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Phospholipid Head Groups as Sensors of Electric Charge in Membranes†

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The functional roles of lipid molecules are still quite poorly understood. This statement may come as a surprise since it is common knowledge that lipids are the essential building blocks of biological membranes. But it is equally certain that those tasks most basic to membrane lipids, namely, the formation of a permeability barrier for ions and macromolecules and the provision of a fluidlike environment for membrane proteins, could be accomplished without resorting to the multiple variations in lipid structure and the compositional heterogeneities found in biological membranes.

Although the chemical structures of most lipids are simple and well-defined, this simplicity is deceptive since even such well-established properties as the polymorphic phase behavior of lipids in water [cf. Luzzati (1968), Shipley (1973), and Cullis and de Kruijff (1979)]—much less specific functional roles—do not follow immediately from the chemical structure. It would be of extreme interest then to understand fully the functional consequences of such different chemical substituents as the choline, ethanolamine, glycerol, and serine polar head groups, to name only those occurring most abundantly.

In this paper we shall focus on the electrical properties of the membrane surface. We shall describe interactions of phospholipid polar groups with other charged or dipolar molecules that modify the membrane surface and could constitute basic regulatory mechanisms via the membrane surface potential. Three aspects of the relationship between polar head groups and the electrical properties of the membrane surface will be discussed. First we summarize recent results concerning the conformation and orientation of lipid polar groups in crystals as well as in model membranes and biological membranes and allude briefly to their dynamic properties. Second, we demonstrate that phospholipid head groups behave as sensors of the electric charge at the membrane surface. This is essentially a new finding although indications of this effect have been obtained already from metal binding studies. Finally, we shall discuss some practical and theoretical consequences of this phenomenon as far as they may possibly modulate the electrical characteristics of the membrane surface.

Orientation and Dynamics of Phospholipid Polar Groups

For many years lipids eluded all attempts at crystallization, and it was only in 1974 that the first crystal structure of a natural phospholipid, namely, phosphatidylethanolamine, was solved (Hitchcock et al., 1974). Since then two further structures, those of phosphatidylcholine (Pearson & Pascher, 1979) and phosphatidylglycerol (Pascher et al., 1987), have been analyzed, and these three molecular conformations are compared in Figure 1. Note that in all three cases the head group dipole is aligned approximately at a right angle to the hydrocarbon chains, the latter crystallizing in the all-trans conformation, except for the beginning of the *sn*-2 chain, which is bent. An additional property common to all three molecular structures is the existence of two different head group conformations in the crystal [for a comprehensive discussion, see Hauser et al. (1981)].

Figure 1 illustrates the structure of crystalline phospholipids with crystals grown in the absence of water. What do we know then about the structure and dynamics of natural phospholipids in an environment as complex as a biological membrane, exposed to an aqueous interface? More specifically, what are the orientations of the phospholipid polar groups under these conditions?

One possible approach to this problem is to use neutron diffraction in combination with deuteriated lipids. Deuterons have a much higher scattering factor for neutrons than do protons, and the position of the deuteriated segment in the scattering profile can be identified with rather high precision. The feasibility of this approach was first demonstrated for the phosphocholine head group and has since then been applied to model membranes composed of either phosphatidylethanolamine or phosphatidylglycerol (Büldt et al., 1978; Büldt & Seelig, 1980; Mischel et al., 1987). One can conclude from

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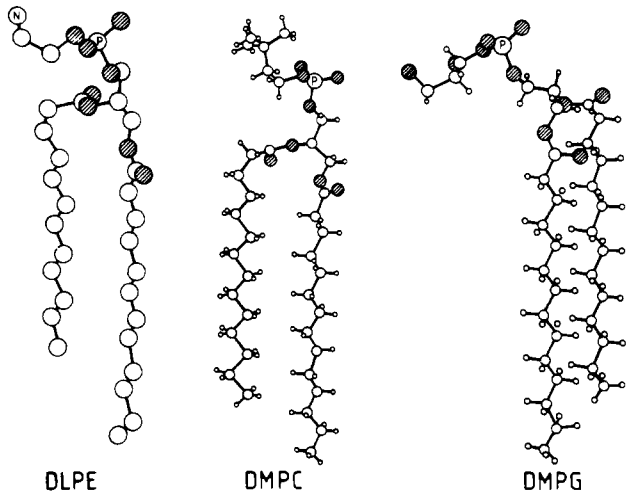


FIGURE 1: Single crystal structures of three phospholipids. The lipids are 1,2-dilauroyl-*sn*-glycero-3-phosphoethanolamine (DLPE) (Hitchcock et al., 1974), 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC) (Pearson & Pascher, 1979), and 1,2-dimyristoyl-*sn*-glycero-3-phosphoglycerol (DMPG) (Pascher et al., 1987). Structural features which are carried over into liquid-crystalline membranes: (1) the polar groups are oriented at approximately a right angle to the hydrocarbon chains; (2) in DLPE and DMPC the *sn*-2 fatty acid chain is bent at the C-2 segment while the *sn*-1 chain is straight. A bent *sn*-2 chain is a common property of phospholipids in biomembranes. Only one of two possible conformations is shown for each lipid.

such studies that fully hydrated phosphocholine, phosphoethanolamine, and phosphoglycerol polar groups are aligned essentially parallel (within 30°) to the plane of the membrane. Earlier speculations which constructed differences between phosphatidylcholine and phosphatidylethanolamine with the phosphocholine dipole perpendicular to the plane of the membrane could not be verified.

The application of neutron diffraction techniques requires the availability of oriented membranes so that the method is limited to pure lipid model membranes that can be stacked with sufficient quality between planar quartz plates. If the head group orientation in biological membranes is to be studied, neutron diffraction is not so helpful. Instead, the method of choice appears to be deuterium nuclear magnetic resonance (^{2}H NMR).[1] Again the starting point is the selective deuteriation of phospholipids by either chemical or biochemical means. This isotopic substitution introduces a nonperturbing probe into the lipid, and in the ^{2}H NMR spectrum the deuteriated lipid is singled out from among the pool of nondeuteriated neighbors. By attachment of the deuterium label at various parts of the phospholipid molecule, structural and dynamic information is obtained with a segment-to-segment resolution.

The ^{2}H NMR technique has been described at various levels of sophistication in recent reviews (Seelig, 1977; Griffin, 1981; Davis, 1983; Seelig & Macdonald, 1987). The method, though originally conceived for lipids, has now been extended to studies on peptides and proteins and synthetic macromolecules (Griffin, 1981; Oldfield et al., 1982; Opella, 1982; Torchia, 1984; Datema et al., 1986; Rice et al., 1981, 1987; Macdonald & Seelig, 1987c). The basic experimental parameters include

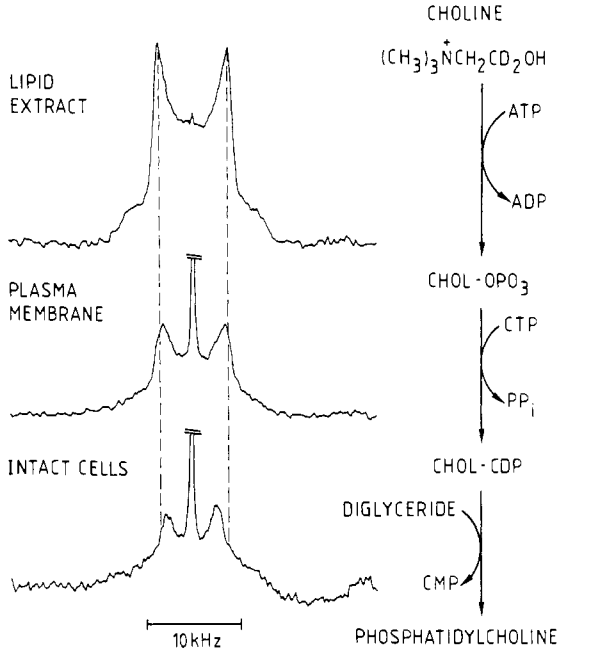


FIGURE 2: Deuterium NMR of mouse fibroblast LM-2 cells. α-Deuteriated choline was added to the growth medium of LM-2 cells and was incorporated into the cell membrane lipids via the indicated pathway. ^{2}H NMR spectra were recorded for intact cells, crude plasma membrane preparations, and lipid dispersions prepared from extracted lipids. The residual quadrupole splitting (indicated by dashed lines) was found to be approximately constant for all three membrane systems. From these and related studies with β-deuteriated choline it follows that the choline head group conformation is rather similar in the three systems. The choline dipole is approximately parallel (within 30°) to the plane of the membrane (Scherer & Seelig, 1987).

the so-called deuterium quadrupole splitting, providing structural information, and the deuterium relaxation times, which bear on the dynamics of the system. In the following we shall use the deuterium quadrupole splitting essentially as an empirical parameter—much as a change in the optical density is used as an indicator in biochemical reactions. However, it should be realized that the deuterium quadrupole splitting is a well-defined physical quantity and that the size of the quadrupole splitting as well as the shape and the intensity of the whole spectrum can be fully understood by a proper physical analysis.

The potential of this approach is demonstrated in Figure 2. Mouse fibroblast LM-2 cells were grown on a medium containing various deuteriated cholines or ethanolamines (Scherer & Seelig, 1987). As indicated by the schematic pathway of the figure, the deuteriated precursors are incorporated into the phosphatidylcholine or phosphatidylethanolamine lipids of the LM-2 cell membranes by the biosynthetic machinery of the cell. Figure 2 then compares the corresponding ^{2}H NMR spectra of intact cells, crude plasma membranes, and liposomes prepared from the extracted lipids. As indicated by the dashed lines, the residual quadrupole splittings are similar for the three membrane preparations. Since the quadrupole splitting is extremely sensitive to even small conformational changes, the results of Figure 2 and those obtained with β-deuteriated choline demonstrate quite clearly that the phosphocholine head group adopts a similar conformation in all three environments. Corresponding results hold true for LM-2 cells grown on ethanolamine. A third lipid head group that we investigated was phosphatidylglycerol, this time in a bacterial membrane (Borle & Seelig, 1983). Again the head group conformation was found to be similar in pure lipid model membranes and intact biological

[1] Abbreviations: ^{2}H NMR, deuterium nuclear magnetic resonance; POPC, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine; POPG, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphoglycerol; DPPC, 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine; DPPE, 1,2-dipalmitoyl-*sn*-glycero-3-phosphoethanolamine; CL, cardiolipin; TPP^+, tetraphenylphosphonium ion; TPB^-, tetraphenylborate ion.

membranes. The small but systematic differences between membranes with protein and pure lipid bilayers will be discussed further below.

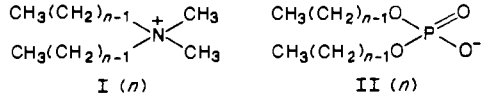
Dielectric measurements (Shepherd & Büldt, 1978) and measurements of the phosphorus and deuterium T_1 relaxation times have led to the surprising finding that the rate of reorientation of the lipid head groups at the membrane–water interface is 1–2 orders of magnitude slower than that expected for small molecules of similar size in water (Seelig et al., 1981; Borle & Seelig, 1983; Scherer & Seelig, 1987). This result is indicative of an extensive hydrogen-bonding network at the membrane surface, which restricts the movement of the lipid head groups.

In summarizing this section we may conclude the following: (1) The phospholipid polar groups align parallel (within 30°) to the plane of the membrane at least in the case of phosphocholine, phosphoethanolamine, and phosphoglycerol. (2) The head group orientation of these three phospholipids is identical in model membranes and biological membranes. (3) The rate of head group rotation at the membrane surface is hindered due to hydrogen bonding. (4) The orientation of other phospholipid head groups is essentially unknown. This holds true for chemically simple lipids such as phosphatidylserine and phosphatidylinositol and also for complex glycolipids. However, reports concerning the head group structure of carbohydrate-containing lipids have now been published (Skarjune & Oldefield, 1982; Jarrel et al., 1987).

Phospholipid Polar Groups as Sensors of Electric Surface Charges

An obvious and often-heard explanation for lipid diversity assigns an enzyme–substrate-like relationship between membrane-bound proteins and their surrounding lipids. However, the lipid specificity of most reconstituted membrane proteins is rather broad; i.e., their functional properties can be restored with quite different lipids (Sandermann, 1978). We believe therefore that specific, cofactor-type interactions, such as those involving inositol phospholipids (Sekar & Hokin, 1986), are the exception rather than the rule and that, instead, lipids act collectively by modulating the physicochemical properties of membranes. In this regard phospholipid polar groups are of particular importance since they form the molecular basis for the electrical behavior of the membrane surface. Therefore, we have explored the possibility that phospholipid polar groups may respond to the presence of electric surface charges.

In a first stage, we have mixed a neutral (i.e., zwitterionic) phosphatidylcholine bilayer with positively and negatively charged amphiphiles (P. Scherer and J. Seelig, unpublished results). As amphiphiles we have used either the cationic dialkyldimethylammonium bromides (I) or the anionic dialkyl phosphates (II). For long hydrocarbon chains ($n \geq 12$) the



I (n) II (n)

solubility of these molecules in water is negligible; instead, they spontaneously aggregate into bilayers, which have been characterized in detail by thermodynamic and spectroscopic techniques (Kunitake, 1979; Fendler, 1980; Kunitake et al., 1986). The chemical structure of the synthetic amphiphiles is simpler than those of the naturally occurring phospholipids. Of particular relevance are the cationic quaternary ammonium salts (I) since they allow one to confer a *positive* charge upon an otherwise neutral phospholipid bilayer without destroying the long-range organization of the membrane. All membranes composed of lipids of a biological origin carry exclusively

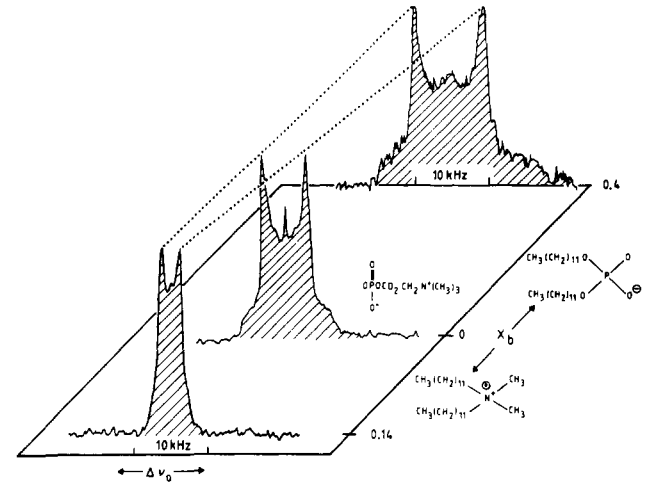
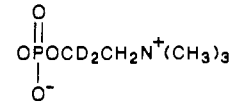


FIGURE 3: Charged amphiphiles in lecithin membranes. The figure shows three deuterium NMR spectra of α-deuteriated 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (α-CD_2-POPC; choline head group structure given in figure), namely, those of pure POPC membranes ($X_b = 0$), POPC with cationic amphiphile ($X_b = 0.14$), and POPC with anionic amphiphile ($X_b = 0.4$). Positive charges decrease the quadrupole splitting of the α-segment; negative charges increase it (P. Scherer and J. Seelig, unpublished results).

negative charges or are electrically neutral. By deuteriation of individual head group segments, as, for example, the choline α- and β-segment, it is then possible to monitor the influence



of the charged amphiphiles on the choline head group conformation. Figure 3 displays the outcome of such an experiment. The measurements were made with 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) deuteriated at the α-segment. POPC mimics most closely the fatty acid composition of naturally occurring phospholipids since it contains a cis-unsaturated fatty acid at the *sn*-2 position and has a gel-to-liquid crystal phase transition at −5 °C. The electrically neutral POPC membrane exhibits a quadrupole splitting of $\Delta\nu_\alpha = 5.9$ kHz (at 25 °C and 0.1 M NaCl). Upon addition of I (12) at a mole fraction of $X_b = 0.14$ the quadrupole splitting is *decreased* to $\Delta\nu_\alpha = 2.6$ kHz whereas addition of the anionic amphiphile II (12) induces an *increase* to $\Delta\nu_\alpha = 9.4$ kHz at $X_b = 0.4$. Indeed, even larger changes in the quadrupole splitting have been observed. For the α-segment of the choline moiety, quadrupole splittings in the range of (+ electric charge) -11.6 kHz $\leq \Delta\nu_\alpha \leq +10$ kHz (− electric charge) have been measured, depending on the sign of the electric charge and the amount of amphiphile added.[2] In contrast, when the β-segment of the choline moiety was probed, the addition of the same amphiphiles gave rise to exactly opposite changes; i.e., the β-splitting *increased* with *positive* electric charge and *decreased* with *negative* charge. Again very large variations of the quadrupole splitting were observed: (+ electric charge) 14.4 kHz $\geq \Delta\nu_\beta \geq -2$ kHz (− electric

[2] The sign of the quadrupole splitting cannot be determined from the conventional ^{2}H NMR experiment. The assignment of positive quadrupole splittings to the α- and β-segments of neutral POPC membranes is hence arbitrary. A sign reversal of the quadrupole splitting is invoked to describe the experimental observation that the quadrupole splitting decreases smoothly to zero and increases again upon continuous addition of amphiphile.

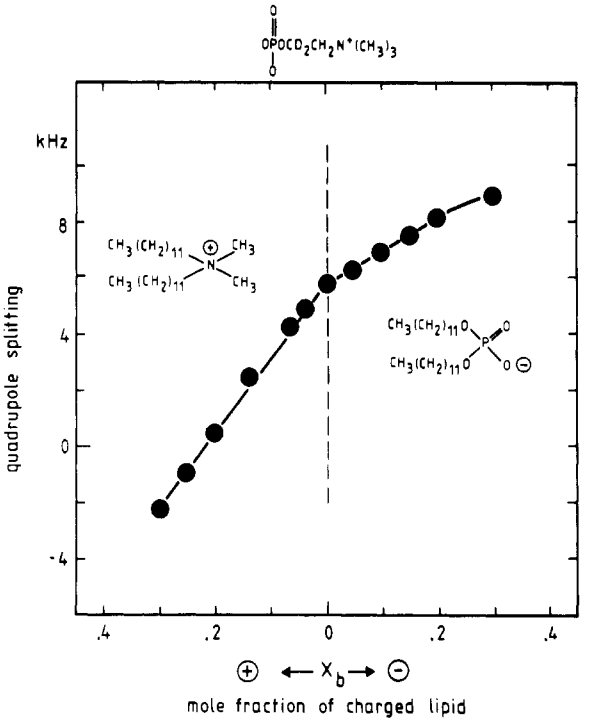


FIGURE 4: Influence of electric charge on phosphatidylcholine head group. The figure summarizes the spectra of Figure 3 and additional data in terms of their quadrupole splittings, i.e., the separation of the most intense peaks in the spectrum. Cationic and anionic amphiphiles were added to 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine deuteriated at the α-choline segment. Up to a mole fraction of $X_b \approx 0.3$, the quadrupole splittings varied linearly with the amount of added amphiphile. At higher concentrations the quadrupole splittings leveled off, probably due to counterion condensation (P. Scherer and J. Seelig, unpublished results).

charge) with the neutral lipid at 5.5 kHz. The counter-directional change of the α- and β-splitting with the same amphiphile excludes a general increase in the head group flexibility and can only be explained by a conformational change of the choline head group.

At low and medium electric charge densities ($\sigma \leq 0.3$ e_0/lipid) the quadrupole splittings vary linearly with the amount of added amphiphile. This is demonstrated in Figure 4 for the two synthetic molecules discussed above. It may be noted that the relative change (slope) of $\Delta\nu_\alpha$ is larger for positively than for negatively charged amphiphiles, indicating that the geometric location of the added electric charge with respect to the choline dipole is also of importance.

It could be argued that the observed charge effects on the choline dipole are, in fact, due to some specific properties of the synthetic amphiphiles employed. However, this objection can easily be refuted because chemically quite different substances lead to qualitatively and quantitatively similar conclusions. This is demonstrated in Figure 5 for the two hydrophobic ions tetraphenylphosphonium (TPP^+) and tetraphenylborate (TPB^-) (Altenbach & Seelig, 1985; M. Malthaner and J. Seelig, unpublished results). Both ions are water soluble and partition into the lipid bilayers with quite different affinities. Since both ions show characteristic UV spectra, the amount of ion bound to the POPC bilayer can be easily measured. As is obvious from Figure 5, the behavior of the phosphocholine head group in the presence of these ions parallels to a large extent that observed for the synthetic bilayer compounds discussed above. In the limited concentration range shown in the figure the quadrupole splittings vary linearly with the amount of bound ion X_b; positively charged TPP^+ decreases $\Delta\nu_\alpha$; negatively charged TPB^- increases $\Delta\nu_\alpha$.

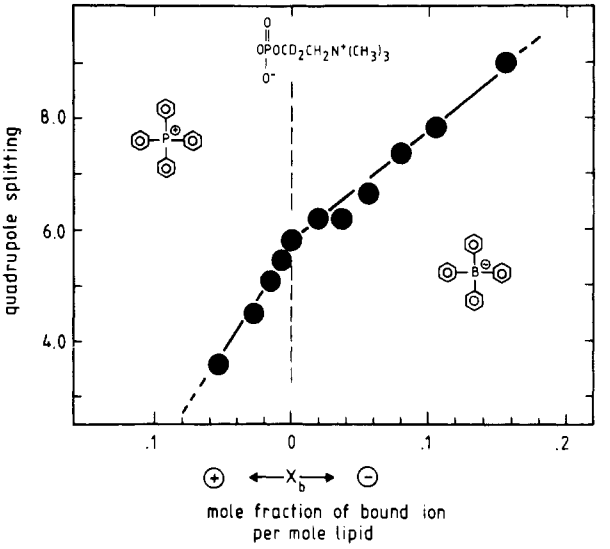


FIGURE 5: Binding of hydrophobic ions to POPC membranes. α-Deuteriated POPC membranes were bathed in solutions containing various concentrations of tetraphenylphosphonium or tetraphenylborate. The amount of membrane-adsorbed hydrophobic ion was determined with UV spectroscopy and the quadrupole splitting of the membrane measured with ^2H NMR. X_b denotes the mole fraction of ions bound per lipid, and a linear relationship between the quadrupole splitting and X_b was observed for low charge densities [TPP^+, data taken from Altenbach and Seelig (1985); TPB^-, data taken from M. Malthaner and J. Seelig, unpublished results].

For the β-segment exactly opposite variations are observed, again in accordance with the amphiphilic lipids.

Local anesthetics in their charged form exhibit structural similarities to hydrophobic ions. Not surprisingly, their effects on the phospholipid head group conformation are also quite similar (Boulanger et al., 1981; A. Seelig, P. Allegrini, and J. Seelig, unpublished results).

As a third example we have chosen negatively charged phospholipids, namely, phosphatidylglycerol and cardiolipin, which carry one and two electric charges, respectively (Macdonald & Seelig, 1987a,b; Scherer & Seelig, 1987). It had been noted before that these lipids induce conformational changes in the choline head group, but the effect was attributed to an alteration of the hydrogen-bonding properties or a modification of the water layer at the membrane surface (Sixl & Watts, 1982, 1983). We have investigated mixtures of these lipids with POPC membranes, and Figure 6 summarizes some essential results. At low concentrations of negatively charged phospholipid the quadrupole splitting varies linearly with the mole fraction of PG or CL (Figure 6A) with the initial slope being twice as large for cardiolipin as for phosphatidylglycerol. However, taking into account that CL has not only two negative charges but also twice the cross section of PG, i.e., expressing the quadrupole splitting as a function of the surface charge density, leads to a coincidence of the two sets of data (Figure 6B).

In summary then we find the following: (1) The phosphocholine head group is sensitive to the electric surface charge. The conformational change of the phosphocholine dipole with positive surface charges is inverse of that occurring with negative surface charge. (2) The conformational change is reflected in the ^2H NMR spectra. The latter may be used to determine the sign of the surface charge and to estimate its size. (3) The quadrupole splitting varies linearly with the surface charge up to a charge density of about $\sigma \simeq 0.3$ e_0/lipid ($\simeq 70$ mC/m^2). At higher charge densities counterion condensation or other mechanisms of charge screening appear to come into play. (4) The response of other polar head groups

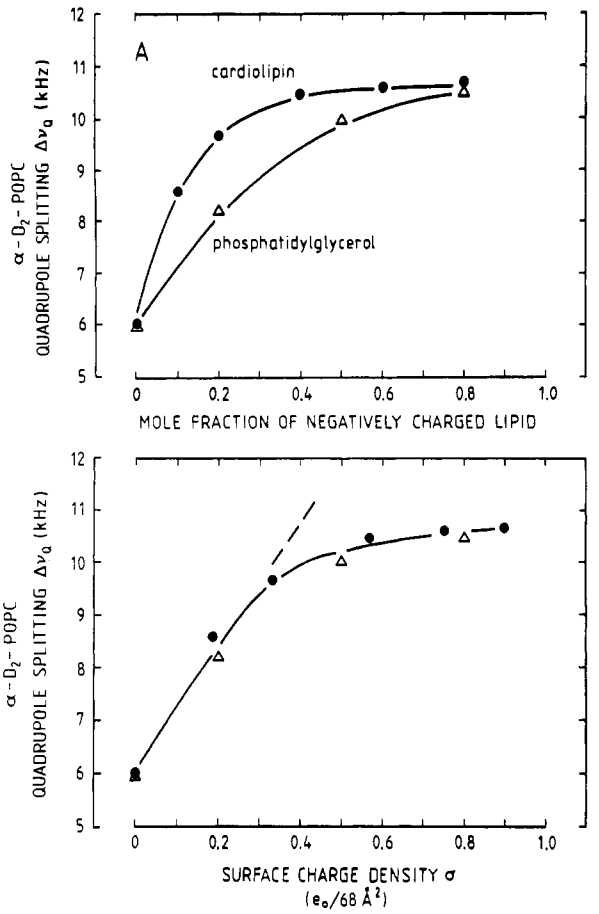


FIGURE 6: Influence of negative lipid surface charge on POPC membranes. α-Deuteriated POPC was mixed with cardiolipin (CL) or 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphoglycerol (POPG), the former carrying two negative electric charges and the latter only one. (A) The initial increase of the quadrupole splitting was linear with the mole fraction X_b of the added lipid. CL was twice as effective as POPG. (B) The CL molecule has four hydrocarbon chains and its cross section is approximately doubled compared to POPG. One CL molecule hence creates the same increase in surface change density as two PG molecules. When plotted as a function of surface charge density, both CL and POPG induce the same variation of the quadrupole splitting [data taken from Macdonald and Seelig (1987a,b); cf. also Scherer and Seelig (1987)].

to the electrical charge has not yet been investigated in detail.

Biological Perspectives

Lipid polar groups should act as "molecular electrometers" responding not only to molecules that partition into the lipid bilayer but also to any process that modifies the electrical properties of the membrane surface. In the following we shall discuss several phenomena which corroborate this contention, namely, (1) metal ion binding to membrane surfaces, (2) influence of membrane dipole potential, and (3) negative effects due to neutral molecules and proteins. Finally, we shall briefly allude to the molecular nature of the observed conformational change of the choline dipole.

Metal Ion Binding. A particularly conspicuous example is the binding of multivalent metals ions, since they cannot penetrate into the nonpolar part of the bilayer. Metal ions must remain superficially adsorbed to the membrane, but nevertheless quite distinct conformational changes have been observed with NMR and other spectroscopic techniques (McLaughlin et al., 1971; Hauser et al., 1977; Hauser & Phillips, 1979; McLaughlin, 1977; MacLaughlin et al., 1978; Westman & Eriksson, 1979; Crzeszczyk et al., 1981; Hauser & Shipley, 1983, 1984; Casal et al., 1987). Particularly large effects are noted with deuterium NMR. As a representative

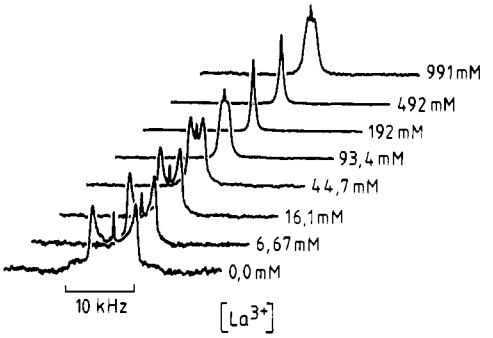


FIGURE 7: Binding of La^{3+} ions to POPC membranes. La^{3+} ions (in 0.1 M NaCl) are added in various concentrations to membranes composed of α-deuteriated POPC. With increasing concentration of La^{3+} the quadrupole spliting decreases, passes through zero, and increases again. Since the quadrupole splitting is a signed quantity, the splitting at the highest La^{3+} concentration has a sign opposite to that of the other spectra (data taken from the Ph.D. work of C. Altenbach, 1985).

example, a concentration series for the binding of La^{3+} to α-CD_2-POPC is displayed in Figure 7. The change in the quadrupole splittings parallels that observed for the positively charged quaternary ammonium amphiphiles and for TPP^+. The residual quadrupole splitting, $\Delta\nu_\alpha$ ($\Delta\nu_\beta$), decreases (increases) linearly with the amount of bound La^{3+}, and by proper calibration the mole fraction of bound La^{3+} and the membrane surface charge density can be determined. The method also works for other multivalent metal ions such as Mg^{2+} and Ca^{2+} (Brown & Seelig, 1977; Akutsu & Seelig, 1981); in particular, the Ca^{2+} binding to zwitterionic and charged phospholipid bilayers has been investigated in detail (Altenbach & Seelig, 1984; Borle & Seelig, 1985; Macdonald & Seelig, 1987a,b). The relative change in the quadrupole splitting depends on the valency of the ions and increases with the charge of the ion. Monovalent ions give rise to only small variations of $\Delta\nu_Q$ whereas trivalent ions exhibit the largest effects.

Metals ion binding to a membrane surface is a complex phenomenon that is best described by considering chemical binding and electrostatic adsorption separately [cf. McLaughlin (1977)]. The chemical aspects are obvious for a membrane surface that carries no net electric charge such as the POPC membrane but nevertheless binds ions. This process must be driven by some chemical affinity. On the other hand, once the first few metal ions have been bound, the membrane surface becomes positively charged and the binding of further metal ions will be increasingly difficult. This illustrates the second, electrical aspect, which is of relevance in metal ion binding. Electrostatic adsorption becomes even more pronounced if the membrane contains negatively charged lipids. Then the rather large negative surface potential will be the primary driving force for metal ion attraction.

The formal distinction between chemical binding and electrostatic adsorption is not reflected, however, at the molecular level. The ^2H NMR spectra always give rise to just one quadrupole splitting, indicating a single, time-averaged head group conformation. With none of the metal ions and phospholipids employed has it been possible to observe ion-complexed and ion-free head groups separately. Apparently, the residence time of a metal ion at an individual binding site is rather short ($\ll 10^{-6}$ s), and the ions move freely from head group to head group. On a molecular basis the metal ions may thus be viewed as creating a layer of positive surface charge that influences the choline dipole in the same manner as hydrophobic ions on charged amphiphiles.

^2H NMR studies on ion binding have also been performed for other polar groups. The behavior of the phospho-

ethanolamine head group is qualitatively and quantitatively similar to that of the phosphocholine group upon binding of charged local anesthetics (Kelusky & Smith, 1984) or multivalent metal ions (H. Akutsu and J. Seelig, unpublished results). Likewise, the β-segment of POPG decreases upon addition of Ca^{2+} although no systematic studies have been performed as yet (Borle & Seelig, 1985).

In conclusion, (1) ^{2}H NMR monitors the binding of multivalent metal ions to a membrane surface. For a given metal ion the quadrupole splitting is linearly related to the amount of the bound/adsorbed ion, and ^{2}H NMR provides a unique means to determine the surface charge density over a large concentration range. (2) The binding/adsorption of metal ions modifies the membrane surface potential. Correspondingly, by changing the surface potential, metal ions may be attracted to or released from the membrane surface.

Membrane Dipole Potential. Another mechanism that could influence electrostatically the lipid head group conformation is the membrane dipole field. The latter arises from the superposition of the electric fields of molecular dipoles at the membrane surface, and three major sources are usually considered: surface water molecules, the lipid head groups themselves, and lipid carbonyls [cf. Flewelling and Hubbell (1986)]. Among these, the ester groups that link the two fatty acyl chains to the glycerol backbone have been suggested to make the largest contribution. The ester group has a dipole moment of 1.8 D, and as indicated by the single crystal structures of DMPC (Figure 1), the C=O vector of the bent *sn*-2 chain is oriented approximately perpendicular to the membrane surface. For lipid membranes dispersed in water the dipole field is positive inside the membrane and negative outside, which requires that the oxygen end of the C=O dipole points toward the lipid–water interface. The ester group of the *sn*-1 chain is less effective because it is positioned deeper inside the membrane and because its carbonyl dipole is almost parallel to the membrane surface. However, in membranes composed of 1,3-dipalmitoyl-*sn*-glycero-2-phosphocholine (1,3-DPPC) both fatty acyl chains adopt a bent configuration and both dipole fields should then influence the phosphocholine head group. Indeed, synthetic 1,3-DPPC exhibits a larger α-splitting (+2 kHz) and a smaller β-splitting (−1 kHz) than 1,2-DPPC just as if a small negative charge had been imparted to the 1,3-DPPC membrane (Seelig et al., 1980). Theoretically, it can be expected that the influence of dipole fields is distinctly smaller than that of net electric charges. The effect of the dipole field might be further tested by replacing the ester linkages by ether bonds. Naturally occurring plasmalogens, for instance, contain an α,β-unsaturated ether linkage at the *sn*-1 position.

Neutral Molecules and Proteins. Addition of phosphatidylethanolamine or sphingomyelin to membranes composed of α- or β-deuteriated POPC induces only very small changes in the phosphocholine conformation (Scherer & Seelig, 1987; Sixl & Watts, 1982, 1983). This is in accordance with the electrostatic model since both lipids carry no net electrical charge and their head group dipoles rotate in approximately the same plane as the phosphocholine dipoles of POPC. Likewise, polyhydroxyl compounds such as trehalose, sorbitol, and glycerol and also noncharged glycolipids such as mono- and digalactosyldiglycerides have little effect on the head group structure (B. Bechinger, P. M. Macdonald, and J. Seelig, unpublished results).

Most proteins possess charged amino acid side chains and could thus interact electrostatically with lipid polar groups. However, these expectations are not substantiated by the available experimental results. The conformation of different lipid polar groups has been investigated with ^{2}H NMR in biological membranes (cf. above) as well as in reconstituted membrane systems containing intrinsic or extrinsic membrane proteins (Tamm & Seelig, 1983; Sixl et al., 1984; Sixl & Watts, 1985; Deveaux et al., 1986). The ^{2}H NMR spectra of LM-2 (Figure 2) may serve as a representative example to illustrate the basic differences. Most conspicuously, the line shapes of biological membranes and reconstituted membrane systems are broadened compared to those of the pure lipid bilayers (cf. top and bottom of Figure 2). However, this effect is not unique to lipid polar groups, and a similar broadening has also been observed for selectively deuteriated fatty acyl chains [cf. Seelig and Seelig (1980), Tamm and Seelig (1983), and Deveaux and Seigneuret (1985)]. The molecular origin of this line broadening is complex and not well understood, but it may be caused by some general reduction in the rates of lipid motion. The second effect generally observed in studies on lipid–protein interactions is a small reduction of $\Delta\nu_Q$ in the order of 1–2 kHz in membranes with proteins (cf. dashed lines in Figure 2). Again head group segments and hydrocarbon chain segments are affected approximately equally. The reduction in $\Delta\nu_Q$ may be only apparent and could be caused by an increase in the intrinsic line width (Paddy et al., 1981). However, if the quantitative differences in $\Delta\nu_Q$ are real, they indicate a general disordering effect of membrane proteins on the average lipid structure and are not consistent with a specific electrostatic effect at the head group level. The only exception could be the myelin basic protein–phosphatidylglycerol system, which shows variations up to 5 kHz (Sixl et al., 1984). The results obtained on lipid–protein interactions are rather puzzling and need further attention.

Addition of up to 50 mol % cholesterol to DPPC membranes leaves the α-choline splitting constant but decreases the β-splitting by about 2 kHz (Brown & Seelig, 1978). Cholesterol thus exerts a specific effect (perhaps via the electric field of the –OH dipole) on the torsion angle characterizing the C_α–C_β linkage.

Conclusions

We have summarized experimental evidence that demonstrates that phospholipid head groups are sensitive to electric charges and dipole fields. The electrostatic model explains the similar action of chemically quite diverse molecules. Conversely, by virtue of their large dipole moment, lipid polar groups may influence the membrane surface potential. The dipole moment of the phosphocholine group has been estimated to be 19 D (Shepherd & Büldt, 1978) and only if the dipole is exactly parallel to the membrane surface will its contribution to the surface potential vanish. On the other hand, even a small inclination, by 20°, will produce a dipole component of $\mu \simeq 6.5$ D perpendicular to the plane of the membrane. The corresponding dipole potential, V_{DIP}, can be approximated crudely by $V_{DIP} \approx n\mu/(\epsilon_0\epsilon_r)$ (Adamson, 1976), where n is the surface density of molecular dipoles, ϵ_0 the permittivity of free space, and ϵ_r the dielectric constant. Assuming a surface area of 68 Å^2 per lipid and dielectric constant of $\epsilon_r \approx 40$ for the boundary region of the head group dipoles, one calculates a minimum value of $V_{DIP} \simeq 90$ mV. Depending on the direction of the dipole moment, this potential will enhance or reduce existing electric potentials and is sufficiently large to trigger conformational changes in membrane proteins or to facilitate protein insertion into membranes [cf. Honig et al. (1986)].

The precise nature of the conformational changes revealed by ^{2}H NMR is only partially understood. Quite small bond rotations are sufficient to bring about large variations in the

quadrupole splittings (Akutsu & Seelig, 1981). Hence the assumption of a ±20° change in the average orientation of the head group dipole is consistent with the experimental data. In fact, the combined analysis of all variations discussed above should lead to a unique picture for the head group conformation and allow a quantitative discussion of the influence of positive and negative surface charges.

Changes in the transmembrane potential should also affect the orientation of the phospholipid head group dipoles. This should be another interesting area of future research.

Registry No. POPC, 26853-31-6; TPP^+, 18198-39-5; TPB^-, 4358-26-3; La, 7439-91-0; $[Me(CH_2)_{11}]_2NMe_2^+$, 13146-86-6; $[Me(CH_2)_{11}O]_2PO_2^-$, 45300-74-1.

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