains occupying a larger volume or head groups which are smaller, and are less hydrated or more strongly self-associated. Such lipids can form lipidic particles, inverted micelles, cubic phase or the inverted hexagonal (H_{II}) phase. In addition to this type of bilayer destabilization, however, it is also possible to have the opposite effect, i.e. relatively larger, more hydrated or strongly self-repelling head groups and/or hydrophobic acyl chains occupying a smaller volume. Here, we demonstrate that certain anionic phospholipids at low ionic strength destabilize bilayers in such a manner.

2. EXPERIMENTAL

The thermal transition properties of the lipid preparations were measured with a Microcal MC-2 differential scanning calorimeter having sample cell volumes of 1.42 ml. Generally a scan rate of 0.65 $K \cdot min^{-1}$ was employed, however, scans done at 0.2–0.9 $K \cdot min^{-1}$ gave similar results as did repeated scans of the same sample. All lipids were purchased from Avanti Polar Lipids (Birmingham, AL). Samples in distilled water were adjusted to

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pH 7 with small amounts of NaOH or HCl. In the cases of dimyristoylphosphatidylglycerol (DMPG) and dimyristoylphosphatidylserine (DMPS), solutions of these lipids in distilled water appeared transparent. Samples of lipid suspended in 0.5 M NaCl, 10 mM sodium phosphate, pH 7.0, were also scanned.

3. RESULTS AND DISCUSSION

The presence of 0.5 M NaCl has a marked effect on the transition of DMPG (fig.1). At high ionic strength DMPG exhibits the well characterized premelt and main transitions at 15.0 and 22.9°C, respectively, with enthalpies of 0.86 and 5.7 kcal/mol. In distilled water the transition is greatly broadened and occurs over the temperature range from about 12 to about 30°C with a transition enthalpy of 6.2 kcal/mol. In 10 mM sodium phosphate, pH 7.0 (no NaCl), a complex DSC curve is obtained containing several transitions [1].

The thermal transitions of other anionic phospholipids are also affected by ionic strength. In the case of DMPS the transition is broadened in the absence of NaCl (fig.2). In this case, there is a more marked shift of the transition to higher temperatures at low ionic strength and a small decrease in the transition enthalpy from 7.5 kcal/mol in 0.5 M NaCl to 6.3 kcal/mol in

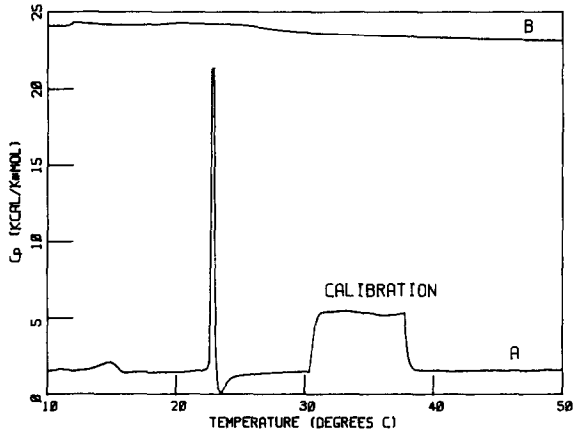

Fig.1. Differential scanning calorimetry of DMPG. Heating scan at 0.65 $K \cdot min^{-1}$. Lipid concentration 3 mM. Curve A, lipid suspended in 10 mM sodium phosphate, 0.5 M NaCl, pH 7.0. Curve B, lipid 'dissolved' in distilled water, pH adjusted to 7.

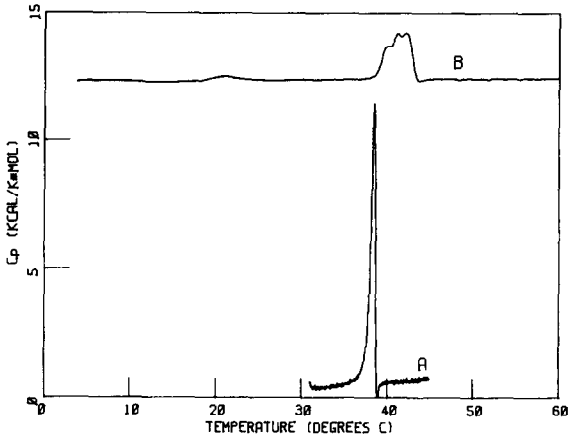

Fig.2. Differential scanning calorimetry of DMPS. For conditions, see fig.1.

distilled water. For dimyristoylphosphatidic acid (DMPA) the transition temperature is raised about 5°C in going from 10 mM sodium phosphate, pH 7.0, 0.5 M NaCl to distilled water but in this case the cooperativity of the transition is little affected (not shown). This effect of NaCl on the transition of DMPA has been previously observed and has been ascribed to an effect of surface electrostatic charge on the pK of the phosphatidic acid [2].

The most pronounced alterations in phase transition properties caused by the removal of salts occur with DMPG. This lipid has the weakest interaction among head groups since it has the lowest melting temperature. In contrast, DMPA has strong hydrogen bonding among head groups and has the highest melting temperature. It also has the smallest head group. Of the three lipids studied, therefore, DMPA should be least affected by increased electrostatic repulsion, as is found.

We have studied how ionic strength affects the morphology of phosphatidylglycerol preparations. Freeze-fracture electron microscopy shows a typical $P_{\beta'}$ multilamellar DMPG bilayer in 0.1 M NaCl (fig.3A). In distilled water these large structures become fragmented (fig.3B). The large and small particles formed from egg phosphatidylglycerol (prepared by transphosphorylation of egg phosphatidylcholine) in distilled water are partially separable by centrifugation at 27000 × g for 30 min (fig.3C,D). All of the samples were freeze-quenched at room temperature. Fig.3B,D shows the presence of vesicles, half-shells and particles.

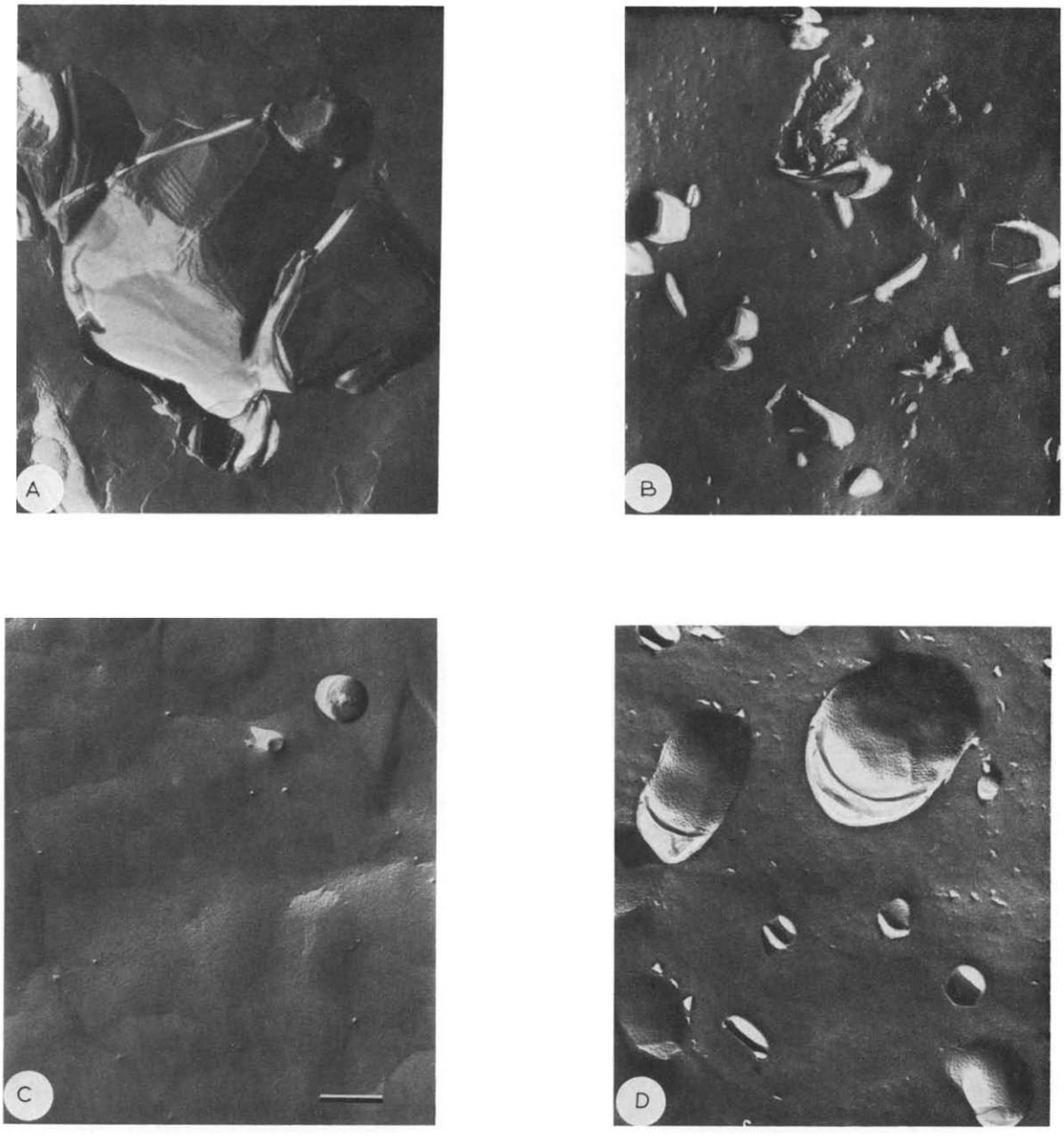

Fig.3. Freeze-fracture of phosphatidylglycerol samples. Specimens sandwiched between 75 μm thick copper foils were rapidly frozen by being plunged into liquid propane from room temperature. Calibration bar in C, 0.1 μm. (A) DMPG in 10 mM sodium phosphate, 100 mM NaCl, pH 7.0; (B) DMPG in distilled water adjusted to pH 7; (C) the supernate of a solution of egg phosphatidylglycerol after centrifugation at 27000 × g for 30 min; (D) the pellet formed after this centrifugation.

The vesicles are less abundant and one has to choose a particular field to show their presence. The half-shells are about 100–200 nm along the longest dimension. The shells seem to have an open edge. The smaller particles are fairly uniform in size, about 20–30 nm in diameter. This is about the size of sonicated liposomes but these particles have a distinct elliptical shape. The supernate from the centrifugation (fig.3C) has even smaller, round particles of 10 nm diameter. These may be micelles.

Thus, at low ionic strength anionic lipids may destabilize bilayers to produce smaller sized particles with altered morphology and phase transition properties. The effect on the phase transition properties is greatest for DMPG. In this case, head group-head group repulsion may allow the hydrophilic surface to acquire a high curvature to seal the bilayer edge of the discs or shells. Therefore the formation of a closed vesicle is no longer mandatory for energy minimization. In distilled water small particles and half-shells replace large bilayer vesicles as the predominant form. As a result, the cooperativity of the phase transition is significantly reduced. The charge repulsion between phosphatidylglycerol head groups in distilled water results in the lipid behaving like lysolecithin and other detergents. As a result, the lipid appears visually to 'dissolve' in distilled water. In the presence of high salt concentration this repulsion is diminished and the curvature of the hydrophilic surface is limited, requiring the lipid to form large, sealed vesicular structures. A similar effect of salt has been observed in studies of dilysocardiolipin [3]. Electrostatic effects are also responsible for ionic-strength-dependent changes in the motional properties of DMPS head groups [4] and for the spontaneous vesiculation of lipid dispersions in the presence of anionic detergents [5].

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