Bolalipid Membrane Structure Revealed by Solid-State ^{2}H NMR Spectroscopy

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The development of membrane-protein based biotechnologies necessitates the identification and design of new membrane materials with enhanced stability. Bolalipids are an interesting class of bipolar lipids that have been proposed for these applications. *Archaea* are unicellular organisms capable of thriving under extreme conditions, such as high temperature, high salt concentration, or low pH. *Archaea* often possess significant amounts of bolalipids that contain a hydrolysis resistant ether-linkage, as opposed to the more labile ester-linked monopolar phospholipids of *Eukaryota* and *Bacteria*. One distinctive feature of bolalipids is the presence of membrane spanning alkyl chains, which are believed to be responsible for their enhanced physical stability. This stability has been utilized to develop planar supported membranes, to reconstitute and study integral membrane proteins, and as gene and vaccine delivery vehicles. Most of these efforts have focused on the use of synthetic archaeal lipid analogues due to the difficulties of obtaining naturally occurring bolalipids in pure form., Given the growing importance of these analogues, a better understanding of their structural and dynamical properties is needed.

Solid-state ^{2}H NMR spectroscopy is commonly used to probe membrane structure and dynamics. ^{2}H NMR spectroscopy can probe individual C–[2]H bonds in a membrane lipid or protein, providing atomically resolved information on the local environment of the deuteron. The observable quadrupolar couplings arise from the interaction of the quadrupole moment of the ^{2}H nucleus with the electric field gradient of the C–[2]H bond. These couplings can be used to calculate segmental order parameters (S_{CD}), which provide insight into the local membrane organization at the position of the C–[2]H bond. Here we utilized ^{2}H NMR spectroscopy to analyze three deuterium-labeled variants of a previously synthesized bolalipid analogue, C_{20}BAS-PC.6 The three compounds, $[1',1',20',-20'-^{2}H_4]C_{20}$BAS-PC (**1**), $[2',2',19',19'-^{2}H_4]C_{20}$BAS-PC (**2**), and $[10',11'-^{2}H_2]C_{20}$BAS-PC (**3**) (Figure 1), were synthesized with deuterium labels on the *sn*-1 chain (Supporting Information). Residual quadrupolar couplings were calculated from the de-Paked ^{2}H NMR spectra. These splittings were used to calculate S_{CD}, the approximate hydrophobic layer thickness for half of the

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membrane (D_c), and the area per lipid $\langle A \rangle$ using a first-order mean-torque model. The data were compared to a monopolar lipid, dilauryl phosphatidylcholine (DLPC).

In the high-temperature, liquid-crystalline state, **2** and **3** (Figure 1) yielded single quadrupolar splittings due to the equivalence of all deuterium atoms in both compounds, resulting in order parameters of $|S_{CD}| = 0.21$ and 0.23 at 25 °C, respectively. However, **1** (Figure 1) gave two distinct quadrupolar splittings, indicating the existence of two distinct environments for deuterium in this molecule. These two components were equal in intensity at two different temperatures (25 and 45 °C) in the liquid-crystalline phase. Similar findings have been reported for 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) at the *sn*-2 C2 position and the C1 position of both the *sn*-1 and *sn*-2 chains in the ether-linked monopolar phospholipid, 1,2-dihexadecyl-*sn*-glycero-3-phospho-ethanolamine (DHPE). It is believed that the inequivalence of the two deuterium atoms arises from different glycerol H–C1–O–C1′–^{2}H alkyl dihedral orientations of the alkyl chain near the bilayer interface.

The segmental order parameters of the bolalipid can be used to determine the conformation of the lipid within the membrane. We were specifically interested in whether the bolalipids adopt a transmembrane conformation, with the two polar head groups on opposite sides of the membrane, or a looping conformer, with the polar head groups on the same side of the membrane. The centermost C–^{2}H bonds should exhibit drastically different orientations with respect to the membrane normal between the two conformers. This difference will be observed as a change in the magnitude of the quadrupolar splitting, where the transmembrane conformer generates a much larger splitting than that of the looping conformer. A previous study of ester-linked bolaamphiphiles resulted in mostly transmembrane conformers with a small (<10%) percentage of looping conformers. The ^{2}H NMR spectrum of **3**, however, exhibits a single large quadrupolar splitting. Based on this finding, we infer that $C_{20}BAS$-PC adopts transmembrane conformers, with no detectable presence of looping conformers.

Powder-type ^{2}H NMR spectra of **3** in the liquid-crystalline phase are well-defined and give a distinct quadrupolar splitting (Figure 1C). As the temperature decreases, the bolalipid motion also decreases upon entering the gel phase ($T_m = 15$ °C), generating a single broad line. This spectrum arises because the *trans-gauche* isomerization occurs at a rate similar to the NMR time scale, leading to an observed loss of axial symmetry of the lipid with respect to the membrane normal. The triangular shape of the ^{2}H NMR spectrum at intermediate temperatures is reminiscent of the $\eta = 1$ powder pattern expected for restricted 2-site rotational isomerism. As the temperature is further decreased, the membrane adopts an all *trans* conformation, and the powder spectrum becomes resolved again due to the reemergence of axial symmetry. The temperature dependence of the quadrupolar splitting of **3** (Figure 2A) displays a large difference in order between the liquid-crystalline and gel phases, the latter of which approaches the 250 MHz rigid lattice limit for the C–^{2}H bond parallel to the $\mathbf{B_0}$ field., Interestingly, there is a large observed increase in segmental order between the C1′(20′) and C2′(19′) positions, and then very little change is observed between the C2′(19′) and C9′(10′) positions. From this we infer that the alkyl chain is highly ordered between C2′ and C19′, in stark contrast to monopolar lipids such as DLPC,

where the order is known to decrease drastically as a function of distance from the glycerol backbone (Figure 2B).,

It has been established that D_c, $\langle A \rangle$, and thermal expansion coefficients can be calculated from the residual quadrupolar splittings (cf. Supporting Information). Our data show that the values of D_c and $\langle A \rangle$ of C_{20}BAS-PC at 30 °C are 9.1 Å and 60.5 Å^2, respectively (Figure 3). While DLPC has a larger value of D_c than C_{20}BAS-PC (10.5 vs 9.1 Å), the area per lipid is quite similar (62.6 vs 60.5 Å^2). Taking into consideration that a DLPC bilayer consists of 22 methylene segments as opposed to 20 in C_{20}BAS-PC, these results demonstrate that the additional mass from the DLPC membrane contributes to a thicker hydrophobic layer while having a much smaller impact on $\langle A \rangle$. The isobaric thermal expansion coefficients for D_c and $\langle A \rangle$ ($\alpha_\parallel$ and $\alpha_\perp$) are given by the slopes of $\ln D_c$ and $\ln \langle A \rangle$ as a function of temperature. We report $\alpha_\parallel$ and $\alpha_\perp$ for C_{20}BAS-PC as -2.4×10^{-3} and 3.6×10^{-3} K^{-1}, respectively. These data are very similar to those for DLPC ($\alpha_\parallel = -3.0 \times 10^{-3}$ and $\alpha_\perp = 4.1 \times 10^{-3}$ K^{-1}), despite the very different molecular structures and segmental order profiles.

These results provide insight into the structural and functional properties of bolalipids and may aid our understanding of their interactions with associated proteins. We are currently utilizing the ^{2}H NMR spectroscopy results reported here to investigate the mixing behavior of bolalipid/monopolar binary membrane systems.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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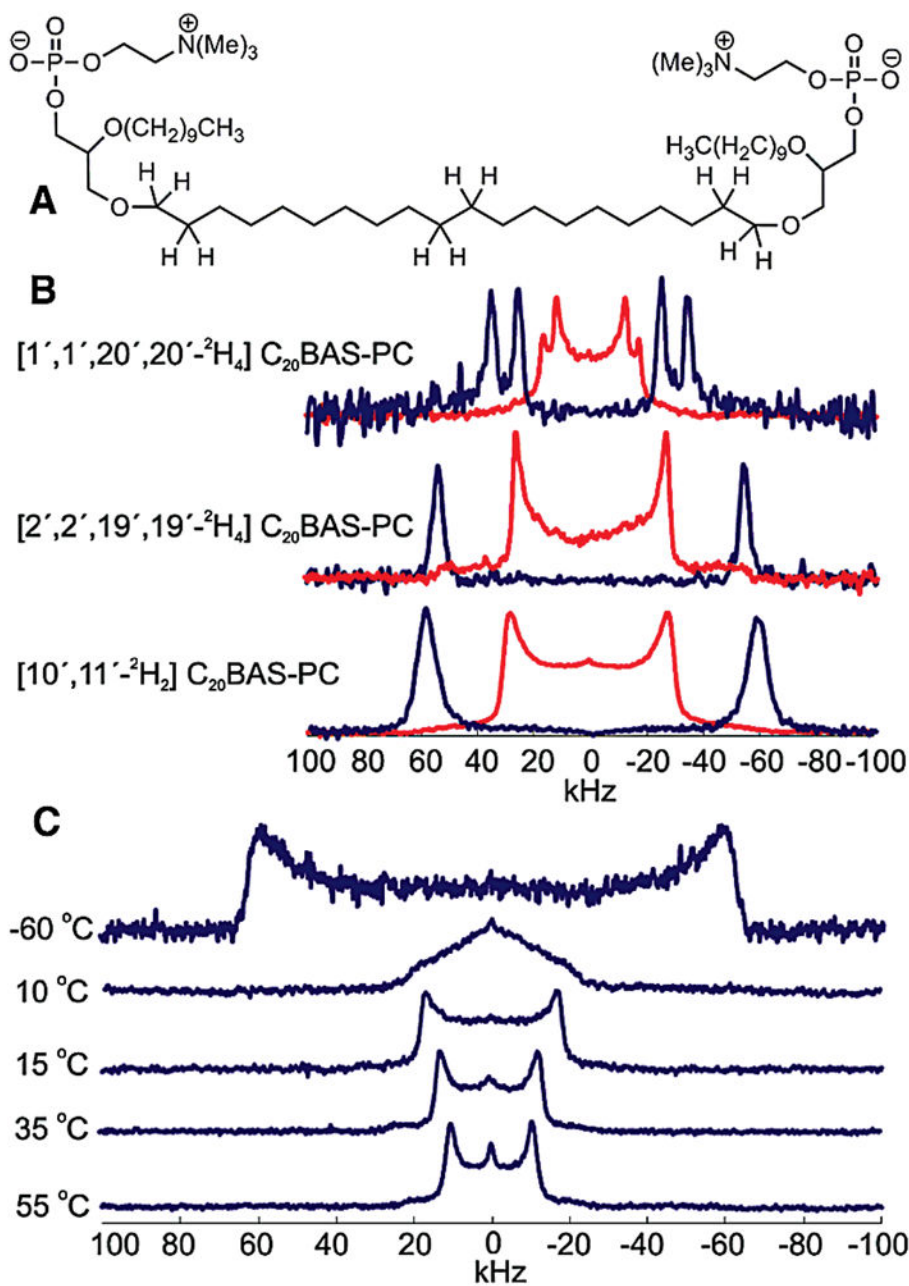
Figure 1.
(A) Structure of non-deuterated $C_{20}BAS$-PC. (B) Powder-type (red) and de-Paked (blue) ^2H NMR spectra of $[1',1',20',20'-^2H_4]C_{20}BAS$-PC (**1**), $[2',2',19',19'-^2H_4]C_{20}BAS$-PC (**2**), and $[10',11'-^2H_2]C_{20}BAS$-PC (**3**) at 25 °C. (C) Powder-type ^2H NMR spectra of $[10', 11'-^2H_2]C_{20}BAS$-PC at −60, 10, 15, 35, and 55 °C.
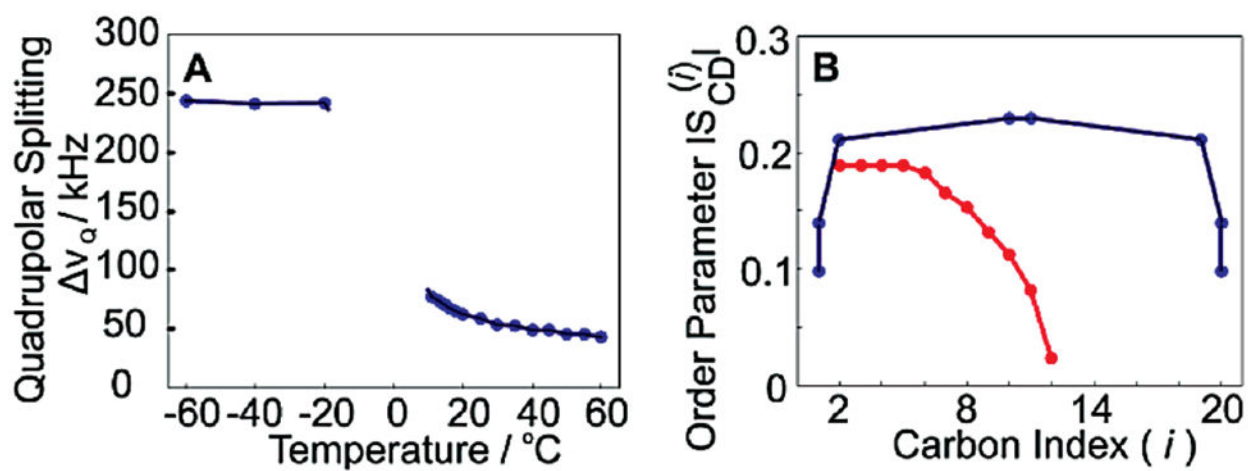
Figure 2.
(A) De-Paked quadrupolar splittings (ν_Q) of $[10',11'-^2H_2]C_{20}$-BAS-PC as a function of temperature. (B) Order parameters $|S_{CD}^{(i)}|$ of C_{20}-BAS-PC (blue) at 25 °C and DLPC (red) at 30 °C as a function of carbon number.

Figure 3.
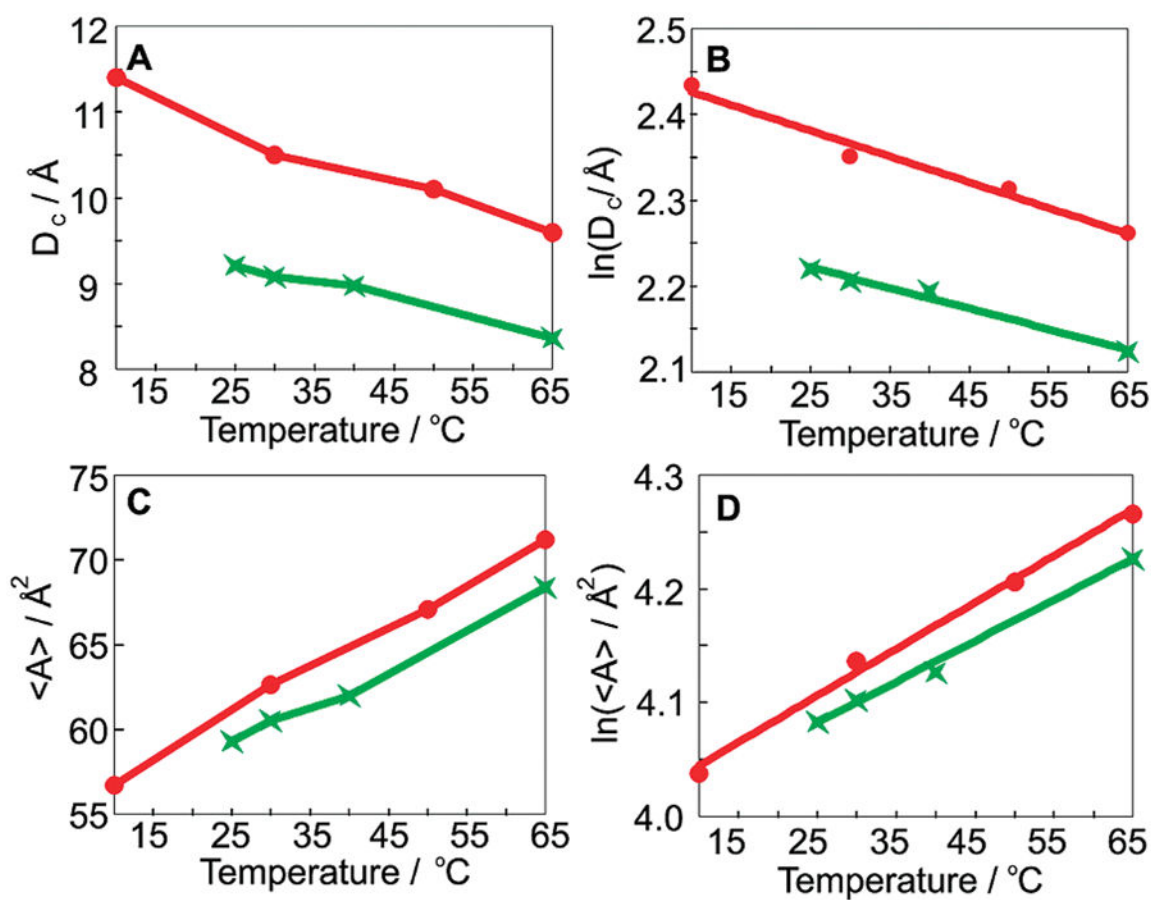(A) Hydrophobic layer thickness (D_C) for [2′,2′,19′,19′-^{2}H$_4$]C$_{20}$-BAS-PC (green) and DLPC (red) as a function of temperature. (B) Natural logarithm of hydrophobic layer thickness, (C) area per lipid, and (D) natural logarithm of area per lipid as a function of temperature.