Phospholipid Bicelles with Positive Anisotropy of the Magnetic Susceptibility

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This communication describes a new type of bicelles which align with their major axes parallel to an external magentic field. Phospholipid bicelles are disk-shaped bilayered micelles[1] formed by mixing appropriate ratios of a long-chain phospholipid, such as 1,2-ditetradecanoyl-*sn*-glycero-3-phosphocholine (DMPC, where M stands for myristoyl) or 1,2-didodecyl-*sn*-glycero-3-phosphocholine (DLPC, where L stands for lauroyl) with a short-chain phospholipid, such as 1,2-dihexanoyl-*sn*-glycero-3-phosphocholine (DHPC), in an aqueous medium. Normal bicellar systems contain about 5−40% w/v phosphatidylcholine in water and exhibit a nematic phase at ambient temperature. When the ester linkages in diacyl phospholipids are replaced by ether linkages, the bicelles are more stable to pH changes.[2]

Magnetically aligned bicelles have been used as model membranes for structural studies of membrane-bound proteins[3] and as an ordering medium for water-soluble proteins and nucleic acids[4] by NMR. For normal bicelles, the anisotropy of the magnetic susceptibility ($\Delta\chi$) is negative, and their principal axes align in all directions perpendicular to the external field, $\mathbf{B}_o$. As a result, membrane constituents which do not possess axial symmetry would not have a unique alignment with respect to $\mathbf{B}_o$; therefore, the NMR peaks show cylindrically symmetrical powder patterns rather than sharp peaks unless the molecule undergoes fast axially symmetric motion. Therefore, it is desirable to force the bicelles to align with their principal axes parallel to $\mathbf{B}_o$. Two approaches have been used to achieve this goal. The first method is to use amphiphilic aromatic compounds to mix with phosphatidylcholine[5] because the phenyl ring has a large positive $\Delta\chi$.[6] The second method is to use paramagnetic lanthanide ions to change the sign of $\Delta\chi$ to make the bicelles turn around.[7] Here we present another approach to achieve parallel alignment of the bicelle axes without adding excessive nonlipid aromatic compounds or using paramagnetic ions. For this purpose, a phospholipid containing a biphenyl group, 1-dodecanoyl-2-(biphenyl-4-acetyl)-*sn*-glycero-3-phosphocholine (DBPC),[8] was synthesized, and the characteristics of the bicelles formed by mixing DBPC and DHPC were monitored by using ^{31}P NMR spectroscopy.

Unlike DLPC/DHPC or DMPC/DHPC systems, the formation DBPC/DHPC bicelles is very sensitive to the ratio (q) of long-chain phospholipid to DHPC, and these bicelles can only be prepared when q is very close to 6. However, they are stable within a reasonably wide temperature range (8−40 °C; Figure 1, B−D). For comparison, the spectrum of a 2.5:1 DLPC/DHPC bicellar system is shown in Figure 1, A. The patterns of the two types of spectra are consistent with parallel and perpendicular alignments of the major axes of the bicelles, respectively.[1,5,9,10] The spectra of the DBPC/DHPC bicelles at 45 and 50 °C (Figure 1, E and F) can be interpreted as arising from three coexisting species (bicelles with $\Delta\chi > 0$, bicelles with $\Delta\chi < 0$, and isotropic micelles) in chemical exchange with moderate rates. At 55 °C, only the isotropic peak remains (Figure 1, G).

From a theoretical consideration,[11] the diameter of DMPC/DHPC bicelles with $q = 3$ was estimated to be about 20 nm. If this theory is applicable to the DBPC/DHPC bicelles with $q = 6$, their diameter would be about 50 nm. However, this is only a rough estimate because the two chains in DBPC are quite different, and thus the molecular conformations in the two types of bicelles may not be the same. This point is further explored by considering the following.

A simulation of the ^{31}P spectra of phospholipid bicelles indicated that, when normal bicelles flip from perpendicular alignment to parallel alignment without adding paramagnetic ions, the ^{31}P peaks would have twice the chemical shifts (with respect to the isotropic peak, which is not necessarily at zero ppm) and opposite signs.[10] The chemical shift difference between the major and minor peaks in the DBPC/DHPC bicelles is +15.4 ppm at 20 °C and +10.9 ppm at 40 °C, whereas the corresponding differences for the DDPC/DHPC bicelles are −5.3 and −4.6 ppm, respectively. In other words, the difference for the "flipped" bicelles is much more than a factor of −2 compared with the normal bicelles, and its temperature dependence is larger. The most likely reason for this is that the conformation of the polar headgroup in the two types of bicelles is different. Studies of phosphatidylcholine bilayers using ^{31}P NMR, X-ray diffraction, and neutron diffraction indicated that the choline dipole is aligned more or less parallel to the bilayer surface,[12] and the same situation can be expected for normal bicelles. However, because of the inequivalence of the two chains in DBPC, the choline dipole could be tilted in the flipped bicelles. This postulation is substantiated by a study of the NMR spectra of small ions in the systems.

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(8) Dissolve 1-lauroyl-2-hydroxy-*sn*-glycero-3-phosphocholine (0.315 mmol) and biphenylacetic acid (0.98 mmol) in CH_2Cl_2 (5 mL). Add 4-(dimethylamino)-pyridine (DMAP) (0.30 mmol) and a solution of 1,3-dicyclohexylcarbodiimide (0.99 mmol) in CH_2Cl_2 (2 mL). Stir at room temperature for 2 days. Remove the white precipitate by filtration through a small cotton wool plug in a 6 in. pipet. Rotoevaporate the filtrate. Purify twice by column chromatography (65:35:4 $CHCl_3/CH_3OH/H_2O$) to yield = 60% based on 1-lauroyl-2-hydroxy-*sn*-glycero-3-phosphocholine loaded initially: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.4 (m, 4H, Ar), 7.3 (m, 2H, Ar), 7.2 (m, 3H, Ar), 5.15 (m, 1H, CH), 4.3 (dd, 1H, CH), 4.2 (m, 2H, $POCH_2CH_2N$), 4.1 (dd, 1H, CH), 3.9 (m, 2H, CH_2OCO), 3.64 (m, 2H, CH_2N), 3.6 (s, 2H, $OCOCH_2Ar$), 3.2 (s, 9H, $N(CH_3)_3$), 2.1 (t, 2H, $OCOCH_2Ar$), 1.4 (m, 2H, $-CH_2-$), 1.16 (s, 16H, fatty CH_2), 0.8 (t, 3H, $-CH_3$); mass spectrum (FAB) *m/z* 634.4 (calculated 633.78).

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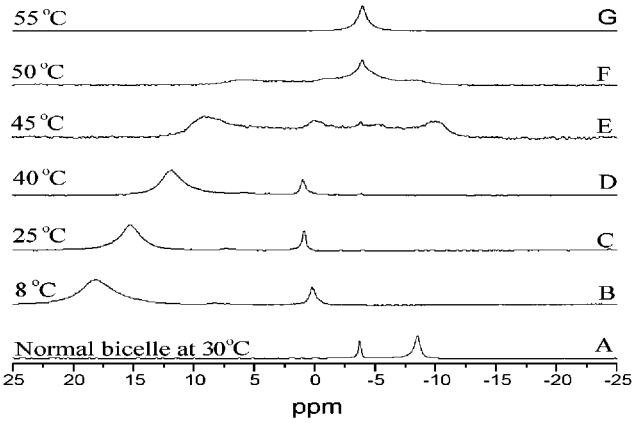

Figure 1. ^{31}P spectra (162 MHz) of (A) 2.5:1 DLPC/DHPC mixture (21% w/v with 0.1 M NaCl) at 30 °C, and (B−G) 6:1 DBPC/DHPC mixture (15% w/v with 0.1 M NaCl) at different temperatures. Concentrated H_3PO_4 was used as external reference.

The monovalent salts LiCl, NaCl, and KCl have no effect on the repeat distance of the lamellar phase of phospatidylcholines.[13] However, the cations do interact with the polar headgroup, with the trend $Ca^{2+} > Mg^{2+} > Li^+ > K^+, Na^+$.[14] Because the close approach of the cations to the bilayer surface can induce an asymmetry in their hydration sheath, ^{23}Na and ^{7}Li quadrupole splittings can be observed.[15] The splittings are expected to decrease rapidly with the decrease of phospholipid concentration. Indeed, the ^{23}Na NMR spectrum of Na^+ in the aqueous solution of normal bicelles show a single peak without quadrupole splitting. On the other hand, the ^{23}Na NMR spectra of Na^+ in the DBPC/DHPC bicellar system show a triplet with quadrupole splitting up to 250 Hz. This is an indication that the Na^+ ions interact with the surface of the phospholipid bilayers differently in the two bicellar systems. Similarly, the ^{19}F NMR spectra of the DBPC/DHPC bicellar solutions containing 0.1 M of $NaCF_3COO$ instead of NaCl show dipolar splittings of about 50 Hz, but those for the normal bicellar solutions show no splitting. These observations indicate that the conformation of the polar headgroup in the DBPC/DHPC bicelles is different from that in the DMPC/DHPC or DLPC/DHPC bicelles. Obviously the replacement of one of the long aliphatic chains by a biphenylacetyl unit is responsible for the conformational change of the polar headgroup, but the details of the change awaits further investigation.

Table 1. Some ^{1}H−^{13}C Dipolar Coupling Constants (D, in Hz) of 30 mM Maltotriose in Two Bicellar Solutions in D_2O[a]

peak assignments	1β	1α	3β	4″	5α	2β
normal bicelles[b]	2.5	−4.0	1.7	−8.0	2.6	4.6
flipped bicelles[c]	−2.7	10.2	−3.2	18.3	−2.1	−7.9

[a] The values were calculated from the splittings (Δ) using the formular $\Delta = 2D + J$, where J's are scalar coupling constants determined from a solution in pure D_2O. The peak assignments follow those in ref 16 and 17. [b] DLPC/DHPC (3/1, 10% w/v, 30 °C). [c] DBPC/DHPC (6/1, 11% w/v, 25 °C).

To test whether the new DBPC/DHPC bicelles can be used for NMR studies of biological molecules, we carried out HMQC experiments for a trisaccharide, maltotriose (from Sigma), in DBPC/DHPC and DDPC/DHPC bicellar solutions. Although the ^{31}P spectra (Figure 1, B−D) of the DBPC/DHPC bicelles and the ^{1}H spectra of the solute molecules are slightly broader, they did not affect the determination of the ^{1}H−^{13}C dipolar coupling constants. The results for the best resolved ^{13}C peaks are listed in Table 1. The data in the table show that the dipolar coupling constants of the trisaccharide in the two liquid crystalline solutions have opposite signs. Therefore, the new DBPC/DHPC bicellar system indeed has a different orientation in the magnetic field and can complement the conventional bicellar systems.

The results for maltotriose show that the DBPC/DHPC bicellar system is suitable as an ordering medium for water-soluble species and may offer certain advantages in controlling the orientational ordering of these species. On the other hand, if they are to be used as model membranes for lipid-soluble species, one must ascertain that the biphenyl unit would not change the conformations and activities of the biomolecules.

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