The Influence of Surface Charge Density of Phosphatides on the Binding of Some Cations*

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SUMMARY

Surface potentials, \( \psi_0 \), of mixtures of synthetic phosphatides dispersed in uni-univalent electrolyte have been calculated from the limiting molecular areas through use of the Gouy equation. Electrophoretic mobilities of the dispersed particles were measured and used to calculate \( \zeta \) potentials from the Helmholtz-Smoluchowski equation. In analogy with several structurally related systems, the ratio \( \zeta/\psi_0 \) was found to be unity at low surface charge density \( (e < 20,000 \text{ e.s.u. cm}^{-2}) \), decreasing progressively at higher surface charge density to a minimum of 0.58. The \( \zeta \) potential at low surface charge density is appreciably affected by the composition of the fatty acyl substituents to the extent that this determines the cross-sectional area per net negative charge in the bilayer.

Charge reversal concentrations have been measured for various metal ions on several phosphatide surfaces differing in charge density. Inserting the conditions for charge reversal into the Stern equation, the standard free energy of adsorption for each cation was calculated and used to obtain a value for the logarithm of the apparent association constant, \( K' \). At an initial surface charge density below 20,000 e.s.u. cm\(^{-2}\), the sequence of values of log \( K' \) was \( Ag < Ba < Sr < Ni < Mg < Ca < Co < Zn < Cu < Mn < Pb < Cd < La < Ce < Th < UO_2^{2+} \). Comparison of log \( K' \) for magnesium, calcium, strontium, and barium as a function of surface charge density suggests that the larger cations are able to form ion triplets at high surface charge density while the smaller cations are unable to do so. The high affinity of \( UO_2^{2+} \) for phosphatide surfaces is also considered.

The results obtained are discussed in terms of their potential applicability to several problems of current biochemical interest.
by acylation in pyridine of sn-glycero-3-phosphorylcholine originating from egg lecithin (17-19). 1-Stearyl-2-oleoyl-sn-glycero-3-phosphorylcholine, prepared as described by de Haas and van Deenen (19), was converted to the corresponding phosphatidic acid by hydrolysis with cabbage leaf phospholipase (20, 21). 1-Stearyl-2-oleoyl-sn-glycero-3-phosphoryl ethanolamine and 1-oleoyl-2-stearoyl-sn-glycero-3-phosphorylserine were obtained by synthesis (22, 23). The fatty acid composition in each case was checked by gas-liquid chromatography of the derived methyl esters on columns of 20% ethylene glycol succinate on 60/70 AS Anakrom support in a Barber-Colman series 5000 gas chromatograph utilizing a hydrogen flame detector. Individual and mixed phosphatide dispersions were prepared by shaking dried lipid films with 0.145 M NaCl, and in some cases they were exposed briefly (30 to 60 sec) to ultrasonic irradiation (Raytheon). The final concentration in each case was 0.05%, w/v.

Inorganic Salts—The following inorganic salts were dissolved in 0.145 M NaCl to a concentration of approximately 1 M: LiCl, NaCl, KCl, CaCl₂, MgCl₂, CaCl₃, SrCl₂, BaCl₂, MnCl₂, NiCl₂, CuCl₂, ZnCl₂, and CdCl₂. Solutions of CaCl₂, Tb(NO₃)₆, La(NO₃)₃, and UO₂(NO₃)₂ were 0.1 M concentration.

Microelectrophoresis—A cylindrical microelectrophoresis apparatus described by Bangham, Flemans, Heard, and Seaman (24) was obtained from Rank Brothers, Cambridge, England. The chamber was modified by removal of the Quickfit B10 socket joints and replacement with B7 joints. Measurements could then be carried out on volumes of the phosphatide dispersions as small as 2 ml. The stationary level was determined by measurement of the velocity of washed human erythrocytes at pH 5.4 and 8.5 in the presence of 1% hemoglobin solution and was found to be at a distance 285 µ from the side of the tube. The potential gradient was calculated from the applied potential and the tube length of 152 mm. Mobility determinations were usually made by timing 10 focused particles in each direction. All measurements were made at 20° ± 0.2° or at 40° ± 0.2°, as indicated.

The effects of the different cations on electrophoretic mobility were examined by making successive additions of the concentrated salt solutions to single samples of the dispersion and re-measuring the mobility. Additions were made from a 10-µl or 100-µl Hamilton syringe and were followed by thorough mixing and incubation at 20° for 10 to 15 min. Each charge reversal point was determined from a series of mobility determinations over a range of cation concentrations appropriate for each.

RESULTS

Relationship between Potential and Surface Potential—The relationship between the two potentials, ζ and φₒ, was studied with a series of defined mixtures of phosphatides. In each case the potential in the shear plane, ζ, was calculated from the measured electrophoretic mobility, u, by application of an appropriate form of the well known Helmholtz-Smoluchowski equation (25) relating these two parameters.

\[
\zeta = \frac{6\pi n \eta}{\varepsilon R F D_u} \tag{1}
\]

where \(n\) is the bulk viscosity coefficient of 0.145 M NaCl (0.010100 poise at 20°; 0.006610 poise at 40°), \(D_u\) is the bulk dielectric constant (80 at 20°; 73 at 40°), \(u\) is the electrophoretic mobility (square centimeters per second), and \(R\) and \(F\) are correction factors for delayed relaxation of the ionic atmosphere and for surface conductance, respectively. Since in the present case \(a \gg 1/k\), where \(a\) is the mean radius of the particles and \(k\) is the Debye-Hückel function, \(R \cong 1\) and \(F \cong 1.33\) and hence

\[
\zeta = \frac{4\pi n \eta}{D_u} \tag{2}
\]

\[
\phi_0 = \frac{2kT}{\varepsilon} \sinh^{-1} \frac{\sigma}{c_i^{1/2}} \left(\frac{500\pi}{D_u RT}\right)^{1/3} \tag{3}
\]

where \(\sigma\) is the net surface charge density, \(c_i\) is the concentration of uni-univalent electrolyte, \(k\) is the Boltzmann constant, \(\varepsilon\) is the electronic charge, \(R\) is the gas constant, and \(T\) is the absolute temperature (degrees Kelvin).

At 20° in 0.145 M NaCl, this reduces to

\[
\phi_0 = 50.4 \sinh^{-1} \left(\frac{352}{A_i}\right) \tag{4}
\]

where \(A_i\) is the mean area per electronic charge. At 40°,

\[
\phi_0 = 53.8 \sinh^{-1} \left(\frac{331}{A_i}\right) \tag{5}
\]

If \(A_1\) and \(A_2\) are the mean areas per molecule of the acidic and zwitterionic species and \(N\) is the mole fraction of acidic species, then, when \(A_2 > A_1\),

\[
A_i = \frac{A_1 A_2}{N[A_1 + N(A_1 - A_2)]} \tag{6}
\]

where, when \(A_1 > A_2\),

\[
A_i = \frac{A_1 A_2}{N[A_1 + N'(A_1 - A_2)]} \tag{7}
\]

where \(N'\) is the mole fraction of zwitterionic species. In Figs. 1 and 2 \(A_1\) and \(A_2\) are kept constant while \(N\) is varied, while in Fig. 3 \(A_1\) and \(A_2\) are varied while \(N\) is kept constant. \(\sigma\) is then given by

\[
\sigma = \frac{10\%}{A_i} \tag{8}
\]

The values of \(A_1\) and \(A_2\) which have been used to calculate \(A_i\) are the limiting areas of the molecules measured from the force-area curves of monolayers (15, 16). These values correspond to the closest possible packing arrangement of the aligned molecules. A similar arrangement may be supposed to exist in the predominating bilayer structure of phospholipid-water dispersions, since the most cohesive, parallel alignment of the molecules is also likely to be the most stable structure in this form. Some implications of this assumption are discussed in a later section. Values obtained from the literature (15, 16) are given below.

1,2-Distearoyl sn-glycero-3-phosphorylcholine (18:0/18:0 PC); 39 Å²

1 Abbreviations provide information on the fatty acid constituents as well as on the particular phosphatide class; e.g. 18:0 represents a straight chain 18-carbon fatty acid with no double bonds; 18:1 represents an 18-carbon acid with one double bond; 18:0/18:1 is a diacyl derivative, position 1 being specified first and position 2 second. PC, phosphatidylcholine; PE, phosphatidylethanolamine; PS, phosphatidylserine; PA, phosphatidic acid.
Fig. 1. Plots of \( \bar{\varepsilon} \) potential (- - -) and surface potential, \( \psi_0 \) (---), with respect to surface charge density for mixtures of phosphatides in 0.145 M NaCl at 20°. The mole fraction of acidic species was varied, while the mean area per molecule was kept approximately constant (53 to 59 Å²). The experimentally measured values of \( \bar{\varepsilon} \) correspond to mole fractions of the acidic lipid equal to 0.1, 0.2, 0.4, 0.6, 0.8, and 1.0.

![Diagram of plots](https://example.com/diagram1.png)

Fig. 2. Plots of \( \bar{\varepsilon} \) potential (- - -) and surface potential, \( \psi_0 \) (---), with respect to surface charge density for mixtures of 18:0/18:1 PS + 18:0/18:1 PC in 0.145 M Tris-NaCl (pH 7.4) at 20° and 40°. In each curve, the mole fraction of acidic species was varied while the mean area per molecule was kept constant (57 to 59 Å²). Experimental points again represent \( x = 0.1, 0.2, 0.4, 0.6, 0.8, \) and 1.0.

1,2-Dioleoyl-sn-glycero-3-phosphorylcholine (18:1/18:1 PC): 83 Å²
1-Stearoyl-2-oleoyl-sn-glycero-3-phosphorylcholine (18:0/18:1 PC): 59 Å²
1-Stearoyl-2-oleoyl-sn-glycero-3-phosphorylethanolamine (18:0/18:1 PE): 53 Å²
1-Oleoyl-2-stearoyl-sn-glycero-3-phosphorylserine (18:1/18:0 PS): 57 Å² at pH 4.8 and 59 Å² at pH 7.4
1-Stearoyl-2-oleoyl-sn-glycero-3-phosphoric acid (18:0/18:1 PA): 53 Å²

Fig. 3. Plots of \( \bar{\varepsilon} \) potential (- - -) and surface potential (---) with respect to surface charge density for mixtures of PA and PC in 0.145 M NaCl at 20°. The mole fraction of PA was kept at 0.4, while the mean area per molecule was varied by the introduction of a higher or lower content of unsaturated fatty acids. Molar ratios of various phosphatides used were (18:0/18:1 PA) (18:0/18:0 PA) (18:0/18:0 PC) (18:0/18:1 PC) (18:0/18:1 PA) (18:0/18:0 PC) = 2:2:3:3, (18:0/18:1 PA) (18:0/18:1 PC) = 2:3, (18:0/18:1 PA) (18:1/18:1 PA) (18:0/18:1 PC) = 2:2:3:3, and (18:1/18:1 PA) (18:1/18:1 PC) = 2:3.

![Diagram of plots](https://example.com/diagram2.png)

Fig. 4. The effect of surface charge density on log \( K' \) for magnesium (O), calcium (●), strontium (▲), and barium (■) on mixtures of phosphatidic acid and phosphatidylethanolamine.

1,2-Distearoyl-sn-glycero-3-phosphoric acid (18:0/18:0 PA): 35 Å²
1,2-Dioleoyl-sn-glycero-3-phosphoric acid (18:1/18:1 PA): 77 Å²

Fig. 1 shows the relationship between \( \bar{\varepsilon} \) and \( \psi_0 \) for three series of phospholipid mixtures with different charged groups in the interface. In one case (18:0/18:1 PS-18:0/18:1 PC), measurements were also made at pH 7.4 and at 40° (Fig. 2). Replac-
ment of the fatty acid substituents by more saturated or less saturated chains also permitted examination of this relationship, but over a more limited range (Fig. 3).

**Binding of Cations**—Charge reversal concentrations, $c_v$, were used to compute values of $\log K'$, where $K'$ is the apparent association constant for the reaction

$$M + L \rightarrow ML$$

$L$ represents a fixed ligand in the interface, and $M$ represents the adsorbing metal ion.

$$K' = \frac{[ML]}{[M][L]}$$

### Table I

**Logarithms of apparent association constants of various metal ion phosphatide complexes**

$N$ represents the mole fraction of phosphatidic acid.

<table>
<thead>
<tr>
<th>Cation</th>
<th>$N = 0.2$</th>
<th>$N = 0.5$</th>
<th>$N = 0.8$</th>
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<tbody>
<tr>
<td>Li</td>
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<td>4.02</td>
<td>4.02</td>
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<td>4.48</td>
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<tr>
<td>Th</td>
<td>7.7</td>
<td>7.63</td>
<td>7.72</td>
</tr>
</tbody>
</table>

### Table II

**Association constants for binding of calcium and magnesium to phosphoric acid and phosphorylcholine surfaces**

<table>
<thead>
<tr>
<th>Method used</th>
<th>Material</th>
<th>Logarithm of association constant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Titration*</td>
<td>PS</td>
<td>$\text{Ca}^+$: 4.03 $\text{Mg}^+$: 3.74 $\text{Mg}^+$: 3.01</td>
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<tr>
<td>Turbidity*</td>
<td>PA</td>
<td>$\text{Ca}^+$: 4.08 $\text{Mg}^+$: 3.89</td>
</tr>
<tr>
<td>Titration*</td>
<td>PA</td>
<td>$\text{Ca}^+$: 4.08 $\text{Mg}^+$: 4.3</td>
</tr>
<tr>
<td>Charge reversal</td>
<td>PA</td>
<td>$\text{Ca}^+$: 4.02 $\text{Mg}^+$: 3.70</td>
</tr>
<tr>
<td>Charge reversal</td>
<td>PS</td>
<td>$\text{Ca}^+$: 4.07 $\text{Mg}^+$: 3.79</td>
</tr>
</tbody>
</table>

* Data of Hendrickson and Fullington (27).
* Data of Abramson, Katzman, and Curei (28).
* Data of Abramson, Katzman, Gregor, and Curei (29).

**Fig. 5.** Relative inhibition of binding of calcium to phosphatidylserine plotted as a function of $\log K'$ calculated from the charge reversal concentrations. Data plotted on the ordinate are taken from Blaustein (8).

The number of counterions, $n_v$, of valency $z_i$ adsorbing from aqueous solution onto an area of surface carrying $n_s$ ligands is given by the Stern equation,

$$n_v = 0.018c_i(n_s - n_v) \exp \left(\frac{-z_i\psi_s + \lambda_p + W}{kT}\right)$$

where $\psi_s$ is the potential at the plane of the adsorbed counterions, $\lambda_p$ is the energy of polarization, and $W$ is the van der Waals energy. We assume the equilibrium value of $c_i$ at the charge reversal point to be identical with the initial value of $c_i$ and hence $c_i = c_v$, at which point $\psi_s = 0$ and $n_v = n_vN/z_i$. Substitution of these conditions in Equation 10 gives

$$N = 0.018c_vz_i \left(1 - \frac{N}{n_s}\right) \exp \left(\frac{\lambda_p + W}{kT}\right)$$

Measurement of $c_v$ at a given value of $N$ allows calculation of the standard free energy of adsorption ($\Delta G_0$) given by the term $(\lambda_p + W)/kT$. From $\Delta G_0$ we can obtain a value of $\log K'$ by using Equation 12.

$$\Delta G_0 = -RT \ln K'$$

Measurements of charge reversal were carried out on dispersions containing 0.2, 0.5, and 0.8 mole of 18:0/18:1 PA per mole
of 18:0/18:1 PC. Values of log $K'$ obtained by the above calculations are shown in Table I. Some of these results are also compared with values obtained from the literature (Table II). By using Equations 6 and 8 to calculate $\sigma$, it was then possible to demonstrate an effect of charge density on values of log $K'$ for the series of cations, magnesium, calcium, strontium, and barium (Fig. 4).

The adsorption of cations onto mixed dispersions containing 18:1/18:0 PS and 18:0/18:1 PC was studied in a like manner. Extrapolation of the values of log $K'$ to $N = 1$ (100% PS) allowed a comparison of the charge reversal technique, with the method of Blaustein (8) as a means of measuring cation binding. In Fig. 5, values of log $K'$ calculated from $c_*$, are plotted against the relative inhibition of Ca$^{2+}$ binding measured by the solvent partition technique.

**Discussion**

Electrophoretic mobility measurements on mixtures of naturally occurring phosphatides have been reported by Papahadjopoulos, Housie, and Hanahan (7). Although the measured mobilities were similar to those found in the present work, an exact comparison of $\xi$ and $\psi_0$ is not possible because the limiting areas of the natural phosphatides were not reported in the earlier work.

It is evident from Fig. 1 that, regardless of the nature of the charged groups in the phospholipid mixtures, there is a close correspondence of $\xi$ and $\psi_0$ over a range of surface charge density from 0 to 20,000 e.s.u. cm$^{-2}$ (i.e. $\xi/\psi_0 = 1$), whereas at higher charge density $\xi/\psi_0$ decreases to a minimum value of 0.58. The agreement between $\xi$ and $\psi_0$ at low surface charge density reported here appears to validate the assumptions made in applying the Helmholtz and Gouy equations. Systems studied at different pH and temperature show very similar behavior (Fig. 2). It should be noted here that the fatty acid composition of the phosphatides can influence the surface charge density over a small range. This is a consequence of the altered spacings of the aligned molecules in the bilayer as a result of the introduction or elimination of double bonds. Through selection of an appropriate phospholipid mixture, the decreasing value of $\xi/\psi_0$ at high charge density can be demonstrated (Fig. 3).

In order to compute values for the surface charge densities and surface potentials of the various colloidal particles, it has been necessary to assume that the area occupied per molecule is equal to the area occupied in a monolayer at the collapse pressure. This assumption is probably valid for fully saturated phospholipids such as 1,2-dioleoyl-sn-glycero-3-phosphorylcholine, which have higher melting points than their unsaturated analogues and which, even in the hydrated state, form solid structures at room temperature. The bilayer structure of these compounds is therefore likely to be similar to the structure in the compressed monolayer. On the other hand, unsaturated compounds such as 1,2-dioleoyl-sn-glycero-3-phosphorylcholine when in the hydrated state form liquid-like structures at ambient temperature (30). Consequently, the bilayer structures for these compounds are likely to be more expanded than the corresponding structures in compressed monolayers. Thus, the calculated surface charge densities are likely to be somewhat too high for mixed acid phospholipids and for phospholipids containing two unsaturated acyl residues per molecule. A similar correction may be required for some nonlamellar structures. Since, however, a closely packed and cohesive molecular structure is always required to exclude bulk water from the hydrocarbon chains, the magnitude of these effects should be quite small. In neither case could this type of correction be expected to account for the large decrease in the ratio $\xi/\psi_0$ at high surface charge density.

The discrepancy between $\xi$ and $\psi_0$ at high charge density, evident in Figs. 1 to 3, has now been observed in at least four distinct systems. Films of octadecyltrimethylammonium bromide spread at the air-water interface show a marked increase in surface viscosity with increasing concentrations of NaCl in the aqueous phase (31). While this effect is quite marked with films spread on an area of 85 A$^2$ per molecule, it is not evident in films spread at 180 A$^2$. The increase in surface viscosity is coincident with a decrease in the ratio $\xi/\psi_0$ to 0.55. Micelles of sodium dodecyl sulfate exhibit a discrepancy in $\xi/\psi_0$ of the same order when $\psi_0$ is made sufficiently large by the addition of NaCl (32). Studies with sodium dodecyl sulfate- and dodecyltrimethylammonium bromide-stabilized oil drops (33) indicate that the ratio $\xi/\psi_0$ equals unity only at low surface charge density, while at higher charge density $\xi/\psi_0$ decreases to about 0.5, an effect again considered to be due to an enhanced viscosity in the ionic double layer. It can be concluded, therefore, that these effects form a general feature of highly charged interfaces and must be explained by a nonspecific mechanism.

Stigter and Mysels (32) have suggested that roughness of the micellar surface leads to entanglement of counterions in the interface, with a consequent decrease in the value of $\xi$. This explanation could be relevant also to the phosphatide surface, since a noncoplanar arrangement may be required to attain maximum adhesion of adjacent molecules (34). It is more likely, however, that an error is introduced into Equation 1 by the use at high electrical field strengths of the bulk values $\eta_0$ and $D_0$ (33). Under these conditions $\eta_1 > \eta_0$ and $D_1 > D_0$, where the subscript 1 refers to the values of these parameters in the interface. Hence, Davies and Ricle (25) obtained the true value of $\xi$ from the equation

$$\xi = \frac{\eta_0}{D_0} \int_0^{\xi_0} \frac{D_1}{\eta_1} d\psi$$

Estimates of $D_1$ and $\eta_1$ at known field strengths can be obtained from data in the literature (35, 36) and, when substituted in Equation 1, give values of $\xi$ which are coincident with the calculated values of $\psi_0$ over the measurable range of charge density. This type of correction will be required in a number of other biologically important cases in which the surface charge greatly exceeds 20,000 e.s.u. cm$^{-2}$, such as may be encountered in helical polynucleotides, polypeptide, and polyguanidic acids.

The adsorption of cations onto negatively charged groups in the interface will lead to a reduction in the net surface charge density or, at sufficiently high concentrations of cations, to a complete reversal of charge, owing to the presence of an excess of positively charged primary and quaternary amine groups. The charge reversal concentration, $c_*$, corresponds to the point of zero charge, at which positive and negative charges are equally balanced. In this paper the charge reversal concentration has been related to the apparent association constant, $K'$, which is characteristic for a given cation on a given type of surface. The cation sequences shown in Table I are in the main those characteristic of phosphate surfaces (5). This is indicated especially by the high value of $K'$ for the divalent uranyl ion. The technique of determining cation sequences by charge reversal has
occasionally been used as a fingerprint method to identify the charged groups on the surfaces of blood cells (6) and bacterial cells (37). The apparent association constants for calcium and magnesium on phosphatidic acid and phosphatidylethanolamine dispersions have also been measured by titrimetric and turbidimetric methods. Table II shows that excellent agreement is obtained when the reported values of log $K'$ are compared with those calculated from the charge reversal concentrations. Replacement of phosphate by phosphorylethanolamine in the head group of the lipid appears to have little or no effect on the affinity of these cations for the surface.

The experimentally measured charge reversal concentration, $c_r$, depends both on the association constant, $K$, for ions in solution and on the surface charge density. Generally, $c_r$ decreases and $K'$ increases with increasing valency of the cation. In earlier work (38), the surface charge density was represented by the "reciprocal hexol number" determined experimentally for each colloid. This was also shown to be related to $c_r$ for cations having a lower affinity than hexol for the surface groups. In estimating $K'$, the apparent association constant, allowance is made in Equation 11 for the charge density factor in the term $N$, and hence, for an ideal system with 1:1 metal ligand complexing, $K'$ should be independent of $\sigma$. This interpretation appears to be valid (Fig. 4) only for metals of small ionic radius (Mg$^{2+} = 0.66$). Increasing the ionic radius from Ca$^{2+}$ (1.02) to Sr$^{2+}$ (1.12) to Ba$^{2+}$ (1.34) produces a progressively greater deviation from this condition. The results illustrated in Fig. 4 can most easily be explained in terms of the greater stability of chelate structures having the stoichiometry $M_{1n}$ as contrasted with the simple ion pair $ML$. Clearly, the formation of ion triplets is limited in the first place by the spacings between the ligands in the interface and by the ionic radius of the adsorbing cation. This situation would favor ion triplet formation with the larger metal ions at higher surface charge densities, as is actually observed. It follows from this that the coincidence of cation sequence between an unknown cell surface and a model surface does not constitute rigorous proof of their identity unless the initial surface charge densities are also shown to be closely similar.

Hausten (8) has studied the binding of cations to phosphatidylethanolamine by measuring the release of $^{46}$Ca from the phospholipid in the presence of various metal ions. Since PS was shown to be present exclusively in the organic phase, it is assumed that the lipid molecules existed in a nonaggregated state. Fig. 5 shows that, with one exception (UO$_2$$^{2+}$), there is nevertheless a good measure of agreement between the transport (charge reversal) and equilibrium measurements. When the two techniques are compared for UO$_2$$^{2+}$, a discrepancy in $K'$ of about $10^4$ is seen, reflecting a much higher affinity of the uranyl ion for the aggregated form than for the monomolecular form of PS. Uranyl phosphate in the naturally occurring mineral, autunite, has a strong tendency to form a layer lattice in which the U-O$_3$ bonds are linear, although not equibranched, and perpendicular to the square formed around the uranium atom by the 4 oxygen atoms of four adjacent phosphate tetrahedrons (39-41). The dimensions of the tetragonal lattice are $a = 6.96$ A and $c = 8.40$ A, requiring an area per phosphate group of 58.5 A$^2$, a figure which lies very close to the limiting area for the various mixed acid phosphates (58 to 69 A$^2$). Thus, for the adsorption of UO$_2$$^{2+}$ onto a phosphate surface, there should be a large contribution to the lattice energy of the uranyl phosphate crystal arising from the existing structural arrangement of the ligands in the interface. The formation of an equiplanar uranyl phosphate lattice may therefore account for the special affinity of UO$_2$$^{2+}$ for this surface.

Many of the biological effects of metal ions can be attributed to the formation of complex ions with the lipid and protein components of the cell membrane. There can be no doubt that the ion exchange properties of phospholipids play an important role in determining the selective permeability of biological membranes to ions, and that quantitative data on ion binding will be required to evaluate these effects. The possibility has been raised that liquid crystals of phospholipids can serve as models for membrane structure and function (42). The liquid crystals contain water cavities sandwiched between concentric bimolecular leaflets and hence can bind or capture both metal ions (43) and nonionic substances (44). Trapped ions can diffuse into the external aqueous phase at characteristic rates, depending on the sign of the charge on the ion and on the sign and magnitude of the surface charge. Although the cations studied were mainly univalent, it was found that ions of higher valency have a large effect on the permeability properties of the acidic phospholipids, phosphatidylethanolamine, phosphatidylcholine, and phosphatidic acid (45). In conjunction with ion exchange effects, there is a large contribution to the over-all permeability of the lipid structures, arising from the precise physical structure of the dispersed particles (45, 46). For example, the volume of trapped water, and hence the amount of captured ion, is dependent on the surface charge density (43). Another possibility is that leakage rates are controlled by subtle changes in the phase structure, since small changes in environment will readily produce phase changes in which the water cavities may become exposed to the external aqueous phase. Ion binding and physical structure of the phospholipids therefore provide supplementary contributions to the over-all permeability properties.

The mechanism of participation of phospholipids in blood coagulation is incompletely understood. However, an important contribution to this area was the suggestion that surface charge of the phospholipids is critical in determining their coagulant activity (7, 11). Various coagulant proteins form high molecular weight complexes with phospholipids. For example, it has been found that in the presence of an optimal concentration of calcium ions prothrombin will form an adsorption complex with a mixture of phosphatidylcholine and phosphatidylethanolamine in a 1:1 molar ratio (47), but not with phosphatidylethanolamine alone. Nor is any complex formed in the absence of calcium ions. These results have been discussed in terms of the ion binding and charge reversal effects of calcium ions and the formation of mixed chelates, in which the water and protein components both contribute ligands to the complex ion (48). Preincubation of the systems with very low concentrations of uranyl and thorium salts prevents the formation of such mixed chelates, and this can readily be understood in terms of a perturbation of charge effects resulting from the much higher affinities of these cations for the phosphatide surface (Table II).

Recent studies with phospholipases indicate that the hydrolytic activity of these enzymes is also dependent on the sign and magnitude of the surface charge on the colloidal substrate particles. Many lipases are metal ion-dependent, and one aspect of this requirement may be the necessity for a reduction or reversal of the surface charge. This question has been discussed in considerable detail (40). It may be remarked, however, that several

1 P. G. Barton, unpublished observations.
of these systems could function as useful models for explaining the association of many enzymes with lipid structures.

REFERENCES

The Influence of Surface Charge Density of Phosphatides on the Binding of Some Cations

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