Evidence for uncorrelated tilted layer structure and electrically polarized bilayers in amphiphilic glycolipids

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A strong low-frequency dielectric relaxation mode spanning from below 100 Hz at low temperatures to 100 kHz at high temperature, x-ray diffraction studies, and optical microscopic observations show, contrary to the currently accepted models, that the glycolipid molecules are tilted with respect to the layer normal in the smectic phase but the tilt direction is not correlated between the bilayers. The tilted glycolipid bilayers are electrically polarized. The tilted structure and the electric polarization of amphiphilic glycolipids may play an important role in biological cell membrane.

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INTRODUCTION

One of the most important roles liquid crystalline structures of amphiphilic lipids play in living organisms can be found in biological cell membranes. The structure of generally accepted fluid mosaic model of the membrane [1] is a two-dimensional smectic *A* liquid crystalline bilayers consisted of amphiphilic lipids and proteins embedded in them [2]. On the other hand, based on the optical microscopic observations which show the uniaxiality and the x-ray diffraction data which show consistently that the thickness of the amphiphilic lipid bilayers are significantly smaller than twice the length of the individual molecules, it is also believed that the amphiphilic lipids form an interdigitated structure [3] where the alkyl chains partially overlap.

The chiral amphiphilic lipids are known to show ferroelectric liquid crystalline properties in the biological cell membranes. The propagation of action potential in nerve and muscle cell and retinal photoreceptors have been attributed to the ferroelectric properties arising from chiral building blocks [4]. A Curie point and current-voltage hysteresis typical of ferroelectric substances have been observed in cell membranes [5,6]. Temperature dependent current has been induced in frog of Ranvier by laser suggesting pyroelectric effect [7]. Swelling of membranes in response to a voltage application which indicates piezoelectric effect has been reported [8]. It has been suggested that ferroelectricity may be common in cell components [9,10] and relationship between liquid crystalline ferroelectricity and nerve and muscle impulses has been predicted [11] but possible origin of the ferroelectric structure in the cell membrane has not been shown.

Glycolipid is one of the three lipids found in the biological cell membranes. Glycolipid molecules contain polar (hydrophilic) sugar headgroups and apolar (hydrophobic) alkyl chains. The length of the alkyl chain and the number of sugar headgroups determine the polymorphism in both lyotropic and thermotropic structures of glycolipids. Lyotropic properties of synthetic glycolipids have been extensively studied in

the last decade but so far their thermotropic states have not been assessed properly [12]. The shorter glycolipids (with eight to ten carbon atoms) are completely soluble in water and mainly used as detergents [13]. Glycolipids with longer alkyl chains are not water soluble and tend to form columnar structures in pure form and stable liposome and bilayers in aqueous medium. The phase behavior of glycolipids is known to involve in cell fusion processes [14,15] and membrane traffic, for example, during exocytosis or the virus-cell fusion in the course of an infection [16,17]. The more complex glycolipids (starting with three sugar head groups) are involved in cell surface recognition processes [18].

The structure of the glycolipid bilayers was studied by powder diffraction measurements [19] which showed diffused rings of scattering commonly associated with smectic *A* and *C* phases. In all such materials, the measured layer spacing was larger than the length of one molecule indicating formation of double layers. However, the length of the double layers was less than the length of two molecules. It is generally accepted that [20] the hydrogen-bond forming ability of the polar moiety is crucial to the formation of mesophases in the amphiphilic derivatives. Therefore it was concluded that polar moieties overlap to produce interdigitated smectic *A* phase known as smectic A_d [3]. Later it was suggested that the stability of the hydrogen bonds of hydroxyl groups is particularly sensitive to the temperature due to their orientational freedom [21]. This observation led to the now widely accepted structure [3] in which the alkyl chains partially overlap to form the core of the smectic A_d bilayers and polar moieties are on the outside.

Recently it was found that the glycolipid layers became electrically polarized by the antiferroelectric bent-core liquid crystal layers [22] in the supramolecular structures of glycolipid dodecyl-β-*D*-glucopyranoside ($C_{12}G_1$) and bent-core ("banana-shape") liquid crystals. Because the pure $C_{12}G_1$ molecules form macroscopically nonpolar structures, it was deduced that in the bent-core environment the glycolipid double layers exhibit a tilted configuration with the polar heads tilted with respect to each other. It has also been found that the glycolipids have shown spontaneous polarization
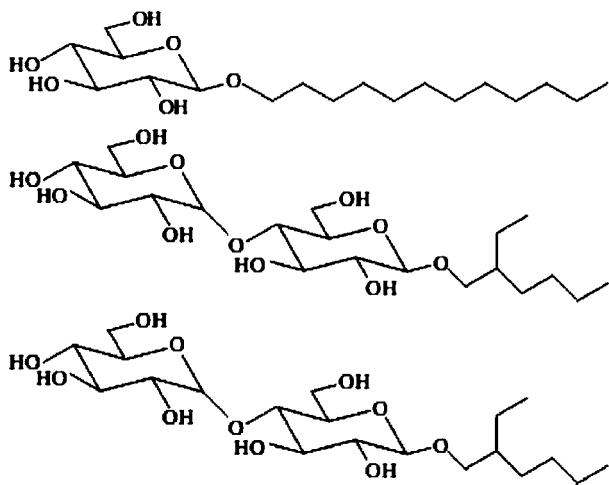
FIG. 1. Molecular structures of the studied materials.

[23] when mixed with straight-core chiral SmC^* substances but show no electric polarization when mixed with straight-core nonchiral SmC substances [23]. This indicates that the tilt direction of the double layers of lipids may be correlated with the tilt direction of the host and the hydrophobic-hydrophilic interactions play a dominant role in the layer structure. It is important to note that these substances do not mix with typical SmA substances.

One possibility to distinguish these structures is to study their dielectric behavior, because it provides information about orientation dependent response of the liquid crystals to the electric field. The interdigitated structure cannot be easily polarized, because a tilting of the constituent molecules would require a change in the layer spacing, which is a hard deformation. Relaxation due to the rotation of the molecules around their long axes can take place but the strong hydrogen bonding may prevent this rotation. However, if the chiral polar molecules are already tilted with respect to the layer normal resulting permanent dipole moments [24], the electric field couple with the dipoles can easily bias the tilt direction. Since this coupling requires the tilt direction to rotate with the field, this response is damped by the rotational viscosity of the fluid, in practice disappear above a few hundred Hz. The dielectric coupling, which is quadratic in field, produces a static response which remains at much higher frequency.

EXPERIMENTAL

Here we report differential scanning calorimetric (DSC), optical microscopy, x-ray diffraction, and dielectric measurements studies of three different glycolipids with varying chemical structure shown in Fig. 1. We have carried out DSC scan to determine the phase transition temperatures and observed the polarizing microscopic textures to identify the liquid crystalline phases. We heated the substances to their isotropic phase and filled them into 4 μm cells (from Displaytech, Inc.) The inner surfaces of the cells were coated with transparent ITO electrodes overcoated with polyimide layers rubbed in antiparallel directions. After cooling from the isotropic phase, the textures of $C_{12}G_1$ consisted of small

(10–20 μm) diameter focal conic fan-shaped domains, indicating bookshelf type alignment, where the layers are normal to the substrates. The focal conic domains of both $C_{6-2}G_2$ and $C_{8-4}G_2$ were much smaller (only visible with larger magnification). The glycolipid $C_{12}G_1$ exhibit a broad smectic phase from 80 to 142 °C, $C_{6-2}G_2$ exhibit a broad smectic phase from room temperature to 129 °C, and $C_{8-4}G_2$ exhibit a broad smectic phase from room temperature to 188 °C. They do not exhibit any other thermotropic mesophases in both heating and cooling.

We also prepared samples in untreated 8 μm cells. As the samples in these cells were cooled down from isotropic liquid to liquid crystalline phase bâtonnets were formed but they rapidly disappeared giving rise to homeotropic texture. Interference figures typical of homeotrically aligned smectic phase could not be observed in conoscopic studies of these samples.

To elucidate the molecular arrangements, high resolution x-ray measurements [25] were carried out. The bilayer thicknesses were determined to be 34.2 Å for $C_{12}G_1$, 27.6 Å for $C_{6-2}G_2$, and 31.5 Å for $C_{8-4}G_2$. The fully stretched molecular bond lengths of $C_{12}G_1$, $C_{6-2}G_2$, and $C_{8-4}G_2$ are 19.2, 15.62, and 20.23 Å, respectively. They are significantly larger than the half of their bilayer thickness.

To determine the complex dielectric constant $\varepsilon^* = \varepsilon' - i\varepsilon''$, we measured the impedance and the phase angle using Quadtech 1920 precision LCR meter (frequency range from 100 Hz to 1 MHz). We controlled the temperature of the samples using Instec heat stage HS 2000. The dielectric spectra (ε') for all three compounds at different temperatures are shown in Figs. 2(a)–2(c). The dielectric relaxations of all three compounds continue into the isotropic phase. The dielectric constant of $C_{12}G_1$, $C_{6-2}G_2$, and $C_{8-4}G_2$ are 54, 102, and 100, respectively, and relaxes to about 6 at high frequencies. The temperature dependency of the relaxation frequencies is shown in Fig. 2(d) indicating Arrhenius-type temperature dependence both in smectic phase and in the isotropic phase. The smaller slopes in the isotropic range show that the activation energies in the isotropic phase are lower than those in the smectic phase for all three compounds. We took only a few data points in the isotropic phase because these compounds can be unstable at higher temperatures above isotropic phase transition. The relaxation frequencies were out of the measurement range below 80 °C.

The activation energies in the smectic phase determined from the slope of the $\ln(f_r)$ vs $1/T$ curves are 81, 118, and 105 kJ/mol for $C_{12}G_1$, $C_{6-2}G_2$, and $C_{8-4}G_2$, respectively. The glycolipids with two head groups and shorter carbon chains have significantly larger activation energies. This indicates that activation energy is determined mainly by the hydrogen bonding between the molecules. The hydrogen bonding of the molecules in adjacent layers may be related more to the activation energy than the hydrogen bonding of the molecules in the same layers.

To determine the type of the relaxation process we prepared the Cole-Cole plots as shown in Fig. 3 and fitted to the following equation:
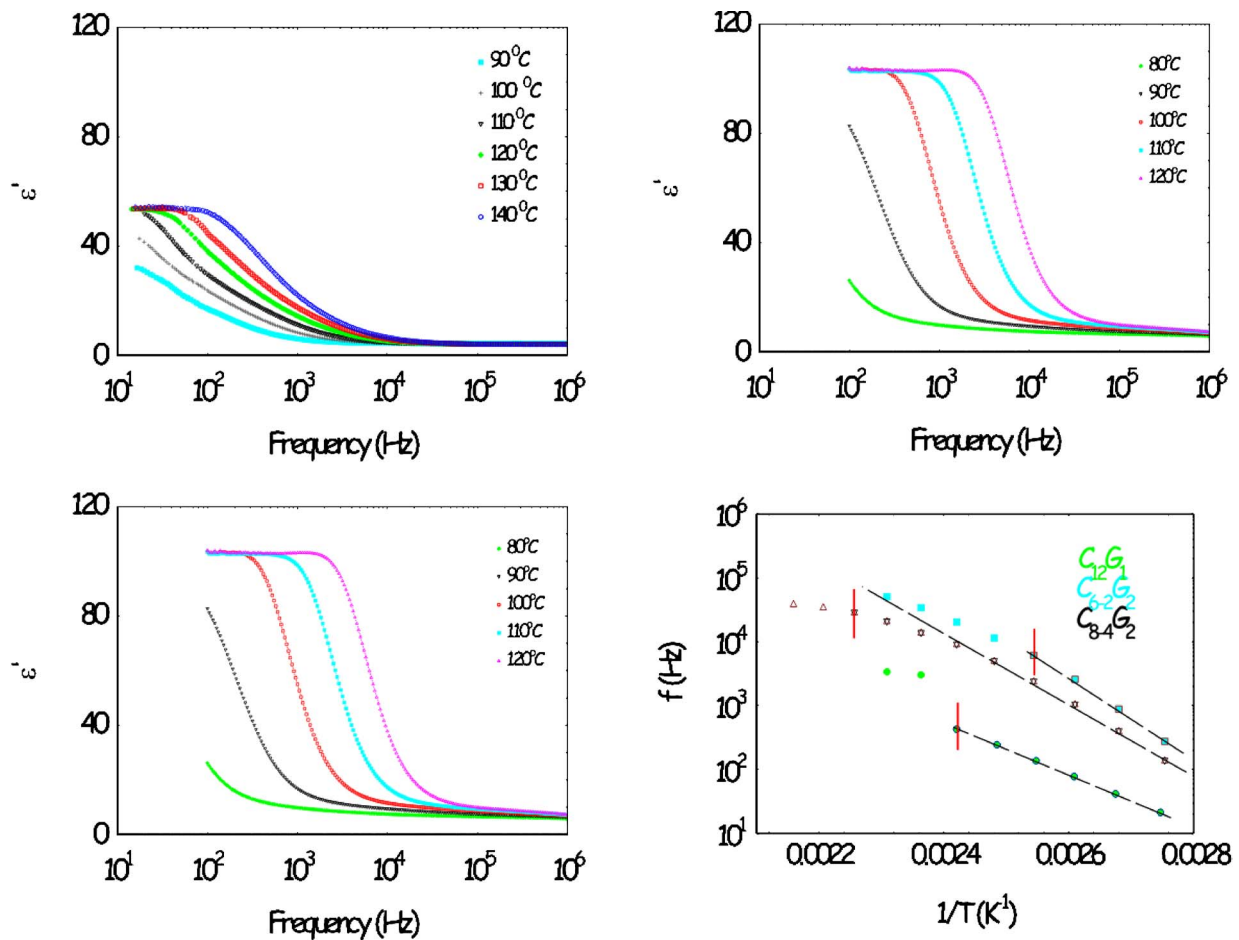
FIG. 2. (Color online) Dielectric spectra of materials (a) $C_{12}G_1$, (b) $C_{6-2}G_2$, (c) $C_{8-4}G_2$, and (d) temperature dependences of the relaxation frequencies for all three materials. Data in the isotropic phase are to the left of the vertical bar.

$$\left[\varepsilon' - \left(\varepsilon'(\infty) + \frac{\chi}{2}\right)\right]^2 + \left[\varepsilon'' + \frac{\chi}{2}\tan\left(\frac{\alpha\pi}{2}\right)\right]^2$$

$$= \left(\frac{\chi}{2\cos\left(\frac{\alpha\pi}{2}\right)}\right)^2. \tag{1}$$

In this equation, χ is the low-frequency dielectric susceptibility, α is the distribution parameter as introduced by Cole and Cole [26], and $\varepsilon'(\infty)$ is the high-frequency dielectric constants. Equation (1) corresponds to a relaxation process with symmetric distribution as described by the equation

$$\varepsilon^*(\omega) - \varepsilon'(\infty) = \frac{\varepsilon'(0) - \varepsilon'(\infty)}{1 + (i\omega\tau)^{1-\alpha}}. \tag{2}$$

The parameters determined from the best fits shown by dashed lines in Fig. 3 are the following. The dielectric susceptibilities $\chi = 60.5, 109, 107$; the distribution parameter $\alpha = 0.39, 0.05, 0.01$; and the high-frequency dielectric constants $\varepsilon'(\infty) = 3.67, 9, 8.5$ for $C_{12}G_1$, $C_{6-2}G_2$, and $C_{8-4}G_2$, respectively.

These values show that both the dielectric constant and the susceptibilities of glycolipids with two sugar head groups are almost the same and much larger than the glycolipid with

one sugar head group. These results are consistent with the calculations which show that the dipoles of the substances with two polar heads have roughly twice as large dipoles. The distribution parameters are also similar to that of typical liquid crystals. The high-frequency permittivity indicates some additional relaxation modes at higher frequencies, especially for the two-head-two tail materials. This mode most probably is related to the rotation around the long axis explaining why it is very weak for the $C_{12}G_1$, which has the smallest transverse dipole.

DISCUSSION

Dielectric spectra for all three glycolipids show a temperature-dependent relaxation mode with relatively large dielectric susceptibility taking place between 100 Hz and 100 kHz range in the smectic phase. The relaxation frequency and the strength of the mode resemble the Goldstone mode of the ferroelectric tilted SmC^* liquid crystals [27]. The low-frequency relaxation associated with Goldstone mode is due to collective rotation of molecules in the helical structure and usually temperature independent. There is no evidence of a helical structure in glycolipids. There are other hydrogen bonding substances showing low frequency relax-
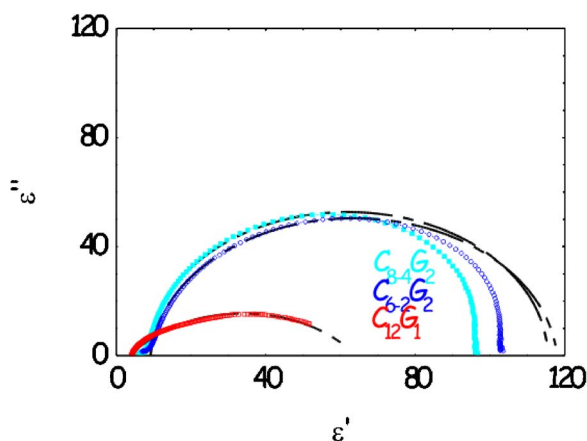
FIG. 3. (Color online) Cole-Cole plots of the glycolipids studied at 100 °C.

ation in their liquid crystalline phases [28]. However, they do not form extensive and strong three-dimensional networks via hydrogen bonding as the amphiphilic glycolipids do [29]. The rotation of glycolipid molecules about their long axis may be restricted by the hydrogen bonding between the molecules in the same layer.

The origin of the observed low frequency relaxation of the glycolipids in the smectic phase can be accounted for if the lipids molecules are taken to be tilted in their layers. This can be expected because average area occupied by polar moiety is significantly larger than the cross section of the alkyl chains in which the tilted structure gives more favorable packing. The glycolipid molecules are chiral and optically active. In spite of this, they form either homeotropic or focal-conic fan textures upon cooling from the isotropic phase but exhibit no helical structure or spontaneous polarization. In a homeotropically aligned texture of SmA phase, where the molecular director is normal to the glass surface, no birefringence is observed because the phase is uniaxial and the optic axis is normal to the substrates. The conoscopic observations of homeotropically aligned SmA texture show interference figures [30] indicating that the texture is not isotropic but uniaxial. Conoscopic observations of the homeotropic textures of amphiphilic glycolipids do not show interference figures typical of SmA phase. This is possible in the homeotropically aligned SmC phase, however, if the tilt direction is randomly oriented about the layer normal. The tilt information is contained in the alkyl chains and communicated via long-range interactions. Those long-range interactions of the amphiphilic molecules are dominated by the strong short-range interactions between the sugar head groups. This indicates that the direction of the tilt is not correlated between the bilayers because the tilt information of the alkyl chains is screened off by the polar head groups.

The relaxation mode in the glycolipids, unlike in the Goldstone mode in the SmC^* phase, is highly temperature dependent. This can be expected because there is no helical structure formed in these bilayers. Instead, the bilayers of glycolipids are weakly connected by the temperature-dependent hydrogen bonding of the polar head groups between adjacent layers [29]. Since the tilt direction of bilayers randomly oriented about the layer normal, each configuration
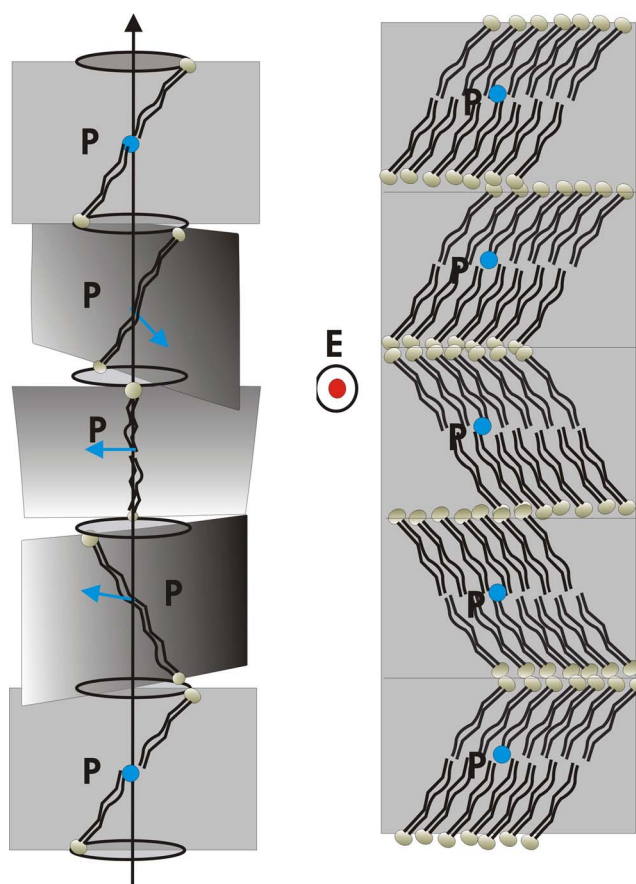

FIG. 4. (Color online) Sketch of the proposed model of the glycolipid bilayers. (a) The resultant permanent dipole moments along the twofold axis randomly oriented about the layer normal in the absence of the electric field and (b) with the electric field (pointing outward) applied in the plane of the layers.

the molecules take during the rotation about the layer normal has a different energy state. The temperature dependence of the relaxation frequency increases as the number of hydrogen bonding of the polar head groups between the adjacent layers increases as indicated by the activation energies.

Layers of tilted molecules have the monoclinic symmetry. The point group for which contains only a twofold rotation axis parallel to the layers and perpendicular to the twofold axis, a reflection plane normal to the twofold axis and center of inversion. When the molecules are chiral, the mirror plane and the center of inversion are eliminated. This results in a permanent dipole moment parallel to the twofold axis. But when the molecules are amphiphilic, the rotation of molecules around twofold axis is prevented due to hydrophobic-hydrophilic interactions and the symmetry is further reduced. Therefore the direction of permanent dipole moment is not determined only by the symmetry but also by the conformation of the molecules.

However, the formation of double layers introduces a situation similar to the rotation of molecules around twofold axis. Existence of twofold symmetry axis enables the molecules with permanent dipole moments to have two degenerated minimum energy states in monoclinic environment. In double layers, those two states are shifted perpendicular to

the twofold axis and molecules correspond to those two states lie in two separate layers. So the double layer structure establishes the permanent dipole moments perpendicular to the tilt direction in the plane of the double layers. Since the tilt direction of the bilayers is not correlated, the polarization is averaged out in the bulk [Fig. 4(a)].

Amphiphilic double layers should have a number of unusual properties and the layers of amphiphilic molecules behave quite different from the layers of monophilic Sc* molecules. Mainly, two effects tend to strengthen the magnitude of the spontaneous polarization in amphiphilic layers. First, in monophilic SmC* layers, the coupling of the molecules to monoclinic symmetry may be weak so that molecules are almost free to rotate about its long axis reducing the magnitude of the spontaneous polarization. In amphiphilic layers, however, this rotation is restricted due to the hydrogen bonding between the polar head groups. Second, since the chiral parts of the molecule occupy the amphiphilic head group, it reduces the internal rotation of the molecules further strengthening the polarization. Even though each layer in smectic phase is energetically the same, in a mixture consisting of lipids with different chain length, handness and twisting power may separate into local domains and different layers for optimal packing. It is also important to note that the fundamental phase behavior of the amphiphilic double layers will not be affected by the presence of an aqueous interface.

The dielectric relaxation of all the substances continues into their isotropic phase. Similar phenomena have also been observed with other liquid crystalline substances. For example, in nematic phase, there are two relaxation processes each related to $\varepsilon_\perp$ and $\varepsilon_\parallel$ which takes place in radio frequency range. These relaxation processes can be observed in their isotropic phase as well [31]. The relaxation process of amphiphilic molecules in the isotropic phase may be due to the fact that they become aggregated clusters in the isotropic phase well above their phase transition because the thermal motion can not overcome the hydrophobic-hydrophilic effect. These clusters may have strong head to head correlation due to hydrogen bonding while they are randomly oriented with respect to their alkyl chains so that their response to an electric field is quite similar to that of liquid crystalline phase.

In summary, our results of dielectric, x-ray diffraction studies, and optical microscopic observations of glycolipids show, contrary to the current models, that the glycolipid molecules are tilted in their bilayers in the smectic phase. The tilted supramolecular structures they form in both bent-core and straight-core liquid crystals further confirm this assertion. The bilayers of tilted chiral glycolipid molecules are electrically polarized. However, the direction of the tilt is not correlated between the bilayers and therefore, no helical structure or spontaneous electric polarization is observed. Due to hydrophobic-hydrophilic interactions and the strong hydrogen bonding between the polar head groups, these amphiphilic molecules seem to remain in aggregated clusters even in the isotropic phase well above their transition. It is possible that, similar to these glycolipids, other amphiphilic lipids also have a tilted structure in the smectic phase. Since the fundamental phase behavior of the amphiphilic bilayers is not affected by the presence of an aqueous interface, the amphiphilic lipid bilayers may be polarized even in the aqueous medium. Therefore, it is possible that the tilted lipids will give rise to ferroelectric domains in the biological cell membranes as well. In-plane anisotropy and the ferroelectricity in the cell membrane may play a crucial role on transport properties, excitability, and ATP-synthesis of the cell membrane.

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