Bilayer Membranes Containing the Ganglioside G_{M1}: Models for Electrostatic Potentials Adjacent to Biological Membranes†

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ABSTRACT: Although the Gouy-Chapman-Stern theory of the aqueous diffuse double layer describes well the electrostatic potential adjacent to negatively charged phospholipid bilayer membranes, it does not describe adequately the ζ potential of biological membranes: the ζ potential of an erythrocyte is about half the value predicted from the theory by using the known density of negatively charged sialic acid residues. To investigate the factors responsible for this low electrophoretic mobility, we formed membranes from mixtures of the zwitterionic lipid phosphatidylcholine, PC, and the glycolipid galactosyl-*N*-acetylgalactosaminyl(*N*-acetylneuraminyl)-galactosylglucosylceramide, G_{M1}. This glycolipid differs from phospholipids in two respects. First, the negative charge on G_{M1} is located about 1 nm from the surface, which tends to increase the electrophoretic mobility of vesicles. Second, the head group of G_{M1} contains five sugar groups that exert a hydrodynamic drag, which tends to decrease the mobility of the vesicles. In a decimolar monovalent salt solution, where the Debye length is about 1 nm, the electrophoretic mobility of the PC-G_{M1} vesicles is about half the mobility of PC-phosphatidylserine or PC-phosphatidylglycerol vesicles of equivalent composition. In addition, conductance measurements with planar bilayer membranes as well as ^{31}P nuclear magnetic resonance and fluorescence measurements with sonicated vesicles indicate that the potential at the surface of PC-G_{M1} membranes is about half the value measured for PC-phosphatidylserine membranes in a 0.1 M monovalent salt solution.

The Gouy-Chapman-Stern theory describes adequately the electrostatic potential adjacent to a phospholipid bilayer (McLaughlin, 1977, 1983; Ohki & Kurland, 1981; Lakhdar-ghazal et al., 1983): as the theory requires, the charges on a negative phospholipid, such as phosphatidylglycerol (PG),[1] are located in a plane at the bilayer surface. The Helmholtz-Smoluchowski equation relates the electrophoretic mobility of a phospholipid vesicle to the ζ potential, the potential at the hydrodynamic plane of shear, which is about 0.2 nm from the membrane surface in a 0.1 M salt solution (Eisenberg et al., 1979; Alvarez et al., 1983; Rooney et al., 1983). However, a combination of the Helmholtz-Smoluchowski and the Gouy-Chapman equations cannot describe the mobility of an erythrocyte with the known surface charge density of the cell. Three similar theories (Donath & Pastushenko, 1979; Wunderlich, 1982; Levine et al., 1983) describe the mobility of an erythrocyte in terms of structural parameters of the cell surface. These theories differ from the classical treatment in two respects: they assume that independent spherical elements (e.g., sugars) in the glycocalyx generate additional frictional forces and that the charged elements (e.g., sialic acid) are either spread uniformly over a volume or concentrated in a plane some distance from the surface. The additional frictional drag decreases the electrophoretic mobility; the location of charged groups away from the bilayer increases the mobility.[2] We examined these two effects experimentally in a model system.

We formed membranes from mixtures of the ganglioside G_{M1}, which has four neutral sugars and one charged sialic acid residue in its head group, and the zwitterionic phospholipid phosphatidylcholine (PC). When the head group of G_{M1} is extended maximally, the charge is about 1 nm from the surface and the head group protrudes about 2 nm (Figure 1). These distances are comparable to the Debye length in a 0.1 M NaCl solution, which is about 1 nm. Thus PC-G_{M1} membranes should be useful for testing the new theories.

There were two additional reasons for choosing G_{M1} for these studies. First, it does not phase separate when mixed with egg PC at concentrations less than 30 mol % (Hill & Lester, 1972; Bach et al., 1982). Second, glycolipids such as G_{M1} are ubiquitous components of biological membranes; they are biologically important blood group and tumor antigens, as well as hormone and toxin receptors (Fishman & Brady, 1976; Hakomori, 1981; Fishman, 1982; Tillack et al., 1982; Felgner et al., 1982; van Heyningen & Seal, 1983).

Materials and Methods

Ganglioside G_{M1}, obtained from Supelco (Bellefonte, PA), ran as a single spot on thin-layer chromatograms with silica gel G plates (Supelco) and a solvent system of $CHCl_3$-MeOH-H_2O (60:40:7.7) with 1.2% 14.8 N NH_4OH. The chromatograms were sprayed with ganglioside spray (Supelco) or 5% $K_2Cr_2O_7$ in 40% H_2SO_4 or exposed to iodine vapors. Egg phosphatidylcholine or diphytanoylphosphatidylcholine (PC), bovine brain phosphatidylserine (PS), and egg phosphatidylglycerol (PG) were obtained from Avanti Biochemicals (Birmingham, AL). Aqueous solutions, prepared with 18 MΩ

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[1] Abbreviations: FCCP, carbonyl cyanide *p*-(trifluoromethoxy)-phenylhydrazone; G_{M1}, galactosyl-*N*-acetylgalactosaminyl(*N*-acetylneuraminyl)galactosylglucosylceramide; MES, 2-(*N*-morpholino)-ethanesulfonate; MOPS, 3-(*N*-morpholino)propanesulfonate; PC, phosphatidylcholine; PG, phosphatidylglycerol; PS, phosphatidylserine; TNS, 6-*p*-toluidinylnaphthalene-2-sulfonate; EDTA, ethylenediaminetetraacetic acid.

[2] Levine et al. (1983) discuss why the electrophoretic mobility of a vesicle should increase when the fixed charge moves away from the surface. Assume the surface of a vesicle is fixed, and consider the fluid velocity far from the surface produced by an electric field E. This velocity is equal to the velocity of a free vesicle in the electric field. The force F exerted by E on a small volume of fluid dV located a distance x from the membrane is $F = E\rho(x)dV$, where $\rho(x)$ is the space charge density in the volume dV. When the fixed charge is located at the surface, $\rho(x)$ and F are largest at the surface. However, the hydrodynamic boundary condition requires that the fluid velocity be zero at the surface. When the fixed charge is located a distance d from the surface, the fluid velocity far from the surface increases because F is largest at $x = d$.

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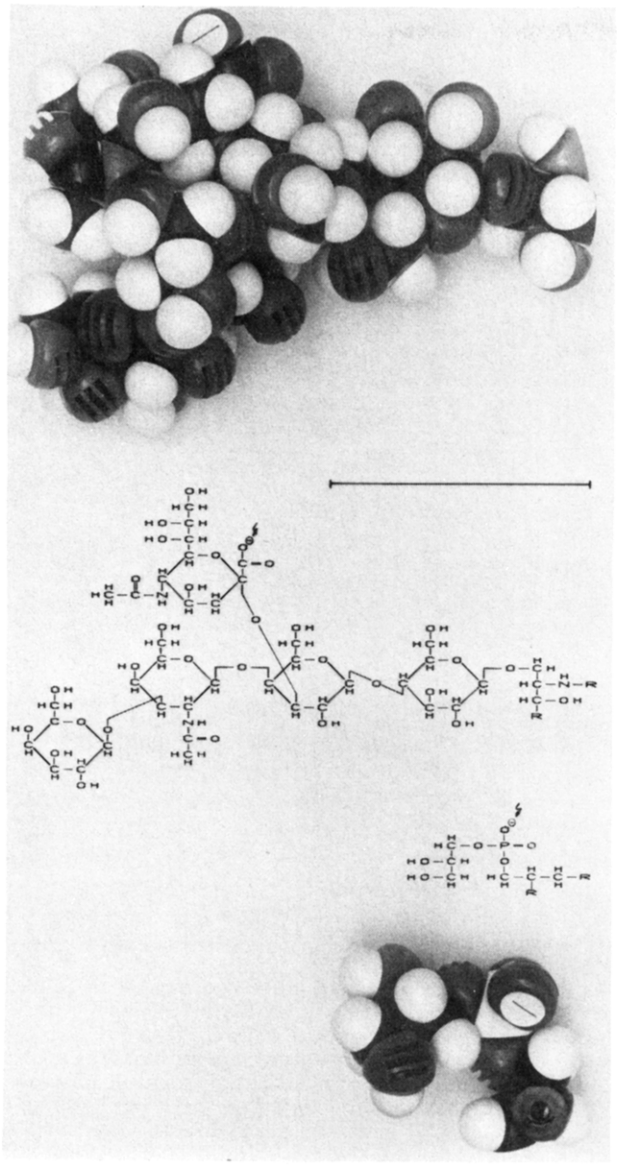

FIGURE 1: Corey–Pauling–Koltun models and primary structures of the G_{M1} (upper) and PG (lower) polar head groups. The circled minus signs denote the negative charges on the sialic acid and phosphate moieties. R represents the hydrocarbon tails of the lipids. The scale bar represents 1.0 nm.

cm water (Super-Q, Millipore Corp., Bedford, MA), were buffered to pH 7.5 with 0.1–10 mM MOPS (P-L Biochemicals, Milwaukee, WI).

We used the following lipid molecular weights in our calculations: G_{M1}, 1545; PS, 786; PG, 775; egg PC, 787; diphytanoyl-PC, 846. Sialic acid analysis of the G_{M1} lot used for these experiments, performed by the supplier, yielded 0.95 mol of sialic acid/mol of G_{M1}. Thin-layer chromatography, performed by us, showed no free sialic acid spots and a single resorcinol-reactive spot. Thus, loss of sialic acid is probably not responsible for the fact that bilayers containing G_{M1} have less negative electrostatic potentials and lower electrophoretic mobilities than bilayers containing PS.

Electrophoretic Mobility Measurements. Multilamellar vesicles for the microelectrophoresis experiments were prepared according to Bangham et al. (1974). Egg PC and defined mixtures of ganglioside G_{M1} and egg PC in chloroform and chloroform–methanol mixtures were vacuum dried in glass round-bottom flasks. The electrophoretic mobilities of PC vesicles in 0.1, 0.01, and 0.001 M NaCl were 0. The mobility of the PC–G_{M1} vesicles was independent of the proportion of methanol in chloroform. For example, in 0.001 M NaCl, the electrophoretic mobilities of vesicles (1 mol % G_{M1} in PC) formed by drying from chloroform, methanol, or 5, 16, 33, and 50 volume % methanol in chloroform were identical within experimental error. The mobilities of the vesicles were measured in a Rank Brothers Mark I instrument (Bottisham, Cambridge, U.K.) as described in detail previously (McLaughlin et al., 1981). We calculated the ζ potential, ζ, from the electrophoretic mobility, u, using the Helmholtz–Smoluchowski equation (Aveyard & Haydon, 1973):

$$\zeta = u\eta/(\epsilon_0\epsilon_r) \quad (1)$$

where ϵ_r is the dielectric constant of the aqueous solution, ϵ_0 is the permittivity of free space, and η is the viscosity of the aqueous solution.

We describe the results for membranes containing PS with the Gouy–Chapman–Stern theory, assuming that the distance from the plane of shear to the charge plane is 0.2, 0.4, and 1.0 nm in 0.1, 0.01, and 0.001 M NaCl, respectively (McLaughlin et al., 1983). We also assume that the intrinsic association constant of Na with PS is 1.0 M^{-1} (Nir et al., 1978; Kurland et al., 1979; Eisenberg et al., 1979; Alvarez et al., 1983; McLaughlin et al., 1983) and that the intrinsic association constant of K with PS is 0.5 M^{-1}.

NMR Measurements. When vesicles were made from mixtures of lipids, known weights of each lipid were mixed in chloroform–methanol (2:1), the phospholipid solutions dried under a stream of nitrogen, and final traces of solvent removed under high vacuum overnight. Samples were rehydrated with 0.1 M NaCl–5 mM MOPS, pH 7.4, and sonicated as described previously (McLaughlin et al., 1981). Membranes formed from PC and from mixtures of PC and G_{M1} were sonicated in an ice bath, while membranes formed from mixtures of PC and PS were sonicated in a room-temperature water bath. After sonication, the pH of the sample was adjusted to 7.4, and an aliquot of a concentrated manganese solution was added. The free manganese concentration in the sample was established by passing the sample over a Sephadex column equilibrated with a solution containing 0.1 M NaCl, 5 mM MOPS, pH 7.4, and the desired manganese concentration.

Manganese has a large effect on the line width of the ^{31}P NMR signal from phosphodiester groups (Shulman et al., 1965; Sternlicht et al., 1965). In our experiments, manganese is added after sonication and is present only in the bulk aqueous medium. The broad ^{31}P NMR signal from phosphodiester groups in the outer monolayer of the vesicles was distinguished from the narrow ^{31}P NMR signal from phosphodiester groups on the inner monolayer by using the $\pi-\tau-\pi/2$ radio-frequency pulse sequence with a value of τ that nulled the ^{31}P NMR signal from phosphodiester groups on the inner monolayer (Lau et al., 1981). $1/T_{2P}$ was calculated from the formula

$$1/T_{2P} = \pi\Delta\nu_P \quad (2)$$

where $\Delta\nu_P$ is the effect of manganese on the line width of the ^{31}P NMR signal from phosphodiester groups on the outer monolayer.

We assume that the PC to PS ratio in the outer monolayer is the same as the overall PC to PS ratio. This assumption is based on previous ^{31}P NMR determinations of the inside to outside ratio of PC and PS in sonicated vesicles formed from mixtures of these two lipids (Berden et al., 1975). We also assume that the PC to G_{M1} ratio in the outer monolayer of sonicated vesicles is equal to the overall ratio of PC to G_{M1}. This assumption is consistent with EPR studies (Sharom &

Grant, 1978), neuraminidase digestion studies (Felgner et al., 1982), and experiments with a glycolipid exchange protein (T. E. Thompson, personal communication), although no direct measurement of the distribution has been made.

The ^{31}P NMR signal from phosphodiester groups in the outer monolayer of PC vesicles and PC–G_{M1} vesicles arises only from PC molecules, whereas in PC–PS vesicles the signal arises from both the PC and PS molecules. However, in the latter case the line width of the observed ^{31}P NMR signal is a good approximation to the line width of the ^{31}P NMR signal from PC molecules in the outer monolayer because more than 90% of the molecules are PC.

Fluorescence Measurements. Sonicated unilamellar vesicles were formed as described by Eisenberg et al. (1979). Briefly, lipids were dried from their stock solutions under vacuum overnight at room temperature, resuspended in 0.1 M NaCl, 0.1 mM EDTA, and 5 mM MES, pH 7.4, and sonicated under nitrogen for about 30 min at 15 °C. Ultracentrifugation at 100000*g* removed the multilamellar lipid vesicles (Barenholz et al., 1977), and phosphate analyses were done to determine the final phospholipid concentrations (Rouser et al., 1970). The anionic fluorescent probe TNS (Sigma, St. Louis, MO) was added to the vesicle preparations from a stock solution. Fluorescence intensities were measured (Spex Fluorocomp, Metuchen, NJ) at 321-nm excitation and 446-nm emission wavelengths. Dark counts were four photon counts/s, and typical signal levels for 1.0 μM TNS and 0.1 mg/mL lipid were about 1000 photon counts/s. TNS can be used as a probe of the electrostatic potential at the hydrocarbon–water interface (Eisenberg et al., 1979). This surface potential was calculated from the relation

$$f(-)/f(0) = \exp[F\psi_0/(RT)] \quad (3)$$

where F is the Faraday constant, R is the gas constant, T is the absolute temperature, and $f(-)$ and $f(0)$ are the net fluorescence intensities for the negatively charged lipid mixtures and the PC vesicles, respectively. The net fluorescence intensity, calculated by subtracting the appropriate backgrounds from the measured intensity, is proportional to the number of TNS molecules adsorbed to the membrane. The values of $f(-)/f(0)$ were independent of vesicle concentrations from 0.01 to 0.1 mg/mL and TNS concentrations from 0.1 to 2.0 μM. Thus, the adsorbed TNS anions did not affect the surface potential significantly, and the adsorption of TNS to vesicles did not decrease the bulk aqueous TNS concentration significantly. We assume that the quantum yield of TNS adsorbed to PC, PC–G_{M1}, and PC–PS vesicles is the same for two reasons. First, fluorescence lifetime measurements show only a 2% higher quantum yield for TNS adsorbed to PC than to PS vesicles (Eisenberg et al., 1979). Second, corrected emission spectra of TNS adsorbed to PC, PC–PS, and PC–G_{M1} vesicles are nearly identical (data not shown).

Conductance Measurements. Planar lipid bilayer membranes were formed from a 1–2% solution of lipid in *n*-decane (McLaughlin et al., 1971). The Teflon chambers have two compartments (20 mL each) separated by a thin wall with a 1.6-mm diameter aperture. Steady-state conductance was measured at voltages less than 25 mV, where conductance is independent of voltage. Nonactin was a gift of B. Stearnes (Squibb, New Brunswick, NJ). Nonactin or FCCP (Pierce Chemical Co., Rockford, IL) was added symmetrically to each compartment as a 1 mM ethanolic solution, with constant stirring, to a final concentration of 0.5 μM.

Results

Electrophoretic Mobility Measurements. Figure 2 illustrates the dependence of the electrophoretic mobility of multilamellar vesicles on lipid composition. The triangles and filled circles refer to vesicles formed from PC–PG and PC–PS mixtures, respectively. The open circles refer to PC–G_{M1} mixtures. The curves illustrate the predictions of Gouy–Chapman–Stern theory. Figure 2 has two salient features. First, the theory provides a reasonable description of the ζ potentials of the vesicles that contain PS or PG, as reported previously (McLaughlin et al., 1981, 1983). Second, the electrophoretic mobilities of vesicles containing G_{M1} are generally less negative than the mobilities of vesicles containing the same mole fraction of PS. For example, consider PC–G_{M1} vesicles containing 17 mol % G_{M1}; these vesicles have charge densities and ζ potentials comparable with human erythrocytes (Levine et al., 1983). In 0.1 M NaCl, their mobilities are about 2-fold lower than those of PC–PS or PC–PG vesicles (Figure 2A). It is unlikely that loss of sialic acid from G_{M1} is responsible for this difference.[3]

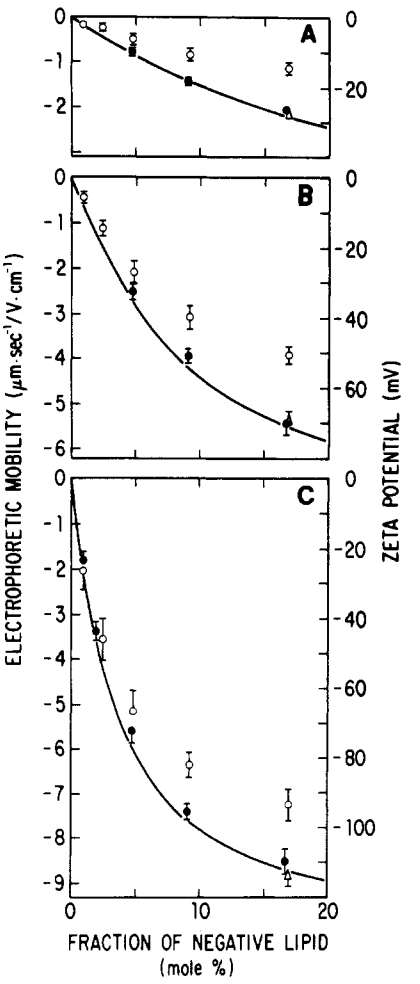

FIGURE 2: Electrophoretic mobility and ζ potential of multilamellar vesicles formed from mixtures of the zwitterionic lipid phosphatidylcholine and the negative lipid G_{M1} (open circles), PS (filled circles), or PG (triangles). The curves illustrate the predictions of the Gouy–Chapman–Stern theory if the intrinsic association constant of Na with the negative lipid is 1 M^{-1}. The error bars illustrate the standard deviations of measurements on at least 20 vesicles. The aqueous solutions contained (A) 0.1 M NaCl, (B) 0.01 M NaCl, and (C) 0.001 M NaCl buffered to pH 7.5 at 25 °C with 10^{-2}, 10^{-3}, and 10^{-4} M MOPS, respectively.

[3] Sialic acid analysis (see Materials and Methods) of G_{M1} shows that one negative charge is on each G_{M1} molecule. Furthermore, if PS and G_{M1} each have one net negative charge, the ζ potentials of PC–G_{M1} vesicles should asymptotically approach those of PC–PS vesicles when the salt concentration and charge density decrease. They do (Figure 2C).

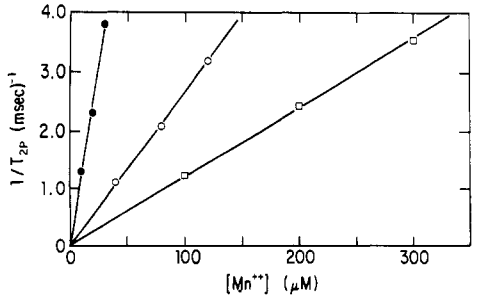

FIGURE 3: Effect of manganese on the line width of the ^{31}P NMR signal, $1/T_{2P}$, of PC molecules in the outer monolayer of sonicated vesicles containing 9.1 mol % PS in PC (top), 8.9 mol % G_{M1} in PC (middle), and PC (bottom). The aqueous solutions contained 0.1 M NaCl buffered to pH 7.4 with 5 mM MOPS at 25 °C.

Sodium binding to G_{M1} is probably not responsible for the difference between phospholipid vesicle mobility and PC–G_{M1} vesicle mobility for two reasons. First, vesicles formed from PG or PS have the same ζ potentials (Eisenberg et al., 1979; McLaughlin et al., 1981; Lau et al., 1981). Vesicles formed from either 5:1 PC–PG or 5:1 PC–PS mixtures also have similar ζ potentials (see Figure 2). Both PS and G_{M1} contain a carboxylate group. Both PG and PS contain a phosphate group that binds Na with an association constant of about 1 M^{-1}. Sodium binding by the carboxylate group on PS cannot be much stronger than this, or the ζ potentials of PS vesicles would be less negative than those of PG vesicles. Thus, it is unlikely that the carboxylate group on G_{M1} binds sodium ions with an association constant greater than 1 M^{-1}. Second, the mobility of PS vesicles is higher in Cs than in Na solutions (Eisenberg et al., 1979; McLaughlin et al., 1983), presumably because the phosphate and carboxylate groups of PS bind Cs less strongly than they bind Na. If the low mobility of vesicles containing G_{M1} were due to strong binding of sodium to the carboxylate group, we would expect the mobility to increase when sodium was replaced with cesium. We repeated the PC–G_{M1} measurements illustrated in Figure 2 with CsCl solutions: the results agreed with the NaCl results within experimental error. Thus, it is unlikely that cesium and sodium bind more strongly to G_{M1} than to PS or PG.

NMR Measurements. Figure 3 shows the effect of manganese on the line width of the ^{31}P NMR signal from PC, PC–G_{M1}, and PC–PS sonicated vesicles. The paramagnetic effect of manganese on the line width of the ^{31}P NMR signal from PC molecules on the outer monolayer of the sonicated vesicles is proportional to the number of phosphodiester groups bound to manganese in inner sphere complexes, which is proportional to the free concentration of manganese in the aqueous phase adjacent to the phosphodiester group.[4] This free concentration is proportional to the Boltzmann factor $\exp[-2F\psi_p/(RT)]$, where ψ_p is the potential at the phosphodiester group of PC. If we assume that $\psi_p = 0$ in the PC vesicles, the data in Figure 3 can be used to calculate ψ_p in the PC–G_{M1} vesicles and the PC–PS vesicles by using the equation

$$[1/T_{2P}(\mathrm{Mn})]/[1/T^0_{2P}(\mathrm{Mn})] = \exp[-2F\psi_p/(RT)] \quad (4)$$

where $1/T_{2P}(\mathrm{Mn})$ is the effect of manganese on the line width of the ^{31}P NMR signal from PC molecules in the PC–G_{M1} or the PC–PS vesicles and $1/T^0_{2P}(\mathrm{Mn})$ is the effect of the same concentration of manganese on the line width of the ^{31}P NMR signal from PC molecules in PC vesicles. ψ_p was calculated to be −10 mV for the PC–G_{M1} vesicles and −30 mV for the PC–PS vesicles.

[4] The paramagnetic effect of manganese on the line width of the ^{31}P NMR signal from PC molecules is also inversely proportional to τ_m, the lifetime of the inner sphere complex between manganese and the phosphodiester group of PC. We assume that τ_m is the same for PC membranes and for membranes formed from mixtures of PC and either PS or G_{M1}.

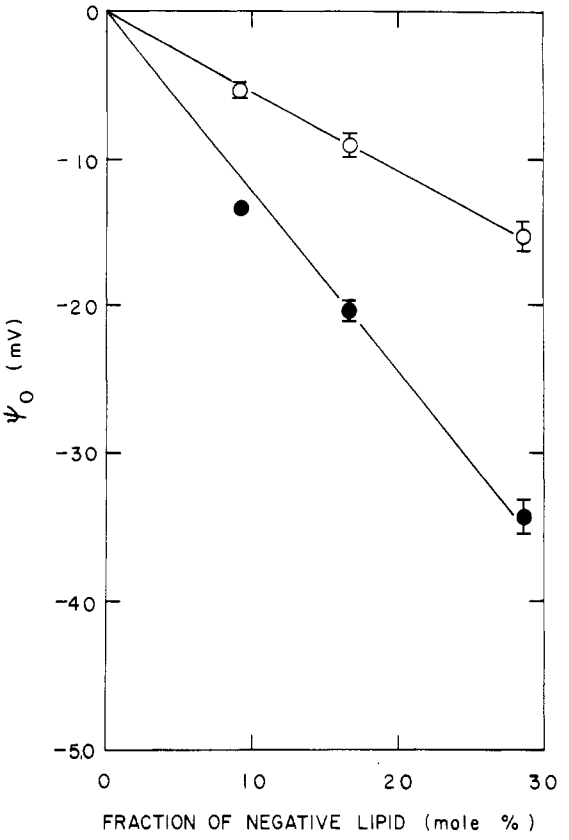

FIGURE 4: Dependence of surface potential on lipid composition in sonicated vesicles formed from either PC–G_{M1} (open circles) or PC–PS (filled circles) mixtures, as determined from fluorescence measurements with the anionic probe TNS at 22 °C. The lines are least-squares fits to each set of points. The aqueous solutions contained 0.1 M NaCl, 0.1 mM EDTA, and 5 mM MES, pH 7.4.

Fluorescence Measurements. Figure 4 shows the electrostatic surface potential of sonicated vesicles formed from mixtures of either G_{M1} (open circles) or PS (filled circles) with PC, calculated from fluorescence data (not shown) by using eq 3. In agreement with the NMR results, the potentials of vesicles formed from PC–G_{M1} mixtures are less negative than the potentials of the vesicles formed from PC–PS mixtures.

Conductance Measurements. We made conductance measurements on decane-containing PC and PC–G_{M1} bilayers to estimate the effect of G_{M1} on the potential within the membrane. We measured the steady-state conductance due to either the positively charged nonactin–K complex or the negatively charged FCCP anion. The change in electrostatic potential in the center of the membrane upon adding G_{M1} to PC was calculated from the Boltzmann relation (McLaughlin et al., 1970, 1971; Szabo et al., 1972; Szabo, 1974). As the G_{M1} content increased, the conductance induced by nonactin increased and the conductance induced by FCCP decreased by approximately the same extent. The observation that the conductance changes are similar in magnitude but opposite in direction implies that these changes are due to a change in electrostatic potential and not, for example, to an effect of G_{M1} on the viscosity or dielectric constant. The change in electrostatic potential within the membrane upon addition of G_{M1} is calculated from

$$\Delta\psi = \pm(RT/F) \ln (G/G_0) \quad (5)$$

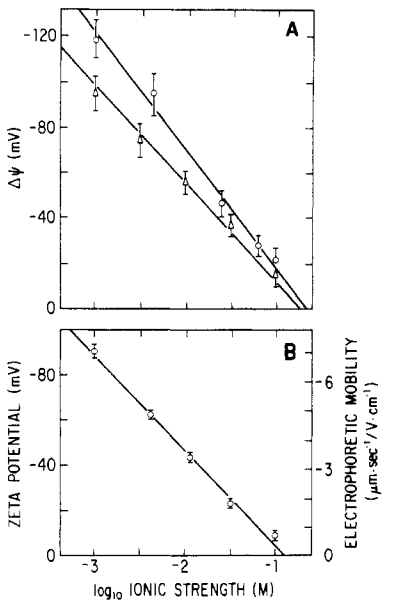

FIGURE 5: Dependence of electrostatic potential on ionic strength. The ionic strength was increased by adding LiCl to a solution containing 0.001 M KCl buffered to pH 7.5 with 0.1 mM MOPS at 22 °C. The planar bilayers and multilamellar vesicles were formed from 17 mol % G_{M1} in diphytanoyl-PC (A) or in egg PC (B). (A) Dependence of the electrostatic potential within planar bilayers on ionic strength. The triangles illustrate the results obtained with the positively charged nonactin–K probe. The circles illustrate the results obtained with the negatively charged FCCP probe. The two lines are least-squares fits to the nonactin and FCCP data: the average slope is 47 mV per decade. (B) Dependence of the ζ potential, calculated from eq 1, on ionic strength. The line, which is a least-squares fit to the points, has a slope of 42 mV/decade.

where G is the conductance of a planar bilayer containing G_{M1} and G_0 is the conductance of a bilayer containing only PC.[5] The + sign denotes the conductance induced by nonactin, and the − sign denotes the conductance induced by FCCP. To show that decane did not affect the PC–G_{M1} ratio at the membrane–solution interface, we measured the electrophoretic mobility of droplets formed by vortexing the decane solution of lipids used to form the planar membranes with an aqueous solution of 0.1 M KCl. The mobilities of the droplets were identical, within experimental error, to the mobilities of solvent-free multilamellar vesicles formed from the same lipid mixture.

The electrostatic potential within PC–G_{M1} planar bilayers becomes more negative when the G_{M1} content increases. On average, the potential changes by −13 mV per 9 mol % G_{M1}, in good agreement with the NMR results. (When the mole fractions of G_{M1} are 5, 17, and 30 mol %, the potential changes by −12, −22, and −40 mV. The aqueous solutions contained 0.1 M KCl, buffered to pH 7.5 with 5 mM MOPS at 22 °C.)

Figure 5 illustrates the effect of ionic strength on the electrostatic potential within planar bilayers and on the mobility of multilamellar vesicles containing 17 mol % G_{M1} in PC. We measured nonactin conductance, FCCP conductance, and electrophoretic mobility in solutions containing 0.001 M KCl and concentrations of LiCl between 0 and 0.1 M. Both the surface potential and the ζ potential become less negative as the concentration of lithium increases. For each 10-fold change in monovalent salt concentration, our conductance measurements indicate that the electrostatic potential within planar bilayers changes by 47 mV (Figure 5A). The surface potential above a monolayer (4.8 mol % monosialoganglioside in PC) changes 52 mV for a 10-fold change in salt concentration, a value that agrees well with the predictions of Gouy–Chapman theory (Hill & Lester, 1972). However, the ζ potential changes by only 42 mV (Figure 5B). Analogous discrepancies between surface and ζ potential results have been observed with phospholipids. For monolayers formed from PG (Lakhador-ghazal et al., 1983), the change in surface potential is 53 mV per decade salt concentration. For multilamellar vesicles formed from either 17 mol % PS or PG in PC, the change in ζ potential is only 42 mV per decade change in monovalent cation concentration [Figure 2 and McLaughlin et al. (1983)].

Discussion

We observed that in 0.1 M NaCl the electrophoretic mobilities of PC–G_{M1} vesicles are lower than the mobilities of either PC–PS or PC–PG vesicles and lower than the mobilities predicted by the Gouy–Chapman–Stern theory (Figure 2A). A human erythrocyte also has a lower mobility than a phospholipid vesicle with an equivalent surface charge density [e.g., Levine et al. (1983)]. Donath & Pastushenko (1979), Wunderlich (1982), and Levine et al. (1983) calculated the hydrodynamic drag exerted by macromolecules that extend from the surface of the red blood cell and concluded that these interactions could account for the low value of the mobility. We attempted to describe the mobility of PC–G_{M1} vesicles using their procedure. We represented the five sugars in a G_{M1} molecule as independent spheres with Stokes radii of 0.35 nm. This value agrees with the size of the molecular model (Figure 1) and with the radius calculated from the Stokes–Einstein equation by using the measured values for the diffusion coefficients of sucrose and glucose (Weast, 1974). We assumed that the spheres were distributed uniformly within a layer of thickness 2 nm, a value consistent with the extended form of the molecular model shown in Figure 1. In other words, we consider the maximal drag that the sugars could exert. Finally, we assumed that the charges were either (a) distributed uniformly within this layer or (b) located in a plane at the hydrocarbon–water interface. We calculated from eq 21 and 23 of Levine et al. (1983) that the electrophoretic mobility, compared to the mobility predicted by the combination of Helmholtz–Smoluchowski and Gouy–Chapman theories, should be reduced by a factor of either (a) 2.0 or (b) 3.5. The electrophoretic mobility of a 17 mol % PC–G_{M1} vesicle in 0.1 M NaCl is a factor of about 2 lower than the electrophoretic mobility of a 17 mol % PC–PS vesicle (Figure 2A). Thus, the new theories can account for the data obtained with PC–G_{M1} vesicles in 0.1 M NaCl. A more complete comparison of the results with the predictions of the existing theories will involve assumptions about the conformation of the head group, the association constant of cations with the carboxyl moiety, and the location of the hydrodynamic plane of shear: this analysis will appear elsewhere (R. V. McDaniel, K. A. Sharp, D. E. Brooks, A. McLaughlin, and S. McLaughlin, unpublished results).

While the existing analyses can account, at least qualitatively, for the data we have obtained with PC–G_{M1} vesicles in 0.1 M NaCl, they do not account correctly for the dependence of the ζ potential on salt concentration. The ζ potentials (calculated from the mobility using eq 1) of erythrocytes and vesicles containing PS, PG, or G_{M1} become more negative as the salt concentration decreases (Figures 2 and 5B). However, the ζ potential does not change as rapidly as predicted by the existing theories. For example, the Gouy–Chapman–Stern

[5] We note that the total electrostatic potential within the membrane includes a contribution from the dipole potentials of both PC and G_{M1}. Maggio et al. (1981) have shown that the dipole potential of a G_{M1} monolayer is less positive than the dipole potential of a PC monolayer.

theory predicts that the ζ potential should change 58 mV per decade salt concentration when the ζ potential is large. The maximum change in ζ potential we observe with vesicles containing PS, PG, or G_{M1} is 42 mV per decade salt concentration (Figures 2 and 5B). The reason for this discrepancy is not clear. McLaughlin et al. (1983) summarize the evidence that the *surface* potential of membranes formed from negative phospholipids does change by about the value predicted by diffuse double layer theory (58 mV per decade salt concentration) when the salt concentration changes. They suggest that the 42 mV per decade change in the ζ potential of phospholipid vesicles could be due to the plane of shear shifting away from the surface as the salt concentration decreases and the Debye length increases. Indeed, the theoretical predictions of the Gouy–Chapman–Stern theory in Figure 2 were calculated by assuming that the plane of shear is 0.2, 0.4, and 1.0 nm from the surface in 0.1, 0.01, and 0.001 M salt solutions, respectively. The theoretical curves account satisfactorily for the PC–PS data obtained at all charge densities.

We made NMR and fluorescence measurements on sonicated vesicles to estimate the surface potential and made conductance measurements on planar membranes to estimate the potential within the membrane. When the vesicles were formed in 0.1 M NaCl from a 9 mol % mixture of G_{M1} in PC, the NMR and fluorescence results indicate the potentials on the outer surface are −10 mV (cationic manganese probe) and −5 mV (anionic TNS probe). The artifacts that can occur with the two techniques are, in general, quite different. However, an unequal distribution of negative lipids between the inner and outer monolayers of the sonicated vesicles would affect the measurements in a similar manner. For this reason, we also studied planar bilayer membranes. The potential within a planar bilayer formed from a 9 mol % mixture of G_{M1} and PC, as deduced from both the anionic FCCP and cationic nonactin–K conductance measurements, is 13 mV more negative than the potential within a PC membrane. If the dipole potentials of PC and a 9 mol % mixture of G_{M1} and PC are identical, this is also the surface potential of the PC–G_{M1} membrane.[5] If we average the results of the three different techniques, we conclude that the surface potential of a membrane consisting of a 9 mol % mixture of G_{M1} and PC is about −9 mV in 0.1 M NaCl.

We now compare the surface potentials of bilayers that contain G_{M1} with those that contain PS. We estimated the surface potential of a phospholipid bilayer formed in 0.1 M salt from a 9 mol % mixture of PS in PC in four ways. First, we measured the ζ potential of multilamellar vesicles (ζ = −19 mV, Figure 2), assumed that the plane of shear is 0.2 nm from the bilayer surface (Eisenberg et al., 1979; Alvarez et al., 1983; Rooney et al., 1983), and used the Gouy–Chapman theory to calculate a surface potential of −23 mV. Second, we measured the ^{31}P NMR spectrum of sonicated vesicles and deduced a surface potential of −30 mV (Figure 3). Third, we measured the TNS fluorescence of sonicated vesicles and calculated a surface potential of −11 mV (Figure 4). Fourth, we measured the FCCP and nonactin conductance of planar bilayers and estimated a surface potential of −23 mV (data not shown). The average surface potential deduced from these four techniques is −22 mV. In contrast, we estimated an average surface potential of −9 mV for sonicated vesicles and planar bilayers containing 9 mol % G_{M1} in PC. The 2-fold difference in surface potentials is presumably due to the fact that the charge on G_{M1} can be located about a Debye length from the surface whereas the charge on PS is close to the surface. A quantitative analysis of the surface potential expected for different charge distributions has been presented by several authors, who used the Poisson–Boltzmann equation and made the conventional assumptions inherent in the classical theory of the diffuse double layer (Hill & Lester, 1972; Parsegian, 1974; Levine et al., 1983). We calculated the electrostatic potential at the bilayer surface by assuming that the charge in the G_{M1} head group was either distributed uniformly in a slab of thickness s [eq 13 of Levine et al. (1983)] or located in a plane a distance d from the surface [eq A10 of Hill & Lester (1972)]. We can account for the 2-fold difference in the surface potentials of PC–G_{M1} and PC–PS membranes containing 9 mol % negative lipid by postulating that either s = 1.5 nm or that d = 0.67 nm. These numbers are not inconsistent with the molecular structure of G_{M1}.

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Kinetics of Phosphatidylcholine and Lysophosphatidylcholine Exchange between Unilamellar Vesicles†

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ABSTRACT: The rates of exchange of phosphatidylcholine and lysophosphatidylcholine from unilamellar donor vesicles to acceptor vesicles of similar composition were followed in a protein-free system to establish the relationship between the rate of exchange and the aqueous-phase solubility of the lipid. Further, the rate of exchange of dimyristoylphosphatidylcholine (DMPC) between vesicles was examined over a range of temperatures to determine the effect of the lipid phase transition on the rate of lipid exchange. Intervesicular exchange of DMPC is faster than transbilayer exchange; lipid molecules in the outer monolayer of the bilayer exchange with $t_{1/2}$ = 2.0 h at 37 °C. A discontinuity is observed in Arrhenius plots of DMPC exchange; the activation energy over the temperature range 27–45 °C is 70 kJ mol^{-1}. The $t_{1/2}$ for DMPC exchange extrapolated to 24.5 °C (the phase transition temperature of the donor bilayer) is 6.5 h and from temperatures below 24 °C is 82.6 h. The differences in the thermodynamic parameters of activation for DMPC exchange above and below 24.5 °C are 25 kJ mol^{-1} for the activation enthalpy and 197 J mol^{-1} K^{-1} for the activation entropy. These differences are similar to the enthalpy and entropy changes associated with the gel to liquid-crystalline phase transition of DMPC. The rate of exchange of lysopalmitoylphosphatidylcholine (LPPC) was difficult to measure since LPPC transfers rapidly to the columns used for separating donor and acceptor vesicles; the $t_{1/2}$ for transfer is <2 min. LPPC at 5 mol % in cholesterol–egg PC vesicles does not affect the rate of cholesterol exchange. The rates of exchange of cholesterol, LPPC, DMPC, dipalmitoylphosphatidylcholine, and 1-palmitoyl-2-oleoylphosphatidylcholine were used to calculate activation free energies for exchange of each molecule. The activation free energies and free energies of transfer from self-micelles to water increase by 2.2 and 2.1 kJ mol^{-1} per methylene group, respectively. Thus, the free energy of transfer is a good predictor of the relative exchange rates of lipid molecules. However, the activation free energies are 30 ± 1 kJ mol^{-1} greater than the free energies of transfer. This excess free energy is proposed to be associated with restriction of the lipid molecule to the surface of the vesicle in the transition-state complex.

Lipid transfers between biological membranes, plasma lipoproteins, and bile acid micelles play an important role in lipid metabolism. Recently, a number of laboratories have shown that cholesterol and phosphatidylcholine exchange between unilamellar vesicles [Martin & MacDonald, 1976; Duckwitz-Peterlein et al., 1977; Thilo, 1977; Duckwitz-Peterlein & Moral, 1978; McLean & Phillips, 1981, 1982; Backer & Dawidowicz, 1981; De Cuyper et al., 1983; but see Kremer et al. (1977)], cholesterol exchange between cells in culture and acceptors in the medium (Phillips et al., 1980; Rothblat & Phillips, 1982), and cholesterol exchange between lipoproteins (Lund-Katz et al. 1982) and between red blood cells and lipoproteins (Bojeson, 1982; Lange et al., 1983) proceed through the aqueous phase. Other reports have demonstrated an aqueous diffusion mechanism for exchange of fluorescently

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