PRELIMINARY NOTE

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The ionic structure of sphingomyelin monolayers

Phospholipid monolayers have been studied by measuring the surface pressure, surface potential[1,2] and surface radioactivity[3,4] in order to understand the physicochemical interactions presumably occurring at the cell surfaces. From the studies of erythrocytes of various species, it has been shown[5,6] that a decrease of lecithin is compensated by an increase of sphingomyelin in the erythrocyte membrane; this is also accompanied by changes in the permeability properties of the erythrocyte membrane. In relation to these observations, this preliminary note presents our results regarding the ionic properties of sphingomyelin and lecithin monolayers.

L-α-Dipalmitoyl lecithin was purchased from Mann Research Laboratories (New York). Beef heart sphingomyelin was supplied by Sylvana Chemical Company (Millburn, N.J.). Both samples showed single spots on the thin-layer chromatography plate with the solvent system chloroform–methanol–water (80:35:5, v/v/v). Lipid solutions were prepared in hexane–methanol–chloroform (3:1:1, v/v/v). Inorganic chemicals of reagent grade and twice-distilled water were used in all experiments.

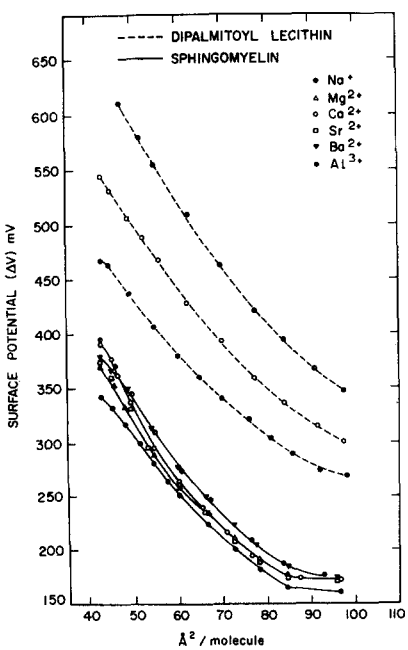


Fig. 1. Surface potential–area curves of sphingomyelin monolayers on subsolutions of 0.02 M NaCl, 0.01 M $CaCl_2$, $MgCl_2$, $SrCl_2$, or $BaCl_2$, and 0.0066 M $AlCl_3$ at pH 5.6 and 25°. The surface potentials of dipalmitoyl lecithin monolayers on the subsolutions of NaCl, $CaCl_2$ or $AlCl_3$ are shown for a comparison.

The method of measuring surface pressures by a modified Wilhelmy plate, and surface potentials (ΔV) by a radioactive electrode has been described previously[1]. The surface measurements were taken on subsolutions of 0.02 M NaCl, 0.01 M $CaCl_2$, $MgCl_2$, $SrCl_2$, or $BaCl_2$, and 0.0066 M $AlCl_3$ at pH 5.6 and 25°.

The interaction of metal ions with the phosphate groups in monolayers results in an increase of surface potential; the magnitude of this increase indicates the extent of interaction between metal ions and the phosphate groups[1]. Fig. 1 shows the surface potential–area curves of sphingomyelin on subsolutions containing various metal ions. For a comparison, the surface potential–area curves of dipalmitoyl lecithin monolayers on subsolutions of NaCl, $CaCl_2$ and $AlCl_3$ are also shown in Fig. 1. It is evident from the increase in surface potentials that the interaction of metal ions is considerably greater with dipalmitoyl lecithin monolayers than with sphingomyelin monolayers; this is strikingly seen in the case of Al^{3+}. In relation to the interaction of metal ions with the phosphate groups in monolayers (Fig. 1), a comparison of the molecular structure of lecithin with that of sphingomyelin suggests that the presence of a hydroxyl group vicinal to the phosphate group strikingly reduces the interaction of metal ions with sphingomyelin monolayers.

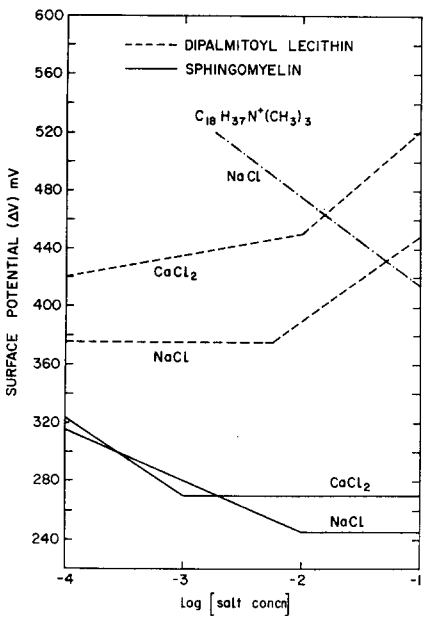
The surface properties of sphingomyelin are compared with those of dipalmitoyl lecithin because they have approximately the same limiting area (42–44 $Å^2$ per molecule)[7] and hence the same intermolecular spacing in monolayers (*i.e.*, the average distance between adjacent phosphate groups). Since the interaction of metal ions with the phosphate groups is coulombic, it is necessary for meaningful comparison of the influence of metal ions that the intermolecular spacing be the same in the two cases; this condition is fulfilled by the monolayers of dipalmitoyl lecithin and sphingomyelin. In contrast to surface potential–area curves (Fig. 1), the surface pressure–area curves of dipalmitoyl lecithin and sphingomyelin are unaltered by the presence of metal ions in the subsolution.

The surface pressures and potentials of lecithin and sphingomyelin monolayers were measured on subsolutions containing NaCl (10^{-1}–10^{-4} M) and $CaCl_2$ (10^{-1}–10^{-4} M) at pH 5.6 and 25° in order to plot ΔV–log c curves (where c is the concentration of electrolyte in the subsolution). The ΔV–log c plots of sphingomyelin and dipalmitoyl lecithin monolayers have opposite slopes (Fig. 2); this indicates a striking difference between the ionic properties of sphingomyelin and lecithin monolayers.

A charged monolayer causes a diffuse layer of counterions in the subsolution, whose thickness depends upon the electrolyte concentration of the subsolution[8]. The slope of the ΔV–log c plot of sphingomyelin resembles that of alkyltrimethylammonium ($C_{18}H_{37}N^+(CH_3)_3$) monolayers[9] which indicates that the sphingomyelin monolayer possesses a net positive surface charge. Thus, the interaction of metal ions (Fig. 1) and the positive surface charge of sphingomyelin (Fig. 2) can satisfactorily be explained in terms of an ion–dipole association between the hydroxyl group and ionic oxygen of the phosphate group of sphingomyelin (Fig. 3). A study of the three-dimensional molecular model (Fisher–Hirschfelder–Taylor model) of sphingomyelin showed that such an ion–dipole association is sterically possible. This association in the sphingomyelin molecule reduces the unit negative charge of the oxygen of the phosphate group to a partial ionic charge (δ_-), which consequently decreases the interaction of the phosphate group with metal ions and leaves the net positive surface charge of the cationic trimethylammonium group (Fig. 3). It should be pointed out that micro-

electrophoresis of aqueous dispersions of sphingomyelin also showed a positive surface charge on the sphingomyelin particles[10].

On the other hand, it is known from microelectrophoresis of lecithin dispersions[11] as well as the ΔV–pH plot of lecithin monolayers[2] that the lecithin molecule possesses a net zero surface charge. This suggests that the phosphate and the trimethylammonium groups act as counterions for each other. Thus, a lecithin monolayer, owing to its net zero charge, does not cause a diffuse layer of ions in the subsolution. Upon increasing the electrolyte concentration, the cations of the subsolution compete with the trimethylammonium group for the anionic phosphate of lecithin. Therefore, the increase in surface potential of the lecithin monolayer upon increasing the electro-



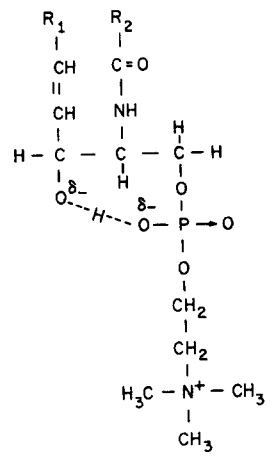


Fig. 2. The surface potential–log (salt conc.) plot of sphingomyelin and dipalmitoyl lecithin on subsolutions containing NaCl, or $CaCl_2$ in various concentrations at pH 5.6 and 25°. The plot for $C_{18}H_{37}N^+(CH_3)_3$ is taken from ref. 8.

Fig. 3. The ion–dipole association between the hydroxyl group and the ionic oxygen of the phosphate group of sphingomyelin. δ_- represents the partial ionic charge on the oxygen atoms.

lyte concentration is due to the binding of metal ions with the phosphate groups (*i.e.*, due to a change in the Śtern layer) and not due to a change in the thickness of the diffuse layer (Gouy layer), as in the case of sphingomyelin monolayers.

The abrupt change in the slope of ΔV–log c plot of lecithin monolayers can be explained as follows. At low concentrations (-<0.005 M NaCl) the binding of Na^+ to the phosphate group would be influence by the repulsion due to the cationic trimethylammonium groups. At concentrations higher than 0.005 M NaCl, there would be a sufficient number of Cl^- to screen the cationic trimethylammonium groups, diminishing their inhibitory effect and consequently permitting substantial binding of Na^+ to the phosphate groups. Similar effect is also observe for subsolutions contain-

ing $CaCl_2$. Recently, similar studies on phosphatidylserine and various lecithin monolayers have been reported[12,13].

For sphingomyelin monolayers the plateau in the ΔV–log c plot at higher concentrations occurs because of elimination of the diffuse layer by high electrolyte concentration, since it is known that the thickness of the diffuse layer decreases as electrolyte concentration increases[8]. The ionic structure of sphingomyelin reported in this note is of considerable interest in relation to lipid–protein interaction in myelin, as well as the cell membrane in general.

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