Bicelles: a model membrane system for all seasons?

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Introduction

Bicelles are a versatile class of model membranes known to align in magnetic fields. Bicelles were originally developed for use in solid-state NMR studies of membrane-associated molecules. More recently, they have been adapted for use in solution NMR as a way of obtaining access to rich classes of structural data for water soluble molecules and complexes. Additional innovations have brought this class of model membranes to the point where they may also be ripe for exploitation in non-NMR-based structural studies.

What are bicelles?

Bicelles bridge the gap between micelles and model bilayer systems

Bicelles (bilayered micelles; Figure 1) are thought to be aqueous lipid–detergent assemblies in which discrete bilayer fragments are edge-stabilized by certain detergents [1,2]. The systems that have emerged as practical tools for structural studies involve mixtures of dimyristoylphosphatidylcholine (DMPC) with either dihexanoylphosphatidylcholine (DHPC) or the bile-salt derivative, 3-(cholamidopropyl)dimethylammonio-2-hydroxy-1-propanesulfonate (CHAPSO) [3–5]. Mixtures of DMPC with these detergents form bicelles that are magnetically orientable and exhibit liquid crystalline like (L_α phase) bilayer properties over wide ranges of detergent:lipid ratio (roughly 1:2–1:5), water content (roughly 60–97%), buffer pH and composition, and temperature (~30–50°C).

Bicelles represent an intermediate morphology between lipid vesicles and classical mixed micelles, combining some of the attractive properties of both of these model membrane systems. Like micelles, bicelles are noncompartmentalized, optically transparent, and effectively monodisperse. Consequently, it is much easier to achieve homogeneous mixing in bicelles than in lipid vesicles. On the other hand, bicelles have a much lower detergent content than classical mixed micelles and maintain some key bilayer properties that are absent in the latter systems.

How well do bicelles mimic membrane bilayers?

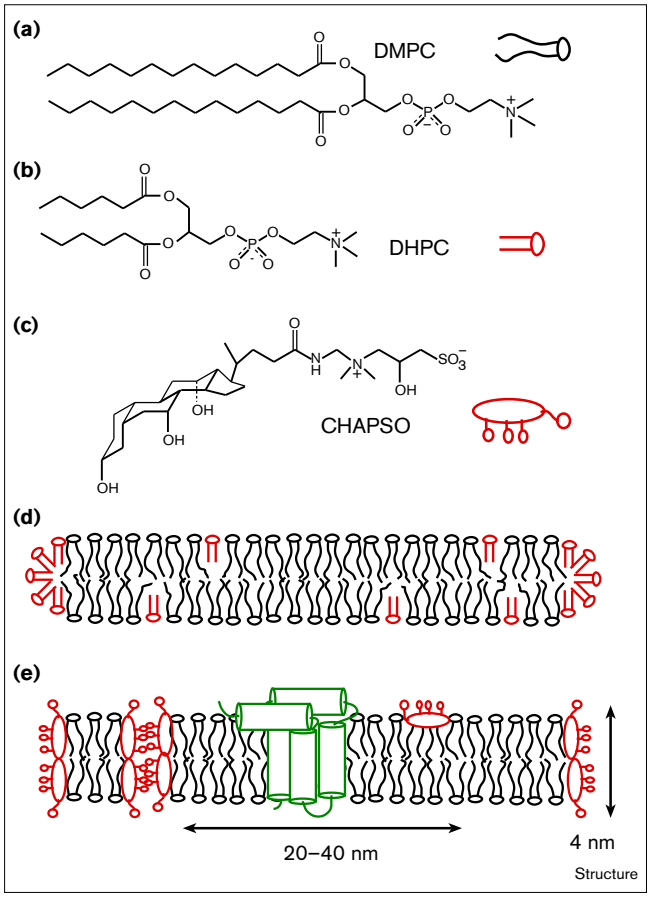
Biological membranes must provide a suitable environment in which to solubilize and maintain membrane protein function, while at the same time presenting an inert, nondenaturing surface to water soluble proteins. The original choices of CHAPSO or DHPC for use in generating bicelles was based, in part, upon the fact that these molecules are mild detergents, because of the zwitterionic nature of their head groups. There is now considerable NMR data [5–7] to suggest that CHAPSO–DMPC and DHPC–DMPC bicelles do indeed provide a generally inert environment for water soluble proteins. In addition, the lactic dehydrogenase/pyruvate kinase reaction coupling system, which is frequently used in kinetic studies of ADP-producing enzymes (kinases, etc.), is catalytically functional in bicelles (CRS, unpublished data). In the case of membrane proteins, there is at least one well-documented example of an integral membrane protein that can be functionally reconstituted into bicelles. *Escherichia coli* diacylglycerol kinase is an integral membrane protein that has three transmembrane spans. This enzyme can be reconstituted into bicelles at levels as high as 1.5 mM, where it is catalytically active and stable for at least 48 h at 40°C [2] (CRS, unpublished data). Nevertheless, it is probable that the existing DMPC-based bicelle mixtures will not prove to be universally suitable for the reconstitution of all integral membrane proteins: some proteins may prefer other classes of lipids, such as those with longer acyl chains and higher degrees of chain unsaturation than DMPC.

The DMPC-rich bilayered domains of bicelles have been demonstrated by NMR to be conformationally and dynamically very similar to DMPC in liquid crystalline phase vesicles [3,4]. In addition, upon cooling, DMPC bicelles undergo a phase transition at room temperature near the liquid crystalline to gel phase transition temperature which occurs for pure DMPC bilayers near 24°C [3–5]. However, the phase that bicelle-forming mixtures adopt below room temperature is isotropic and is not well characterized.

Bicelles provide a solution to the long-standing problem of detergent-induced artifacts in structural studies

Although one might argue that mixed micelles provide a suitable model membrane mimic for structural studies, it has traditionally been very difficult to prove that the presence of detergent is not leading to artifactual structural perturbations. Bicelles offer a solution to this old problem by virtue of the fact that the concentration of detergents present in bicelles is low and, more importantly, can be

Figure 1



Components and cross-section models for ideal bicelles. The chemical structures of **(a)** DMPC, **(b)** DHPC and **(c)** CHAPSO. **(d)** A model DHPC–DMPC bicelle. **(e)** A model CHAPSO–DMPC bicelle depicted to contain a reconstituted integral membrane protein (in green). For both bicelle types it is believed that some detergent partitions into the bilayer domain. In the case of CHAPSO, there are at least two possible modes of bilayer interaction [48]: surface-associated and transmembrane (in the form of an oligomer in which the hydroxyl groups line the inside of a pore). An attempt has been made to draw the bicelles approximately to scale on the basis of the known dimensions of α helices, liquid crystalline phosphatidylcholine molecules [55], the thickness of DMPC bilayers [55] and the probable disc diameter of bicelles [39,40]. The disc diameter illustrated is 250 Å (to scale), but can be varied experimentally by adjusting the detergent:lipid ratio.

varied by a factor of two or more without significantly altering essential bicelle properties [1,5]. Structural measurements can therefore be made at multiple detergent:lipid ratios to verify that key observations are independent of detergent concentration. Even in cases where a detergent concentration dependence is observed, it is possible to minimize artifacts by extrapolating key observations to zero detergent content. This approach has been demonstrated in studies of both membrane lipids [8,9] and of surface-associated proteins/peptides [2].

Bicelles can be magnetically aligned

Much of the original impetus for developing the DHPC–DMPC and CHAPSO–DMPC bicelle systems originated from the knowledge that discoidal hydrocarbon assemblies can be uniaxially oriented in a strong magnetic field with their bilayer normals perpendicular to the magnetic field [10–12] (see Figure 2). Alignment is a consequence of the interaction of the field with the aggregate diamagnetic susceptibility tensor of bicelles, and is critically related both to the number of lipids per assembly and to interassembly interactions. Alignment can be accomplished even at the very modest field strengths attainable with small permanent magnets [13].

Bicelles bridge the gap between solid-state NMR and solution NMR

Solid-state NMR parameters for structural determination and refinement

The emergence of bicelles as a tool for use in structural biology is coupled to the access their use provides to the facile measurement of two structurally useful NMR spin interactions that are central to solid-state NMR (for a broader review, see [14]). The first of these is dipolar coupling. Dipolar coupling is very different from scalar coupling because it is through space, rather than through bond, in nature. For any pair of spin 1/2 nuclei, dipolar coupling can be described:

$$\text{observed coupling} = D_{ij} \cdot \langle(3\cos^2\theta - 1)/r^3\rangle \quad (1)$$

where D_{ij} is a constant depending primarily upon the isotopic pair involved, r is the internuclear distance and θ is the angle between the internuclear vector and the experimental director (which is either the magnetic field direction or the bilayer normal, depending upon the particular experiment). The brackets (< >) indicate that any motions more rapid than D_{ij} (typically in the order of 10 kHz) will lead to averaging of the term. With rapid isotropic motion, this term averages to zero; hence, dipolar coupling is not usually directly observed in solution NMR.

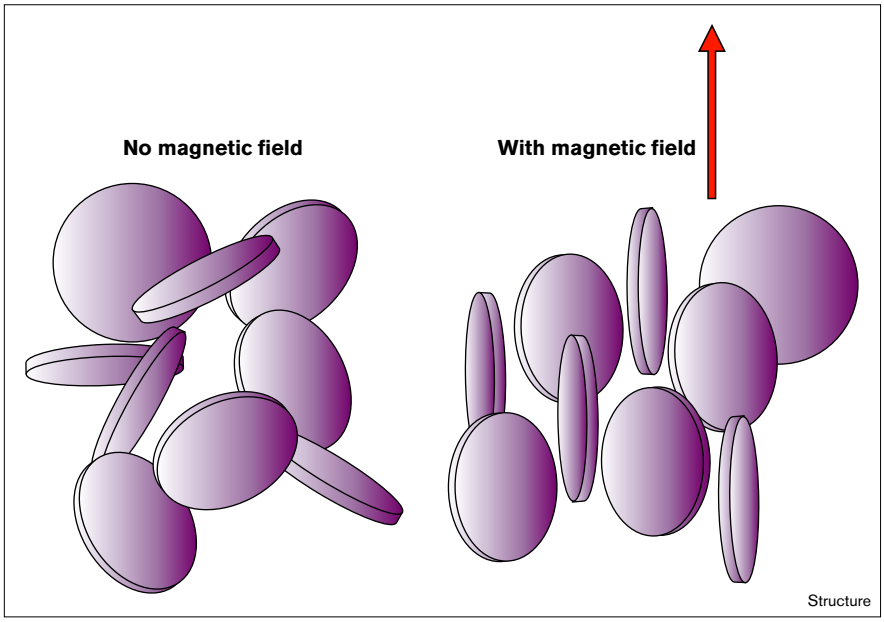
The other spin interaction of particular relevance to this discussion is chemical shift anisotropy (CSA). The observed chemical shift of any nucleus can be described:

$$\text{observed shift} = \langle\cos^2\theta_1\rangle \cdot \sigma_{11} + \langle\cos^2\theta_2\rangle \cdot \sigma_{22} + \langle\cos^2\theta_3\rangle \cdot \sigma_{33} \quad (2)$$

where the three σ are the eigenvalues for the principle axes of the chemical shift tensor associated with any given nucleus and the θ are the angles made by each tensor axis with respect to the experimental director. Again, motional averaging profoundly influences the observed shift. For example, in the presence of isotropic motion the observed shift is equal to $1/3 \cdot (\sigma_{11} + \sigma_{22} + \sigma_{33})$. The CSA is simply

Figure 2

The alignment of ideal bicelles in an applied magnetic field. By doping bicelles with certain lanthanide ions it is also possible to induce alignment so that the bilayer normals are parallel to the field [42,43]. In this case, however, it is believed that the 'ideal bicelle' model for the liquid crystal is not a very accurate description (see text for details).



the difference between the chemical shift from an oriented sample and that in solution. The orientation of the shift tensor in the local chemical frame is generally predictable or experimentally determinable [15,16].

Because of their orientation dependence, observed chemical shifts and dipolar couplings can be the source of rich structural information. The use of orientation-dependent data to yield structural information is perhaps most easily visualized by considering sending a blindfolded graduate student on a roller coaster ride through a helical track that is parallel to the ground: as the student translates through space her/his inner ear gravity detector will collect orientational data that will allow the student to perceive both the conformation of the coaster track (helical) and its overall orientation (i.e., orthogonal to the gravitational field). In a similar fashion, orientation-dependent measurements for internuclear pairs and nuclear shift tensors provide information that can provide both the conformation and the orientation of structural elements with respect to a fixed director frame. Such measurements are potentially extremely powerful both for *de novo* structural determination [17,18] and as a tool for structural refinement [6].

Finally, it should be noted that equations (1) and (2) explain the origins of 'powder patterns' often associated with solid-state NMR. In a sample in which molecular alignment is random but where isotropic motion is not present, all orientations will be populated. Thus, the resulting spectra will not yield discrete chemical shifts and dipolar couplings, but instead will yield the geometrically weighted distribution of overlapping spectra from all orientations — powder patterns.

Alignment of biomolecules in bicelles and their NMR spectra

Bicelles provide a method for avoiding the poor spectral resolution of powder patterns without eliminating useful dipolar couplings and CSA.

Water-soluble molecules achieve a net molecular alignment by being entrapped in the oriented matrix represented by bicelles. As neither the shape nor the various terms of the surface interactive energy potential of real biomolecules will be exactly spherically symmetric, all biomolecules will have a preferred orientation in an aligned matrix. Membrane-associating molecules behave differently, however. Molecules of this class are oriented by virtue of direct binding to bicelle surfaces. Consequently, molecular alignment is with respect to the bilayer normal. For standard 90° aligned bicelles, membrane-associated molecules must execute rapid rotation about the bilayer normal in order for powder patterns to be eliminated. To visualize this, consider a C–H bond vector on a hydrocarbon chain aligned with the bilayer normal. When bicelles are aligned orthogonal to the magnetic field, the C–H vector will populate all possible orientations with respect to the field. In the absence of rapid rotation of the hydrocarbon chain about the bilayer normal, all orientations will be directly observed and powder patterns will result from ^{13}C–^{1}H dipolar coupling and CSA. However, if the chain can execute rapid axial rotation about the bilayer normal then the orientational

dependence of the carbon and proton spin interactions will be averaged about the rotational axis, which is uniformly aligned with respect to the field, resulting in an oriented sample spectrum. In practice, the condition of rapid axial rotation holds for most lipids and smaller membrane proteins, but may fail to be met by larger membrane proteins. For this latter class, bicelles must be aligned with their bilayer normals parallel to the field in order to eliminate powder patterns (see below). For example, when bilayers are aligned with the field, the C–H vectors along an inserted hydrocarbon chain will maintain the same orientation with respect to the field, regardless of the rotational state about the bilayer normal.

Figure 3 shows ^{13}C spectra of DMPC under three different conditions. In randomly oriented multilayers, powder patterns are observed, with CSA and dipolar couplings being obscured by asymmetry and poor spectral resolution. In isotropic mixed micelles, only the isotropic chemical shifts are observed. In oriented bicelles, carbon resonances are split because of dipolar coupling between the dilute ^{13}C nuclei and the ^{31}P of the head group. Frequency shifts are observed relative to the isotropic sample due to chemical shift anisotropy. In addition, the DMPC and DHPC peaks are now resolved, with the dipolar couplings and CSA for DHPC being smaller than for DMPC, because of the extra modes of orientational averaging accessible to the detergent-like DHPC.

Single molecule alignment in solution by a magnetic field is sometimes observable at very high fields and/or by paramagnetically doping the molecule (e.g., [19–21]). The advantage of bicelles to free molecule alignment of water soluble species is that the net degree of orientation achievable at reasonable field strength is much higher, making detection and measurement of dipolar couplings and CSA much easier [6,7]. In addition, when bicelles are used the average degree of alignment (the sample orientational 'order') is, to some degree, scaleable at will by the observer.

Scaling of order using bicelles

Bicelles are not the only method available for aligning biomolecules for NMR or other purposes (c.f., [19–25]). However, bicelles are rather unique in that they provide a method to uniformly and systematically scale sample order, while making only minor changes in sample composition/conditions. By 'scaling of sample order' we refer to any operation that leads to a uniform scaling of all dipolar couplings and CSAs within a given molecule. For isotropic motion, a scaling factor of 0.0 pertains, whereas for rigidly aligned molecules a scaling factor of 1.0 applies.

The ability to scale sample order can be a very powerful tool. For example, by reducing the sizes of NMR proton dipolar coupling to heteronuclei in membranous samples by a factor of two or more (relative to fixed bilayers), employment of bicelles makes efficient proton decoupling feasible at much lower powers than would otherwise be possible. This dramatically reduces the potential for unwanted heating of aqueous samples by the decoupling radiofrequency pulses. The ability to scale the order of bicelles also provides an effective continuum between solution (isotropic conditions) NMR and solid-state NMR. Among other applications, systematic scaling of order from zero to much higher values provides a method for correlating oriented sample resonances with their much more easily assignable isotropic resonances [2].

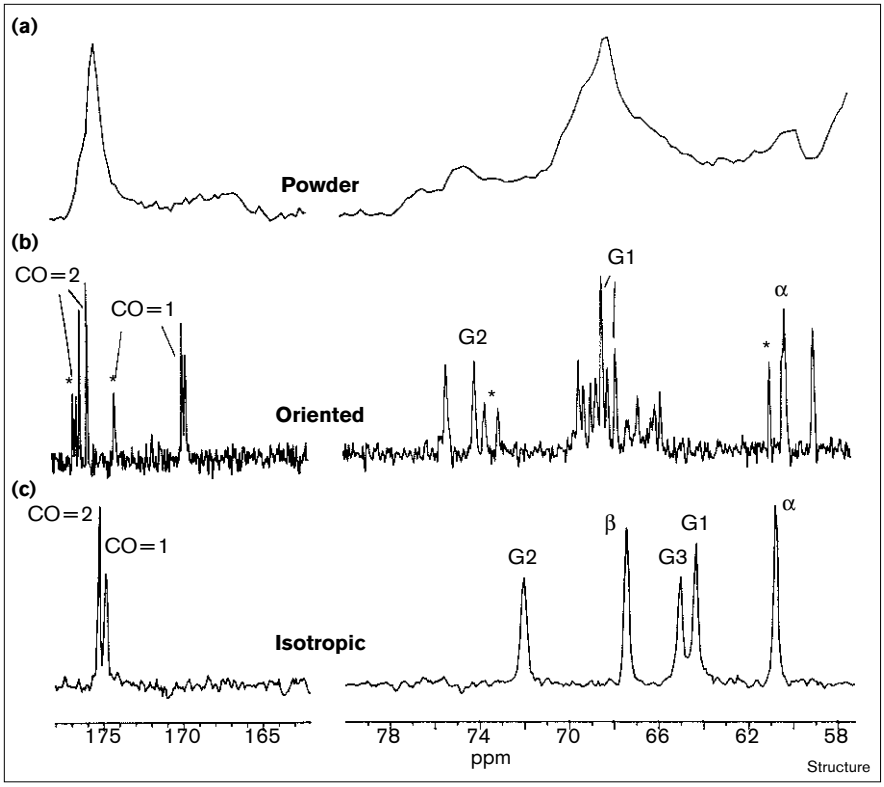
Variations in the scaling of order for water-soluble biomolecules can be achieved simply by varying the percentage of water in a bicellar sample [6,7,26]. At lower levels of hydration, soluble molecules have less room to exercise orientational excursions from the preferred molecular alignment, such that the scaling factor is relatively high, leading to relatively large CSA and dipolar couplings. To date, studies of water-soluble molecules in bicelles have generally been carried out in proton-detection mode. For this reason, very dilute bicelle samples have been used in order to tune the scaling factor so that it is in the range of 0.001, where only highly proximal (typically directly bonded) spin pairs exhibit significant dipolar couplings. In more concentrated samples, higher scaling factors can lead to severe proton line broadening in nondeuterated samples due to the upscaling of ubiquitous long range ^{1}H–^{1}H dipolar coupling.

Another method for conferring additional order to a water-soluble biomolecule is to endow it with affinity for bicelle surfaces [2]. This has been shown for cytochrome *c*. The ordering of this protein can be dramatically increased by doping bicelles with a negatively charged lipid so that the affinity of cytochrome *c* for the bicelle surface is enhanced via electrostatic interactions.

There are two methods for varying the order of bicelles themselves and, thereby, all associated molecules. One method is to vary the temperature. Some bicelle mixtures exhibit a gradual reduction in sample order as the temperature is lowered towards the phase transition temperature [9]. A more well-characterized method involves scaling the order by changing the detergent:lipid ratio [2,5]. As detergent is titrated, the bicelles are broken down into smaller and smaller discs, with a corresponding reduction in the overall alignment torque, leading to reduced order. In practice, scaling factors of 0–0.7 are readily accessible for both DHPC–DMPC and CHAPSO–DMPC systems, although scaling for bicelle-inserted molecules in the narrow > 0 to 0.2 range is very difficult to attain.

Figure 3

^{13}C NMR spectra of DMPC in **(a)** randomly dispersed multilamellar vesicles, **(b)** oriented DHPC–DMPC bicelles and **(c)** isotropic DHPC–DMPC bicelles. The temperature is 40°C such that L_α phase liquid crystalline conditions pertain. In all cases, ^1H decoupling has been employed. Samples are ^{13}C-labeled at natural abundance (no ^{13}C–^{13}C coupling is present). The small resonances labeled with an asterisk are from DHPC. DHPC and DMPC resonances are not resolved in the isotropic spectrum. G1–3 represent the three glycerol carbons, CO=1 and CO=2 represent the ester carbonyl carbons, and α and β represent the choline methylene carbons. (This figure is reproduced from [3] with permission.)



Use of bicelles in structural studies of water-soluble biomolecules

Tjandra and Bax have recently used bicelles [6,7] to achieve a desired degree of sample alignment of ubiquitin and other water-soluble biomolecules and complexes. The goal of these efforts was to be able to measure dipolar couplings for use in structural determination/refinement under spectroscopic conditions in which proton detection could be employed. In order to achieve this goal, they had to find conditions in which the order of the protein could be upscaled to the point where dipolar couplings between directly bonded spin 1/2 pairs could be conveniently measured, while keeping ^1H–^1H dipolar couplings small enough such that severe line-broadening of proton resonances due to numerous unresolved ^1H–^1H interactions was kept at bay. Such conditions were met using a DHPC : DMPC ratio of 1:2.9 and using rather dilute mixtures (95% water). Under these conditions, they were able to measure more than 150 dipolar couplings for ubiquitin and showed that these measurements agreed rather well with the X-ray crystal structure of this relatively rigid protein. A more recent study involving similar sample conditions led to the measurement of over 300 dipolar couplings that were used with other data in the *de novo* structural determination of cyanovirin N [27]. This approach is now being applied to many other proteins, nucleic acids, oligosaccharides and complexes, as reported in preliminary form by the Bax laboratory and others at various international meetings in 1998 (see also [14]). The potential for routinely measuring a large number of dipolar couplings and CSAs for water-soluble biomolecules is a particularly welcome advance in the structural determination of molecules such as nucleic acids, oligosaccharides, loop regions of proteins, multidomain proteins, and larger proteins/complexes. In these cases, the number of measurements that can be made using classical solution NMR methods is often insufficient for reliable *de novo* structural determination or for establishing the nature of structural ensembles for flexible molecules. It should also be noted that, given the through-space nature of dipolar coupling, this form of data is by no means limited only to directly bonded spin pairs. Indeed, Tjandra and Bax were able to observe a few long-range ^1H–^1H dipolar couplings in their original study [6,7], foretelling what promises to be a bounteous future.

Use of bicelles in structural studies of membranous biomolecules

Virtually all early applications of bicelles involved lipids and membrane peptides/proteins. The use of magnetically aligned bicelles has promoted the combined use of isotopic labeling and two-dimensional (2D) NMR, yielding

structural insight into membrane systems, which is hard to come by using any other method. Examples include the generation of quantitative structural models for a myristoyl-anchored fragment of the ADP ribosylation factor [28] and for a ganglioside–lectin complex [29,30]. Significant structural insight has also been provided for a variety of lipids (e.g. [1,8,31–34]), including the second messenger diacylglycerol [9], and for bicelle-associated polypeptides [2,35–37]. In the case of membrane-associated molecules, however, complete atomic resolution structural determination in studies employing bicelles has been difficult. This is because in these studies it is has not been possible to exploit the proton-detected NMR methods that lie at the heart of solution NMR structural determination (including the excursion into ordered samples made by Bax and others). For membranous molecules, it is presently extremely difficult to generate samples in which the order is extremely low, but nonisotropic (scaling << 0.1). Thus, ^{1}H–^{1}H dipolar broadening of proton resonances is generally very severe for bicelle-associated molecules. Moreover, sample dilution will not eliminate this problem as it will for water-soluble molecules. Thus, measurements must typically be made in X-nucleus-detection mode (e.g., ^{13}C). Nevertheless, given the very high spectral resolution which bicelles can sometimes yield for membrane-associated molecules (e.g. [2]), and the continued development of multinuclear multidimensional NMR methods for both solutions and solids, high resolution structural determination for bicelle-associated proteins is most likely a goal within reach. In addition, the eventual use of proton-detection of membranous biomolecules in such studies cannot be ruled out. The combination of highly sophisticated solid-state NMR pulse sequences, such as MREV-8 [38], with random fractional deuteration can, in principle, help to overcome the undesirable affects of strong ^{1}H–^{1}H dipolar couplings in bicelles.

Directions for further development and application of bicellar systems

The need for continued basic characterization of DMPC-based bicellar systems

Continued characterization of existing bicellar systems is imperative, both to illuminate the basic properties of these systems and to provide insight that will enhance their practical utilization. The original model for the aggregate morphology of DMPC bicellar assemblies with CHAPSO or DHPC (Figure 1) is consistent with a substantial body of NMR data [1,3–5,39,40]. Even for 90° aligned systems, however, there is reason to believe that bicelles are more complex than the simple model of monodisperse bilayered discs that interact only through non-sticky collisions. For example, this model does not readily account for the high viscosity of bicellar mixtures or for the fact that alignment in a magnetic field and disalignment upon removal from a field are relatively slow processes — typically taking many seconds to minutes. Furthermore, recent measurements made using a polarizing microscope suggest that bicelles are not strict nematics involving completely monodisperse discs. Instead, bicelles may be interconnected in the manner of 'sponge phase' liquid crystals [41], where the bilayers are knit together by detergent-rich curved defects (SP, unpublished results). Additional characterization is clearly needed. In the meantime, it should be kept in mind that although the 'ideal bilayered disc' model for bicelle structure accounts for much data and provides much practical insight regarding the ways in which bicelles can be used, it remains a model subject to revision.

Another uncertainty in the use of DMPC-based bicelles has arisen as investigators have sought to employ very dilute bicelle mixtures (3–10% lipid plus detergent by weight). Unlike the situation for more concentrated mixtures, the morphological properties of dilute mixtures (as discerned by NMR and light scattering experiments) appear to be sensitive to variations in exact conditions (pH, salt, batch of lipid used, etc.), complicating their practical application in NMR studies (unpublished results from several laboratories). Work to resolve the source(s) of these complicating factors is presently underway in a number of laboratories, with notable advances already being made [26].

The need for novel bicellar systems

There are applications that are generally appropriate for bicelles, but for which the existing DMPC-based bicelles are not satisfactory. These include studies that must be carried out at room temperature or below (where DMPC-based systems do not orient), studies of integral membrane proteins (where DMPC forms bilayers which are not as thick as preferred by some proteins of interest), and studies of phospholipases (which bind DMPC surfaces, but then chemically degrade the lipid components). It is fortunate that there is a vast literature dealing with the physical chemical characterization of lyotropic membrane systems for investigators to draw upon in formulating strategies for improved, or application-customized bicelles. Work is on-going in a number of laboratories to develop novel bicellar systems, and also to develop alternate sample orientation strategies.

Optimizing methods for achieving parallel magnetic alignment

Recently, it was discovered that phospholipid bicelles can be aligned with their bilayer normals parallel to the magnetic field by doping standard mixtures with certain lanthanide ion:lipid molar ratios in the range of 1:50–1:150 [42,43]. This is a major development because, as noted earlier, this orientation is required to eliminate NMR powder patterns in the case of larger membrane proteins. Zero degree alignment is also preferred for many projected non-NMR-based applications of bicelles (see below).

The liquid crystalline phase that is formed when lanthanides are added to bicelles does not strictly conform to

the classical bicelle model. Instead, a combination of low-angle neutron diffraction [44] and NMR results [43] suggest that both DHPC and DMPC are incorporated into non-vesicular multilayers in which the bilayers are evenly spaced, being separated from one another by layers of water. The thickness of the intervening water layers was determined for one composition to be ~75 Å. The thickness of the water layer can be varied at will simply by altering the overall percentage of sample water. These mixtures maintain optical clarity.

More recently, the potential drawbacks associated with the presence of the lanthanides (namely paramagnetic broadening and binding of the paramagnetic ion to the protein of interest) have been minimized by introducing a phospholipid chelate into the bilayer component of bicelles, the task of which is to sequester the lanthanides [45,46]. Using this chelate, it has been possible to generate samples that have been shown to undergo parallel alignment over a large temperature range (35–90°C). Alignment by this method has also been successfully demonstrated in the presence of membrane-associated peptides [46]. Work is presently under way to extend the temperature range of these mixtures to very low temperatures. This may ultimately lead to much higher NMR sensitivity and, in some cases, spectral resolution.

Prospects for exploiting bicelles using non-NMR forms of spectroscopy

One of the most intriguing possibilities for bicelles and lanthanide-doped membranes is their potential for use in a wide range of physical techniques, such as electron paramagnetic resonance and optical and vibrational spectroscopies. Because alignment can be accomplished at very modest fields, the integration of magnets with a variety of commercial spectrometers is feasible. Unlike oriented multilayers, bicelles are usually optically transparent and can therefore be employed in experiments requiring sample clarity.

Magnetically aligned lanthanide-doped membranes may also be ideal for studies of membrane proteins by low-angle diffraction (i.e., X-ray or neutron). Their suitability for such studies arises from the symmetry of this multilamellar phase, the high degree of alignment observed in such systems, and the ease with which hydration can be controlled. The ability to systematically vary interbilayer spacing may be particularly attractive as a route to phasing in such studies. The degree of association or penetration of a membrane-associated molecule can, in principle, be quantitatively assessed from a one-dimensional (1D) scattering profile along the membrane normal [47].

Conclusions

Bicelles combine a number of the attractive features of both micellar and lipid vesicular model membrane systems, while eliminating some of the drawbacks of both. Thus, from a strictly biochemical standpoint, bicelles appear to be well suited for wide application. From a spectroscopic point of view, bicelles now appear to be well on their way to becoming a standard tool in biological NMR. This development is being paralleled by continued basic development and characterization of bicelles and their derived systems. The time now appears to be ripe for their extension to non-NMR-based biophysical studies. The impact of bicelles upon structural biology will hopefully be greatest where new tools are needed most, as in studies of integral membrane proteins.

Finally, it is worth noting that bicelles represent an example of the importance of basic research in laying the foundation for novel and useful biotechnology. Important early work related to the development of the DMPC-based bicelle systems included the characterization of mixtures of phosphatidylcholine with bile salts and with short-chain phosphatidylcholine (e.g., [48–50]), the magnetic alignment of lipid vesicles and small molecules (e.g., [21,51,52]), and the development and application of nematic liquid crystals in NMR (e.g., [10,53,54]).

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