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Sodium–Calcium-Ion Exchange in Phosphatidyl Serine Monolayer

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Abstract □ The effect of replacement of sodium chloride subphase solution with calcium chloride subphase solution and the effect of replacement of calcium chloride subphase solution with sodium chloride subphase solution were studied on monolayers of phosphatidyl serine. When the sodium chloride subphase was replaced with calcium chloride solution, the π–A curve shifted to almost the same position as the π–A plot of a film spread on calcium chloride without subphase replacement. This showed that almost all sodium ions had been replaced by calcium ions in the film. When the calcium chloride subphase was replaced by a sodium chloride solution, the film expanded somewhat, showing that some calcium ions had been replaced by sodium ions. This study gives evidence for the existence of cation exchange in phosphatidyl serine films, and sodium seems to be in competition with calcium for the negatively charged sites in the film. It is possible that this competition is concentration dependent.

Keyphrases □ Sodium-calcium subphase exchange—effects on phosphatidyl serine monolayers □ Calcium-sodium subphase exchange—effects on phosphatidyl serine monolayers □ Phosphatidyl serine monolayers—effects of sodium-calcium and calcium-sodium subphase exchange, sodium-calcium competition, cation-exchange evidence □ Monolayers, phosphatidyl serine—effects of sodium-calcium and calcium-sodium subphase exchange, sodium-calcium competition, cation-exchange evidence

A previous article (1) reported an apparatus and technique for studying monomolecular films by which the subphase can be exchanged without disturbing the film. Data were presented to demonstrate the utility of this system for studying protein and enzyme monolayers. This article will show the applicability of the system to the study of ion-exchange properties of phospholipid monolayers.

DISCUSSION

Binding of cations by phospholipids and fatty acids has been studied extensively (2–26). Calcium ions were found (3) to produce little reduction in area per molecule of cephalin, spread at an air–water interface at a given surface pressure. Studies (4, 5) on the dependence on surface pressure of the penetration rate of water molecules through lipid monolayers found that calcium ions, at low concentration, decreased such penetration. The association of synthetic lecithin with ions was studied (6), showing indirectly that, with dilute solutions, there is no binding of sodium, potassium, or lithium. However, the same study reported that magnesium seemed to be adsorbed from dilute solutions. Vilallonga *et al.* (7) observed that the presence of monovalent ions increases the area per molecule, the surface potential, and the surface dipole moment of dipalmitoyl lecithin monolayers in the order sodium > potassium ≈ lithium > water. It was reported (8) that lecithin monolayers bind calcium (0.1 mM) and that sodium or potassium ions (112 mM) each displace the same fraction of this calcium (about 50%). Many workers reported interaction of divalent and monovalent cations with phosphatidyl serine (2, 9–13).

Shah and Schulman (14) reported that the π–A curves of lecithins, phosphatidyl choline, and dicetyl phosphate are not affected by the presence of divalent metal ions. However, dipalmitoyl lecithin shows a higher surface potential in the presence of divalent metal ions than in the presence of monovalent ions; the increase is greatest for the fully saturated dipalmitoyl lecithin and the smallest for a highly unsaturated lecithin. They suggested that the divalent metal ion–lecithin monolayer interaction is dependent on the packing of the hydrocarbon chains. The large intermolecular separation permits water, hydrated monovalent ions, and nitrogen ion of the same molecule to associate with the phosphate ion; this prevents the calcium interaction with the phosphate ion. On compression, some water molecules and hydrated ions are squeezed out and the conditions become more favorable for calcium, because one calcium ion between two phosphate groups is smaller than two monovalent ions. These authors also postulated a position for calcium in dicetyl phosphate monolayers that would not affect the area of the film but would increase the surface potential.

In a later publication, Shah and Schulman (15) suggested that there is an internal salt linkage between the phosphate and trimethylammonium group on the same lecithin molecule, which prevents the interaction of the phosphate group with calcium. An increase in unsaturation of the fatty acyl chains increases the intermolecular spacing, which reduces the ionic repulsion between polar groups and hence strengthens the internal salt linkage. Thus, increasing unsaturation decreases calcium binding in lecithin monolayers. They suggested a vertical rather than a coplanar orientation of the phosphoryl choline group with respect to the interface and proposed a position for calcium in the lecithin films. It was suggested, based on surface potential studies, that calcium forms an ionic dipole ($Ca^{+2} \leftrightarrow O^{-}$).

Colacicco (16) showed that the reported (14, 15) surface poten-

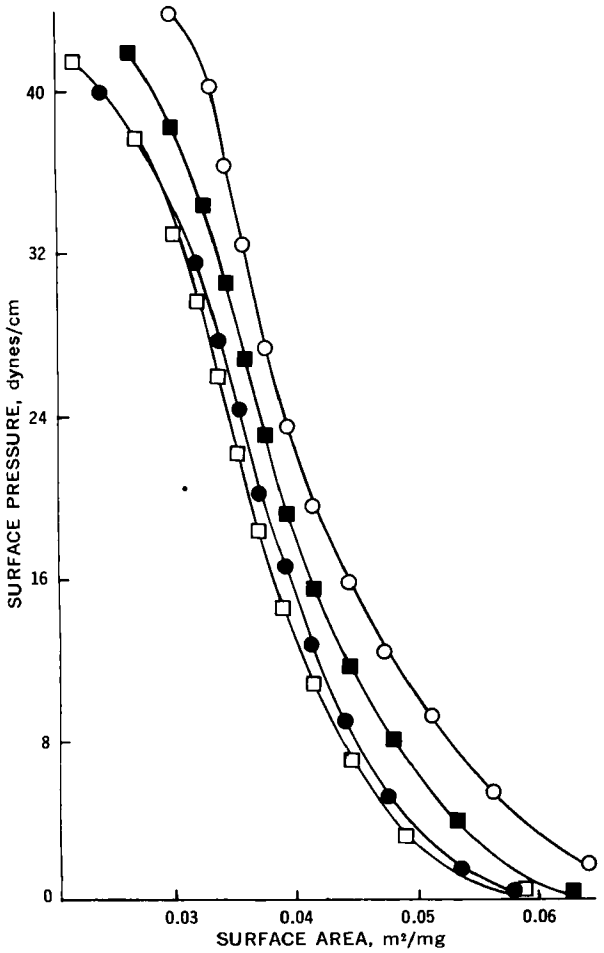

Figure 1—*The π–A curves of phosphatidyl serine films. Key:* ○, *spread on 0.1* M *sodium chloride solution;* □, *spread on 0.033* M *calcium chloride solution;* ●, *spread on 0.1* M *sodium chloride solution and subphase replaced with 1500 ml of 0.033* M *calcium chloride solution; and* ■, *spread on 0.033* M *calcium chloride solution and subphase replaced with 1500 ml of 0.1* M *sodium chloride solution.*

tial increase in dipalmitoyl lecithin monolayers due to calcium ions was actually due to acidic contaminants in the impure lecithins. Thus, the increased potentials of calcium chloride solutions may be due to the effects of interaction of calcium with fixed charges of the acidic contaminants. The potentials that the previous workers attributed to fixed dipoles of the lipid may not exist, and the vertical orientation proposed (14, 15) for the phosphoryl choline is questionable. From studies of electrokinetic potentials, Hanai *et al.* (17, 18) concluded that the phosphatidyl choline head groups of lecithin in bimolecular leaflets are probably oriented so that the trimethylammonium and phosphate ionic groups are both in a plane parallel to the leaflet. Measurements of surface dipole moments and surface pK values (19–21) also indicate that this configuration exists in monolayers of lecithins and phosphatidyl ethanolamine. However, on the grounds of X-ray long-spacing studies (22), it was suggested that the preferred orientation of the polar group in lecithin has the zwitterion extended normal to the bilayer.

Many workers believe that acidic phospholipids provide negatively charged sites for cation exchange. Blaustein (2) demonstrated ion exchange in phosphatidyl serine by using distribution ratios between a chloroform–methanol phase as the criterion. Abramson *et al.* (11) showed interaction of cations with aqueous dispersions of phosphatidyl serine. Phosphatidyl serine and phosphatidyl glycerol have been shown to exhibit discrimination between sodium and potassium (12). Antagonistic effects of calcium and local anesthetics on the permeability of phosphatidyl serine vesicles have been reported (23). Nielson (24) reported that lipids show evidence of cation exchange when eluted down a silicic acid column. Association of inorganic cations with acidic phospholipid monolayers has been studied by many workers (9–13).

Rojas and Tobias (9) reported that calcium condenses phosphatidyl serine monolayers and monovalent cations (sodium, potassium, and hydrogen) are in competition with calcium ions for the negatively charged sites in the film. The extent of this competition depends on the competing ion concentration. More calcium is displaced by either sodium or potassium ions at low surface pressure than at high pressures; this they interpreted in terms of peripolar group volume available for ion intrusion. By isotope techniques, they revealed that there is one calcium ion adsorbed per phosphatidyl serine and 0.35 calcium ion adsorbed per phosphatidyl ethanolamine molecule in their monolayers at pH 7. At this pH, the phosphoric groups of phosphatidyl serine and phosphatidyl ethanolamine and the carboxyl group of phosphatidyl serine seem to be ionized, thus generating monolayers with negatively charged sites for cation exchange. The cations, of course, condense the phosphatidyl serine and phosphatidyl ethanolamine monolayers. In striking contrast, however, the π–A curves of phosphatidyl choline were independent of hypophase sodium-, potassium-, or calcium-ion concentration. It was concluded that within the pH range used, the phosphatidyl choline monolayers were not negatively charged. However, as previously mentioned, some workers have reported condensation of lecithin monolayers in the presence of calcium. Hauser and Dawson (13) also reported the displacement of adsorbed calcium from phosphatidyl serine monolayers by sodium and potassium.

Papahadjopoulos (10) reported that acidic phospholipids interact strongly with bivalent metal ions at low concentrations, the interaction being accompanied by a condensation of the monolayer and an increase in the surface potential. Phosphatidyl serine and phosphatidic acid films exhibit the highest affinity above pH 7, since both possess two negative groups per molecule at these pH values. The relative affinity for the different bivalent cations was calcium > barium > magnesium. Based on these studies, a model was proposed for the complex of phosphatidyl serine and calcium whereby the phosphatidyl serine molecules are packed in such a way in the film that the calcium can form six coordination bonds with four phosphatidyl serine molecules to form a linear polymeric arrangement. Addition of bivalent ions under neutral phospholipid films (phosphatidyl choline and phosphatidyl ethanolamine) had very little effect on π or ΔV. These studies were carried out in the presence of physiological concentrations (10^{-1} M) of univalent salts, sodium chloride or potassium chloride.

The importance of acidic lipids as tissue ion-exchange agents has been emphasized by a number of workers. In addition, there is evidence that these lipids may play a role in transmembrane cation transport (27–29). Reported sodium–potassium discrimination by phosphatidyl serine and phosphatidyl glycerol (12) seems highly relevant to the physiological mechanism of transmembrane ion movement. Phosphatidyl serine constitutes 10–20% of the total phosphatidyl content of most cell membranes (30) and phospholipids may contain the ion-exchange sites controlling neuronal excitation (31). Therefore, ionic binding and ion-exchange capabilities of the purified acidic lipids are very important.

There does not seem to be general agreement on how cations bind to phospholipid monolayers. Whether or not the acidic phospholipid films (*e.g.*, phosphatidyl serine) exhibit ion exchange between divalent cations and monovalent cations has not yet been fully established.

The purpose of this investigation was to examine the subphase exchange system (1) as a means of studying the sodium–calcium-ion exchange characteristics of phosphatidyl serine films.

EXPERIMENTAL

Bovine phosphatidyl serine (25 mg/ml in chloroform) was purchased[1]. TLC analysis yielded a single spot. Spreading solutions of phosphatidyl serine were prepared with hexane to yield a solution containing 0.625 mg/ml. Then 0.08 ml of this solution was spread on either a 0.1 M NaCl tromethamine–hydrochloric acid-buffered (pH 7.4) subphase or a 0.033 M $CaCl_2$ tromethamine–hydrochloric acid-buffered (pH 7.4) subphase. A pH value of 7.4

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was chosen since the π–A curves of phosphatidyl serine are independent of pH in the 6–8 pH range (10).

The π–A plots were then constructed for phosphatidyl serine films on the sodium chloride and calcium chloride subphases. In addition, the sodium chloride subphase was exchanged with calcium chloride, while the calcium chloride subphase was exchanged with sodium chloride. The ionic strength of the subphase remained constant throughout the exchange process. The π–A plots were also constructed for each system. In all experiments, the π–A plots were reproducible to within 0.1 dyne/cm.

The details for the apparatus used and the method of subphase exchange were given previously (1).

RESULTS

The π–A plots of phosphatidyl serine films spread on buffer solution containing 0.1 M sodium chloride or 0.033 M calcium chloride are shown in Fig. 1. The two π–A plots are almost parallel, but the film on calcium chloride is much more condensed.

Figure 1 also shows the π–A plots of phosphatidyl serine when it was spread on 0.1 M sodium chloride subphase and the subphase was replaced by 1500 ml of 0.033 M calcium chloride solution. The π–A plot of the film has shifted very close to the π–A plot of phosphatidyl serine film spread on calcium chloride solution. Therefore, by replacing the sodium chloride subphase with calcium chloride solution of the same ionic strength, almost all of the sodium is replaced by calcium in the film. The replacement of sodium by calcium in the film is expected in light of the many reports that affinity of phosphatidyl serine for calcium is much greater than for sodium (9, 13).

Figure 1 also shows the π–A plot of phosphatidyl serine when it was spread on calcium chloride solution and the subphase was replaced with 1500 ml of sodium chloride solution of the same ionic strength. The π–A plot has shifted midway between the plot for a film spread on calcium chloride solution and a film spread on sodium chloride solution. This suggests that some calcium in the film has been replaced by sodium as a result of the subphase exchange. This demonstrates that cation exchange between sodium and calcium does occur in phosphatidyl serine films. If the phosphatidyl serine film interacted with calcium so as to form very stable complexes, as suggested (10), the observed replacement of calcium by sodium would not be expected.

As the subphase exchange proceeds, the calcium-ion concentration in the subphase is decreasing while that of sodium is increasing. The exchange in the film may also depend on the rate of diffusion of the sodium ions from the bulk to the interfacial region. If the competition was not concentration dependent, it would be expected that the π–A plot would shift completely to the π–A plot of the film spread on sodium chloride solution, as was observed in the replacement of the sodium chloride subphase solution with calcium chloride solution. It can be seen clearly from the results that calcium interacts preferentially and more strongly than sodium with phosphatidyl serine films. However, this study also gives definite evidence for the existence of sodium–calcium exchange in phosphatidyl serine films in which sodium, at relatively high concentrations, competes with calcium for the negatively charged sites in the film.

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