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Review

Membrane curvature modulation of protein activity determined by NMR☆

Richard M. Epand [a,*], Kenneth D'Souza [a], Bob Berno [b], Michael Schlame [c]

[a] Department of Biochemistry and Biomedical Sciences, McMaster University, 1280 Main Street West, Hamilton, Ontario L8S 4K1, Canada
[b] Department of Chemistry and Chemical Biology, McMaster University, 1280 Main Street West, Hamilton, Ontario L8S 4K1, Canada
[c] Department of Anesthesiology and Cell Biology, New York University School of Medicine, 550 First Avenue, New York, NY 10016, USA

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ABSTRACT

In addition to specific intermolecular interactions, biological processes at membranes are also modulated by the physical properties of the membrane. One of these properties is membrane curvature. NMR methods are useful for studying how membrane curvature affects the binding and insertion of proteins into membranes as well as how proteins can affect membrane curvature properties. In many cases these interactions result in a marked change in protein activity. We have reviewed examples from a range of systems having varied mechanisms by which membrane curvature is linked to protein activity. Among the examples discussed are antimicrobial peptides, proteins affecting membrane fusion, rhodopsin, protein kinase C, phospholipase C-delta1, phosphatidylinositol-3 kinase-related kinases and tafazzin. This article is part of a Special Issue entitled: NMR Spectroscopy for Atomistic Views of Biomembranes and Cell Surfaces. Guest Editors: Lynette Cegelski and David P. Weliky.

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Abbreviations: CSA, chemical shift anisotropy; MAS, magic angle spinning; PIKKs, phosphatidylinositol-3 kinase-related kinases; PLCδ1, phospholipase C-delta1; PKC, protein kinase C; SSB, spinning side band; CL, cardiolipin

☆ This article is part of a Special Issue entitled: NMR Spectroscopy for Atomistic Views of Biomembranes and Cell Surfaces. Guest Editors: Lynette Cegelski and David P. Weliky.

* Corresponding author. Tel.: +1 905 525 9140x22073; fax: +1 905 521 1397.
E-mail address: epand@mcmaster.ca (R.M. Epand).

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1. Introduction and background

1.1. Membrane curvature

The word curvature in relation to membrane structure and properties can be used in two contexts. Most directly, it is used to describe the physical shape of a structure, such as the round shape of a sphere, with the curvature dependent on the size of the sphere. The larger the sphere, the smaller is the physical curvature. Another type of curvature is the intrinsic curvature. This curvature is the one that would be adopted if there was no force or physical constraint limiting the shape of the object. For example, if one had a ball or sphere made of an elastic material and the sphere was stretched into an oblate ellipsoid, it would have altered physical shape, but the intrinsic curvature, which is a property of the material, would remain the same. The difference being that as a sphere the material is "relaxed" and the physical curvature is similar to the intrinsic curvature, but under stress as an oblate ellipsoid the system is no longer at equilibrium, but is under stress and will eventually relax back to a sphere.

The difference between physical and intrinsic curvature has particular ramifications for biological membranes. The predominant structure formed by polar lipids in biological membranes is a bilayer. A phospholipid bilayer is composed of two juxtaposed monolayers having the phospholipid polar headgroups oriented at the membrane–water interface on opposite sides of the bilayer. If the bilayer is symmetrical in lipid composition, then the entire bilayer will tend to have a flat, planar structure with no tendency to bend, since bending in one direction will produce opposite kinds of curvature for each monolayer. Hence, if the intrinsic curvature of the component lipids drives them to form a curved structure, such a structure cannot be achieved as long as a bilayer structure is maintained, since relieving the curvature strain on one monolayer will cause increased strain on the other.

1.1.1. Mean and Gaussian curvature

For each point on a curved surface, two principle curvatures can be defined by cross-sectioning the point by two planes that are oriented along the two principle directions. For the two principle directions, the intercepts between the planes and the surface are nearly circular in proximity to the point under consideration. The two principle curvatures κ_1 and κ_2 are defined as the reciprocal of the radii of the two circles [1]. A diagram illustrating the principle curvatures at a saddle point is illustrated in Fig. 1. The mean curvature, H, is $(\kappa_1 + \kappa_2) / 2$. The Gaussian curvature, K, is the product of the two principle curvatures, i.e. $K = \kappa_1 \kappa_2$. The Gaussian curvatures of simple objects are shown in Fig. 2. The mean curvature is easier to measure than the Gaussian curvature [2,3] and most discussion about the curvature modulation of membrane events refers to mean curvature. However, Gaussian curvature plays a particularly important role in the membrane fusion and in the formation of bicontinuous cubic phases (see below). The signs of H and K for different lipid phases are shown in Table 1.

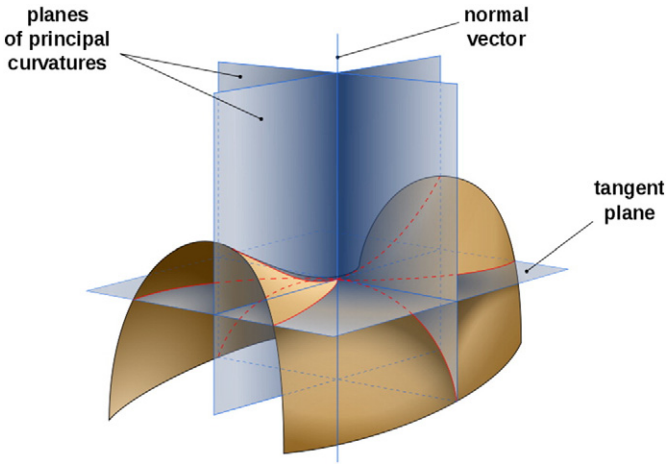

Fig. 1. Diagram showing principle axes of curvature of a saddle point having negative Gaussian curvature. Taken from http://en.wikipedia.org/wiki/Principal_curvature.

1.1.2. Membrane curvature and lipid polymorphism

The bilayer is a flat structure with no mean or Gaussian curvature. However, when the intrinsic curvature of each monolayer becomes sufficiently great, the bilayer will become destabilized and the structure will rearrange to one in which each monolayer acquires curvature. The most common new phases that are formed include the micellar phase, the cubic phase and the inverted hexagonal phase. Phases can be classified as type I, also called "normal" phases, or type II, also called "inverted" phases. The difference between the two is that in type I phases each phospholipid molecule is oriented with the polar headgroup facing away from the structure, i.e. at the lipid–water interface. In contrast, the orientation of the lipid is opposite in type II phases, with the headgroup pointing inward toward the interior of the structure. While many morphologies of both type I and type II phases are possible and several have been observed, we will describe the most common phases observed.

A common type I phase is that of spherical micelles. This phase is formed in excess water from lipids having a high positive intrinsic curvature. The polar headgroups of the lipids are on the surface of the micelle.

Among type II phases, the hexagonal or H_{II} phase and the cubic phase have been studied most extensively. The H_{II} phase is useful as a tool for measuring the mean curvature of a monolayer from the

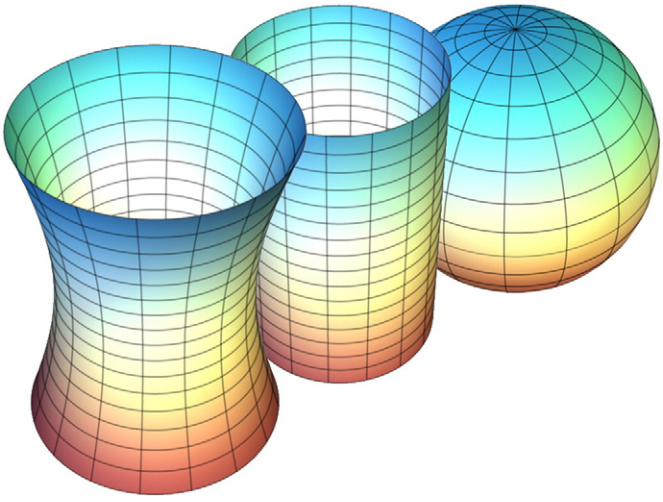

Fig. 2. Shapes of objects and Gaussian curvature. From left to right: a surface of negative Gaussian curvature (hyperboloid), a surface of zero Gaussian curvature (cylinder), and a surface of positive Gaussian curvature (sphere). Taken from http://en.wikipedia.org/wiki/Gaussian_curvature.

Table 1
Mean (H) and Gaussian (K) curvatures of commonly observed lipid phases.

Phase	H	K
Bilayer	Zero	Zero
Micelle	Positive	Positive
Inverted hexagonal	Negative	Zero
Inverted cubic	Zero	Negative

diameter of the hexagonal phase cylinder. To do this precisely, a correction must also be made for hydrocarbon packing constraints. The elastic bending modulus can also be determined from experiments in which the osmotic pressure of the solution surrounding the aggregate of hexagonal phase cylinders is changed [4].

The cubic phase is really a group of structures having in common that the overall pattern exhibits cubic symmetry, as can be shown by diffraction methods [5]. Type II cubic phase can be formed from an assembly of inverted micelles arranged with cubic symmetry or as a bicontinuous cubic phase. In bicontinuous cubic phases the lipid is arranged as a curved bilayer in one of the several geometric arrangements having cubic symmetry. An example of a bicontinuous cubic phase is shown in Fig. 3. Among the most common bicontinuous cubic phases are those with symmetry elements belonging to the space groups Pm3n or Ia3d. Cubic phases are often formed as intermediates between bilayer and hexagonal phases. Unlike the H_{II} phase, the cubic phase has no hydrocarbon packing voids. Bicontinuous cubic phases have a large Gaussian curvature. The energetics of cubic phase formation has been studied as a model for membrane fusion intermediates [6–8].

1.1.3. Enzyme activity and membrane curvature

The importance of membrane curvature in the modulation of the activity of enzymes and more generally of biological processes, has been of interest for several years [9]. Our understanding of the mechanism by which membrane curvature is coupled to enzymatic activity has been evolving. Some of the physical properties of membranes that may be coupled with activity include the physical and intrinsic curvatures of the membrane as well as properties that are affected by membrane curvature, including membrane defects and membrane hydration (Fig. 4). The most direct control of enzyme activity by membrane intrinsic curvature has been shown for CTP: phosphocholine cytidylyltransferase [10,11] and rhodopsin [12–14]. There are also other enzymes, such as protein kinase C, whose activity is affected by binding to membranes with different curvature properties [15,16]. However, in the case of protein kinase C the correlation appears to be mediated through a curvature modulation of membrane interfacial polarity [17]. Interfacial polarity refers to the dielectric constant at the membrane–water interface. It is a property that is indirectly related to membrane curvature. Lipid headgroups that do not strongly bind to water, such as phosphatidylethanolamine, often exhibit inter-lipid H-bonding. Such interfaces have low polarity and are more compressed, hence contributing to the negative curvature of the lipid. The opposite happens with polar membrane interfaces, such as phosphatidylcholine, that expand by binding more water and hence have more positive curvature. Lastly, there is an example of tafazzin whose activity and substrate specificity are determined by the curvature properties of the membrane, apparently driven by changes in lipid packing [18]. It was shown that tafazzin was more active in micelles or in hexagonal phase aggregates than in flat bilayers. Thus tafazzin is active only in membranes with either positive or negative physical or intrinsic curvature. It was suggested that this behavior could be explained by the presence of "defects". The term defect is not very specific and more study is required of the relationship between tafazzin activity and membrane properties. The present review will focus on the application of NMR methods to the understanding of the relationship of membrane curvature properties and enzymatic and other biological activities.

1.1.4. Membrane fusion and curvature

Membrane fusion is an important biological process. It is necessary for the entry of enveloped viruses into cells, for the fertilization of an egg by sperm and for the maturation of bone and muscle resulting in the fusion of cells to form polykaryocytes, to name only a few examples. Biological fusion processes are generally facilitated by proteins. In order for two planar bilayers to fuse there must be bending of their constituent monolayers [19]. NMR is a good technique to measure changes in lipid arrangements and also to monitor how proteins and peptides

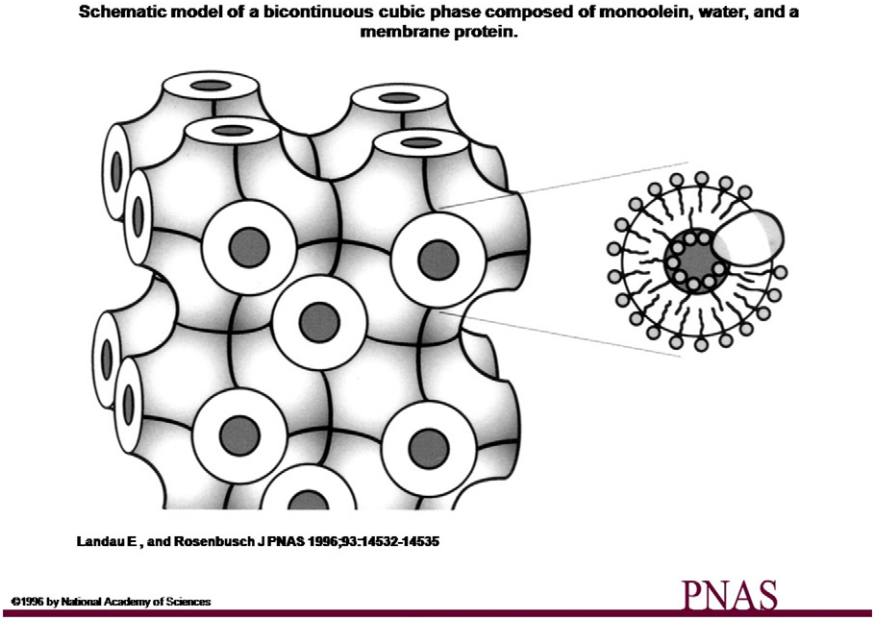

Fig. 3. Schematic model of a bicontinuous cubic phase composed of monoolein, water, and a membrane protein. The matrix consists of two compartments, a membrane system with an infinite three-dimensional periodic minimal surface (*Left*), interpenetrated by a system of continuous aqueous channels (shown in black). The enlarged section (*Right*) shows the curved lipid bilayer (with an inserted membrane protein molecule) enveloping a water conduit. In a cubic phase consisting of 60–70% (wt/wt) monoolein or monopalmitolein and water, hydrophobic proteins diffuse laterally in the bilayer, while water-soluble components diffuse freely through the intercommunicating aqueous channel system. Taken from Proc. Natl. Acad. Sci. USA, 93, 14532-14535 (1996).

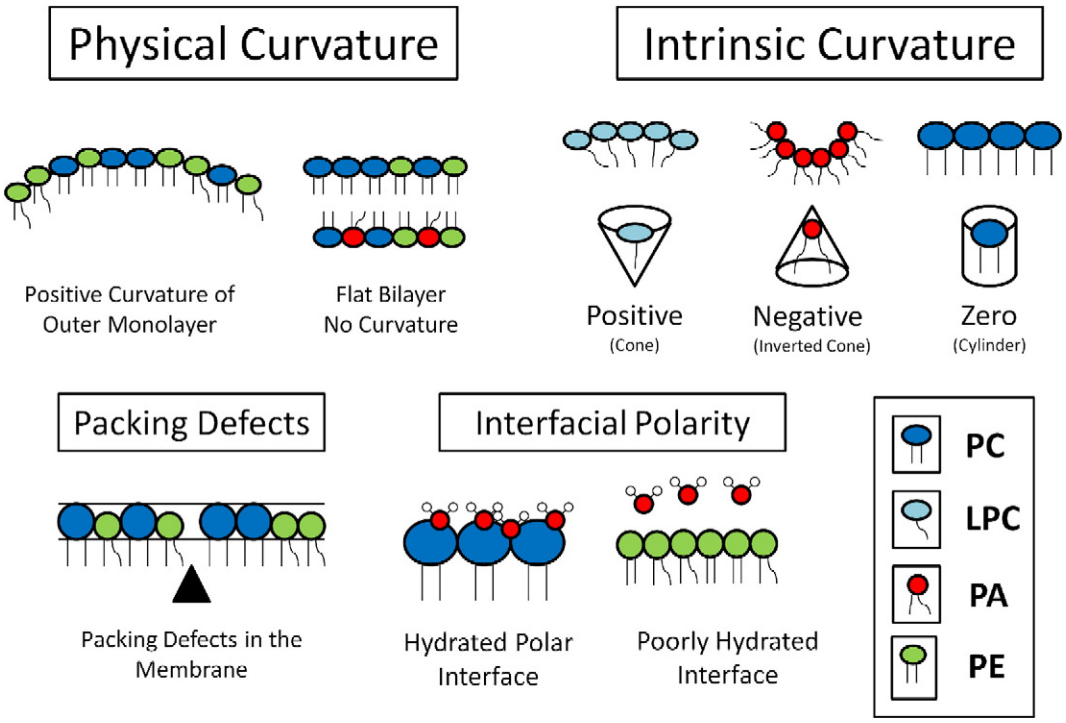

Fig. 4. Direct and indirect relationships between membrane curvature properties and physical properties of membranes that may affect biological activity. Physical curvature relates to the shape of the whole membrane particle. Some proteins are sensitive to physical curvature. Intrinsic curvature of a lipid in the bilayer phase indicates the shape one monolayer of the bilayer would adopt if it were not constrained to be juxtaposed opposite another monolayer. Intrinsic curvature can contribute elastic bending energy that can modulate membrane function. Packing defects can arise when there are components of bilayers that have propensity for forming other phases because of their intrinsic curvature. Interfacial polarity is affected by the extent to which lipid headgroups interact with water. This feature also affects the intrinsic curvature.

affect some lipid properties as well as how lipid binding affects protein conformation. This will allow for the elucidation of the nature of the interaction of fusion peptides with membranes as well as monitoring the appearance of non-lamellar intermediates. In this review we will discuss some specific examples of such systems.

1.2. Lipid polymorphism and ^{31}P NMR

There are many ways to measure lipid polymorphism including X-ray diffraction, NMR, DSC and others. In the current review we will focus on NMR methods. ^{31}P NMR is particularly attractive for the measurements of lipid organization. Most lipid components of membranes contain one phosphorous atom, so that the assignment of the resonance is generally trivial. In addition, ^{31}P NMR has a very large Chemical Shift Anisotropy (CSA) that is sensitive to the rate of motion of the lipid as well as the geometrical properties of the membrane. The CSA is averaged out for rapidly tumbling molecules, leaving a comparatively narrow isotropic peak. Thus the sign and magnitude of the CSA can be used to identify the nature of the lipid curvature and phase (Fig. 5).

1.2.1. MAS and static powder patterns

There are two classes of NMR measurements that can be used for measuring the ^{31}P NMR of suspensions of lipids or lipid–protein mixtures as non-oriented samples, static measurements and measurements with magic angle spinning (MAS). In the latter method, the sample is placed in a rotor that is made to spin on its axis at the "magic angle" with respect to the magnetic field direction. This fixed angle is 54.74° with respect to the direction of the external magnetic field. By spinning the sample at the magic angle, the (x, y, z) orientations of the nuclei are averaged out (similar to rapidly tumbling molecules), so that spin–spin interactions, that scale as 3 $\cos^2\theta$-1, go to zero. Under very rapid spinning conditions, the spectrum resembles a solution spectrum where only the isotropic chemical shift is observed. When the spinning rate is slower than the frequency width of the CSA, spinning side bands (SSBs) are observed on either side of the isotropic peak. The advantage of MAS NMR is that NMR resonances of solids become sharper and as a result the detection becomes more sensitive. In addition, the chemical shift anisotropy (CSA) can be more accurately determined from the position and magnitude of a series of spinning side bands (SSB) that also varies as a function of the rate of spinning. A recent study of the phase behavior of lipid mixtures using ^{31}P MAS NMR was based on measuring the CSA [18].

The CSA can also be measured from the width of a static powder pattern (Fig. 5), but because these powder patterns are often very broad it can be difficult to precisely determine the limits of the powder pattern that will also be sensitive to the phasing of the spectra. In addition, it is more difficult to resolve contributing components in overlapping broad resonances found in static powder patterns.

Nevertheless, there are some advantages to using static powder patterns [20–23]. NMR instruments capable of measuring MAS spectra are less common than static NMR machines and they are generally of lower field strength and do not have cryo-probes. All of these factors contribute to making static powder patterns measured in liquid state NMR instruments more sensitive and of higher resolution. There is also a second advantage of using static powder patterns. It is for detecting minor components having a CSA close to zero. With MAS NMR, the center peak and all the side bands will be sharp peaks with a high intensity, making it more difficult to distinguish a single peak resulting from isotropic motion. In contrast, with static powder patterns, most phospholipid phases will give rise to broad peaks so that a single sharp component can readily be detected because of its greater intensity, even though the area of this peak may be much less than that of an accompanying broad powder pattern.

1.2.2. Positive and negative CSA and CSA conventions

The CSA is described mathematically by a second rank tensor. Generally, one is able to describe the CSA tensor using three principal components in a coordinate axis frame. There are three main conventions used

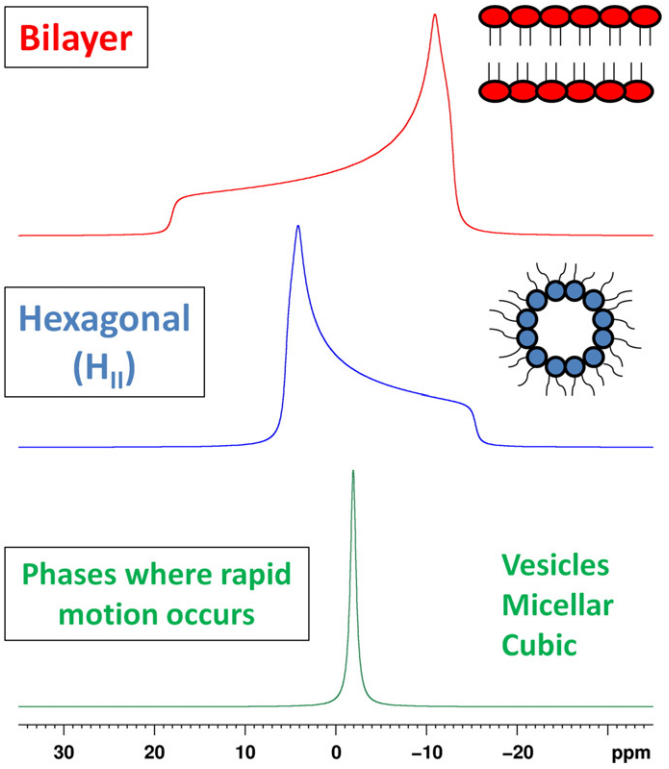

Fig. 5. ^{31}P NMR spectra of different lipid phases. In the case of an isotropic signal, it can arise from a variety of structures.

to describe CSA; we use the Haeberlen convention [24–26]. Using this convention, the sign of the CSA tensor can be either positive or negative. In general, flat bilayers have a positive CSA as do structures having a positive physical curvature. A bilayer static powder pattern has a characteristic shape and the CSA from static or MAS NMR is about +40 ppm. In contrast, the CSA from the inverted hexagonal, H_{II} phase, has a negative CSA of about −20 ppm. While the relationship between CSA and lipid phase is well established, it should be kept in mind that the CSA is determined by the rate of motion of the phospholipid around the long axis of the molecules and by the angle of the P–O bonds of the phosphate with respect to the aqueous interface. It has been shown that certain headgroup motions can give rise to alterations in the classical CSA patterns [27–29].

1.2.3. Origins of isotropic peaks

Narrow single ^{31}P NMR resonances having a low CSA and no side bands in MAS, are often referred to as isotropic peaks, indicating that they arise from isotropic motion. Such peaks have a similar appearance in both MAS and static NMR. They suggest that the CSA has been averaged to zero by molecular motion. This motion can be of the entire lipid-containing particle. Therefore, structures such as micelles, small unilamellar vesicles and small membrane fragments will give rise to an isotropic ^{31}P NMR signal. In addition, rapid spatial reorientation by diffusion of a molecule within the structure, rather than by the structure itself, will also result in a sharp line NMR peak with a CSA of zero. This will occur in the cubic phase or in a highly curved structure that is part of a larger structure. This is part of the problem with isotropic peaks. Although they can represent structures that are functionally important, they can arise from very different molecular arrangements. The only requirement is that there is a complete motional averaging of the CSA within the lifetime of the spin state.

1.2.4. Oriented membranes

Static powder patterns are generally broad spectra that are the result of an overlap of many sharper spectra of individual molecules, each molecule having an orientation at a different angle with respect to the external magnetic field. Not all orientations are equally probable, but depend on the shape of the entire particle. The familiar shape of a powder pattern that is characteristic of the lamellar phase requires certain conditions to be met. The lipid bilayer must be arranged as a sphere or as a series of concentric spheres as in an MLV. This assures a particular relative probability of the spatial orientation of phospholipid molecules with respect to the external magnetic field, e.g. there will be more phospholipid molecules around the rim of the sphere than in a dimension along the direction of the magnetic field. Thus the number of phospholipid molecules at each orientation will not be the same, giving rise to an asymmetric powder pattern. The shape of the powder pattern also requires that there is a rapid rotation of individual molecules around their long axis. These broad spectra can be sharpened by using a sample in which the molecules are all oriented in the same way. There are two general methods for preparing oriented membrane samples. One method is by depositing membrane bilayers on the surface of a solid support [30]. The solid support can then be mounted inside the cavity of the NMR magnet and oriented at any desired angle. Because NMR is not a very sensitive method, in order to have sufficient sample, stacks of membrane-coated solid supports are used. The other method for getting oriented samples is with the use of bicelles [31]. These bicelles are discoidal fragments of membrane bilayer that are stabilized at the edge of the disk by some amphiphile that can provide positive curvature. A common bicelle used for NMR is composed of a flat disk containing a long chain phospholipid(s) with a smaller fraction of a short chain phospholipid that will sequester to the edge of the disk. These bicelles will spontaneously orient in the presence of a strong external magnetic field because of the dipole moment of the lipid. Hence they are more convenient than physically oriented bilayers and can easily be scaled up to get more material into the NMR cavity. There are many variations of these bicelles, including the use of lanthanide ions to alter the dipole of the bicelle and hence its orientation in the magnetic field. The shortcoming of this method is that it requires all of the amphiphile with positive curvature to be at the edge of the bicelle and not part of the planar membrane bilayer. Furthermore, it requires that the added material of interest to be studied (e.g. a protein) partitions into the bilayer portion of the bicelle and is not sequestered to the edge. Two problems would arise if the molecule of interest would sequester to the edge of the micelle. First the molecule of interest would be in the environment of the minor lipid having positive curvature and not the major lipid that makes up the oriented bilayer. Furthermore, the edge of the disk is rounded; thus the molecule would not have a unique orientation with respect to the external magnetic field.

1.3. Lipid polymorphism and ^{2}H NMR

In addition to ^{31}P NMR, ^{2}H NMR can also be used for membrane phase determination that can be related to membrane curvature tendencies. For the study of phospholipid phase behavior, most often lipids with completely deuterated acyl chains are used. The order parameter at each position in the acyl chain can then be determined from the nuclear-quadrupole splitting. A model study using membrane bilayers with different curvatures as a result of them adhering to glass beads of different diameters has shown that changes in membrane physical curvature can be determined from ^{2}H NMR [32].

2. Examples of curvature modulation detected by NMR

We will review several classes of membrane proteins and discuss how their function relates to the manner in which they modulate membrane curvature tendencies or conversely, how membrane curvature affects the properties of a membrane protein. We have chosen several examples to illustrate diverse relationships of protein activity to membrane curvature and how NMR methods helped to elucidate this. The

examples discussed are not comprehensive of all relevant systems reported in the literature, but rather are illustrative.

2.1. Antimicrobial peptides

Studies with ^{2}H NMR and ^{31}P NMR showed that the synthetic peptide MSI-367, [$(KFAKKFA)_3$-NH_2] is sequestered at the water–membrane interface. Preferential increase in the cross-sectional area at the membrane interface/phospholipid headgroup region, would expand this region to produce positive curvature. This was confirmed by DSC measurements showing that the peptide inhibited the conversion of the lamellar phase of phosphatidylethanolamine to the hexagonal phase [33], demonstrating the ability of the peptide to inhibit the formation of structures with negative curvature.

Another study compared the 33-residue-long cationic peptide, Dermaseptin B2, with a C-terminal truncated analog of this peptide having only 23 residues [34]. It was shown that in a membrane with strong positive curvature, the full length 33-residue peptide could adopt a flexible helix-hinge-helix structure that allows the peptide greater insertion into the membrane than occurs with the truncated version. The pivotal plane of the bilayer, i.e. the localization in the monolayer that does not experience a change in cross-sectional area when the monolayer bends, is located near the headgroup. Hence if a longer peptide has more penetration below the pivotal plane, it will then promote greater negative curvature by preferentially expanding the center of the bilayer. The observation that the shorter 23-residue peptide is virtually inactive against bacteria, could be explained by a loss of curvature effect on the bacterial membrane.

Another feature of peptides that can result in the changes in membrane curvature is mismatch between the length of the peptide and the thickness of the bilayer. This feature has been shown, in part, by ^{31}P NMR to result in the promotion of negative curvature with a model WALP peptide [35] and in another study using gramicidin [36].

NMR measurements of peptides interacting with oriented planar bilayers can be used to measure the orientation of the peptide with respect to the plane of the membrane. One such study used ^{2}H NMR to determine the insertion of MSI-103 (sequence [$KIAGKIA]_3$-NH_2) in the membrane as a function of the properties of the membrane lipids [37]. It was found that with membranes having a negative curvature tendency, the peptide remained in a surface-bound state, while in membranes with positive curvature lipids the peptide adopted an oblique conformation with the peptide embedded in the membrane. It was concluded that curvature properties determined the orientation of the peptide and its insertion into the membrane, while electrostatic interactions promoted membrane binding but did not affect peptide orientation.

Another application of using NMR to study peptide helix orientation was undertaken to study the mechanistic basis of the synergistic activity between PGLa and magainin 2 [38]. ^{15}N NMR was used to study the orientation of these amphipathic helical cationic peptides alone and in combination using solid state NMR with oriented membranes. Using lipids with negative spontaneous curvature these peptides, either alone or in combination, remained on the membrane surface parallel to the plane of the membrane. However, in membranes with positive spontaneous curvature, PGLa alone has a tilted orientation, but in combination with magainin 2 it inserts into the bilayer with a transmembrane orientation. In these membranes magainin 2 remains largely on the membrane surface either by itself or in combination with PGLa. It was concluded that curvature-dependent helix orientation is a general feature of membrane-bound peptides that also influences synergistic intermolecular interactions [38].

^{31}P MAS/NMR has been used to demonstrate lipid clustering [39]. This rearrangement of anionic lipids on the bacterial membrane surface has been suggested to contribute to the antimicrobial action of peptides and their species specificity [40]. The segregation of lipid components may contribute to promoting membrane curvature and the conversion of bilayers into cochleates [41]. The complexes of antimicrobial peptides and lipids in the form of cochleates have been shown to be effective in reversing multidrug resistance in bacteria [42,43].

2.2. Membrane fusion

Membrane curvature is essential to allow membrane fusion to progress. However, it is difficult, if not impossible, to obtain information about the molecular details of fusion intermediates using NMR. These intermediates are generally transient and of higher energy. However, the nature of the membrane insertion of fusion peptides can be determined by NMR. In addition, the effect of a fusion peptide or protein on the physical properties of the membrane can also be assessed by NMR. The changes in properties are usually measured with ^{31}P NMR and are reflected by either an increase in the amount of isotropic signal or by measuring a change in the temperature at which the lamellar phase converts to the inverted hexagonal phase. The appearance of an isotropic peak has often been shown to correlate with increased rates of membrane fusion. Similarly, a lowering of the bilayer to hexagonal phase transition temperature, as can be measured by ^{31}P NMR, has also been associated with increased fusion rates [44]. In contrast, positive curvature agents can inhibit fusion [45].

Intermediates in membrane fusion have been suggested to resemble structures joining unit cells in bicontinuous cubic phases [46]. Such phases, as well as the proposed fusion intermediates, would give rise to narrow ^{31}P NMR spectra. The effects of single chain lipids on the rates of membrane fusion promoted by phospholipase C was measured [47]. These fusion rates were compared with the effects of these single chain lipids on the formation of isotropic phases. It was found that membrane fusion inhibitors raise the transition temperature to the isotropic phase, while lipids that promote fusion decrease this transition temperature [47]. Furthermore, fusion rates showed a maximum at the lamellar to isotropic transition temperature. These results support the involvement of inverted lipid structures that give rise to isotropic ^{31}P NMR signals as intermediates in membrane fusion [47]. There are also viral fusion peptides that promote isotropic ^{31}P NMR signals, including simian immunodeficiency virus [48], feline leukemia virus [49] and Sendai virus [50]. It has been shown for the influenza virus fusion peptide that effects of varying pH or changing amino acid residues have effects on the formation of inverted phases that parallel their effects on fusion rates [51]. Interestingly, in one study it was shown that the extent of lowering the phase transition temperature to the inverted hexagonal phase correlated better with increased rates of membrane fusion than did the appearance of an isotropic phase [52].

The segregation of phosphatidylserine by Ca^{2+} in the presence of segments of synaptotagmin 1 can be shown by specific changes in the ^{13}C chemical shifts of phosphatidylserine acyl resonances that do not occur with phosphatidylcholine, the second lipid in the mixture [53]. These findings were used as a basis for suggesting that synaptotagmin 1 could facilitate SNARE-mediated membrane fusion by separating phosphatidylserine from other lipids, which may result in local changes in membrane curvature.

The M2 protein of influenza A is a proton channel that facilitates viral budding from the host cell. Using ^{31}P and ^{13}C NMR with oriented membranes, it was shown that the segment of residues 21–61 causes a high curvature isotropic phase in both virus mimetic, cholesterol-rich model membranes as well as in simple dimyristoylphosphatidylcholine bilayers [54]. Both the lamellar and isotropic domains have distinct isotropic ^{31}P NMR chemical shifts, suggesting perturbation of the lipid headgroup conformation by the amphipathic helix component of this protein. Using proton T_2 relaxation rate measurements, it was demonstrated that the peptide binds preferentially to the high curvature membrane domain. A strong negative correlation was found between high membrane curvature and drug binding of the transmembrane segment of this protein. It was concluded that the amphipathic helical segment promotes membrane curvature, which perturbs the transmembrane helical conformation and abolishes drug binding [54].

2.3. Rhodopsin

There has been considerable interest in understanding how the curvature properties of the membrane environment affect the photoactivation of rhodopsin. Rhodopsin belongs to a group of membrane proteins that have seven transmembrane helical segments. Order parameter profiles of lipid acyl chains in membranes with rhodopsin, have been derived from nuclear-quadrupole interactions using ^{2}H NMR. It has been found that the surrounding lipids exhibit hydrophobic matching with the non-polar interface of the protein [12,14]. Hydrophobic matching is a phenomenon in which the thickness of the lipid bilayer is adjusted by gauche-trans isomerization of the C – C single bonds in the acyl chain so that the bilayer thickness matches the non-polar interface of the protein. It was concluded from both the ^{2}H NMR data and from molecular modeling, that rhodopsin activation could be explained by a flexible surface model involving spontaneous monolayer curvature [12,14]. However, subsequent studies using membranes containing several different lipids with known curvature properties, suggested that other features contributed to the equilibrium between the MI and MII intermediates in the photocycle [13]. Thus, although membrane curvature is important, additional factors, such as the ability of ethanolamine headgroups to hydrogen bond to the protein, also contributed to the conformational equilibrium of rhodopsin.

2.4. Enzymes

The activities of several enzymes that associate with membranes are modulated by the curvature properties of the membrane. In the present review we have selected some examples that used NMR as part of the evidence for a role of curvature in the membrane. Perhaps the best example of an enzyme activity that is directly coupled to intrinsic curvature strain is CTP: phosphocholine cytidylyltransferase [10,11]. However, this example will not be discussed because NMR was not a primary method for determining the curvature properties. We review several other examples, for which NMR was an important tool to elucidate the mechanism that relates enzyme activity to curvature properties of the membrane.

2.4.1. Protein kinase C (PKC)

Based on the correlation between the effect of membrane additives on the activity of PKC and the effect of these same additives on membrane curvature properties, it was concluded that membrane curvature played a role in the modulation of enzyme activity [16]. This relationship was also used to explain the synergistic effects of diacylglycerol and unsaturated fatty acids on PKC activity [55]. The effects of fatty acids and diacylglycerol on membrane structure were assessed using ^{31}P and ^{2}H NMR [55]. It was subsequently shown that the effects of membrane curvature on the enzyme were not direct, but rather dependent on changes in the interfacial polarity of the membrane (see Fig. 4 for illustrations of the various mechanisms by which membrane physical properties related to curvature may affect function) that accompanied curvature changes [56].

2.4.2. Phospholipase C-delta1 (PLCδ1)

Studies were performed to monitor the effect of curvature on the penetration of a segment of PLCδ1 into the membrane [57]. PLCδ1 has a PH domain that interacts with the headgroup of phosphatidylinositol-4,5-bisphosphate (PIP2) at the membrane interface and plays a major role in the membrane binding of this protein. The PH domain of PLCδ1, which corresponds to the first 140 residues, also contains an amphipathic α2-helix that contributes non-specific hydrophobic interaction with the membrane. A peptide corresponding to this PH domain was biosynthetically labeled with ^{13}C-Ala at position 112. The interaction of this protein fragment with membranes was assessed using changes in the chemical shift of the ^{13}C-labeled peptide as a function of the radius (1/curvature) of the lipid aggregate. There was a progressive change in the chemical shift of the Ala112 ^{13}C resonance in going from micelles to small unilamellar vesicles (SUVs) to large multilamellar vesicles (MLV). The results indicated that the location of the α2-helix was sensitive to changes in the curvature of the lipid bilayer surface. It was suggested that changes in the conformation of the PH domain could contribute to the mechanism determining the intracellular localization of PLCδ1 [57].

2.4.3. Phosphatidylinositol-3 kinase-related kinases (PIKKs)

The family of PIKK enzymes contains a common domain, the FATC domain that is rich in aromatic residues and can interact with membranes. Sensitivity of the conformation of this domain to the organization of lipids was determined by 2-D NMR measurements using ^{13}C, ^{15}N, and ^{1}H NMR methods. Lipid was organized in different morphologies including spherical micelles, bicelles and liposomes [58]. It was concluded that membrane curvature was one of the factors determining the interaction between the protein and the membrane.

2.4.4. Tafazzin

Tafazzin is a non-specific phospholipid-lysophospholipid transacylase. Despite the fact that this enzyme can catalyze a large variety of acyl transfer reactions between different phospholipid and lysophospholipid classes, mutation of this protein in humans results specifically in defective remodeling of cardiolipin (CL). In particular, in mammalian cardiac muscle, acyl transfer processes catalyzed by tafazzin result in the enrichment of CL with linoleic acid. This and other results suggest that the substrate specificity of tafazzin depends on its environment, most likely the physical properties of the membrane to which the enzyme binds.

Recently a study was undertaken to determine the role of membrane curvature on the extent and specificity of the acyl transfer reaction catalyzed by tafazzin [18]. Interestingly, it was found that the extent of reaction was lowest in stable bilayers. However, increased reaction was observed both with lipid mixtures having negative curvature, such as lipids in the hexagonal phase, as well as with lipids having positive curvature such as micelles. It was concluded that the extent of reaction catalyzed by this enzyme required defects in the membrane, i.e. perturbations in the packing order induced by positive or negative curvature. A lower packing order is likely to promote the mixing of acyl donors and acceptors in spite of widely different phase preferences of the two. It was shown that curvature also governed the specificity of acyl transfer when there was competition between two possible acyl donor lipids [18].

The predominant lipid phase in biological membranes is the bilayer. This then raises the question of how tafazzin is activated in the mitochondria. One possibility is that there is a sufficient curvature in the cristae of the inner mitochondrial membrane to promote tafazzin activity. For instance, cristae tips and cristae junctions provide membrane segments with high positive and negative curvature (order of magnitude of 10^{8} m^{-1}). Furthermore, hemifusion intermediates that are produced by the fission-fusion cycle as well as outer–inner membrane contact sites that may contain highly curved domains.

Although it is clear that mitochondria contain membranes with curved shapes, it is not clear whether there are domains that are sufficiently curved to be categorized as a non-bilayer membrane segment. Earlier studies using static ^{31}P NMR on intact mitochondria suggested that there was a small fraction of the organelle that gave rise to an isotropic signal [59,60]. We repeated this experiment using fresh rat liver mitochondria and modern NMR instrumentation with a Bruker AVANCE-III 700 MHz spectrometer (^{31}P frequency, 283.4 MHz) equipped with a QNP-cryoprobe. We observed a minor isotropic component at 37 °C that disappeared when the sample was cooled to 0 °C (~ −0.5 ppm). However, the magnitude of this isotropic component showed some variation in area among preparations. In addition, treating the mitochondria to hypotonic lysis in order to remove ^{31}P NMR peaks from small, water soluble phosphate-containing compounds, did not eliminate the isotropic peak. Since this hypotonic treatment is expected to destroy the morphology of the mitochondria and the regions of high

curvature of the inner membrane, we concluded that it was likely that the peak arose from a membrane fragment or vesicle, whose rotation decreased at low temperature, therefore broadening the peak. In addition, we prepared mitochondria from a rat heart following the procedure of Saks et al. [61]. To our knowledge there are no published ^{31}P NMR spectra of intact heart mitochondria. Heart mitochondria have a much more uniform acyl chain composition of their cardiolipin, with about 85% of the acyl chains of CL being linoleoyl. The larger extent of remodeling of CL in heart mitochondria suggests that tafazzin should be more active in this organ than in liver mitochondria and therefore have more deviations from a stable bilayer. However, the ^{31}P NMR static powder pattern of the heart mitochondria at 37 °C showed almost no presence of an isotropic component (not shown). Despite this result, we believe that the question remains open as to whether mitochondria contain non-bilayer membrane domains.

3. Conclusions

It is clear that membrane curvature plays an important role in determining the activity and interaction with membranes of a variety of unrelated enzymes. NMR methods can play a role in demonstrating a dependence of membrane insertion of a protein or its enzymatic activity on the curvature properties of the system. However, NMR is not the only method that can probe curvature properties of lipid assemblies; for instance DSC is useful to establish the temperature of lipid polymorphic changes. On the other hand, NMR can also be employed to partially characterize polymorphic phase transitions. Indeed, structures giving rise to a minor isotropic resonance in ^{31}P NMR, would be difficult to observe by other methods. In DSC, a transition to an inverted hexagonal phase cannot be distinguished from a transition to a cubic phase, while with ^{31}P NMR this difference is quite clear. Also disordered lipid arrangements will not give rise to diffraction and therefore will give no signal in diffraction experiments, but they may give an isotropic ^{31}P NMR resonance line. However, perhaps the greatest potential role for NMR is in determining the conformation and membrane insertion of proteins or protein segments and how these properties relate to the nature and arrangement of the lipids. Such information will contribute to developing an understanding at the molecular level of the interrelationship between protein structure and activity and the physical properties of the membrane in which they insert.

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