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INTERACTIONS OF La^{3+} WITH PHOSPHATIDYLSERINE VESICLES

BINDING, PHASE TRANSITION, LEAKAGE AND FUSION

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Summary

The interaction of La^{3+} with phosphatidylserine vesicles is elucidated by binding studies, differential scanning calorimetry, X-ray diffraction, freeze fracture electron microscopy, and release of vesicle contents. La^{3+} effectively competes with Ca^{2+} for phosphatidylserine binding sites. The saturation level is close to a La/lipid ratio of 1 : 3. A concentration of 0.1 mM of La^{3+} is sufficient to induce fusion between sonicated vesicles.

Previous studies on phosphatidylserine vesicles demonstrated that fusion [1] induced by Ca^{2+} and Mg^{2+}, resulted in different final structures [2,3]. The larger capacity of Ca^{2+} to promote aggregation and fusion correlated with its greater intrinsic binding constant for phosphatidylserine [2,4]. Our main goal in this study is to shed more light on the question of membrane fusion by studying the effect of trivalent cations, and to correlate their binding capacity to acidic phospholipid vesicles with their ability to promote membrane fusion.

Phosphatidylserine was isolated from beef brain as previously described [1,5,6]. Dispersions of multilamellar vesicles and sonicated unilamellar vesicles of phosphatidylserine were prepared as described before [1] in a buffer adjusted to pH 7.4. Endothermic phase transitions were detected with the Perkin-Elmer DSC-2 differential scanning calorimeter [2,6]. The release of vesicle contents was followed by enhancement of fluorescence intensity that occurred

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upon release and dilution of carboxyfluorescein initially encapsulated within the vesicles at high (self-quenching) concentrations [7]. The extent of La^{3+} binding to phosphatidylserine vesicles was determined by using the radioactive isotope ^{140}La; binding of Ca^{2+} was determined by using ^{45}Ca as described before [2]. An Auto-Gamma Scintillation Spectrometer-Packard was used to detect the γ-radiation. X-ray diffraction was recorded by a microfocusing Frank Camera [8]. Freeze fracture was carried out in a Polaron E 7500 unit under a vacuum of about $5 \cdot 10^{-7}$ Torr [8]. Replicas were viewed in a Siemens 101 microscope.

Measurements in the presence of saturation concentrations of La^{3+} (e.g. 3 mM) gave a binding ratio of 0.37 for La/phosphatidylserine. This value to within the experimental accuracy [2], is close to 1 : 3, and indicates a predominance of the stoichiometric binding of 1 : 3. Binding measurements of Ca^{2+} in the presence of La^{3+} indicate that La^{3+} effectively displaces Ca^{2+} from phosphatidylserine vesicles. For instance, the binding ratio Ca/lipid is 0.29 in the presence of 2 mM Ca^{2+} +0.5 mM La^{3+}, whereas without La^{3+} it is 0.47. Fig. 1 illustrates that the endothermic peak of phosphatidylserine, which occurs at 9°C in buffer only, is about 38°C in the presence of La^{3+} or Gd^{3+}. Fig. 1(f) indicates that in the presence of 0.2 mM Ca^{2+} + 0.2 mM La^{3+} the endothermic peak has the same appearance as with La^{3+} alone, whereas in the presence of Ca^{2+}, or Ca^{2+} and Mg^{2+} the peak shifts to very high temperatures [2].

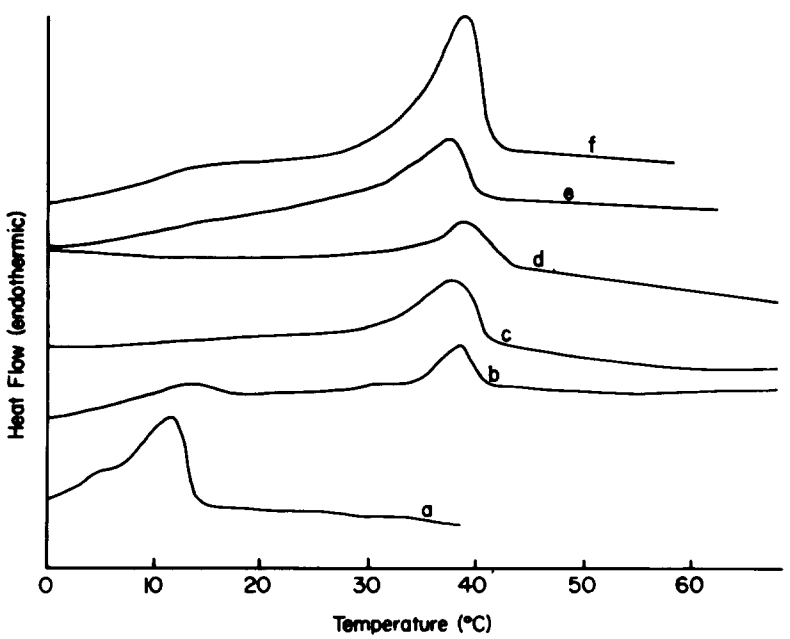

Fig. 1. The phospholipids were suspended by shaking 10 μmol in 2 ml of 100 mM NaCl buffer, pH 7.4, to a final concentration of 1 mM lipids. The cations were added after formation of multilamellae to the concentrations indicated below. The samples were given three brief (15 s) bursts of sonication, and incubated for 30 min at 37° C. After incubation the samples were centrifuged at 100 000 X g for 30 min at 20° C [1,2]. The analysis started 6-12 h after incubation. The solutions used in the incubations were either buffer only (a), or had additional cations: (b) 0.1 mM La^{3+}; (c) 0.2 mM La^{3+} or 0.2 mM Gd^{3+}; (d) 0.5 mM La^{3+} or 0.5 mM Gd^{3+}; (e) 0.2 mM La^{3+} + 0.2 mM Ca^{2+}; (f) 0.2 mM La^{3+} + 3 mM Mg^{2+}.

The fluorescence release results in Fig. 2 demonstrate that a significant degree of leakage occurs rapidly in the presence of small concentrations of La^{3+} less than 0.1 mM. Another point illustrated is that the release of material is enhanced at 38°C which coincides with the transition temperature of the complex phosphatidylserine-La.

In morphological studies sonicated vesicles of phosphatidylserine were incubated for 30 min with various concentrations of La^{3+}. The X-ray diffraction results show that samples incubated at 25°C have lamellar repeat distance of 61 ± 1 Å at all concentrations of La^{3+} up to 1.0 mM, whereas incubation at 39°C gave a transition of lamellar repeat distance from 61 ± 1 Å to 54 ± 1 Å as the concentration of La^{3+} is increased from 0.1 mM to 1 mM. All of these samples show a single high angle diffraction spacing at 4.2 Å. The molecular packing is in the gel state in both these two forms, although it is at the liquid crystalline state before the La^{3+} is added. The 54 Å form does not revert to the 61 Å form by lowering the temperature.

Freeze fracture electron micrographs of these samples also show variations with La^{3+} concentration and temperature of incubation. Cold temperature incubation results in aggregated vesicles whose diameters vary between 300 and 3000 Å (Fig. 3). Incubation at the higher temperature gives multilamellar structures, which resemble giant flattened and aggregated multilamellar vesicles (Fig. 4). The morphology of these structures is distinct from

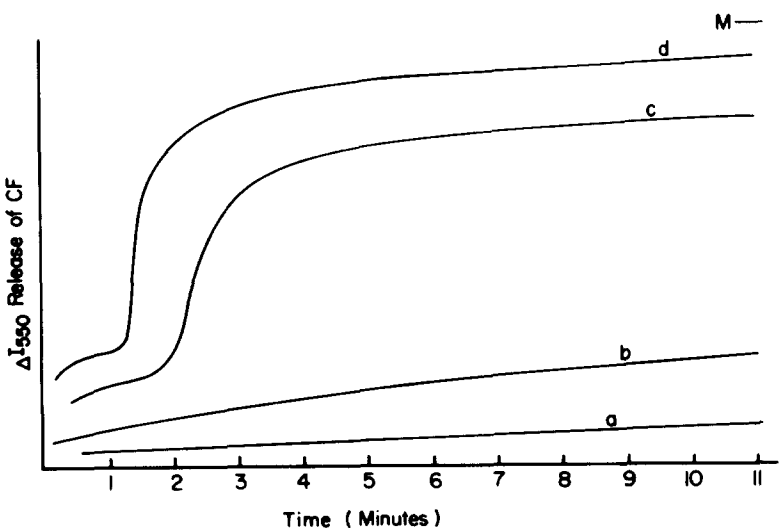

Fig. 2. Carboxyfluorescein-containing vesicles were prepared by sonication of phosphatidylserine in a solution of 100 mM carboxyfluorescein adjusted to pH 7.4. The vesicles were separated from carboxyfluorescein by passing through a Sephadex G-50 column (1.0 X 20 cm) equilibrated with the standard (0.1 M NaCl) buffer without EDTA, diluted and subsequently stored on ice. Fluorescence of the carboxyfluorescein-containing vesicles was measured with an Aminco-Bowman spectrofluorometer (excitation 490 nm, emission 550 nm) using a Corning cut off filter (No. 3-68, ≃520 nm). The figure gives the extent of carboxyfluorescein release from phosphatidylserine vesicles (0.23 mM) after addition of La^{3+} with continuous agitation: (a) with 0.05 mM at 24°C; (b) with 0.05 mM at 38°C; (c) with 0.075 mM at 24°C; (d) with 0.075 mM at 38°C. A modified Fiske-SubbaRow [9] method was used to determine the amount of phosphate in the sample. M indicates maximum fluorescence intensity, which is obtained by the addition of 0.05% Triton X-100.

the cochleate structure produced by adding Ca^{2+} to phosphatidylserine [1,3] at these temperatures. Although the lamellar repeat spacing measured by X-ray diffraction is the same as in cochleates, the wide angle diffraction of these two structures is different.

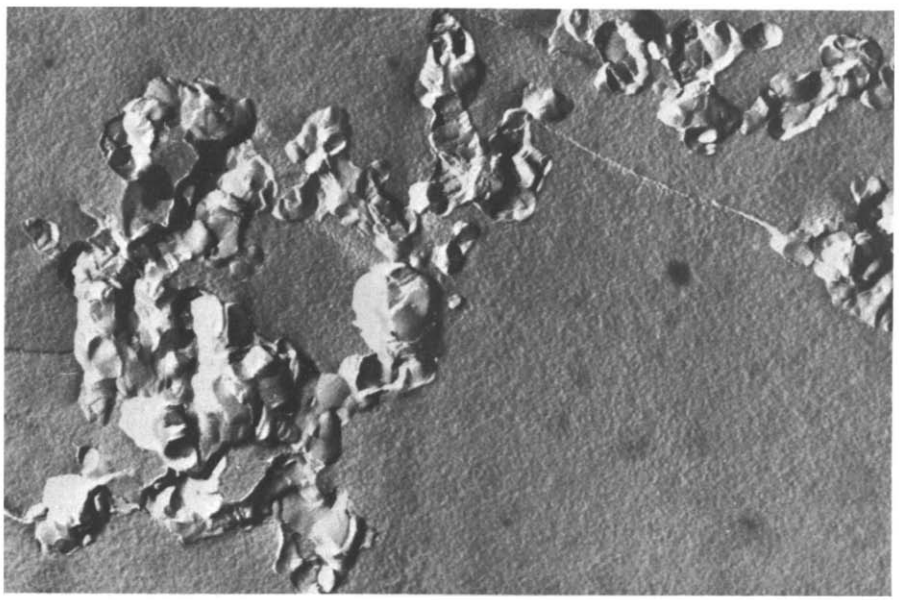

Fig. 3. Freeze-fracture micrograph of sonicated vesicles of bovine brain phosphatidylserine incubated at 25 ± 1 °C for 30 min in the presence of 1.0 mM La^{3+}. Freeze-fracturing was performed as described in Ref. 8. Magnification is 54 000X.

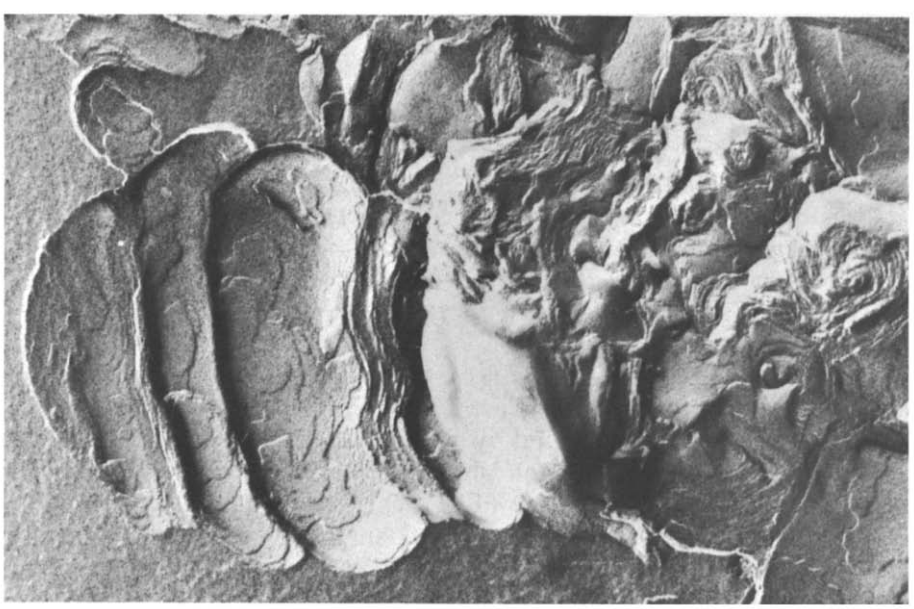

Fig. 4. Same as Fig. 3, but incubation at 39° C.

This study indicates that La^{3+} can promote the fusion process at concentrations significantly smaller than those required with the divalent cations Ca^{2+} or Mg^{2+}. The sequence $La^{3+} > Ca^{2+} > Mg^{2+}$ reflects both the binding capacity of these cations as well as their capacity to induce structural changes of phosphatidylserine vesicles, as seen in differential scanning colorimetry results, leakage and direct binding measurements.

Binding of cations to acidic phospholipids results in charge neutralization and in reduction of the electrostatic potential barrier, and thus it enables a close approach of the vesicles. However, in the presence of more than 500 mM Na^+ rapid aggregation of phosphatidylserine occurs, but not leakage and fusion [10], and little fusion occurs with Ca^{2+} or Mg^{2+} at temperatures below the phase transition, T_c, of pure phosphatidylserine (in NaCl), or (with Mg^{2+}) above the T_c of the complex phosphatidylserine-Mg [1]. Previous studies with other phospholipids [11] showed that maximum fusion occurred in a narrow temperature range around T_c. Recent studies [12] demonstrated that the increase in vesicle size by fusion in the presence of Ca^{2+} was largest at a particular temperature, which might be the T_c of the complex at a prefusion stage. The underlying idea [1,10,12] has been that the process of vesicle fusion requires the existence of a state of transient instability from which the structural changes could proceed rapidly. Our results with La^{3+} induced fusion further support this idea. There is significantly less fusion at room temperature than at 39°C, which is close to T_c of phosphatidylserine in the presence of La^{3+}. The existence of different structures depending on temperature may imply two different stages of fusion. It is to be noted that the differential scanning calorimetry measurements are done on multilamellar vesicles (with brief sonication), whereas the fusion studies pertain to sonicated vesicles. However, X-ray diffraction study on multilamellar vesicles showed similar temperature dependent structural transitions as in sonicated vesicles.

The destabilization of vesicles by Mg^{2+}, Ca^{2+} or La^{3+} is probably due to the fact that divalent and trivalent cations "cross-link" respectively, at least a pair and a triplet of negatively charged phospholipid molecules on the surfaces of vesicles, thus introducing spatial constraints, which results in a partial structural condensation and "crystallization" of lipid molecules. This process destabilizes the original molecular ordering in the bilayer by forming phase-separated patches or domains of ion-lipid complexes. Although the details of molecular rearrangement during the fusion step remain to be elucidated, it is clear that it is facilitated in a state of maximal fluctuations in the membrane, which exists at temperatures in the vicinity of the transition temperature of lipid-cation complexes.

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