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Phospholipid Headgroup Behavior in Biological Assemblies

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A probable prerequisite to development of the most primitive life forms was sequestration of the essential materials and reactions in compartments separate from the primeval sea. What we now recognize as a cell membrane thus has origins as ancient as life itself.

The fundamental physical behavior of a major component of cell membranes echoes that prehistoric need. Phospholipids are characterized by hydrocarbon chains, often 16 to 20 carbon atoms long, attached to polar, charged headgroups such as phosphorylcholine or phosphorylethanolamine. An amphipathic molecule results, with a hydrophilic head and a hydrophobic tail.

Such molecules, in aqueous salt solution, aggregate to form bilayers (see Figure 1) which enclose aqueous compartments. Aggregation organizes the headgroups to oppose the water and allows the hydrocarbon tails to mingle in a somewhat ordered pattern, protected from the water, in a liquid crystalline array. Thus two of the properties of a cell membrane express themselves in one of the major components: the ability to separate one aqueous zone from another and the ability to undergo phase transitions to a gel state as do other liquid crystals.

Phospholipids are by no means the only lipids found in membranes. Cholesterol, another major membrane component, mixes avidly with phospholipids at low to moderate concentrations. A polar hydroxyl bonded to the steroid ring system bestows on cholesterol amphipathic character so that it orients itself in a phospholipid bilayer similarly to the phospholipids. Its role is not well understood. Numerous other lipids, such as glycolipids, inhabit cell membranes, and they add a third feature to our list. They can play roles as recognition sites for the binding of various species, including hormones and perhaps even other cells, to the membrane surface.

Proteins constitute the other major component of cell membranes, sometimes even the dominant component. Membrane proteins appear to form three-dimensional structures, often imbedded in the membrane. They may even completely span the membrane. Integral membrane proteins contact both the hydrophilic and the hydrophobic portions of the lipids surrounding them.

Because of the three-dimensional structure and considerable complexity possible, proteins add considerably to the capabilities of a cell membrane. Enzymes, organized in a functioning complex by a membrane, perform essential biochemical reactions. The electrolyte imbalance between the inside and outside of a cell is maintained by proteins. Membrane proteins mediate development and discharge of energy potentials, important in nerve conduction. Membrane proteins control communication between the outside and inside of the cell, and from compartment to compartment within the cell. Thus membranes play roles in processes as diverse as control of metabolic levels and replication.

Since membranes function so critically in the life process, it is appropriate to ask about the level of understanding of the organization of cell membranes. From a structural viewpoint, which is my present emphasis, the modern model of a membrane is the fluid-mosaic model described in detail by Singer and Nicholson,[1] among others. It envisions proteins floating in a sea of lipid bilayer, often like icebergs with much of the protein submerged in a globular mass in the bilayer.

Phospholipids play an important structural role in this concept of a membrane. This Account emphasizes that portion of the surface of the membrane created by the polar headgroups of the phospholipids. Because of its intermediary position, the membrane surface can mediate communication between the hydrophobic and hydrophilic regions. For example, divalent cation binding to phospholipid headgroups can induce an increase in the temperature of the liquid crystalline to gel-phase transitions associated with bilayers of saturated phospholipids,[2] and cholesterol influences the ordering of water near the membrane surface.[3]

The asymmetry of membranes with respect to phospholipids[4] leads to surfaces of different properties on the two sides of the membrane, due to the different structures and charges of the phospholipid headgroups. Though roles for this asymmetry of composition and

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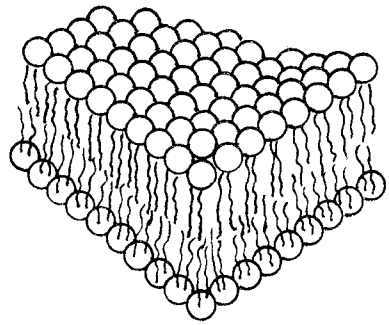

Figure 1. Phospholipid bilayer. The circles represent the polar headgroups of the phospholipids, facing the aqueous medium, and the wavy lines represent the hydrocarbon tails of the phospholipids.

structure are not yet clear, it may become important as our understanding of membrane function expands.

The membrane surface created by phospholipid headgroups is also important to the protein component of the membrane. When integral proteins are included in the phospholipid bilayer, the protein creates three kinds of interfaces in its neighborhood. The area of contact between the lipid and the protein generates one interface which suggests that phospholipid residing next to a protein might experience a different environment from phospholipid with other phospholipids as neighbors. Boundary lipid of this type has been observed[5] and may be crucial to the structural integrity of membrane proteins.

A second interface also lies in the region of phospholipid–protein contact, but in the plane of the membrane at the surface, rather than perpendicular to the surface like the lipid–protein interface. The boundary between the hydrophobic region of the lipid and protein and the hydrophilic region of the lipid and protein may be important in the orientation of membrane proteins. Correct orientations probably are necessary when two integral proteins must collide to react, such as in the cytochrome b_5–cytochrome b_5 reductase reaction.[6] A third interface separates the aqueous medium and the hydrophilic portion of the membrane protein, but does not involve phospholipid headgroups and so will not be discussed here.

An understanding of the structure of the membrane as a whole therefore requires a detailed knowledge of the structure of the surface of the membrane. The following discussion concentrates on the behavior of phospholipid headgroups, which constitute the surface of the phospholipid portion of the membrane, in biological assemblies where phospholipids have other phospholipids as neighbors and where phospholipids have protein as a neighbor.

Phospholipid Headgroup Conformations. The large body of information available on structure and behavior of the hydrocarbon region of pure phospholipid bilayers and biological membranes has not been matched with information on the conformation and behavior of the polar headgroups of phospholipids, probably due to the lack of suitable, nonperturbing probing methods. Because small changes in the structure of the headgroup produce large changes in the behavior of the phospholipids, many of the probing methods used successfully for the hydrocarbon region cannot be adapted for the headgroup region since they involve structural perturbations. Recent work with nuclear magnetic resonance (NMR) and neutron diffraction have provided the most definitive, nonperturbing approaches to phospholipid headgroup conformation.

Three phospholipids will now be considered in detail: phosphatidylcholine (PC), sphingomyelin (SPM), and phosphatidylethanolamine (PE). The chemical structures of the polar headgroups of the three phospholipids are given in Figure 2. These are the zwitterionic phospholipids and three of the most common naturally occurring phospholipids. For example, they make up a majority of the phospholipids of the erythrocyte membrane.

Phosphatidylcholine and Sphingomyelin. One approach to phospholipid headgroup conformation employs phosphorus-31 NMR. At least one phosphorus atom inhabits the polar headgroup of each phospholipid, and no ambiguities confuse which region of the bilayer is sensed. Therefore ^{31}P NMR should serve as an informative, nonperturbing method for the problem of phospholipid headgroup behavior.

Phospholipids in vesicles (200–300 Å diameter spheres with a single phospholipid bilayer as the shell, created by sonication of aqueous dispersions of phospholipids[7] and frequently used as model membrane systems) produce single high-resolution ^{31}P resonances, one arising from each phospholipid. In mixtures of two different phospholipids, it is frequently possible to measure properties of both phospholipids independently, yet simultaneously, while they form a common bilayer.

How then might ^{31}P NMR be helpful in studying phospholipid headgroups in these model systems? A wide variety of phosphorus-containing compounds, including phospholipids, exhibit a real increase in the intensity of the ^{31}P resonance upon decoupling of all the protons in the sample by irradiation at the proton resonance frequencies.[8-10] This increase in intensity is called the nuclear Overhauser effect (NOE), and it arises from protons dipolar coupled to the phosphorus being observed.[11] It is possible to ask which protons

(5) O. H. Griffith and P. C. Jost, in "Spin Labeling", L. Berliner, Ed., Academic Press, New York, N.Y., 1976, p 454.
(6) M. J. Rogers and P. Strittmatter, *J. Biol. Chem.*, **250**, 5713 (1975).
(7) C. Huang, *Biochemistry*, 8, 344 (1969).
(8) P. L. Yeagle, W. C. Hutton, and R. B. Martin, *J. Am. Chem. Soc.*, **97**, 7175 (1975).
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(11) A dipolar interaction between the protons and the phosphorus allows the two spin systems to communicate, even though the protons and the phosphorus resonate in quite different frequency ranges. Therefore, disturbing the disposition of nuclei in one spin system (by decoupling the

$$1/T_1 = K_i \Sigma_i (n_i/r_i^6) f_i(\tau) \quad (1)$$

protons) can affect nuclei in the other spin system, changing populations of energy levels and therefore phosphorus resonance intensities. The extent to which a given set of protons contributes to the ^{31}P NOE is directly proportional to the contribution of those protons to the spin–lattice relaxation rate, $1/T_1$, at the phosphorus, and that can be described by This constitutes a sum of the contributions of all types of protons, i, to $1/T_1$ (where, for example, the N-methyl protons of phosphatidylcholine are a single type of proton), where K is a collection of constants, n_i is the number of protons of type i, a distance r_i away, and the internuclear vector between the phosphorus atom and proton i has motion described by the correlation function, $f_i(\tau)$. The relative internuclear, through-space distance between the various protons and the phosphorus is thus crucial in determining the effectiveness of a given set of protons in causing a NOE, compared to other protons.

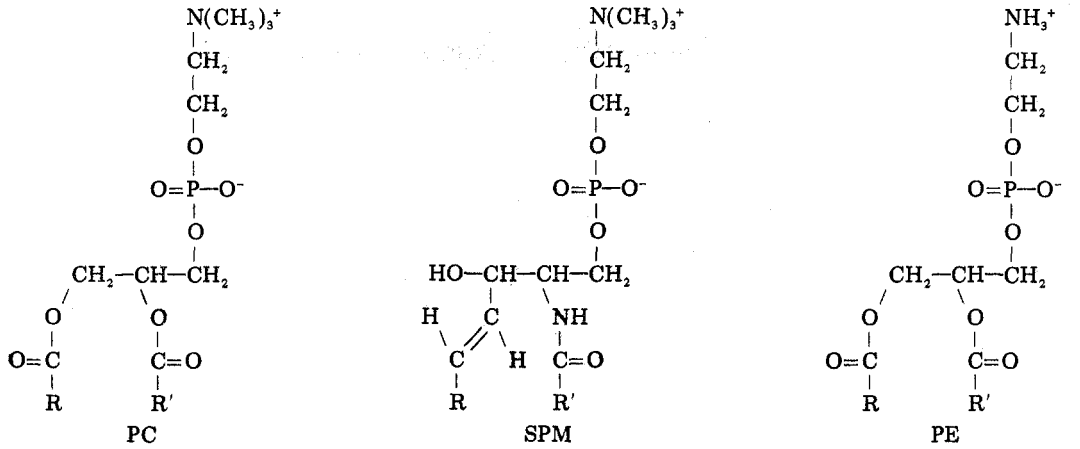

Figure 2. Chemical structures of phosphatidylcholine (PC), sphingomyelin (SPM), and phosphatidylethanolamine (PE). R and R′ represent hydrocarbon chains consisting normally of 16 to 20 carbon atoms in length for natural species. The headgroup, as used in the text, consists of the phosphate and choline or phosphate and ethanolamine moieties.

of all the protons in the phospholipid molecules are responsible for the NOE.[12] When two or more types of protons compete to cause an NOE, generally, the protons closest to the phosphorus will be the most successful.

The combination of these two pieces of information provides the conformational information about the phospholipid headgroup that we seek. The *N*-methyl protons, which contribute to the NOE in phosphatidylcholine,[13] must compete with the methylene protons next to the phosphate (2.6–3 Å away) to cause the NOE and thus must reside near the phosphate. Studies on *N*-methyl deuterated egg phosphatidylcholine (PC) vesicles and on mixed vesicles of egg PC and diphosphatidylglycerol and egg PC and phosphatidylethanolamine, exploiting the ^{31}P NOE as for pure egg PC, have demonstrated that the *N*-methyl protons of PC interact closely with the phosphates of PC, and with other phospholipid phosphates when mixed with them,[13] in an electrostatically favorable interaction. The model for the headgroup conformation of PC which emerges unambiguously from these data requires the headgroup to lie parallel to the surface of the bilayer and engage in intermolecular interactions with neighboring phospholipids (see Figure 3). Similar data lead to the same conclusion for the phosphorylcholine headgroup of sphingomyelin.[13]

Recently experiments with oriented dipalmitoylphosphatidylcholine bilayers at low water content in the gel phase have led to a similar conclusion.[14] By observing the variation in ^{31}P chemical shift as a function of the angle the oriented bilayers made with the magnetic field, and knowing the orientation on a molecular basis of the ^{31}P chemical shift tensor, it was concluded that the plane defined by the two unesterified oxygens and the phosphorus was tilted 50° with

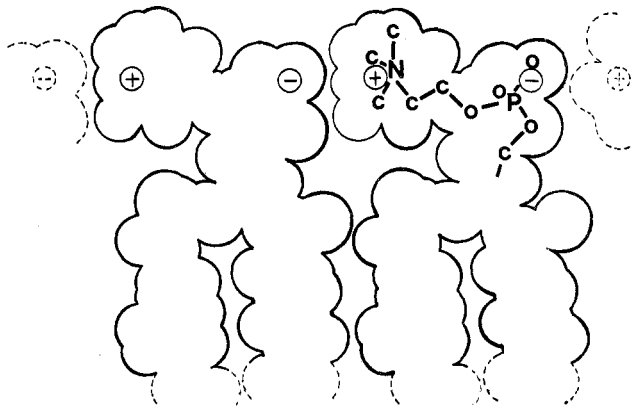

Figure 3. Schematic representation of the preferred headgroup conformation (for phosphatidylcholine) described in the text.

respect to the bilayer surface. This information, along with the gauche conformation in the choline O–C–C–N moiety,[15,16] aligns the phosphorylcholine headgroup approximately parallel to the bilayer surface.

An elegant and definitive approach to this problem employed specifically deuterated dipalmitoylphosphatidylcholine and neutron diffraction. The neutron scattering factors of hydrogen and deuterium are different, so that in the profiles of scattering density the deuterium labels show clearly and their positions can be located. Thus distances from the center of the bilayer to various positions on the choline headgroup of PC were measured, and the results indicated that the deuteriums attached to all three kinds of choline carbon atoms reside at the same distance from the bilayer center.[17] The resulting conclusion that the headgroup must be parallel to the bilayer surface agrees with the results described above.

Another, independent approach to the problem of phospholipid headgroup conformation exploited the chemical shift anisotropy seen in the ^{31}P NMR spectra of unsonicated dispersions of phospholipids.[18] Calculations using motional models of the headgroup show how various motions can "reduce" the static chemical shift tensor to the chemical shift anisotropy observed

(12) Selective irradiation allows the perturbations of only one region of the proton NMR spectrum, that region which resonates with a frequency equal to that of the selective irradiation. If protons which resonate with that frequency are contributing to the NOE, an increase in the phosphorus resonance intensity will be seen. If not, no increase in intensity will be observed. Plotting the magnitude of the NOE as a function of which protons are being irradiated will produce maxima at the protons which cause the NOE, and thus the particular protons in the molecule responsible for the NOE can be identified.

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experimentally, by motionally averaging various elements of the tensor.

Such data have proven most useful by combination with results from deuterium NMR. In the absence of rapid motion, the quadrupole coupling of the deuterium is not averaged to zero, and appears as a doublet in the NMR spectrum. The coupling for a static C–D bond is known, and can be compared to the motionally "reduced" coupling observed from dipalmitoyl-PC specifically deuterated in the headgroup. As with the phosphorus data, calculations invoking various kinds of motion reduce the coupling from the static values, and the results are then compared with experimental observations. A combined approach with phosphorus and deuterium NMR data suggested a model for the phosphorylcholine headgroup which agrees with the model described above.[18] While it has been noted that different motional models can also lead to calculations in agreement with some of the experimental results,[19] the data remain consistent with this model. As an additional consideration, the headgroup undergoes considerable motion, and this conformation should be considered a favored one in a dynamic situation and not a static model.[20]

Electrical Properties. Electrophoretic mobility of the particles in an unsonicated phosphatidylcholine dispersion indicated small, positive values for electrokinetic potentials. Based on Gouy–Chapman double layer potential theory, these data conformed best to the headgroup conformation parallel to the bilayer surface, with the positive and negative charges coplanar.[21]

Calculations of the coulombic interactions between charges in phosphorylcholine show the conformation parallel to the bilayer surface to be most favorable energetically.[22] These calculations also suggest that the interactions between phosphatidylethanolamine headgroups should be stronger than between phosphatidylcholine headgroups.

Phosphatidylethanolamine. Several kinds of evidence are available for phosphatidylethanolamine which suggest that the ethanolamine headgroup is parallel to the membrane surface engaged in intermolecular interactions, like the choline moiety of PC and sphingomyelin. A single-crystal X-ray structure of dilaurylphosphatidylethanolamine shows just such a conformation of the ethanolamine headgroup, with the positively charged ammonio group hydrogen bonded to a neighboring phosphate.[23] The same kind of analysis was performed for dipalmitoylphosphatidylethanolamine as for PC with a combined ^{2}D NMR and ^{31}P NMR study, and the data were found to be consistent with the crystal structure of phosphatidylethanolamine.[24] Infrared dichroism of hydrated, oriented phosphatidylethanolamine multilayers also suggests the same headgroup conformation as found in the crystal structure, and evidence was found for the $-N^+H_3-PO_4^-$ interactions.[25]

Thus the headgroup conformations of the three most common phospholipids, phosphatidylcholine, sphingomyelin, and phosphatidylethanolamine, are all similar, with the headgroup parallel to the bilayer surface, engaged in intermolecular interactions.

Alternate Views. Some investigators have been led to different conclusions about the conformation of the headgroup in phosphatidylcholine. On the basis of the extrapolation of X-ray data from saturated phosphatidylcholines, the contribution to the bilayer thickness of the polar headgroup was determined and an extended conformation inferred.[26] However, due to the long extrapolation required, the considerable uncertainty in the determination of the polar headgroup contribution suggests these data are not necessarily inconsistent with the headgroup conformation advocated here.

Another group proposed both extended conformation of the headgroup and intramolecular trimethylammonio–phosphate interactions, dependent on the area occupied by each headgroup, and based on Ca^{2+} binding.[27] For example, no trimethylammonio–phosphate interactions were permitted in their model for dipalmitoylphosphatidylcholine, which is contrary to the ^{31}P NOE data.[13] Also, intramolecular trimethylammonio–phosphate interactions were proposed to occur in PC bilayers containing cholesterol, but the ^{31}P NOE data (described below) show that cholesterol disrupts these interactions, and that such interactions must be intermolecular.

It has been suggested on the basis of binding of paramagnetic ions to phosphatidylcholine that the phosphorylcholine assumed an orientation perpendicular to the bilayer surface.[28] However, it was incorrectly assumed that a single, rigid conformer of the glycerol moiety existed, so that the conformation in the presence of cations remains uncertain.[13,29]

Perturbations of Headgroup Conformation. One question which is relatively easy to address is whether the nature of the acyl chains has any effect on headgroup conformation. For phosphatidylcholine data obtained from egg PC, a natural species with an average between one and two double bonds per phosphatidylcholine molecule, and dipalmitoylphosphatidylcholine, a saturated species, led to the same molecular model.[13] A related question is whether the headgroup conformation changes during a phase transition. All the above experiments were performed at temperatures above the phase transition temperature of phosphatidylcholine. One X-ray diffraction experiment obtained data below the phase transition temperature and concluded that the phosphate and *N*-methyl groups of dipalmitoylphosphatidylcholine must lie in the same plane,[30] which is like phosphatidylcholine above the phase transition temperature.

Because of the electrostatic interactions between headgroups suggested by this model of headgroup conformation, the effect of salt concentration was investigated. Up to 0.25 M Na_2SO_4, no effect on head-

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group conformation as measured by ^{31}P NOE was observed.[13b] The ^{31}P NOE experiment senses only the bulk of the phospholipid, however, so a majority of the phospholipid must be affected before a change would be detected.

Deuterium NMR studies of water binding to phosphatidylcholine indicated that hydration of the surface increased in 5 M NaCl.[31] As the authors indicate, the headgroup model described here proves useful in explaining these results. As the salt concentration is increased, the intermolecular interactions between headgroups may be weakened, either by binding of the salt to the polar headgroups, or by the increase in ionic strength, and with the headgroup interactions weakened, more water molecules may bind.

The effects of high salt on headgroup behavior have also been investigated with the quadrupole splitting of the deuterium-labeled *N*-methyl moiety of PC. 3 M NaCl reduces the quadrupole splitting, which is what would be expected if high salt were disrupting the *N*-methyl–phosphate interaction and increasing the freedom of motion of the choline.[32]

Cholesterol. The probable location of cholesterol with its polar hydroxyl moiety in the vicinity of the phospholipid ester carbonyls[10,33,34] renders unlikely any direct cholesterol interactions with the phosphorylcholine moiety. However, ^{31}P NOE data indicate that cholesterol has an indirect effect on the phospholipid headgroup. As the cholesterol concentration is increased in the bilayer, the position of maximum NOE shifts, until at concentrations of 40 mol % or greater, the maximum lies in the region of the methylene protons next to the phosphate rather than the *N*-methyl group. This result was interpreted as indicating that cholesterol increases the average distances between phospholipid headgroups so that the intermolecular interactions referred to in the model of headgroup conformation no longer occur, or at the least, are substantially weakened.[13] (Cholesterol apparently perturbs an interaction between sulfatides and sphingomyelin similarly.[35])

The average headgroup conformation, however, appears unchanged. Neutron diffraction data of D_2O binding to cholesterol-containing egg phosphatidylcholine bilayers can best be explained by the same headgroup conformation as described above in the absence of cholesterol.[34] Similar results were obtained from a combined ^{31}P and ^{2}D NMR study. In the presence of high cholesterol, the headgroup relaxed its conformation slightly, due to the spacing effect of cholesterol, but did not change its orientation parallel to the bilayer surface.[36]

Surface Hydration. With the effects of cholesterol on the bilayer surface defined, it is appropriate to return to the discussion of hydration of the bilayer surface: hydration as measured by hydrodynamic and electrical conductivity methods. Water is bound to PC at three main sites: the phosphate, the *N*-methyl group, and the carbonyl of the ester bonds to the hydrocarbon chains.[37,38] One water molecule can have considerable effect on the phospholipid headgroup, as can be seen in the difference in the ^{31}P chemical shift anisotropy between dry and monohydrated phospholipids.[39] The following will argue that the determining factor in the extent of surface hydration is the relative accessibility of these sites to the solvent.

The extent of hydration of phosphatidylcholine bilayers is greater than that for phosphatidylethanolamine bilayers,[40] though ethanolamine is more strongly hydrated than choline. Bilayers of phosphatidylcholine containing cholesterol are more hydrated than pure phosphatidylcholine.[3] The model of headgroup conformation described here explains these results readily. While in all cases the headgroup is predominantly parallel to the bilayer surface, the strength of the intermolecular interactions varies.

The ^{31}P NOE results demonstrate that the intermolecular interactions between headgroups are weaker in PC bilayers with high cholesterol than in pure PC bilayers.[13] It is also reasonable to conclude that phosphatidylethanolamine headgroups engage in stronger intermolecular interactions than PC headgroups. Because of lesser steric effects, the ammonio group can approach the phosphate more closely than can the trimethylammonio group. The ammonio group can hydrogen bond to the phosphate, and the crystal structure contains such a hydrogen bond.[23] ^{31}P and ^{2}D NMR data show less motional freedom in phosphatidylethanolamine headgroups than in PC headgroups.[24,41] Therefore, the extent of hydration can be correlated with the strength of association of the headgroups, and thereby with the accessibility of the charged moieties to the solvent.[13] In other words, in bilayers in which the headgroup associations are the weakest and the charged groups most free to the aqueous medium, the greatest extent of hydration occurs.

Phase Transitions. Arguing in a similar fashion, the model of headgroup conformation offers an explanation for the difference in phase transition temperatures of saturated PC and saturated phosphatidylethanolamine. For instance the phase transition temperature of dimyristoylphosphatidylethanolamine is about 24 °C higher than that for dimyristoyl-PC,[42] even though the two phospholipids differ in structure only in the substituents on the nitrogen in the headgroup. In a study of the transition temperatures of derivatives of dipalmitoylphosphatidylethanolamine, the transition temperature decreased proportionately to the number of methyl substituents on the nitrogen, the lowest transition occurring with the trimethyl derivative, phosphatidylcholine.[43] The binding of divalent cations to the surface of phospholipid bilayers increases the

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phase transition temperature, and this has been explained on the basis of the Gouy–Chapman theory of the electrical double layer and a reduction in charge density on the bilayer surface.[2]

It was argued previously that the headgroups of phosphatidylethanolamine interact more strongly than the headgroups of PC. The positive and negative charges must be closer in phosphatidylethanolamine than in phosphatidylcholine, and the surface charge more effectively neutralized. This interaction involves nearly every headgroup, if not all headgroups, and thus could be relatively effective.[44] Likewise, in the pH dependence at high pH of the phase transition of phosphatidylethanolamine, where an increase in pH from 9 to 11 decreases the transition temperature,[2] the deprotonation of the ammonio group reduces its interaction with the phosphate, leading to a decrease in transition temperature. Theoretical energy calculations also predict a higher transition temperature for phosphatidylethanolamine, based on hydrogen bonding between the ammonio and phosphate moieties.[45]

Comparisons of phase transition temperatures of unsaturated phosphatidylcholines and phosphatidylethanolamines reveal that the higher the degree of unsaturation, the less the difference in phase transition temperature for phospholipids with the same hydrocarbon chains.[42] The addition of double bonds to hydrocarbon chains of phosphatidylcholines increases the surface area occupied by each molecule.[46] If the same is true for phosphatidylethanolamines, then for both phospholipids, distances between headgroups increase with increases in unsaturation, and the resulting weaker intermolecular headgroup interactions must exercise a diminishing influence on the phase transition.

Chemical Shifts in Nuclear Magnetic Resonance. The model described here offers an insight into one additional experimental observation. In the ^{31}P NMR spectra of egg PC vesicles, the resonance arising from phospholipids on the exterior of the vesicle appears slightly downfield of the resonance arising from phospholipids on the interior of the vesicle. It is known that these small PC vesicles are asymmetric with respect to the composition of the two sides of the bilayer shell,[47,48] and recent calculations suggest tighter headgroup packing on the inside surface than on the outside surface.[49] Experiments have shown that binding of the cations Ca^{2+} and La^{2+} to PC vesicles shifts the resonance of lipids with which they have contact upfield,[50] so that bringing positive charge close to the phosphate shifts the ^{31}P resonance upfield. Tighter headgroup packing brings the positive trimethylammonio group closer, on average, to the phosphate, and thus the upfield ^{31}P resonance shift of phospholipids on the inside of the vesicle can be understood.

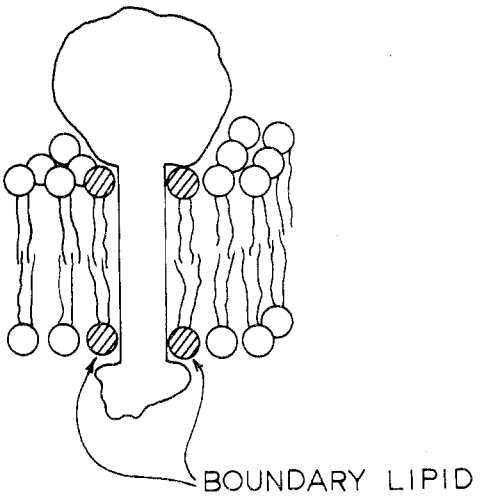

Figure 4. Schematic representation of boundary lipid (with shaded headgroups). Irregular object represents a transmembrane protein.

Phospholipid Headgroup–Protein Interactions. Phospholipid residing next to a protein in a membrane should experience a different environment than phospholipid residing next to other phospholipids. Since the protein is larger, the phospholipid would most likely be immobilized (see Figure 4). This has been observed for the hydrocarbon region, by probing with spin labels, for cytochrome oxidase,[51] cytochrome b_5,[52] Ca^{2+}-ATPase,[53] myelin basic protein,[54] and the hydrophobic segment of glycophorin.[55] Therefore one might expect a similar immobilization in the headgroup region between phospholipids and proteins. In order to discover whether phospholipid headgroups are immobilized by proteins, ^{31}P NMR has been explored as a nonperturbing probe. Three examples will be described.

Vesicular Stomatitus Virus. The membrane of vesicular stomatitus virus is a particularly simple, functioning membrane system, consisting of phospholipids, cholesterol, and a glycoprotein called the G protein. The G protein appears as spikes protruding from the membrane surface in electronmicrographs of the virion. Eighty percent of the phosphorus in the virion is in the phospholipids, so for this and other reasons, ^{31}P NMR provides information on the membrane surface. The spin–lattice relaxation rate ($1/T_1$) proved to be the most useful probing technique, because as indicated in eq 1[9] motion is an important factor in determining the relaxation rate.

The relaxation rate for the intact, unperturbed virus was nearly an order of magnitude faster than for the aqueous dispersion of the total phospholipid extract from the virus.[56] This suggested immobilization of the phospholipid headgroups by some structure in the virus that was not in the total lipid extract. Removal of the

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G protein by trypsin treatment, which otherwise leaves the virion intact, also removed the immobilization. This suggested that the immobilization was due to phospholipid–protein interactions involving the G protein.[56] ESR spin label data (Patzer, Wagner, Yeagle, and Grisham, unpublished observations) and fluorescence data[57] indicate that only the headgroups are immobilized, since there are no effects in the hydrocarbon region.

A Red Blood Cell Membrane Protein. Glycophorin, the human erythrocyte MN glycoprotein, was reconstituted in unsonicated egg phosphatidylcholine bilayers. The ^{31}P NMR spectra of this system exhibited chemical shift anisotropy.[55] The magnitude of the effective chemical shift anisotropy is a measure of the motional order of the phosphate group.[58] Two classes of phospholipids were identified in a 1:20 protein to lipid mole ratio sample. One class exhibited a line width so large as to not contribute significantly to the resonance observed. The broad line width indicated immobilization, and it was possible to estimate that about five phospholipid headgroups were immobilized per protein, in an aggregated state. The other class of lipid actually exhibited less motional order in the headgroup than phospholipids in bilayers not containing protein. Since it has been suggested that glycophorin preferentially attracts phosphatidylserine with its carboxyl terminal segment,[59] phospholipid headgroup–protein interactions involving glycophorin may be important in the phospholipid asymmetry of the erythrocyte membrane.

Plasma Lipoproteins. Human low density lipoprotein (LDL) is a much smaller biological assembly consisting of lipids and protein, which gives rise to high-resolution ^{31}P NMR spectra consisting of two distinguishable resonances from the two major phospholipid components, PC and sphingomyelin. Measurement of the absolute resonance intensities revealed that four-fifths of the phospholipids are contributing to the high-resolution intensity, but one-fifth resides in an environment with resonances so broad that they do not contribute to the high-resolution resonance. Trypsin treatment removed that environment, and the conclusion was reached that one-fifth of the phospholipid in native LDL engaged in lipid–protein interactions immobilizing that phospholipid, probably mediated by the phospholipid headgroups.[60]

In these three examples, at least, lipid–protein interactions may be mediated by phospholipid headgroups, resulting in immobilization of those headgroups, analogous to the interaction of hydrocarbon chains of phospholipids with proteins in the boundary layer. Since the major differences between most phospholipids lie in the headgroup region, specificity of interactions of phospholipids with proteins will most likely arise from the interaction of the phospholipid headgroups with the protein, rather than from hydrophobic interactions with the hydrocarbon chains. Preferential interactions between certain phospholipids and membrane proteins might play a role in the lipid composition and asymmetry of natural membranes. They may also be important as effectors in activity of membrane enzymes.

Thus we have progressed from phospholipids in the primeval sea to specific problems of modern membrane biochemistry, via the vehicle of physical chemistry. However, much of the structure and function of the cell membrane remains to be explored.

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The Use of Insoluble Polymer Supports in General Organic Synthesis

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The concept of performing chemical reactions on solid phases in a heterogeneous medium was enunciated by Merrifield[1] and by Letsinger.[2] It was demonstrated that the classical idea that chemical reactions should be performed in a completely homogeneous medium was not necessarily correct and that reactions can be accomplished even if one of the substrates was insoluble in the reaction media. The methodology of some organic synthesis has been revolutionized by this idea in that the normal procedures associated with the workup of a chemical reaction are obviated and replaced by a simple filtration step.[1,2] This general advantage of solid-phase synthesis has been particularly exploited in polypeptide synthesis,[3] where a polypeptide is syn-

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