Assessing the size, stability, and utility of isotropically tumbling bicelle systems for structural biology

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A R T I C L E   I N F O
Article history:
Received 13 August 2009
Received in revised form 24 October 2009
Accepted 5 November 2009
Available online 13 November 2009

Keywords:
Bicelle
Phospholipid
Membrane mimetic
NMR
31P NMR
2D NMR
AFM

A B S T R A C T
Aqueous phospholipid mixtures that form bilayered micelles (bicelles) have gained wide use by molecular biophysicists during the past 20 years for spectroscopic studies of membrane-bound peptides and structural refinement of soluble protein structures. Nonetheless, the utility of bicelle systems may be compromised by considerations of cost, chemical stability, and preservation of the bicelle aggregate organization under a broad range of temperature, concentration, pH, and ionic strength conditions. In the current work, 31p nuclear magnetic resonance (NMR) and atomic force microscopy (AFM) have been used to monitor the size and morphology of isotropically tumbling small bicelles formed by mixtures of 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC) or 1,2-di-O-tetradecyl-sn-glycero-3-phosphocholine (DIOHPC) with either 1,2-dihexanoyl-sn-glycero-3-phosphocholine (DHPC) or 1,2-di-O-hexyl-sn-glycero-3-phosphocholine (DIOHPC), testing their tolerance of variations in commonly used experimental conditions. 1H–15N 2D NMR has been used to demonstrate the usefulness of the robust DMPC–DIOHPC system for conformational studies of a fatty acid-binding protein that shuttles small ligands to and from biological membranes.
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1. Introduction

Since the late 1980s, bilayered phospholipid micelles (bicelles) have attracted substantial interest in the molecular biophysics community as membrane mimetics for NMR structural studies of amphiphile assemblies and of membrane-bound peptides and proteins [1]. As compared with other model biological membranes such as micelles or small unilamellar vesicles, the principal advantage of bicelles lies in their ability to maintain stable planar bilayers that more closely mimic in vivo membranous structures and thus better preserve the native conformation of the polypeptide under study. Depending on the temperature, total aqueous phospholipid concentration (cL) and molar ratio of long- to short-chain components (q), these assemblies with dislikelike [2,3] or ‘Swiss cheese’ morphologies [4,5] may tumble isotropically or become aligned with respect to a magnetic field. Whereas bicelles with q exceeding a value of 2 will orient in an NMR magnet regardless of whether cL is small (3–5%, w/v) [4] or large (15–25%, w/v) [6], dislikelike bicelles with q less than 2 can tumble isotropically.

Dilute preparations of the high-q assemblies have gained wide usage as magnetically aligned media for the refinement of NMR-based 3D structures of soluble proteins with measurements of residual dipolar couplings [1,4,6]. Solid-state NMR investigations [7–10] have typically made use of more concentrated preparations of these bicelles, for which the direction of magnetic alignment can be tuned by addition of lanthanide ions [11,12] that increase the spectral dispersion. In a number of cases the low-q, isotropically tumbling bicelles have made it possible to conduct high resolution solution-state NMR studies of peptides [13] and transmembrane proteins [14,15] without compromising their biochemical activity; the small size and rapid tumbling of these assemblies permits acquisition of well-resolved NMR spectra, comparable to those seen in micelles [14]. A bicelle organizational model has also been proposed for mixtures of long- and short-chain phospholipids, e.g. dimyristoylphosphatidylcholine (DMPC) and dihexanoylphosphatidylcholine (DHPC), respectively. In the bicelle thus produced, the two lipid species tend to segregate, with the long-chain phospholipids located in a central planar region and the short-chain phospholipids concentrated in a curved rim region [2,3,16] (Fig. 1). One interesting feature of this system is that the two bicellar regions experience different magnetic environments and thus display distinct peaks in the 31p NMR spectrum [2].

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As a practical matter for the design of NMR structural studies using bicellar media, it is important to consider what factors influence their size, magnetic alignment behavior, and stability and how best to measure these properties. The critical micelle concentration (cmc) of each phospholipid controls the concentration of monomers that must be maintained in equilibrium with its associated aggregate. For a short-chain phospholipid such as DHPC with a large cmc of 14–15 mM [2,17], a significant portion will be present in monomeric form if the DMPC–DHPC bicelle solution is dilute, enhancing the molar ratio of the aggregate and thus making it grow in size [2]. The larger head-group volume occupied by phospholipids spread out around the rim compared with those packed tightly in the bilayer is also expected to enlarge the size of the bicelle disk. The presence of salts in the bicelle solution can influence not only the size but also the magnetic alignment of a bicelle system. It is known, for instance, that trivalent lanthanide ions with positive magnetic anisotropy susceptibility (e.g., Er\(^{3+}\), Yb\(^{3+}\), Tm\(^{3+}\), and Eu\(^{3+}\)) can change the bicelle orientation by 90° with respect to the magnetic field [11,12,18]. Bivalent ions such as Ca\(^{2+}\) and Mg\(^{2+}\) have been reported to increase bicelle sizes significantly and also to improve their magnetic alignment [19], whereas monovalent ions (e.g., K\(^+\) and Na\(^+\)) only improve magnetic alignment [19–21]. Finally, the presence of chemically labile phospholipid linkages can compromise bicelle stability, especially at extremes of pH. Many investigators now prefer to use phospholipids such as dihexanoylphosphatidylcholine (DHPC), in which ether (rather than ester) bonds link the glycerol backbone and the aliphatic chains, because ethers are less susceptible to hydrolysis under both acidic and alkaline conditions [22–24].

In the current study we utilize both NMR and atomic force microscopy (AFM) methods to assess stability, aggregate size, and phospholipid organization for these versatile assemblies. We further describe a new isotropically tumbling DMPC–DIOHPC bicelle system that is cost effective, resistant to changes in size and morphology over a wide range of concentrations, temperatures, and physiological salt conditions, and stable enough to serve as a membrane-mimetic medium for NMR structural studies of peripheral membrane proteins. The utility and robustness of this DMPC–DIOHPC bicelle as a membrane mimetic for biological macromolecules is demonstrated by two means. Firstly, NMR of two intrinsically well-separated \(^{31}\)P peaks from central planar and rim regions of the bilayer are shown to provide an accurate monitor of the bicelle morphological and chemical stability. Secondly, 2D \(^{1}H–^{15}N\) NMR correlation data for the bicelle mixed with intestinal fatty acid binding protein (IFABP), a protein that transfers fatty acids to and from biological membranes, suggest the formation of a stable, rapidly tumbling bilayeric complex that offers the potential to yield high-resolution spectra for structural studies of peripheral membrane proteins.

2. Materials and methods

2.1. Preparation of bicelle samples

1,2-Dimyristoyl-sn-glycero-3-phosphocholine (14:0) (DMPC, MW: 677.93), 1,2-dioleoyl-sn-glycero-3-phosphocholine (16:0) (DIOHPC, MW: 649.97) and 1,2-di-O-tetradecyl-sn-glycero-3-phosphocholine (14:0) (DIOHPC, MW: 453.25), 1,2-di-O-tetradecyl-sn-glycero-3-phosphocholine (14:0) (DIOHPC, MW: 453.25), 1,2-di-O-tetradecyl-sn-glycero-3-phosphocholine (14:0) (DIOHPC, MW: 453.25) and 1,2-di-O-tetradecyl-sn-glycero-3-phosphocholine (14:0) (DIOHPC, MW: 453.25) and 1,2-di-O-hexadecyl-sn-glycero-3-phosphocholine (14:0) (DIOHPC, MW: 453.25) were purchased from Avanti Polar Lipids (Alabaster, AL) and used without further purification. Research grade NaCl, KCl, CaCl\(_2\) and MgCl\(_2\) were purchased from Fisher Scientific (Fair Lawn, NJ).

We compared properties of three bicellar systems, DMPC–DIOHPC, DMPC–DIOHPC, and DMPC–DIOHPC. To prepare a DMPC–DIOHPC bicelle stock solution with \(c_{L} = 25\%\) (w/v), the appropriate amount of DMPC was suspended in deionized water and then vortexed at room temperature to form a slurry. An aqueous DIOHPC stock solution (0.5 g/ml in water) was mixed with the DMPC slurry to achieve \(c_{L} = 0.5\), and water was added to maintain an overall lipid concentration of \(c_{L} = 25\%\). The samples were vortexed, centrifuged and vortexed again, then heated briefly at 37 °C in a water bath and cooled down to 0°C in ice water. This protocol was repeated for at least five cycles until a clear, homogeneous bicelle solution was obtained. DMPC–DIOHPC and DMPC–DIOHPC bicelles were handled in the same fashion as DMPC–DIOHPC mixtures.

Samples with \(q = 0.5\) and a series of \(c_{L}\) values were prepared for NMR studies by adding appropriate amounts of the 25% (w/v) bicelle stock solution and D\(_{2}\)O, then diluting with water to a final volume of 500 μl and 10% D\(_{2}\)O. Selected samples contained various salts (NaCl, KCl, CaCl\(_{2}\), and MgCl\(_{2}\)) that were added to yield final concentrations of 50, 100, 150, and 200 mM.

2.2. Preparation of intestinal fatty acid-binding protein

IFABP was expressed in BL21(DE3) E. coli cells and purified by G-50 size exclusion chromatography, DEAE-52 ion exchange chromatography, and delipidation by a Lipidex column, as described previously [25,26]. A 0.3 mM solution of I-FABP was prepared in 50 mM NaH\(_{2}\)PO\(_{4}\), 100 mM NaCl, 5 μM EDTA, 0.02% NaN\(_{3}\), and 5% D\(_{2}\)O at pH 7.0; 20% DMPC/DIOHPC stock bicelle solution in pure water was added directly to the protein solution to achieve a final bicelle concentration of 1% (w/v) in a total NMR sample volume of 500 μl.

2.3. Nuclear magnetic resonance spectroscopy

All data were acquired on Varian spectrometers at either the College of Staten Island or the City College of New York: a UNITYINOVA 600 instrument (Palo Alto, CA) equipped with an IDQG probe and operating at \(^{1}H\) and \(^{31}\)P frequencies of 599.497 and 242.856 MHz, respectively or a UNITYINOVA 600 spectrometer equipped with a cold probe and operating at 599.761 MHz. One-dimensional \(^{31}\)P spectra were recorded at various temperatures using a proton-decoupled single pulse experiment with 32–1024 scans for \(c_{L} = 15\%\) to 0.125%. The spectra were recorded with 4096 complex points and a sweep width of 4882.8 Hz (~20 ppm), producing a digital resolution of <0.01 ppm/point or 2.39 Hz/point. The carrier frequency was set to 0 ppm as the chemical shift reference. The data were processed with Varian VnmrJ software. Two-dimensional \(^{1}H–^{13}N\) Heteronuclear Single Quantum Correlation NMR Spectroscopy (HSQC) [27] was conducted using a gradient-equipped HCN triple resonance probe. The spectra were recorded with 512 × 128 complex points and 64 scans per spectrum. The \(^{1}H\) chemical shift was referenced to the water peak (4.938 ppm at 37 °C).

2.4. Atomic force microscopy

A \(c_{L} = 2\%\) DMPC/DHPC bicelle solution (\(q = 0.5\)) was freshly diluted 10-fold with distilled water; 200–300 μl were put on a cover slip in a liquid cell with a freshly isolated mica surface (Ted Pella, Inc., Redding, CA). The AFM measurements were performed at room temperature on an Agilent 5500 AFM/SPM Microscope (Agilent Technologies, Inc., Santa Clara, CA), using tapping mode with a scanning rate of 0.7 Hz and a...
tapping frequency of ~300 kHz. A cantilever (NSC15/no Al) with a spring constant of 45 N/m (MikroMasch USA, Wilsonville, OR) was used for these measurements.

2.5. Modeling of bicelle size

The bicelle organizational models proposed previously [2,11] were used to relate the phospholipid ratio in the aggregate to its diameter, allowing us to assess the robustness of the DMPC–DIOHPC bicelle under various experimental conditions. Fig. 1 shows a schematic representation of this bicelle, where the long-chain phospholipids occupy the central planar bilayer region and the short-chain phospholipids are present in the curved micelle-like rim [2,16]. Although DIOHPC could be partially miscible with the long-chain lipids in the planar region and undergo fast exchange between rim and planar sites [28–30], this effect was neglected in the absence of diagnostic 31P chemical shift changes (see Section 3.4). Straightforward geometrical considerations provide a relationship between the molar ratio of the two components and the dimensions of the bicelle assembly:

\[ q = \frac{[\text{DMPC}]}{[\text{DIOHPC}]} \]

\[ = \frac{\text{(area of central plane)}}{\text{(area of rim)}} = \frac{R^2}{(\pi R + 2r)r}, \]

where \( R \) is the radius of the central planar region and \( r \) is the radius of the rim area. The thickness of the DMPC bilayer \( h \) has been estimated to be about 4 nm [31]. As the radius of the DHPC micelles has been estimated to be 2 nm [16], we use this value as an approximation for the free-state DIOHPC monomers ([DIOHPC]free) and DIOHPC molecules bound to the bicelle ([DIOHPC]bound = [DIOHPC]total − [DIOHPC]free). Considerations lead to an effective q value for our DMPC–DIOHPC bicelle:

\[ q_{\text{eff}} = \frac{[\text{DMPC}]/[\text{DIOHPC}]_{\text{total}} - [\text{DIOHPC}]_{\text{free}}}{[\text{DMPC}]/[\text{DIOHPC}]_{\text{bound}} = a^{-1}(\text{the area of central plane})} \]

\[ / (\text{the area of rim}) = \frac{R^2}{a(\pi R + 2r)r}, \]

where \( a \) is the ratio of DMPC to DIOHPC (or DHPC) head group areas in planar bilayers and rim micelles, respectively. The head group area of bilayered DMPC has been reported as 0.66 cm² [31], whereas for micellar DHPC it has been reported variously as 0.66 cm² [32] and 1.02 cm² [33]. In conformance with other research reports [2,34], our calculations use the latter value and thus an \( a \) value of 0.6, making the assumption that DHPC and DIOHPC have the same head group area.

Finally, Eq. (2) yields a bicelle diameter as follows:

\[ D_{\text{bicelle}} = 2(R + r), \]

\[ \text{where } R = aq_{\text{eff}} / \left[ \pi + \left( \pi^2 + 8/aq_{\text{eff}} \right)^{1/2} \right]/2. \]
maintained at room temperature to be at least as stable as the DMPC–DHPC system against hydrolysis. Successive NMR examination of the $q = 0.5$, $c_1 = 2\%$ w/v and $q = 0.5$, $c_1 = 8\%$ w/v DMPC–DHPC systems (kept at pH 6.3 for 2 years in a freezer at -20°C or ~3 months at room temperature) gave $3^1P$ spectra that were unchanged and free from degradation products, consistent with chemical stability that is at least comparable to the DMPC–DHPC bimellar system under similar conditions [4]. Moreover, this performance is achieved with significant cost savings, since retaining an ether-linked phospholipid as one of the bimellar components shaves a factor of ~10 off the costs of the chemicals.

3.3. Concentration of short-chain lipid monomers in bimellar solutions

The impact of DIOHPC partitioning between monomeric and bimellar locations on the ratio $q_{fr}$ and the corresponding aggregate size was assessed by determining the monomer concentration $[DIOHPC]_{free}$ from $3^1P$ NMR measurements as a function of the total phospholipid concentration $c_1$ in $q = 0.5$ DMPC–DIOHPC mixtures (Table 1) [2]. Taking 5.03 ppm as the chemical shift of free-state DIOHPC monomer, a plot of shift vs. $[DIOHPC]_{free}$ yielded a straight line ($R^2 = 0.999$) and $[DIOHPC]_{free} = 6$ mM at 37 °C (Fig. 3). Analogous measurements for DMPC–DHPC bimellae (in pure water) give $[DHPC]_{free} = 7$ mM at 37 °C ($R^2 = 0.997$, chemical shift data not shown), in excellent agreement with the result obtained by Glover et al. [2] in the presence of ~200 mM Pr$^{3+}$. This consistency of $[DHPC]_{free}$ shows that neither the $q$ value nor the aggregate size is altered by the presence of lanthanide ions. The comparative values of $[DIOHPC]_{free}$ and $[DHPC]_{free}$ suggest that the ether-linked lipid is slightly more hydrophobic than its ester-linked analog; for that reason it is also likely to have a cmc lower than the 14 mM value determined for DHPC [2] and should exert a smaller impact on the lipid ratio and bimellar size according to Eqs. (2) and (3). The concentration of short-chain phospholipid monomers remained essentially constant at 5.7–6.5 mM over this temperature range (data not shown), in accord with prior reports for DHPC. For instance, dynamic light scattering has been used to show that the variation of hydrodynamic radii with $c_1$ for $q = 0.5$ bimellae is invariant to temperature, with a value of $[DHPC]_{free}$ at about 5 mM in the range of 25–37 °C [34]. Analogous temperature invariance of small angle neutron scattering (SANS) profiles for $q = 0.5$ bimellae has been reported in the temperature range of 10–40 °C [3].

It was also of interest to establish the range of total lipid concentration over which the bimellar size is retained. Subtracting $[DIOHPC]_{free}$ from $[DIOHPC]_{total}$ and calculating the effective $q$ from Eq. (2), we find that $q_{eff}$ remains essentially constant at 0.5 when $c_1 \geq 98$ mM (5% w/v). By comparison, $c_1$ must exceed 130 mM (7% w/v) for the DMPC/DHPC bimellar system in order to keep the $q_{eff}$ value constant at 0.5 [2]. This result demonstrates an augmented versatility for bimellar media containing an ether-linked short-chain phospholipid, since it becomes possible to work with smaller amounts of lipid and either peptide or protein while still maintaining a small, rapidly tumbling bimellar structure and high-resolution NMR spectra.

3.5. Influence of salts on DMPC–DIOHPC isotropic bimellar size

Losonczi and Prestegard [20] first reported the beneficial effects of ionic strength on the stability and lifetime of phospholipid bimellar preparations. Both the results in Section 3.3 and prior experiments

Table 1

<table>
<thead>
<tr>
<th>$c_1$ (%)</th>
<th>Total lipid (mM)</th>
<th>DMPC (mM)</th>
<th>DMPC $\delta$(ppm)$^a$</th>
<th>DIOHPC (mM)</th>
<th>DIOHPC $\delta$(ppm)$^b$</th>
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<td>15</td>
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<td>4.502</td>
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<td>4.502</td>
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<td>4.501</td>
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<td>32.7</td>
<td>4.502</td>
<td>65.4</td>
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<td>0.8175</td>
<td>1.635</td>
<td>5.03</td>
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</tbody>
</table>

$^a$ All mixtures were prepared with a ratio [DMPC]/[DIOHPC]$_{total} = 0.5$ in aqueous solutions containing 10% D$_2$O and examined at 37 °C.
$^b$ Spectra were referenced by setting the carrier frequency to 0 ppm.

Table 2

<table>
<thead>
<tr>
<th>Salt concentration (mM)</th>
<th>KCl$^a$</th>
<th>NaCl$^a$</th>
<th>CaCl$_2$$^b$</th>
<th>MgCl$_2$$^b$</th>
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</tr>
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<td>100</td>
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<td>0.46</td>
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</tr>
<tr>
<td>150</td>
<td>0.46</td>
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</tr>
<tr>
<td>200</td>
<td>0.46</td>
<td>0.46</td>
<td>0.46</td>
<td>0.46</td>
</tr>
</tbody>
</table>

$^a$ The measured $q$ value (defined as [DMPC]/[DIOHPC]) was derived from the ratio of normalized integrated areas for DMPC and DIOHPC peaks in $3^1P$ NMR spectra, with a precision of 2% estimated from duplicate measurements.
$^b$ The mixture was prepared with $c_1 = 10\%$ (w/v), $q = 0.5$, 10% D$_2$O.
$^c$ The mixture was prepared with $c_1 = 2\%$ (w/v), $q = 0.5$, 10% D$_2$O.
$^d$ Not measured.
suggest that trivalent lanthanide ions (e.g., Er$^{3+}$, Yb$^{3+}$, Tm$^{3+}$, and Eu$^{3+}$) do not influence the bicelle size despite their ability to change the orientation of liquid crystalline aggregates by 90° in the magnetic field. As noted above, both monovalent and divalent ions have been shown to impact the size and the magnetic field alignment properties of high-$q$ bicelles [4,19,21]. The sensitivity of bicelle architecture to salt concentration may be important in designing structural studies to approximate particular physiological conditions.

$^{31}$P NMR peak integrals and associated values of $q$ were measured for DMPC–DIOHPC bicelle solutions to which four commonly used physiological salts (NaCl, KCl, CaCl$_2$, MgCl$_2$) were added in independent experiments. Table 2 summarizes the values of $q$ measured at three different temperatures and four concentrations of each salt. The largest perturbation of bicelle size occurred upon addition of up to 200 mM NaCl to the $c_l = 2\%$ DMPC–DIOHPC mixture at 25°C, for which the $q$ value increased from 0.47 to 0.54 (namely 15% increments in $q$ value) and the corresponding bicelle diameter increased from 92 to 98 Å (namely 6.5% increments in size). In other cases the changes in $q$ were no greater than 0.03 (6% increments, KCl at 46 °C) and 0.04 (8% increments, NaCl at 46 °C); thus none of these four salts had a significant influence on bicelle size over the concentration range 0–200 mM and the temperature range 25–46 °C.

Our finding that the bicelle size is invariant to the monovalent ions K$^+$ or Na$^+$ was consistent with previous reports for DMPC–DHPC assemblies [19,21,34]. In contrast, the modest size changes we observed upon addition of the divalent ions Ca$^{2+}$ and Mg$^{2+}$ differed from the findings of Arnold et al., who used solid-state $^{31}$P NMR spectra to characterize high-$q$ ($c_l = 20\%$) magnetically aligned DMPC–DHPC bicelles made with a different sample preparation method [19]. They found that ‘depressed’ $q_{\text{eff}}$ values of 2.6 could be restored to their stoichiometric values of 3.6 upon addition of 100–200 mM CaCl$_2$ or MgCl$_2$, corresponding to an increase in diameter from ~30 to ~50 nm and in accord with the known role of enhanced ionic strength in promoting bilayer and bicelle assembly [20].

### 3.6. Direct visualization of isotropic DMPC–DHPC bicelles by AFM

A direct assessment of bicelle size and shape was also obtained using atomic force microscopy in DMPC–DHPC and DMPC–DIOHPC bicelles. Previously, Arnold et al. [19] used freeze-fracture electron microscopy to visualize disklike DMPC–DHPC bicelles with a $q$ value of 3.5. Glover et al. [2] also observed $q = 0.5$ DMPC–DHPC bicelles using negative staining electron microscopy, whereas Luchette et al. [3] characterized such aggregates by SANS methods. As a more direct alternative that can be implemented under near-physiological conditions, we used AFM to obtain images of nominally similar $q = 0.5$ DMPC–DHPC bicelles, employing tapping mode to minimize destructive frictional forces [35,36]. Fig. 4A–C shows images acquired with scanning ranges of 3–5 μm; the corresponding cross-sectional

![AFM images of DMPC-DHPC bicelles (10-fold diluted of $c_l = 2\%$ w/v, $q = 0.5$) obtained with scanning ranges of 4.6×4.6 μm (A and B), and 3.25×3.25 μm (C). Panel C was scanned along an axis perpendicular to the ones in A and B, producing “shadows” of the individual disks that are oriented differently by 90°. Panels D, E and F are cross-sectional plots along the lines drawn in panels A, B, and C, respectively, yielding thickness estimates (heights) of about 40 Å for the bicelle planar area. The two circled aggregates were used to estimate 87 and 96 Å diameters, respectively.](image)

![An overlay of the amide regions of $^{15}$N–$^1$H HSQC NMR spectra for ~0.3 mM IFABP in 50 mM Na$_2$PO$_4$, 100 mM NaCl, 5 μM EDTA, 0.02% NaN$_3$, and 5% D$_2$O at pH 7.0, showing samples with (red) and without (black) a ($c_l = 1\%$ w/v, $q = 0.5$) DMPC–DIOHPC bicelle solution in water.](image)
plots (Fig. 4D–F) provide disk thickness estimates of about 40 Å in each case, matching that expected for the lipid bilayer [2,3,4]. Fig. 4A reveals at least three kinds of lipid assemblies: large disks, small disks, and worm-like structures. The worm-like structures, observed previously by SAXS in high-q mesophases [37,38], may be cylindrical micelles of DHP (34), whereas the large disks could be lamellar DMPC membranes. We identify the small bilayered disks, which are found most abundantly, as DMPC–DIOHPC bicelles.

Fig. 4B shows that the small disks are not uniform in size, in agreement with prior freeze-fracture EM data on high-q aggregates [34]. This observation may reflect an intrinsic size variation, but it is also possible that the disks grow as a consequence of water evaporation from the bicelles. The “shadows” on the individual disks arise from the expected edge effects of the AFM tip; the cross-sectional plots allow us to estimate a 20 Å thickness for these shadows, corresponding to a lipid monolayer and matching the expected height at the bicelle edge (Fig. 1). Using the thickness measured perpendicular to the scanning plane for the “worm-like” cylindrical structures as a calibration distance, we find the diameters of the two smallest bicelles in Fig. 4A and B to be 87 and 96 Å, respectively. These values are in excellent agreement with the calculated value of 96 Å obtained from Eq. (3) with \( q = 0.5 \), \( a = 0.6 \), and \( r = 2 \) nm; the AFM-derived dimensions are also in accord with fits of the SANS data [3]. Thus, the AFM images can provide a direct quantitative picture of the isotropically tumbling bicelle assemblies.

3.7. Isotopic DMPC–DIOHPC bicelles as membrane mimetics for NMR structural studies

As noted above, DMPC–DHP bicelles have been used widely as membrane mimetic media [1]. The utility of the new DMPC–DIOHPC bicelle system as a model membrane for structural biology research was evaluated using IFABP, an intracellular protein that transfers hydrophobic ligands to and from biological membranes and may be viewed as a model peripheral membrane protein [26]. Fig. 5 shows an overlay of \(^1H-15N\) HSQC spectra for IFABP alone (black) and upon addition of 1% w/v (~60:1 lipid/protein) aqueous DMPC–DIOHPC bicelles (red). It is clear that both spectra exhibit sharp resonances, excellent spectral resolution, and the high degree of chemical shift dispersion typical of \(^\beta\)-sheet structures. These high-quality spectra, maintained without precipitation for more than 45 days at bicelle concentrations up to \( c_1 = 1\% \), indicate formation of small protein–bicelle assemblies that tumble rapidly and do not aggregate together. A single NH resonance appears for each observed backbone site, consistent with rapid exchange between bound and unbound states. Most of the NH peaks are coincident in the two samples, but a small subset showing changes in magnetic environment is likely to be diagnostic for interactions between particular regions of the IFABP protein and the DMPC–DIOHPC bicelles, respectively. Perhaps 10% of the NH resonances disappear upon addition of the bicelles, suggesting that protein–membrane interactions produce bound and unbound states of particular polypeptide sites that are interconverting at rates comparable to their respective chemical shift differences. These observations open the way for detailed studies of the structural and motional requisites of IFABP-facilitated transport to and from biological membranes, which are currently in progress.

4. Conclusions

A new DMPC–DIOHPC isotropic bicelle system has been evaluated as a membrane mimetic medium for biomolecular NMR and related structural biology research. Compared with other similar systems, DMPC–DIOHPC isotropic bicelles exhibit improved hydrolytic stability and superior \(^31P\) NMR peak separation, allowing more accurate measurements of integrals and associated sizes. Moreover, the new system extends the range of experimental conditions over which constant size and aggregate organization can be maintained, including overall phospholipid concentrations down to \( c_2 \) of 5%, temperatures from 25 °C to 46 °C, and the presence of physiologically relevant salts at concentrations up to 200 mM. AFM imaging experiments are demonstrated for direct visualization of bicellar aggregates under near-physiological conditions and comparison with prior EM data, not only confirming the predominance of the expected dislikable assemblies but also revealing the presence of extended bilayers and DHP micelles. Finally, the \( q = 0.5 \) DMPC–DIOHPC bicelle system shows potential as a useful medium for high-resolution NMR studies of peripheral membrane proteins.

Acknowledgements

The IFABP plasmid was a generous gift of Dr. Judith Storch, Rutgers University Department of Nutritional Sciences. The NMR and AFM facilities used in this work are operated by the College of Staten Island, The City College, and the CUNY Institute for Macromolecular Assemblies, a Center of Excellence of the Generating Employment through New York State Science Program. Additional infrastructural support was provided at The City College of New York by NIH 5G12 RR03060 from the National Center for Research Resources. Dr. Sublette is supported by NIMH K08 MH079033-01A2.

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