

perimental conditions (photochemical, electrochemical, thermal, photosensitization)^{91,106} to exploit a given reaction in this class; design of reactions using matrices of activation,⁹¹ approximative correspondence principles,⁹¹ tables of activation,^{57e} application of these processes to reach new structures, activate inert substrates,

or obtain improved selectivities.

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Phospholipids and Proteins in Biological Membranes. ²H NMR as a Method To Study Structure, Dynamics, and Interactions

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Cell membranes are very delicate structures, their average thickness measuring only 5–15 nm. They are invisible in the conventional light microscope but can easily be detected with the electron microscope.¹ Not only the whole cell but also the various organelles within the cell such as the nucleus, the mitochondria, the endoplasmic reticulum, etc., are surrounded by membranes of similar thickness.

The chemical composition of these thin separating walls is extremely complex, comprising a multitude of chemically quite different species. The main membrane constituents are lipids and proteins to which carbohydrates may be affixed covalently.² Each of these major building blocks is further divided into numerous chemical subgroups. The functional purpose of this chemical variability is, however, poorly understood.

The molecular packing of proteins and lipids in biological membranes follows a common pattern.³ The basic structure is a continuous bilayer composed of phospholipids (and other amphiphatic molecules such as cholesterol) with their hydrocarbon chains sequestered toward the bilayer center and their hydrophilic polar groups exposed to the aqueous solution. Lipids are practically insoluble in water, and even though a pure lipid bilayer has a thickness of only 4–5 nm, it provides a sufficiently impermeable separating wall between two aqueous compartments, not so much for water but for charged solutes such as metal ions or macromolecules. The membrane proteins are found partly as insertions in the lipid bilayer, traversing the hydrophobic core of the bilayer, or as surface cover on both sides. The active functional properties of cell

membranes such as substrate transport, signal recognition, and cell–cell interaction are all thought to be mediated by the structurally complex and highly specialized membrane proteins.

The chemical structure of major lipid classes is quite simple, but as yet no unique functional role can be associated with a specific lipid. In a few cases (e.g., phosphatidylinositol) cofactor type of interactions have been postulated between lipids and membrane-bound proteins,⁴ but for the most part, lipids appear to act collectively by modulating and amplifying the physicochemical properties of biomembranes or membrane domains.

Studies on simple membranes, composed of one or two types of lipids, have suggested several physical mechanisms by which lipids could influence membrane-associated protein activity. Firstly, the most obvious function of lipids is to act as a “grease” for the protein machinery, providing a fluidlike, two-dimensional solvent.⁵ Different lipid species perform this role with varying efficacy. Secondly, depending on the chemical structure and the physical state of the fatty acyl chains, the membrane thickness will vary. A mismatch between the hydrophobic regions of the lipid matrix and the trans-membrane proteins could induce inhomogeneities in the lateral packing, leading to phase segregation and protein–protein association.⁶ Thirdly, and perhaps most important, lipids could exert regulatory functions via the membrane surface potential⁷ and via their ion binding properties.⁸ These two parameters are mainly determined by the lipid polar

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groups and, to a lesser extent, also by the lipid backbone region. Again, a large variability exists with respect to the chemical nature of the polar groups, their lateral and transverse distribution in the two half-sides of the bilayer, the average orientation of the headgroup dipoles, the charge and size of the ions adsorbed, the location of the binding sites, etc. From a physiological point of view it suffices to note that some of these features could easily generate changes in the membrane surface potential of several tens of millivolts which would be of the same order of magnitude as, for example, the nerve action potential.⁹ Finally, lipids may be involved in the translocation of proteins across membranes. Protein transport through bilayer membranes requires an amino terminal extension of the polypeptide chain by 20–40 amino acids, termed “signal peptide”. This signal peptide has been suggested to interact directly with the lipid components of the membrane, perhaps inducing transitory nonbilayer states such as micellar structures or inverted hexagonal phase.¹⁰

Any attempt to further characterize these biophysical mechanisms of membrane regulation requires a quantitative description of the average structure of the molecules in the membrane, of their mobility, and of their internal fluctuations. Electron microscopy certainly showed us the approximate arrangement of lipids and proteins, but the molecular picture remained rather diffuse and moreover static. Only with the development of modern spectroscopic techniques such as spin-label EPR¹¹ and ²H NMR¹² did it become possible to achieve a high enough resolution to describe biomembrane conformation and dynamics at the level of individual molecules.

In particular, ²H NMR as a nonperturbing NMR method provides quantitative insight into almost all of the questions raised above. In this Account we will demonstrate the versatility and quantitative precision of ²H NMR with three recent examples: (1) The concept of the order parameter and membrane order profiles will be introduced and applied to the problem of lipid–protein interaction. ²H NMR measures the fluctuations and rates of mobility of the lipid acyl chains at different depths in the bilayer. This information may be exploited to calculate membrane thicknesses, to clarify the concept of membrane “fluidity”, and to discern the effects of proteins on each.^{13–15} (2) We shall describe how deuterium-labeled phospholipid headgroups may be employed to study the binding of metal ions, hydrophobic ions, local anesthetics, and extrinsic proteins to a membrane surface. The proper analysis of the ²H NMR spectra separates chemical and electrostatic binding contributions, allows an evaluation of the binding mechanism and binding constants, and leads to a calculation of the membrane surface potential. (3) We shall focus on the structural and dynamic properties of membrane proteins them-

selves. By selective deuteration of amino acid side chains, membrane proteins have also become amenable to investigation by ²H NMR. Hence, detailed characterizations of the conformation and dynamics of membrane proteins and of the nature of interactions between proteins and lipids are now feasible.

²H NMR of Membranes

The NMR spectra of small- and medium-sized molecules in liquids consist of individual sharp lines due to the rapid and isotropic tumbling of the molecules. In membranes, however, the molecular motions are hindered by the parallel packing of the lipid molecules. In many respects, membranes behave like uniaxial liquid crystals with the normal to the plane of the membrane constituting the optical axis. All molecular movements exhibit rotational symmetry with respect to the axis. Conventional ¹H NMR spectra of even small molecules will become rather complex under these conditions, while larger molecules such as the lipids give rise to broad and featureless resonances. Here the use of ²H NMR in combination with selectively deuterated lipids offers distinct advantages. The replacement of a proton by deuterium is an essentially nonperturbing labeling, the deuterium label can be placed on almost any molecule and at any position within the molecule, the assignment of the resonances is straightforward, and, above all, the interpretation of the ²H NMR spectrum is particularly simple. It provides information about the ordering of the lipid molecules, about the angular fluctuations of the molecules around the optical axis, and about the rate of the segmental reorientations.

Let us illustrate the essential features of ²H NMR with the physically simplest situation, that of homogeneously oriented membranes. Admittedly, this is a rather artificial situation not observed in nature, but the appearance and interpretation of the ²H NMR spectra become particularly simple and straightforward. Macroscopically oriented planar membranes can be prepared by pressing lipid–water dispersions between flat glass plates, thus orienting the membrane microdomains by mechanical shearing forces.¹⁶ Recently, it was discovered that certain lipid membranes align spontaneously in the field of a NMR magnet, providing an even simpler way of orienting membranes.¹⁷ The driving force for the macroscopic ordering is the negative diamagnetic anisotropy of the fatty acyl chains leading to an orientation of the membrane microdomains such that the planes of the membrane are parallel to the magnetic field.

The ²H NMR spectrum of magnetically oriented membranes in which the phosphatidylglycerol component was selectively deuterated in the polar headgroup is shown in Figure 1A. The spectrum is characterized by two sharp lines corresponding to the two allowed NMR transitions of the deuterium $I = 1$ nucleus. The most important parameter which can be read off immediately from the spectrum is the residual deuterium quadrupole splitting, $\Delta\nu_Q$, which is given by the separation between the two resonances. $\Delta\nu_Q$ provides information about the average orientation and the fluctuation

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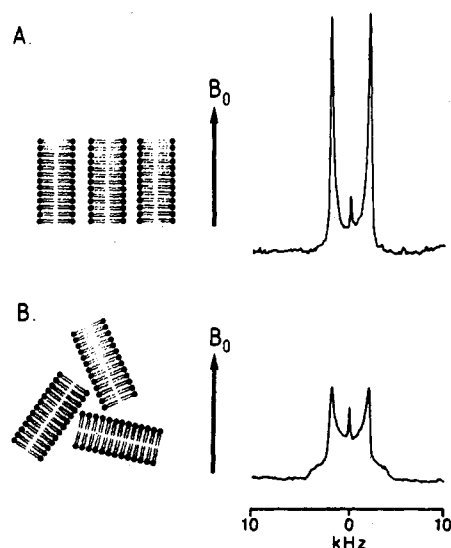


Figure 1. ^2H NMR spectra of a mixture of synthetic lipids: 83% 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphoethanolamine (POPE) and 17% 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphoglycerol (POPG) dispersed in buffer (85 wt %). POPG was deuteriated at the *sn*-2'-position of the glycerol headgroup. Spectrum A demonstrates the orientation of the lipid bilayer by the magnetic field while spectrum B results from vortexing the sample. The orientational distribution of lipid bilayers with respect to the magnetic field B_0 is shown schematically on the left.¹⁷

tuations of the C- ^2H bond vector which is usually expressed in terms of the "deuterium order parameter" S_{CD}

$$\Delta\nu_Q = (3/4)(e^2qQ/h)S_{\text{CD}} \quad (1)$$

(e^2qQ/h is the static quadrupole coupling constant, 170 kHz for an aliphatic C- ^2H bond). Equation 1 takes into account the specific membrane ordering depicted in Figure 1A, i.e., the optical axis being perpendicular to B_0 . The order parameter, S_{CD} , is a well-defined physical quantity, the molecular interpretation of which will be discussed below.

Most biological membranes cannot be ordered this way but occur as random dispersions. Nevertheless, the interpretation of their ^2H NMR spectra is almost equally simple. Figure 1B shows the ^2H NMR spectrum of the same phospholipid membrane as used in Figure 1A, but this time measured with a nonoriented sample. Since all orientations of the bilayer relative to the magnetic field are present simultaneously, the spectrum is fairly broad and the signal intensity reduced. However, the separation of the two maxima in this "power-type" pattern is identical with the quadrupole splitting observed in the oriented bilayers (Figure 1A), and information on the order parameter, S_{CD} , and all that accrues therefrom is again available by application of eq 1.

The intrinsic line width in the ^2H NMR spectra of oriented membranes is fairly small, indicating rapid rotational and translational motions of the lipid molecules. A quantitative characterization of these fast motions can be obtained by measuring the spin-lattice relaxation time, T_1 , which is related to the molecular correlation time, τ_c , according to

$$(1/T_1) = (3\pi^2/2)(e^2qQ/h)^2[1 + (1/2)S_{\text{CD}} - (3/2)S_{\text{CD}}^2]\tau_c \quad (2)$$

Equation 2 is a first approximation valid for the fast

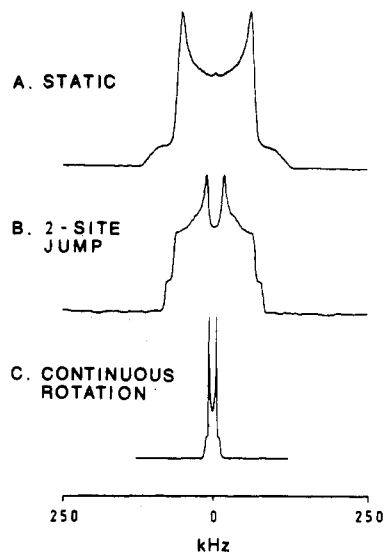


Figure 2. ^2H NMR spectra obtained for various motional regimes. Spectrum A is that of crystalline [$^2\text{H}_5$]tryptophan and exemplifies the static condition. Spectrum B is that of a particular crystalline form of [phenyl- $^2\text{H}_5$]alanine which is capable of 180° ring flipping motions.²¹ Spectrum C is that of crystalline methyl-deuteriated choline iodide where the methyl groups undergo rapid rotational motions.⁴⁰

correlation time regime ($\tau_c < 10^{-8}$ s) and assuming a single correlation time. The last term in parentheses contains the order parameter correction.¹⁸

Deuterium T_1 relaxation times are short because of the large quadrupole moment which determines the relaxation. For pure $^2\text{H}_2\text{O}$ the relaxation time is $T_1 \sim 400$ ms ($\tau_c \sim 6$ ps) which upon hydration with lipids reduces to 10–100 ms.¹⁹ The relaxation times of deuteriated aliphatic segments of the fatty acyl chains vary between 5 and 200 ms.^{15,18}

So far we have discussed the situation in which the motions of the molecules in the membrane, although hindered and anisotropic, are still fast on the ^2H NMR time scale. In many circumstances, for example for membrane proteins, the motions are slow relative to this time scale, and here it is the shape of the ^2H NMR spectrum itself which reveals the character and rate of any motion present.^{20,21} For a completely static C-D bond the very broad ^2H NMR spectrum shown in Figure 2A is observed, and the residual deuterium quadrupole splitting reaches a maximum value of 128 kHz. However, the deuterium line shape changes dramatically to that shown in Figure 2B if the C-D bond vector is executing discontinuous jumps between two orientations relative to the magnetic field. This might correspond to the spectrum observed for an aromatic amino acid side chain undergoing 180° ring flipping motions.²¹ If the motion is continuous, i.e., when the energy barriers between different degrees of rotation about the motional axis are small, the static spectrum narrows markedly to that shown in Figure 2C, which is identical

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in shape with that observed in uniaxial liquidlike membranes. For both types of motion, continuous and discontinuous, the changes in line shape are characteristic as the rate of motion increases.

Lipid-Protein Interactions and Bilayer Order Profiles

Lipids and proteins can interact by a number of different mechanisms. (1) Proteins may bind electrostatically to the membrane surface, i.e., to the lipid polar groups. (2) Proteins may penetrate into the hydrophobic part of the membrane, influence the ordering and packing of the hydrocarbon chains, and vary the membrane thickness. (3) Proteins may modulate the long-range organization of lipids either by inducing nonbilayer types of structures or by stabilizing nonbilayer lipids into a bilayer structure.

The exact physical modes of these interactions are complex and ill-understood; we shall not attempt to review the full range of possibilities here but will focus on the interaction of the fatty acyl chains with membrane spanning proteins. This topic has been studied extensively in the past few years with apparently contradictory results:^{11,14} although spin-label EPR measurements suggested the existence of "immobilized" boundary lipids, i.e., a ring of lipids in direct contact with the hydrophobic surface and with physical properties different from those of bulk lipids, this "immobilized" component was missing in ²H and ³¹P NMR spectra. Originally, the boundary lipids were pictured as being glued rigidly to the protein surface, but in view of the NMR results a rapid exchange between boundary lipid and bulk lipid was postulated. The exchange rate could be narrowed to 10⁶–10⁸ s⁻¹ since this was sufficiently fast to produce exchange-narrowed ²H and ³¹P NMR spectra but at the same time slow enough to yield separate EPR spectra for boundary lipid and bulk lipid.

The physical nature of the so-called boundary lipids is still controversial. We shall, therefore, discuss recent ²H and ³¹P NMR results obtained on a unique membrane system in which, by virtue of the chemical composition, all lipids can be classified as boundary lipids.²² This membrane system is composed of gramicidin A and 1-palmitoyllysophosphatidylcholine in the molar ratio of 1:4. Gramicidin A is a linear hydrophobic polypeptide consisting of 15 amino acids with alternating L and D configurations. Conductance measurements on membranes doped with low concentrations of gramicidin A showed that two molecules of the antibiotic form a channel for monovalent cations. The length of the channel is ~30 Å; the outer and inner diameters are 15 and 4–5 Å, respectively. The channel conformation in membranes (including lysophosphatidylcholine bilayers) is believed to be an amino terminal to amino terminal helical dimer.²³ In the helical conformation gramicidin A has a highly irregular contour with four bulky tryptophan residues sticking out near the carboxy terminus of each polypeptide.

At high concentrations gramicidin A has been found to destabilize the membrane.¹⁰ Likewise, 1-palmitoyl-

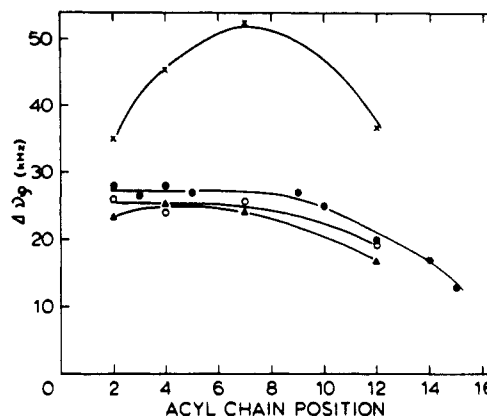


Figure 3. Order profiles of various bilayer membranes at 45 °C. The deuterium quadrupole splitting is shown as a function of the labeled carbon atom in the acyl chain for aqueous dispersions of (●) dipalmitoylphosphatidylcholine, (○) lysophosphatidylcholine/dipalmitoylphosphatidylcholine (1:4), (▲) lysophosphatidylcholine/gramicidin A (4:1), and (×) lysophosphatidylcholine/cholesterol (1:1).²²

lysophosphatidylcholine has detergent-like properties and may be used to lyse membranes. However, when the two compounds are mixed in the stoichiometric ratio of 1:4 gramicidin A/lysophosphatidylcholine, a stable membrane is formed. We deuteriated the fatty acyl chain at several strategic carbon segments and recorded the ²H and ³¹P NMR spectra of the corresponding membrane.²² The results are at first sight surprising but are completely in line with previous studies on reconstituted lipid-protein systems and biological membranes. A comparison of order profiles is reproduced in Figure 3 where we plot the deuterium order parameter as a function of the position of the deuterium for the gramicidin A/1-palmitoyllysophosphatidylcholine membrane and two other membranes composed of lipids only. It may be seen that the order profiles of three membranes are virtually identical, in spite of the fact that two of the membranes contain no protein while in the third system each fatty acyl chain must be in contact with the polypeptide chain. This result demonstrates quite clearly that the average conformation of the "boundary" lipid around the gramicidin A molecule is identical with the chain conformation in comparable pure lipid membranes.

Let us now consider the statistical interpretation of the membrane order profiles. The order parameter is reduced by any motion which changes the orientation of the fatty acyl chain relative to the normal to the bilayer. In addition to whole chain tilt, we are particularly concerned with trans/gauche rotational isomerizations about C–C bonds which alter the fatty acyl configuration. Since these rotational isomerizations occur very quickly, the order parameter is related to the probability that a particular methylene segment assumes either a trans or a gauche conformation. Hence, the actual configuration of the fatty acyl chain may only be described in a statistical sense. From the order profiles of Figure 3 we see that, statistically, the probability of gauche isomerizations increases drastically toward the chain terminus. Furthermore, each gauche conformer tends to shorten the effective chain length (i.e., the "shadow" or projection of the fatty acid onto the membrane normal) so that the thickness of a fluid membrane containing many gauche conformers will be

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much less than that predicted for an all-trans situation. The effective length, $\langle L \rangle$, of a hydrocarbon chain with n segments can then be calculated from the order profile according to^{14,24}

$$\langle L \rangle = 1.25[(n/2) + \sum_{i=1}^n |S_{CD}^i|] \text{ (\AA)} \quad (3)$$

This formula is, of course, based on a specific model for the chain motion, but it has been verified experimentally by measuring the length of the hydrocarbon chain directly with neutron diffraction techniques.²⁵ From the order profile of Figure 3 we thus calculate an effective chain length of the palmitic acyl chain of $\langle L \rangle = 13.5 \text{ \AA}$ ($n = 15$) at 45°C . Taking into account the van der Waals radii of the terminal methyl groups, the total thickness of the hydrocarbon bilayer is $\sim 28 \text{ \AA}$, yielding a perfect match with the length of the gramicidin A dimer channel. The lipid polar groups are extending beyond the gramicidin channel, giving the whole arrangement a funnellike appearance.

Order profiles have been described in both natural and model membranes and for a variety of lipids differing with respect to both their fatty acyl and polar headgroup constituents. Within this diverse grouping individual order parameters and the shape of the order profile remain remarkably constant provided they are compared at an equivalent "reduced" temperature, i.e., at comparable temperatures relative to their respective lipid-phase transitions.²⁶

Can order profiles at all be influenced by changing the membrane composition? As a positive example, we have included in Figure 3 the order profile of a cholesterol-containing membrane. When 1-palmitoyllysophosphatidylcholine is mixed with cholesterol, a common component of eukaryotic membranes, it also forms stable bilayers. However, the order parameters increase dramatically by almost a factor of 2 at a 1:1 molar ratio. This decrease in the chain flexibility is the consequence of the insertion of the rigid sterol structure between the lipids. This same behavior is observed when cholesterol is mixed with other lipids.²⁷

Since gramicidin A has no effect on either the absolute order or the shape of the order profile despite the high protein-to-lipid ratio, the irregular contour of the peptide, and the proven sensitivity of 1-palmitoyllysophosphatidylcholine to changes in molecular order, the question naturally arises as to why no such effects are observed. A number of plausible explanations may be put forward. It may be that the hydrophobic amino acid side chains of the membrane spanning portion of integral membrane proteins are themselves highly fluid and present no obstacle to lipid fatty acyl chain motions. Alternatively, a rigid but irregular protein surface might still permit lipid motions similar to those in a protein-free bilayer. To differentiate these possibilities requires that the motional properties of the proteins

themselves be characterized. This has now become possible, and first results will be summarized in the last section.

Ion Binding to Biological Membranes

So far we have concentrated on the hydrophobic acyl chains of the amphipathic phospholipids and have ignored the role of the hydrophilic polar headgroups. In terms of the order profile and the average chain configuration this appears to be justified since the influence of the polar groups is small. We know, for instance, that biological membranes of different origin and composition give rise to qualitatively and quantitatively similar order profiles.

On the other hand, cell membranes do contain a multitude of chemically different polar lipids. Moreover, the lipid composition of those membranes delineating various subcellular compartments are each unique and different. Furthermore, at least some biological membranes are characterized by an asymmetry of the phospholipid distribution; for example, in the erythrocyte membrane relatively more phosphatidylcholine and sphingomyelin are present at the external than at the internal surface, while the opposite holds true for phosphatidylethanolamine and phosphatidylserine.

Unfortunately, the functional role of this lipid complexity is uncertain. Undoubtedly, some phospholipids function in a geometric capacity, affecting the stability of the overall bilayer configuration. Obviously, others will be involved in very specific enzyme-substrate-like interactions. But the creation of particular physicochemical conditions at the membrane surface could be equally important. The role of the surface in regulating such essential functions as the binding of ligands (metal ions, water-soluble nutrients, peptides, and proteins, to name but a few) has only recently come into focus. In the past such studies were hindered by the fact that no simple method was available to visualize the disposition of individual molecules within the cell membrane.

Here again, ^2H NMR has proven to be helpful. The ^2H NMR method requires that deuterons be introduced specifically into the polar headgroup moiety by either chemical or biochemical synthesis. This has been accomplished for five major phospholipid headgroups, namely, choline, ethanolamine, glycerol, serine, and cardiolipin.¹⁴ The properties of an individual headgroup can then be characterized either in the pure form or in mixture with other lipids as found, for example, in bacterial or mammalian cell membranes. From such studies it has been deduced that the dipole of the choline, ethanolamine, and glycerol headgroups is oriented on average essentially parallel to the membrane surface, a result confirmed by simultaneous neutron diffraction studies.^{14,25} It was also found that the rates of headgroup motion at the membrane surface were reduced by about 2 orders of magnitude relative to small molecules of comparable size tumbling freely in solution. In addition, and more importantly for what follows, it was noted that the mixing of various headgroup types and the binding of various molecules to the membrane surface induced a conformational change in the phospholipid headgroups. Though small in terms of the variation of the bond rotation angles, this conformational change translates into a large change in the quadrupole splitting, providing a sensitive handle for

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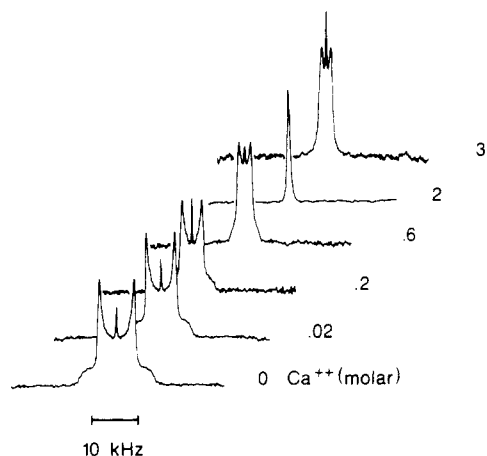


Figure 4. ^2H NMR spectra of aqueous dispersions of 20/80 mixtures of 1-palmitoyl-2-oleoylphosphatidylglycerol/1-palmitoyl-2-oleoylphosphatidylcholine as a function of CaCl_2 concentration. The lipid was deuterated at the α -segment of the choline headgroup.⁹

studying binding equilibria.

To illustrate the approach, we will describe ^2H NMR studies of Ca^{2+} binding to mixtures of negatively charged and neutral lipids, phosphatidylglycerol (PG) plus phosphatidylcholine (PC).⁹ Since most biological membranes contain approximately 10–20% negative lipids, we are mimicking a biologically relevant situation. As well, calcium ions have unique biological importance.

In the first instance, the ^2H NMR spectra inform us that the binding of Ca^{2+} does not alter the fluidlike properties of the membrane lipids. When membranes containing headgroup deuterated PC and nondeuterated PG are exposed to various levels of Ca^{2+} , the spectra remain characteristic of a liquid-crystalline bilayer while the quadrupole splitting progressively decreases with increasing Ca^{2+} (Figure 4). Similar results are obtained if PG is deuterated. So the conformational change induced by calcium binding is one experienced by all the lipids. Since for each lipid type only one quadrupole splitting is observed, we can further conclude that the exchange of lipids between a “ Ca^{2+} -bound” and a “free” state is fast on the ^2H NMR time scale of 10^{-5} – 10^{-6} s.

In the second instance, the ^2H NMR spectra provide us with a quantitative measure of ion binding. The change in quadrupole splitting, when properly calibrated, gives directly the amount of bound ion. Figure 5 shows Ca^{2+} binding isotherms for various mixtures of PC and PG. Since we are dealing with charged ligands binding to a charged surface, electrostatic effects will influence the concentration of ions in the solution adjacent to the membrane surface and available to bind to sites on the surface. These effects are encompassed within the Gouy–Chapman theory.^{7,8}

In brief, the ^2H NMR studies lead to the following conclusions: (1) The presence of negatively charged lipids radically increases the ability of the membrane surface to attract positively charged ions. For example, at 2 mM equilibrium Ca^{2+} concentrations 100% POPC membranes have bound 8 mmol of Ca^{2+} /mol of lipid while a 20/80 mixture of POPG/POPC has bound 50 mmol of Ca^{2+} /mol of lipid. (2) The increased Ca^{2+} binding in the presence of negatively charged lipids is due to electrostatic effects rather than to a greater in-

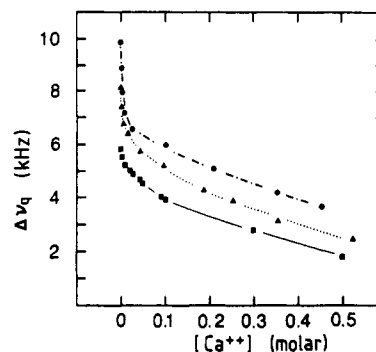


Figure 5. Calcium binding isotherms for various mixtures of phosphatidylglycerol (POPG)/phosphatidylcholine (POPC) from ^2H NMR data: squares, 100% POPC; triangles, 80% POPC plus 20% POPG; circles, 50% POPC plus 50% POPG. Note the sharp decrease in quadrupole splitting (corresponding to a sharp increase in calcium binding) which occurs in the presence of the negatively charged lipid POPG.⁹ The solid lines represent computer fits generated by taking into account the calibration of the change in quadrupole splitting as well as electrostatic effects and the form of the ion binding equilibrium.

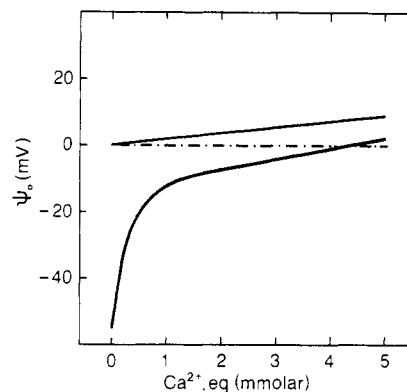


Figure 6. The surface potential, ψ_0 , for an 80/20 mixture of POPC/POPG (lower curve) and for 100% POPC (upper curve) as a function of the solution calcium concentration. The dashed line is a zero reference. This comparison accentuates the sensitivity of the surface potential of membranes containing negative lipids to the binding of calcium.⁹

trinsic affinity of POPG for Ca^{2+} . The Ca^{2+} binding constants for the various membrane compositions were very similar (about 15 – 20 M^{-1}). (3) The mode of Ca^{2+} binding to neutral and negatively charged membranes appears to be different. For the PG/PC mixtures a simple 1:1 binding model sufficed to describe Ca^{2+} binding whereas for pure PC membranes a complex between two lipids and one Ca^{2+} gave a better fit to the data.

We see then that Ca^{2+} ions are attracted toward the surface by the negatively charged lipids and remain trapped in a trough of negative potential, floating freely and transiently binding to the lipid polar headgroups. The membrane surface potential is altered concomitantly, and Figure 6 shows that Ca^{2+} ions even at very low, i.e., physiological, concentrations have a distinct effect on the surface potential of membranes containing negative lipids. Neutral lipid membranes are considerably less affected.

These results suggest two intimately related functional roles of phospholipid in membranes. Firstly, lipids may regulate the concentration of multivalent metal ions in the cellular milieu. For example, a change in the surface potential could induce a release or

binding of Ca^{2+} sufficient to significantly influence the local Ca^{2+} concentration. Secondly, external changes of the metal ion concentration may vary the surface potential and hence reduce or increase the threshold for electrically regulated processes. It is known, for example, that a reduction of the external Ca^{2+} concentration by a factor of 5 reduces the threshold for nerve excitation by about 15 mV.²⁸

With ^2H NMR we can now query the effects of a specific headgroup species on the surface properties of a mixed bilayer, quantitate its influence on binding of a particular ligand, and then discern the surface consequences for the membrane potential. ^2H NMR studies of the binding of ions (both hydrophobic²⁹ and hydrophilic^{9,30,31}), local anaesthetics,^{32,33} and proteins^{34,35} to model membranes of various compositions have now appeared.

Proteins in Membranes

From the perspective of the lipids we have seen that the interactions between the proteins and the lipids do little to perturb the average lipid conformation and freedom of movement. We now address the converse question: i.e., we consider the effects of the lipid matrix on the protein structure and the dynamic properties of the amino acid side chains.

Compared to the wealth of structural information available for water-soluble proteins, relatively little is known about membranous proteins. Only recently has the first X-ray structural analysis of an integral membrane protein, the photoreaction center, been published.³⁶ In addition, electron microscopy studies have provided a fairly detailed picture of the arrangement of bacteriorhodopsin in the purple membrane patches of the bacterium *H. halobium*.³⁷ On the basis of these studies and work with model peptides, it is generally believed that the membrane spanning portions of integral membrane proteins adopt a conformation in which the hydrophobic amino acids are located such as to maximize their contact with the surrounding fatty acyl chains.

^2H NMR may be employed to study protein motion by growing bacteria or phages on media supplemented with selectively deuterated amino acids.³⁸ For small peptides it is also possible to acquire ^2H NMR spectra for the exchangeable hydrogen sites in the presence of excess $^2\text{H}_2\text{O}$.³⁹

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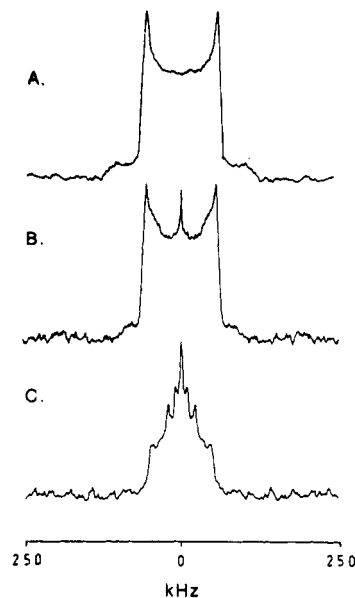


Figure 7. ^2H NMR spectra of $[(\text{Trp-}^2\text{H}_5)_4]\text{gramicidin}$: (A) dry powder of gramicidin; (B) lysophosphatidylcholine/gramicidin (4:1) in aqueous solution at 315 K; (C) dimyristoylphosphatidylcholine/gramicidin (15:1) in aqueous solution at 325 K.⁴⁰

As a prototype experiment to illustrate the information available with ^2H NMR we consider again the model membrane discussed above, i.e., the polypeptide gramicidin A dissolved in different lipids. By chemical methods the gramicidin A molecule was selectively deuterated at the four tryptophan rings ($[(\text{Trp-}^2\text{H}_5)_4]\text{gramicidin A}$).⁴⁰ A dry powder of $[(\text{Trp-}^2\text{H}_5)_4]\text{gramicidin A}$ gives the deuterium spectrum at the top in Figure 7. This spectrum approaches the static limit with virtually no large-amplitude motions of the tryptophan side chains. The spectrum is identical with that of pure tryptophan crystals, and the separation of the most intense peaks in the spectrum equals almost 137 kHz, which is the maximum possible value (based on a quadrupole coupling constant of 183 kHz).

Next, the deuterated gramicidin A was mixed with 1-palmitoyllysophosphatidylcholine in the molar ratio 1:4 in order to yield the fluid bilayer model membrane discussed above. However, the ^2H NMR spectra of this bilayer are also typical of a virtually complete tryptophan side chain immobilization. Moreover, the tryptophan side chains remain static over a range of 70 °C, from -20 to +50 °C. This result is quite unexpected. Despite the demonstrably high fluid nature of the lipids in this bilayer, we observe neither a motion of the tryptophan rings nor a rotation of the peptide as a whole. In contrast, when we incorporated $[(\text{Trp-}^2\text{H}_5)_4]\text{gramicidin A}$ into bilayers of 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (1:15) and raised the temperature above that of the lipid-phase transition, a new motionally averaged component appeared as shown at the bottom of Figure 7. The contribution of this component increased with increasing temperature and disappeared below the lipid-phase transition. This effect can be explained by the onset of rotational diffusion about the peptide long helical axis upon con-

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version of the lipids from the gel state to the liquid crystal state.

The different results obtained with 1-palmitoyllysophosphatidylcholine and 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC) are indicative of differential degrees of self-association of gramicidin A. In the case of the stoichiometric 1-palmitoyllysophosphatidylcholine/gramicidin A model membrane the ^2H NMR data suggest that gramicidin A forms a rigid two-dimensional array of peptides, thereby excluding any lateral or rotational diffusion in the plane of the membrane and also tryptophan ring flipping motions. On the other hand, the interstices between the gramicidin A are apparently large enough to allow an almost unhindered lipid motion and to create a normal lipid bilayer order profile. The tendency of self-association is much reduced in bilayers of 1,2-dimyristoyl-*sn*-glycerol-3-phosphocholine as evidenced by the observation of mobile components in the ^2H NMR spectrum of gramicidin A. Since 1:4 1-palmitoyllysophosphatidylcholine/gramicidin A bilayers form preferentially regardless of the initial lipid/gramicidin ratio,¹⁰ the differences in gramicidin mobility between DMPC and lysophosphatidylcholine membranes must be due to more than simply their different lipid/protein ratios.

At present, ^2H NMR of membrane proteins is at an early stage. The studies on the gramicidin model

membrane suggest that in this particular membrane it is the irregular outer contour of the peptide which matches with the inherent disordering tendency of the lipids. In other cases, yet to be investigated, the outer surface of the protein in the membrane may however be fluidlike, providing a fluid mechanical match with the fluid lipid bilayer.⁶

Most important, however, if a deuterium labeling of the active site of membrane proteins could be achieved, ^2H NMR might provide insight into the mechanistic aspects of such proteins.

Concluding Remarks

^2H NMR can provide insights into the structural and dynamic properties of each membrane component. Studies of the lipid hydrocarbon chains and headgroups have revealed the nature and rates of motions undergone and the effects of proteins, other lipids, and ions. Future work will be directed toward more closely defining the roles of specific interactions in membrane functioning. Studies of membrane proteins, while still relatively new, should provide detailed insights into the motions of proteins and their constituents, how they are affected by lipids and other factors, and, most promisingly, into the mechanism of catalysis and transport.

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Synthesis of Complex Metal Oxides by Novel Routes[†]

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Introduction

A variety of inorganic solids have been prepared in recent years by employing a wide range of conditions such as irradiation by laser or electron beams, use of high pressures, high vacuum, or hydrothermal conditions, rapid quenching of melts, melting in a water-cooled copper container using rf power (skull melting) or in an arc, and so on.¹ Several ways of accomplishing synthesis under mild conditions have been explored,

and of special importance are the energy-saving routes involving low temperatures. In this article we shall present some of the important innovations in the synthesis of complex metal oxides, hoping that the article will also serve to illustrate how solid-state chemists pursue synthesis often employing seemingly simple techniques.

Innovations in the synthesis of complex metal oxides and related materials are necessary for tailor-making solids with the desired structure and properties. It is only by employing ingenious methods that one can prepare solids that are ordinarily metastable or unstable. Low-temperature methods enable the synthesis of several such materials (e.g., ReO_3 -like MoO_3 , $\text{La}_2\text{Co}_2\text{O}_5$, $\text{Ca}_2\text{Mn}_2\text{O}_5$, $\text{Pb}_2\text{Ru}_{2-x}\text{Pb}_x\text{O}_{7-y}$, VS_2 , Mo_6S_8 , and so on). Besides being of academic interest, novel synthesis of metal oxides is of great relevance because of possible applications in crucial sectors such as electronics, communication, and energy. Active metal oxides with controlled particle size are important in catalysis. Metal

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