Binding of metal ions to monolayers of lecithins, plasmalogen, cardiolipin, and dicetyl phosphate

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SUMMARY The surface pressure–area curves of synthetic (dipalmitoyl), egg, and yeast lecithins showed that the limiting areas depend on the degree of unsaturation of the fatty acid residues (dipalmitoyl < egg < yeast lecithin). The surface potential of phosphatidal choline (240 mv) was lower than that of the dipalmitoyl lecithin (380 mv), both at 60 A^2/molecule. This difference in surface potentials is attributed to the presence of an additional induced dipole in the double bond of the vinyl ether linkage of the plasmalogen. The binding of metal ions to lecithin and phosphatidal choline resulted in an increase in the surface potential. With different lecithins, the binding of calcium varied with the degree of unsaturation, suggesting that steric characteristics of fatty acid residues significantly influence the phospholipid–metal ion interaction.

The surface pressure–area curves of lecithin, phosphatidal choline, and dicetyl phosphate were not affected by the presence of the divalent metal ions in the subsolution whereas the surface pressure–area curve of cardiolipin showed 10–13% contraction of the film in their presence. The component dipoles of the dicetyl phosphate and that of the fatty acid are explained. The increase in the surface potential of phospholipids and the contraction–expansion effect of the cardiolipin monolayer is accounted for by postulating a position for the divalent metal ion in the dipole lattice.

KEY WORDS lecithins (egg, yeast, dipalmitoyl) · plasmalogen · cardiolipin · dicetyl phosphate · monolayers · surface pressure · surface potential · dipoles · induced dipole · metal ion interaction · metal ion position · unsaturation effect

PHOSPHOLIPID is a major structural component of the cell membrane. Since Ca^{++} and Mg^{++} are thought to be present in the cell membrane, and further, since these ions markedly influence the rate of enzymic hydrolysis of phospholipids, it seemed desirable to investigate metal ion–phospholipid interactions. This paper describes the application of surface techniques to studies of the influence of Na^+, K^+, Li^+, Ca^{++}, Mg^{++}, Ba^{++}, Sr^{++}, and Al^{+++} on monolayers of synthetic and natural phospholipids.

MATERIALS

"Synthetic lecithin" [phosphatidyl (dipalmitoyl) choline] was purchased from Mann Research Laboratories. Egg lecithin and beef heart cardiolipin prepared according to Pangborn and co-workers (1, 2), were supplied by Sylvana Chemical Company, (Millburn, N. J.). Yeast lecithin and phosphatidal choline were gifts from Dr. M. M. Rapport. Dicetyl phosphate was purchased from K & K Laboratories, (Jamaica, N. Y.) Phospholipid solutions were prepared in hexane–ethanol 4:1 (v/v). Inorganic chemicals of reagent grade and twice distilled water were used. The water was distilled over alkaline $KMnO_4$ and the final conductance was found to be 1.2×10^{-6} ohm^{-1} cm^{-1}. Table 1 shows the

TABLE 1 FATTY ACID COMPOSITIONS OF THE PHOSPHOLIPIDS

Fatty acid*	Yeast Lecithin	Egg Lecithin	Phosphatidal Choline	Cardiolipin
		mole %		
12:0	1.59	—	—	—
14:0	1.86	tr.	—	—
14:0 dma	—	—	1.62	—
14:1	—	—	—	—
16:0	6.38	34.34	—	2.22
16:0 dma	—	—	28.18	4.45
16:1	51.05	tr.	—	1.94
18:0	1.59	13.01	tr.	10.30
18:0 dma	—	—	7.85	—
18:1	32.97	31.85	10.02	10.86
18:2	2.66	17.17	39.56	70.18
20:3	tr.	tr.	5.42	—
20:4	tr.	3.6	7.04	—

* Number of carbon atoms: number of double bonds. dma = dimethylacetal.

fatty acid composition of the phospholipids, analyzed by gas–liquid chromatography by courtesy of the laboratory of Dr. E. H. Ahrens, Jr. (Rockefeller Institute, New York).

METHODS

Surface Pressure Measurements

The Wilhelmy plate method (3) was used with slight modifications. The surface pressure was measured with a sand blasted rectangular platinum plate (5 cm perimeter), suspended from a Roller Smith torsion balance (500 mg max) mounted on an adjustable elevating stand. A lucite trough of 400 ml capacity was used as a Langmuir trough. The temperature of the trough was kept constant at 25° by circulating water from a thermostated water bath through glass tubing immersed in the trough. The lecithin solution (0.025 ml, concn 1 mg/ml) was spread on the surface (240 cm^2) by means of an Agla microsyringe.

Surface Potential Measurements

The surface potential (ΔV) measurements were made with an ionizing (α-radiation) air electrode, the tip of which was covered with a 6 mm × 6 mm gold sandwich containing Ra^{226} (5 μc). The ionizing electrode was held 3–4 mm above the surface. An Ag–AgCl electroplated electrode was dipped in the trough and used as a reference electrode. The two electrodes were connected to a Keithley 610A electrometer, accurate to 0.2 mv. The surface measurements were taken on a 0.02 M NaCl, LiCl, or KCl subsolution, and on a subsolution consisting of 0.02 M NaCl + 0.01 M metal salt [$MgCl_2$, $CaCl_2$, $BaCl_2$, $SrCl_2$ or $Al(NO_3)_3$].

The surface pressure (π) is defined as

$$\pi = \gamma_0 - \gamma \qquad [1]$$

where γ_0 = interfacial tension without the film, and γ = interfacial tension with the film. The surface potential ΔV is defined as

$$\Delta V = V_f - V_0 = \frac{12\pi\mu n}{D} + \psi_0 \qquad [2]$$

where V_0 = interfacial potential without the film and V_f = interfacial potential with the film, μ = resultant vertical component of the dipoles of the molecule, n = number of molecules per cm^2 of film, D = surface dielectric constant, and ψ_0 = potential due to the diffuse ionic double layer. The molecular weights, calculated

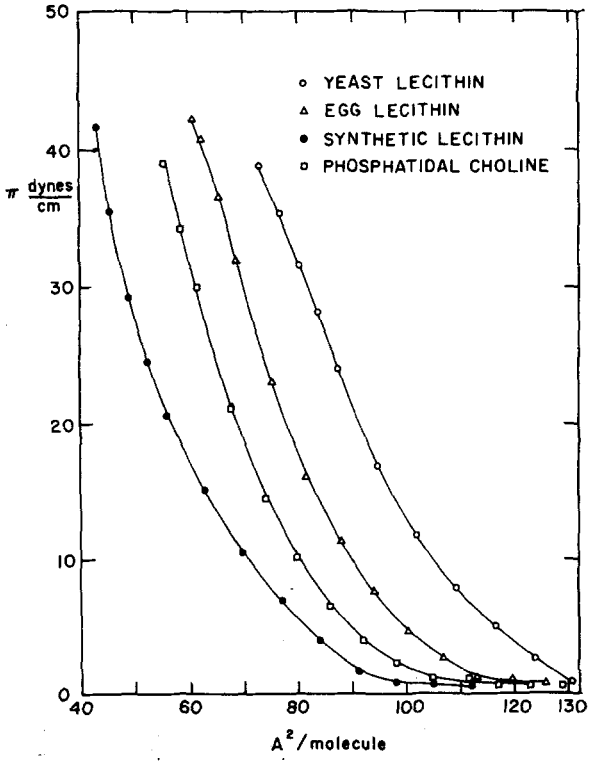


FIG. 1. Surface pressure–area curves of synthetic (dipalmitoyl), egg and yeast lecithins, and phosphatidal choline on 0.02 M NaCl subsolution, pH 5.6, at 25°.

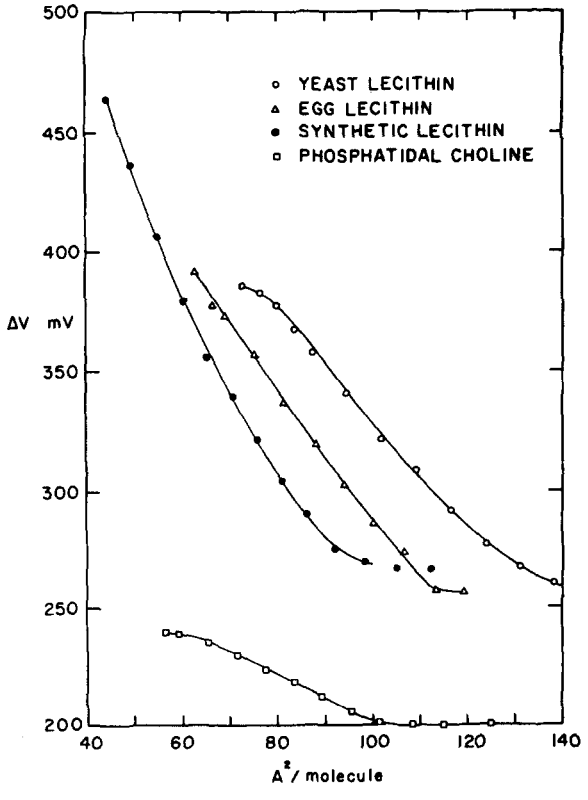


FIG. 2. Surface potential–area curves of synthetic (dipalmitoyl), egg and yeast lecithins, and phosphatidal choline on 0.02 M NaCl subsolution, pH 5.6, at 25°.

from the fatty acid composition, for yeast lecithin, egg lecithin, phosphatidal choline, and cardiolipin are respectively 776, 790, 776, and 1496. The molecular weights of 752 and 546 were used for dipalmitoyl lecithin and dicetyl phosphate.

RESULTS AND DISCUSSION

Surface Pressure Characteristics of Lecithins and Phosphatidal Choline

Figure 1 shows the surface pressure–area curves of dipalmitoyl, egg and yeast lecithins, and that of phosphatidal choline on a subsolution of 0.02 M NaCl. The limiting areas (collapse areas) of different lecithins vary with the degree of unsaturation of the fatty acid residues (dipalmitoyl < egg < yeast lecithin).

It is known (4) that a saturated fatty acid chain (e.g., palmitic acid) and an unsaturated chain (e.g., oleic acid) have the limiting areas 21 A^2 and 32 A^2 respectively. For dipalmitoyl lecithin, the limiting area of 44 A^2/molecule is close to its predicted value (42 A^2/molecule). The fatty acid composition of egg lecithin (Table 1) corresponds approximately to a lecithin containing one saturated and one unsaturated fatty acid chain, which predicts a limiting area of 53 A^2/molecule. The observed value of 62 A^2/molecule is considerably higher than the predicted value, although it is in agreement with the limiting area of α-stearoyl-β-oleoyl L-α-lecithin reported by Van Deenen et al. (5). For yeast lecithin, the limiting area of 72 A^2/molecule is also larger than the predicted value, which would be 64 A^2/molecule for a lecithin containing two unsaturated chains. The fatty acid composition of yeast lecithin (Table 1) indicates that about 88% of the lecithin molecules have both chains unsaturated.

Phosphatidal choline has a limiting area of 55 A^2/molecule. Table 1 shows that the phosphatidal choline has a saturated (except for the vinyl ether double bond) aldehydogenic chain and an unsaturated fatty acyl chain. The difference between the limiting areas of the egg lecithin and the phosphatidal choline could be due to the deficiency of one carbonyl group in the phosphatidal choline.

Surface Pressure Characteristics of Lecithins and Phosphatidal Choline in the Presence of Different Metal Ions

The surface pressure–area curves of dipalmitoyl, egg and yeast lecithins, and phosphatidal choline were not influenced by the nature of the subsolution. The surface pressure–area curves obtained in the presence of different metal ions (Na^+, K^+, Li^+, Mg^{++}, Ca^{++}, Sr^{++}, Ba^{++}, Al^{+++}) were identical with those shown in Fig. 1, which were obtained on subsolutions of 0.02 M NaCl.

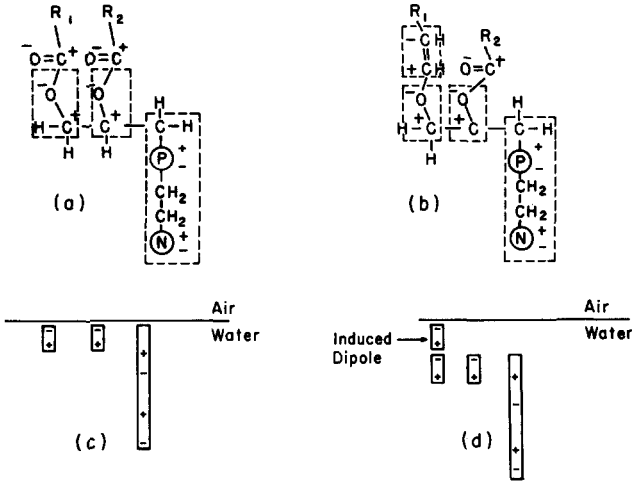


FIG. 3. Structure of lecithin (*a*) and phosphatidal choline (*b*); the corresponding dipole components, (*c*) and (*d*). R_1 and R_2 represent the hydrocarbon chains. P and N represent the phosphate and the trimethylammonium groups. + and − are to be understood as meaning *partial* charges, δ^+ and δ^- respectively.

The surface pressure–area curves of lecithins were also identical on 0.02 M NaCl, 0.01 M Na_2SO_4, and 0.02 M $NaNO_3$ subsolutions.

Surface Potential Characteristics of Lecithins and Phosphatidal Choline

Figure 2 shows the surface potentials of dipalmitoyl, egg and yeast lecithins, and phosphatidal choline on a subsolution of 0.02 M NaCl. Phosphatidal choline has a surface potential which is strikingly different from those of the lecithins.

The surface potential, ΔV, is related to the total surface dipole moment of the molecules in the film. The expression for the surface potential of ionized monolayers was given by Schulman and Rideal (6) and is shown in equation *2*. The term μ in the equation is the algebraic sum of the vertical components of all the dipoles in the molecule. To interpret the ΔV–area curves of lecithin and phosphatidal choline, it is convenient to consider individually each of the dipoles in the molecule.

As shown in Fig. 3 (*a*, *c*), the lecithin molecule is oriented at the air/water interface with its polar end in the water and its fatty acid chains pointing upward. The total surface dipole of lecithin can be considered as the resultant of three component dipoles, each around one of the glycerol carbon atoms. Consider first the ester linkages. As shown in Fig. 3 (*a*, *c*), the two ester bonds of the fatty acids will form two identical dipoles. The ketonic group $C(\delta+)=O(\delta-)$ is approximately horizontally oriented, and therefore will have a negligible vertical component. The carbon of the glycerol and the oxygen of the ester linkage will form a dipole

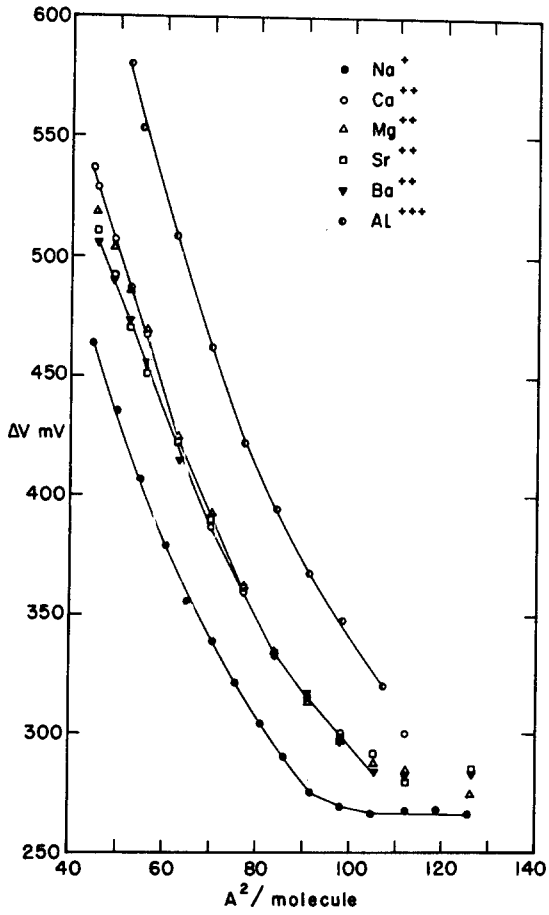


FIG. 4. Surface potential–area curves of synthetic (dipalmitoyl) lecithin on subsolutions of 0.02 M NaCl + 0.01 M metal salt ($CaCl_2$, $MgCl_2$, $SrCl_2$ or $BaCl_2$) at pH 5.6 and $Al(NO_3)_3$ at pH 3.8 and 25°.

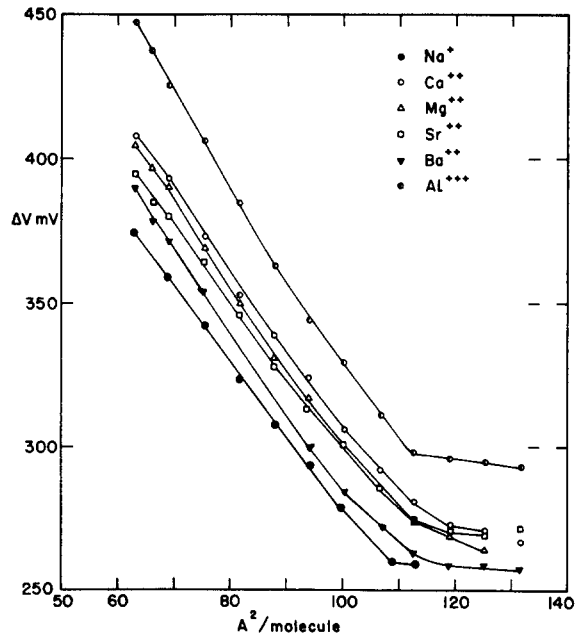


FIG. 5. Surface potential–area curves of egg lecithin on subsolutions of 0.02 M NaCl + 0.01 M metal salt ($CaCl_2$, $MgCl_2$, $SrCl_2$ or $BaCl_2$) at pH 5.6 and $Al(NO_3)_3$ at pH 3.8 and 25°.

with a positive pole at the lower end and a negative pole at the upper end as shown in Fig. 3*c*. This interpretation is supported by a consideration of the three-dimensional model of lecithin, as well as by a consideration of bond angles (7). The third dipole is composed of the third carbon atom of the glycerol, the phosphate group, and the trimethylammonium group.

It is known (8) that the lecithin molecule acts as an uncharged molecule in the pH range 2–8. This can be accounted for by the mutual electrostatic attraction of the phosphate and trimethylammonium groups in the same molecule. Thus there will not be any counter-ion contribution from the subsolution. Since the phosphate group and the trimethylammonium group are part of the molecule and separated by two methylene groups, their dipoles should be considered individually. Surface potential measurements of monolayers of long-chain phosphate (9) indicate that the phosphate group gives rise to a dipole with the upper pole positive and the lower pole negative. It has been shown (10–13) that charged or uncharged amines, as well as substituted amines, similarly give rise to a dipole with the upper pole positive and the lower pole negative. Thus the net magnitude of the third dipole is the sum of the phosphate and the trimethyl ammonium dipoles, placed end to end (Fig. 3*c*).

The polarity of the two dipoles formed by the fatty acid ester bonds is in the opposite direction to that of the third large dipole formed by the phosphoryl choline group. The subtraction of these two dipoles (fatty acid ester bonds) from the third large dipole will give the resultant vertical surface dipole of the lecithin molecule.

It was shown by Hughes and Rideal (14) that in Δ^2-octadecenoic acid, in which a double bond is vicinal to the carboxyl group, the surface dipole is twice that of its isomer, oleic acid. Here the double bond situated immediately above the polar group possesses an induced dipole of the strength equal to that of the polar group, but if situated farther away, the double bond is not influenced by the polar group. The sole difference between phosphatidal choline and lecithin is that the former has a vertically oriented C=C bond instead of the horizontal C=O. As this bond is vicinal to the ether-bond oxygen, a dipole will be induced in it. This induced dipole will further reduce the oppositely oriented dipole of the phosphoryl choline group. Hence the surface potential of phosphatidal choline is lower than that of the lecithins (Fig. 3*d*).

The surface potential is thus a useful way of distinguishing between lecithin and phosphatidal choline molecules, which behave similarly in many other re-

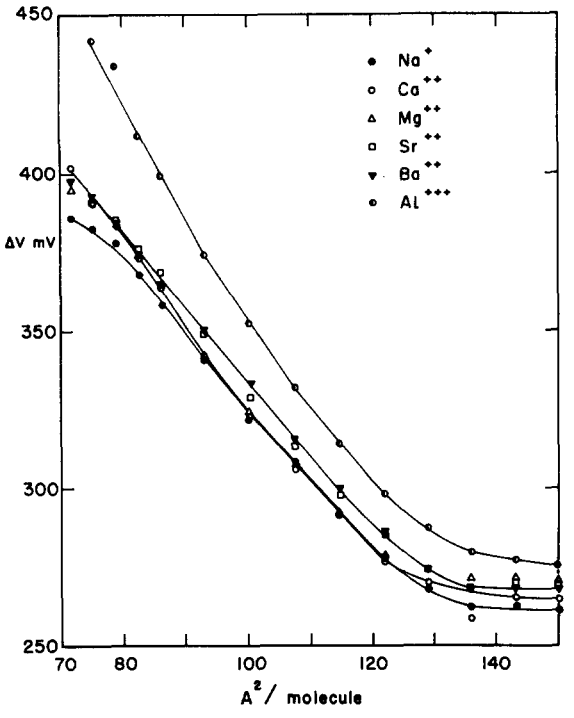


FIG. 6. Surface potential–area curves of yeast lecithin on subsolutions of 0.02 M NaCl + 0.01 M metal salt ($CaCl_2$, $MgCl_2$, $SrCl_2$ or $BaCl_2$) at pH 5.6 and $Al(NO_3)_3$ at pH 3.8 and 25°.

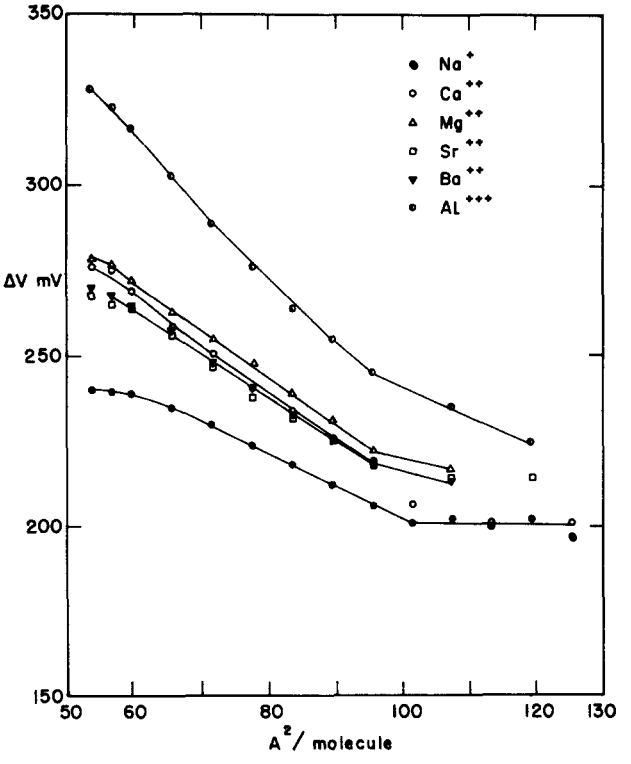


FIG. 7. Surface potential–area curves of phosphatidal choline on subsolutions of 0.02 M NaCl + 0.01 M metal salt ($CaCl_2$, $MgCl_2$, $SrCl_2$, or $BaCl_2$) at pH 5.6 and $Al(NO_3)_3$ at pH 3.8 and 25°.

spects. Mixtures of lecithin and phosphatidal choline extracted from natural sources would, presumably, exhibit intermediate values of surface potential.

Surface Potential Characteristics of Lecithins and Phosphatidal Choline in the Presence of Metal Ions

The ΔV–area curves of lecithin on 0.02 M NaCl, KCl, LiCl, $NaNO_3$, and 0.01 M Na_2SO_4 subsolutions do not show any differences. The surface potential of lecithin in the presence of a divalent metal ion is the same at pH 5.6 as at pH 7.0.

By using the potentiometric titration technique Dervichian (15) showed that natural lecithin was unable to bind Ca^{++} in the pH range 2–8; whereas Anderson and Pethica (8) reported the binding of Mg^{++} to the monolayers of synthetic (distearoyl) lecithin. Kimizuka and Koketsu (16) reported the binding of Ca^{45} to the multilayer films of synthetic (dipalmitoyl) and natural lecithins.

Figures 4–7 show the surface potentials of dipalmitoyl, egg and yeast lecithins, and phosphatidal choline on subsolutions of the different metal ions. Synthetic (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 smallest for the

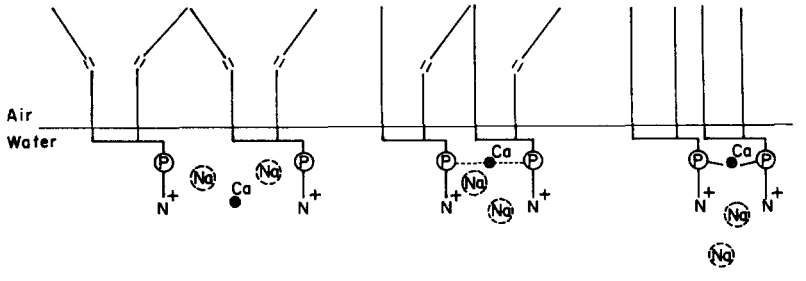


FIG. 8. Schematic representation of interaction of calcium ion with lecithins of different degrees of unsaturation. The dotted line represents weak and the continuous line, strong Ca^{++} interaction.

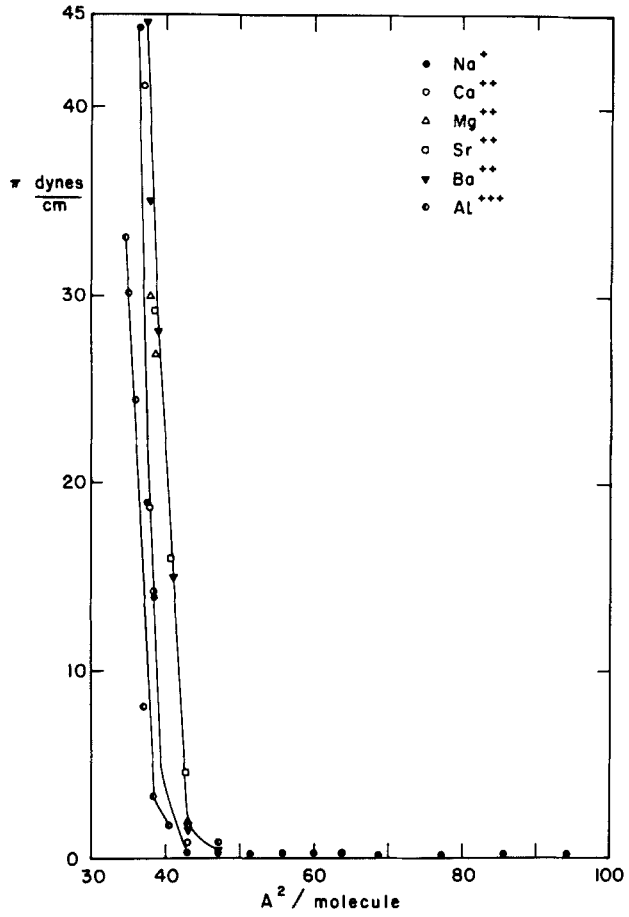


FIG. 9. Surface pressure–area curves of dicetyl phosphate on subsolutions of 0.02 M NaCl + 0.01 M metal salt ($CaCl_2$, $MgCl_2$, $SrCl_2$, or $BaCl_2$) at pH 5.6 and $Al(NO_3)_3$ at pH 3.8 and 25°.

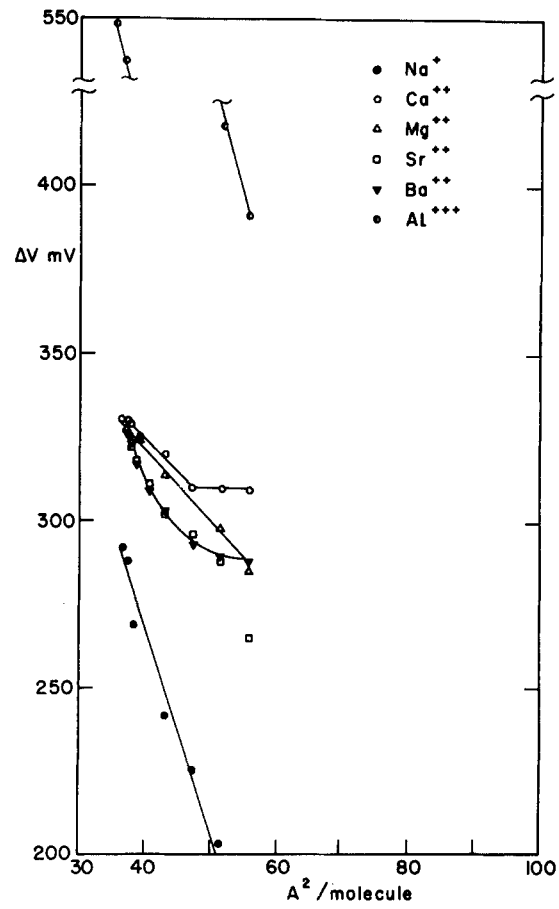


FIG. 10. Surface potential–area curves of dicetyl phosphate on subsolutions of 0.02 M NaCl + 0.01 M metal salt ($CaCl_2$, $MgCl_2$, $SrCl_2$, or $BaCl_2$), at pH 5.6 and $Al(NO_3)_3$ at pH 3.8 and 25°.

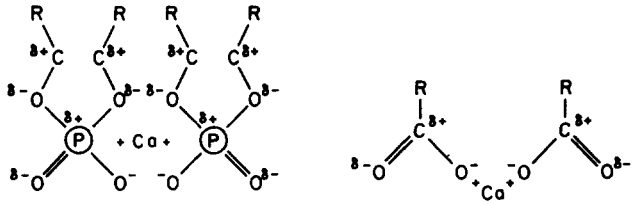
fully unsaturated (yeast) lecithin. In fact, yeast lecithin does not show Ca^{++} binding at all at low surface pressures but shows a little Ca^{++} binding at high monolayer compression.

The data suggest strongly that the interaction of the divalent metal ions with lecithin monolayers is dependent on the packing of the hydrocarbon chains. Figure 8 is a schematic representation of the Ca^{++} interaction with saturated and unsaturated lecithins. The larger intermolecular separation in the monolayers of yeast lecithin (due to unsaturated hydrocarbon chains) permits water, hydrated monovalent ions, and the nitrogen ion of the same molecule to associate with the phosphate ion; this prevents the Ca^{++} interaction with the phosphate ion. On compression of this monolayer, the intermolecular distance decreases, some water molecules and hydrated monovalent ions are squeezed out. Since one Ca^{++} shared between two phosphate groups is smaller than two monovalent ions, conditions are now more favorable for the former; differently stated, for a divalent cation the proximity of the negatively charged groups is an important factor for interaction. Thus the *more unsaturated* the hydrocarbon chains are, the *less reactive* is the lecithin monolayer to the divalent metal ions. The above results indicate for the first time that the interaction of the phosphate group of lecithin with metal ions is influenced by the degree of unsaturation of the hydrocarbon chains.[1]

A strong dependence of the surface potential on the valence of the ion is illustrated by the high potentials

[1] Recent work (unpublished) done in this laboratory showed that the surface potentials of the mixed monolayer of dicetyl phosphate and eicosanoyl trimethylammonium bromide (1:1) were identical on 0.02 M NaCl or 0.01 M $CaCl_2$. Here the trimethylammonium (TMA) group interacts with the phosphate group and the monolayer shows the properties of an uncharged film (i.e., no Ca^{++} binding).

In the case of lecithin, the phosphate and TMA groups of the same molecule interact similarly and produce an uncharged monolayer. In contrast to the mixed monolayer, the two oppositely charged groups are separated vertically by two methylene groups. Normally, this separation between the oppositely charged groups keeps the lecithin molecule uncharged, while still allowing it to bind Ca^{++} upon compression. In this case, the phosphate group interacts more with the vicinal Ca^{++} than with the TMA group. These results indicate the significance of these two methylene groups in the lecithin molecule.



(a) (b)

FIG. 11. The surface dipole components of dicetyl phosphate (*a*) and fatty acid (*b*) in presence of calcium ion. R represents the hydrocarbon chain.

of all three lecithins and phosphatidal choline in the presence of Al^{+++} (Figs. 4–7).

Surface Pressure and Surface Potential Characteristics of Dicetyl Phosphate Monolayers

Surface studies of dicetyl phosphate are helpful in understanding the interaction of the metal ion with the phosphate group. Figure 9 shows a limiting area of 38.5 A^2/molecule for dicetyl phosphate. This agrees with the predicted area of 38 A^2/molecule and that reported by Parreira and Pethica (9). A hydrocarbon chain in dicetyl phosphate is considered to have a cross-sectional area of 19 A^2, whereas the area of a saturated fatty acid residue in the lecithin is somewhat larger (21 A^2), because of the presence of a ketonic group. The addition of Ca^{++} to the subsolution has no effect on the surface pressure–area curve of dicetyl phosphate (Fig. 9). With Sr^{++} and Ba^{++} in the subsolution, the monolayers are slightly expanded; this may be due to the larger diameters of these ions (assuming identical hydration). However, there is a marked increase in the surface potentials of the monolayers when divalent metal ions are in the subsolution (Fig. 10). In contrast, it is known (17, 18) that the addition of Ca^{++} to the subsolution brings about a decrease in the surface potential of a soap (or fatty acid) monolayer at alkaline pH. We explain these differences as follows: an increase or a decrease in the surface potential of the monolayer by Ca^{++} depends upon the position of the Ca^{++} in the dipole lattice of the monolayer. Fig. 11*a* shows the postulated position of the Ca^{++} in the monolayer of dicetyl phosphate. It is attracted above the plane of the negatively charged oxygens by the polarized ether bond oxygens; in this position Ca^{++} will not affect the area of the film (Fig. 9). The dipole produced, with its positive upper pole, increases the total dipole of the molecule and hence the surface potential.[2] In the case of fatty acid or soap monolayers (Fig. 11*b*) Ca^{++} will preferentially take up a position below the negatively charged oxygens because of the repulsive force of $C(\delta+)$. This dipole (due to Ca^{++}) decreases the total surface dipole and hence the surface potential.

Surface Pressure and Surface Potential Characteristics of Cardiolipin Monolayers

Figure 12 shows the molecular structure of cardiolipin, as proposed by Macfarlane and Wheeldon (19) and by Benson and Strickland (20), oriented at the interface. Figure 13 shows that the limiting area for cardiolipin on a 0.02 M NaCl subsolution is 110 A^2/molecule. In contrast to other phospholipids, the surface pressure–area curves of cardiolipin show 10–13% contraction in the presence of divalent metal ions. However, the limiting area is not changed significantly. This shows that the packing of the hydrocarbon chains is the same regardless of the presence or absence of Ca^{++} in the subsolution. This was also observed in the surface pressure–area curves of dicetyl phosphate (Fig. 9).

[2] According to the explanation of the surface potential in relation to the position of the metal ion, it was expected that the binding of Ca^{++} with alkyl sulfate should increase the surface potential because of the similar structure and equal cross-sectional area (25 A^2) of the phosphate and sulfate groups. This was shown by work done recently in this laboratory on the monolayers of sodium cetyl sulfate (unpublished).

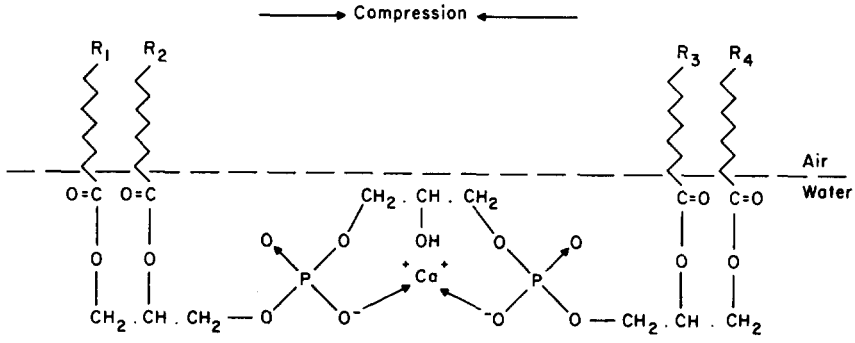


FIG. 12. Orientation of cardiolipin molecule at the air/water interface and calcium ion binding by its two phosphate groups. R_1, R_2, R_3, R_4 represent the hydrocarbon chains.

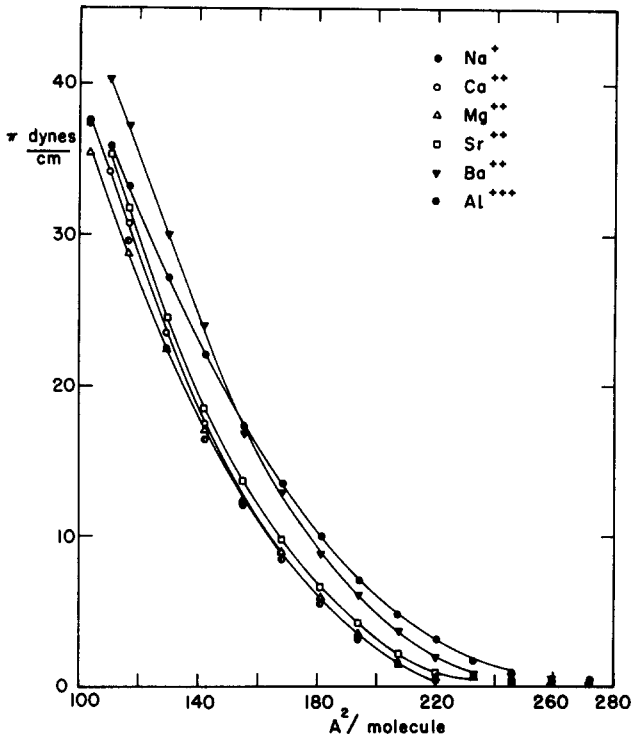


FIG. 13. Surface pressure–area curves of cardiolipin on subsolutions of 0.02 M NaCl + 0.01 M metal salt ($CaCl_2$, $MgCl_2$, $SrCl_2$ or $BaCl_2$) at pH 5.6 and $Al(NO_3)_3$ at pH 3.8 and 25°.

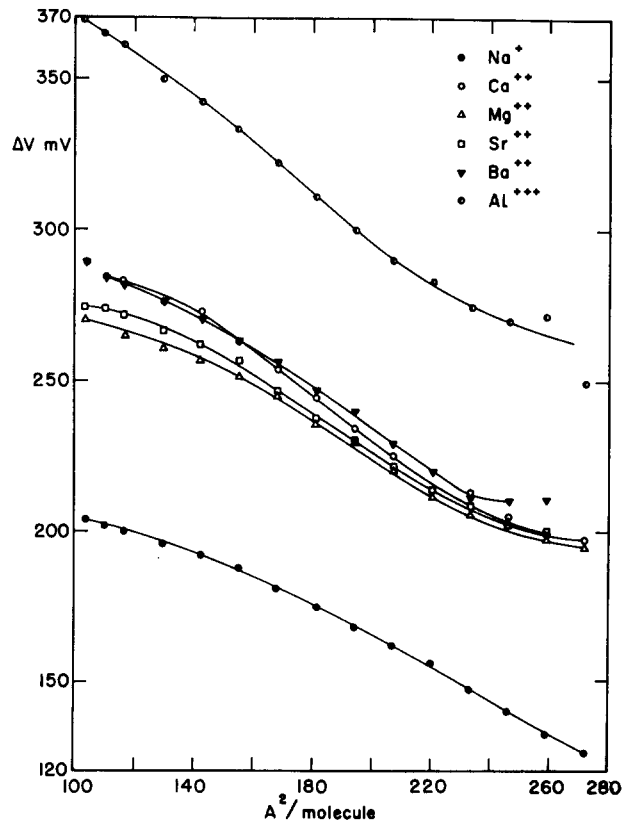


FIG. 14. Surface potential–area curves of cardiolipin on subsolutions of 0.02 M NaCl + 0.01 M metal salt ($CaCl_2$, $MgCl_2$, $SrCl_2$ or $BaCl_2$) at pH 5.6 and $Al(NO_3)_3$ at pH 3.8 and 25°.

Figure 12 affords an explanation of the effect of compression on the cardiolipin monolayers. The two effects of compression are a decrease in the intermolecular spacing, and a decrease in the intramolecular spacing (i.e., cross-sectional area). The latter is brought about by reducing the size of the phosphodiester part of the molecule to a minimum by appropriate rotation of bonds. At low pressures, this portion will be held apart by the mutual repulsion of the phosphate groups and the presence of divalent cations causes a contractile effect (Fig. 13). At high pressures, the cross-sectional area of the hydrocarbon chains becomes the limiting factor and divalent cations do not show significant contraction.

At high compression, the size of the divalent cations constitutes an additional factor influencing the magnitude of surface pressure. This effect is seen markedly with Ba^{++} (Fig. 13). The surface pressures at 120 A^2/molecule in the presence of divalent metal ions are in the order $Mg^{++} < Ca^{++} < Sr^{++} < Ba^{++}$; this is also the order of their ionic radii.

For yeast lecithin the unsaturation in both hydrocarbon chains prevents the interaction of calcium ion with the phosphate groups, whereas in cardiolipin the unsaturated hydrocarbon chains do not prevent such interaction. This can be explained by the fact that in the cardiolipin molecule the two phosphate groups involved in calcium binding are on the same molecule, and the unsaturation of the hydrocarbon chains of cardiolipin does not affect the proximity or orientation of the two phosphate groups (see Fig. 12). Also cardiolipin does not have an oppositely charged nitrogen ion, which in the case of yeast lecithin reduces the interaction of Ca^{++} with the phosphate group.

It is interesting to note that the contractile effect of Ca^{++}, as shown by the surface pressure–area curve, disappears when the subsolution contains Na^+ and Ca^{++} in the ratio 5:1 or higher. The pressure–area curves in these cases are the same as that with Na^+ in the subsolution, shown in Fig. 13. This can be tentatively explained by assuming that the cardiolipin acts as an ion-exchanger and that high concentrations of Na^+ displace the centrally located Ca^{++} from the two phosphate groups.

Figure 14 shows the surface potentials of cardiolipin monolayers in the presence of different metal ions. The effect of divalent and trivalent cations is already apparent at low pressures, presumably due to the absence of a vicinal positively charged group in the molecule.

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