The Neutral Area Surface of the Cubic Mesophase: Location and Properties

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ABSTRACT A neutral area surface can be defined as one whose area remains fixed upon bending. It is assumed that such a surface exists within the amphiphilic monolayers that constitute the bicontinuous cubic mesophases and that it parallels approximately the highly convoluted polar/apolar interface in such systems. Here, we report on how the neutral area surface in the cubic phase (space group Ia3d) of the hydrated monoacylglycerol, monoolein, was determined. It is located at a distance of ~8.8 Å from the methyl terminus of the acyl chain. At 25°C, the surface area per lipid molecule at the neutral area surface is 35.1 ± 0.2 Å^2, which is remarkably similar to the mean cross-sectional area of hydrated monoolein in the lamellar liquid crystalline phase at this same temperature.

INTRODUCTION

The focus of this study can be best understood by considering the bending of a flexible bar. In so doing, one of the surfaces will experience an area expansion while the opposite surface contracts. Somewhere between these two surfaces there exists a neutral area surface, also referred to simply as a neutral surface, whose area remains constant during bending.

Several of the lyotropic mesophases can be viewed as assemblages of flexible monomolecular films of amphiphiles dispersed in a lyotrope. For purposes of this discussion, we will focus on water as the lyotrope and lipid as the amphiphile. Typical mesophases include the lamellar, hexagonal, and cubic phases (Fig. 1). In the case of the inverted hexagonal (H_{II}) phase, the highly curved lipid monolayer coating the hexagonally arranged water-filled cylindrical cores (Fig. 1) can be caused to flex and to bend by adjusting the relative amounts of water and lipid constituting the phase. A pivotal surface, wherein the variation in molecular cross-sectional area as a function of bending is minimal, has been identified in the H_{II} mesophase formed by hydrated phospholipids (Rand et al., 1990). Its stated location is in the apolar part of the mesophase at a distance of approximately one-third of the hydrocarbon chain length from the ester linkage.

Identifying and characterizing the neutral surface in lyotropic mesophases are important because the monolayer radius of curvature should be defined here (Gruner, 1989; Rand et al., 1990; Szleifer et al., 1990; Templer et al., 1993). A complete description of the microstructure of bicontinuous cubic phases (Hyde, 1989) should include the neutral surface. Further, calculations having to do with curvature elasticity in these systems are referenced to the neutral area surface (Szleifer et al., 1990; Templer et al., 1993). Parenthetically, we note that the neutral surface described in this communication is not that of Kozlov and Winterhalter (1991*a*, *b*), which is defined as having its extension and bending deformations uncoupled, i.e., it has a zero modulus of mixed deformation.

In this report, we describe a simple and generally applicable procedure for locating the neutral surface in the cubic phase. It involves calculating the molecular cross-sectional area along the length of the lipid molecule in the cubic phase as the hydration level of the mesophase is changed. By adjusting water content, the unit cell dimension of the phase is changed, which in turn causes flexing of the lipid monolayers within the mesophase matrix. The neutral surface is identified as the surface located at that point along the length of the lipid molecule where the area per molecule remains invariant during the bending (hydration/dehydration) process. It is emphasized that the neutral surface may reposition itself when lipid length changes due to large adjustments in water content and/or temperature. Further, it may cease to exist if bending is coupled with monolayer extension/compression (Templer et al., 1993).

The cubic and hexagonal phases are members of the so-called nonlamellar mesophases. As such, they possess curved polar/apolar interfaces. In the case of the bicontinuous cubics, the surface where the ends of the hydrocarbon chains from adjacent lipid monolayers meet separates the unit cell into two interpenetrating, nonconnected continuous networks (Longley and McIntosh, 1983; Gruner, 1989). It is referred to as an infinite periodic minimal surface (IPMS) (Longley and McIntosh, 1983; Anderson et al., 1988) because its mean curvature is everywhere zero. It is convenient to discuss the bicontinuous cubic phases in terms of IPMS because much is known about the topology of such mathematically well defined surfaces (Andersson et al., 1988). The lipid length throughout the unit cell of such cubic phases can be assumed to be constant (Anderson et al., 1988). Indeed, the deviation of the lipid length from the average value has been estimated to be ~4% in the Pn3m cubic phase when the average lipid length is in the range 15–20 Å (Anderson, 1988). With a view to identifying the neutral surface of the cubic phase as noted above, the following relationship can

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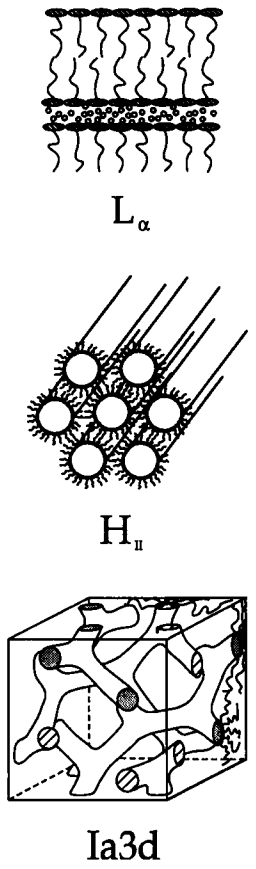

FIGURE 1 A schematic representation of the structure of the lamellar liquid crystalline, inverted hexagonal and the bicontinuous Ia3d cubic (adapted from Seddon (1990) with permission from *Biochim. Biophys. Acta*) phases.

be used to determine the cross-sectional area of a lipid molecule at a distance d from the minimal surface integrated over one of the two monolayers within the unit cell (Anderson et al., 1988):

$$A(d) = A_o a^2 + 2\pi\chi d^2. \quad (1)$$

Here, a is the lattice parameter, χ is the Euler characteristic of the surface, and A_o is the unitless quantity that describes the ratio of the area of the minimal surface in a unit cell to (unit cell volume)$^{2/3}$. Accordingly, the area of the minimal surface is given by a^2A_o when the lattice parameter is a. The space group of the cubic phase determines the type of IPMS associated with the phase and thus, the values of A_o and χ (Table 1). The cross-sectional area per lipid molecule at a distance d from the minimal surface is given by Turner et al. (1992),

$$A_{cs}(d) = \frac{2A(d)v_l}{\phi_l a^3} \quad (2)$$

where v_l and ϕ_l are the molecular volume and the volume fraction of the lipid, respectively. By assuming a lipid specific volume $\bar{v}_l$ of 1 cm^3/g (or 10^{24} Å^3/g), v_l is calculated as $v_l = M\bar{v}_l/N_A$ or 593 Å^3/molecule where M is the molecular weight of the lipid (356 g/mol) and N_A is Avogadro's number. Because the partial specific volume of the lipid and the water components of the cubic phase has not yet been determined, we assume that ϕ_l is equal to the lipid weight fraction, which is known for samples prepared gravimetrically. The final equation used in the neutral surface analysis enables us to calculate the lipid length l for a given cubic phase provided we know ϕ_l and a. It has the following form (Anderson et al., 1988, Turner et al., 1992):

$$\phi_l = 2A_o\left[\frac{l}{a}\right] + \frac{4\pi\chi}{3}\left[\frac{l}{a}\right]^3. \quad (3)$$

The neutral surface analysis involves calculating $A_{cs}(d)$ at different positions along the length of the lipid molecule in the cubic phase as a function of hydration. The required surface is located at that value of d in the cross-sectional area profile where $A_{cs}(d)$ remains constant, i.e., where the profiles cross, in the hydration series.

TABLE 1 The Euler characteristic (χ) and the surface area of the minimal surface per unit volume (A_o) for different types of infinite periodic minimal surfaces

Space group symbol	Space group number*	IPMS Type‡	χ§	A_o§
Pn3m	224	D	−2	1.919
Im3m	229	P	−4	2.345
Ia3d	230	G	−8	3.091

* Number corresponds to the space group of the cubic lattice (Macgillavry and Rieck, 1962).
‡ Type refers to common names used to identify particular IPMS. D, P, and G stand for diamond, primitive, and gyroid, respectively (Andersson et al., 1988).
§ From Anderson et al., 1988.

MATERIALS AND METHODS

Materials

Monoolein (Nu Chek Prep Inc., Elysian, MN) had a measured purity of ≥99% as judged by thin-layer chromatography on silica gel K5F plates (Alltech Assoc., Inc.; Deerfield, IL) of >100 μg of lipid in three solvent systems (petroleum ether/diethyl ether, 94:6, v/v; chloroform/acetone/methanol/acetic acid, 72.5:25:2:0.5, v/v; petroleum ether/diethyl ether/acetic acid, 75:15:1, v/v) and was used as received. Water was purified by using a water purification system (Milli-Q; Millipore Corp., Bedford, MA).

X-ray diffraction

Measurements were made in the X9B station at the Brookhaven National Synchrotron Light Source (NSLS, 1.55 Å, 8.0 keV) and by using a Rigaku RU300 rotating copper (25-μm nickel filter, 1.54 Å, 54 kV, 240 mA) anode generator. In either case, small-angle diffraction patterns were recorded on x-ray-sensitive film (DEF5; Eastman Kodak Co., Rochester, NY).

At NSLS, the x-ray beam was defined initially by upstream X-Y slits and was subsequently focused horizontally and vertically by a double-bounce silicon (111) monochromator followed by a flat, cylindrically bent nickel-coated aluminum mirror. The hutch aperture consisted of computer-controlled X-Y slits used to define the focused x-ray beam. Huber X-Y slits (Crossed slit screen 3013, Huber Diffraktiontechnik, Rimsting, Germany) were used as guard slits. The beam measured 0.5 mm in the horizontal and 0.35 mm in the vertical at the detector. The sample-to-film distance was 26 cm. Exposure times were typically 15 s.

The x-ray beam from the rotating anode was defined by Huber X-Y slits and focused vertically by using a nickel-coated quartz mirror (Charles Supper Co., Inc., Natick, MA). A pair of guard slits was positioned between the sample and the mirror. The sample-to-film distance ranged from 25 to 30 cm. Exposure times were typically 40 min.

Sample preparation

A set of monoolein samples was prepared gravimetrically at different hydration levels. The lipid and water were mixed by using a home-built lipid mixing device consisting of two 100-μl syringes (Hamilton Co., Reno, NV) connected via a coupler that incorporates a 7-mm-long narrow (0.41-mm internal bore, 22 gauge) stainless steel tube. Approximately 50 mg of lipid was added to one of the syringes as a liquid, and an appropriate amount of water was added to the other to achieve the desired composition. Plungers were inserted into the syringe barrels, as much air as possible was expelled from the two syringes and they were then connected via the coupler. The lipid and water were passaged through the coupler from one syringe to the other by working the plungers back and forth at least 500 times at room temperature. Subsequently, the homogenized sample was transferred completely to one of the syringes, and the second empty syringe and the coupler were disconnected. A syringe needle was attached to the first syringe, and part of the sample was injected into the bottom of a 1-mm diameter x-ray capillary tube (Charles Supper Co.). The capillary was immediately flame- and epoxy-sealed. For purposes of determining accurately sample composition, a second fraction of the same homogenized sample, usually representing in excess of 20 mg total, was injected into a preweighed intact plastic bubble (empty bubble weighs ~6–7 mg, bubble is obtained from bubble-wrap packing material). The loaded bubble was weighed immediately, then opened to allow for drying under vacuum at room temperature for more than 12 h. The bubble containing the dry lipid was weighed again. The sensitivity of the balance (Sartorius M3P microbalance; Sartorius Instruments, McGraw Park, IL) used in these measurements is ~3 μg. In the water concentration range used, we estimate that the total error in weight fraction determination is ~0.1–0.2%. The temperature of the samples was controlled by using a home-built Peltier based temperature-regulated sample holder. Temperature was controlled to 25.0 ± 0.1°C for all samples.

RESULTS AND DISCUSSION

The lattice parameter of the Ia3d phase at 25°C was determined by using x-ray diffraction (Table 2) for a set of monoolein samples that were prepared gravimetrically at different hydration levels. For each sample in the set that corresponds to a unique value of ϕ_l, the lipid length was calculated using Eq. 3. The data show that $l = 17.3 \pm 0.1$ Å and that it is insensitive to composition in the range studied.

The dependence of the calculated cross-sectional area per lipid molecule on distance from the terminal methyl group of the hydrocarbon chain, which corresponds to the minimal surface, is shown in Fig. 2 as a function of hydration. In all cases, the cross-sectional area is larger at the methyl terminus of the lipid molecule and smaller at the polar-apolar interface. This disparity highlights the dynamically averaged wedge shape of the constituent molecules. The data in Fig. 2 also show that the wedge shape becomes more pronounced as water content decreases and the respective surfaces become more highly curved. Interestingly, the family of cross-sectional area curves is seen to cross over in the vicinity of

TABLE 2 The hydration-dependence of the lattice parameter of monoolein in the Ia3d cubic phase at 25°C

Weight fraction of water*	Lattice parameter (Å)‡	Weight fraction of water	Lattice parameter (Å)
0.2306	125.35	0.2887	137.36
0.2600	131.56	0.2890	135.47
0.2756	133.93	0.2900	137.08
0.2758	132.79	0.2960	139.73
0.2760	135.16	0.3169	142.77
0.2765	135.43	0.3171	143.36
0.2839	135.39	0.3260	146.61
0.2878	136.06	0.3346	147.59

* The weight fraction of water is defined as the weight of water divided by total sample (water plus lipid) weight. The values reported are accurate to ~±0.001.

‡ The lattice parameter represents the smallest repeating unit of the Ia3d cubic lattice. For instance, if the unit cell is a Å, then the volume of the unit cell is a^3 Å^3, and the area of the corresponding minimal surface per unit volume is a^2A_0 in units of Å^2.

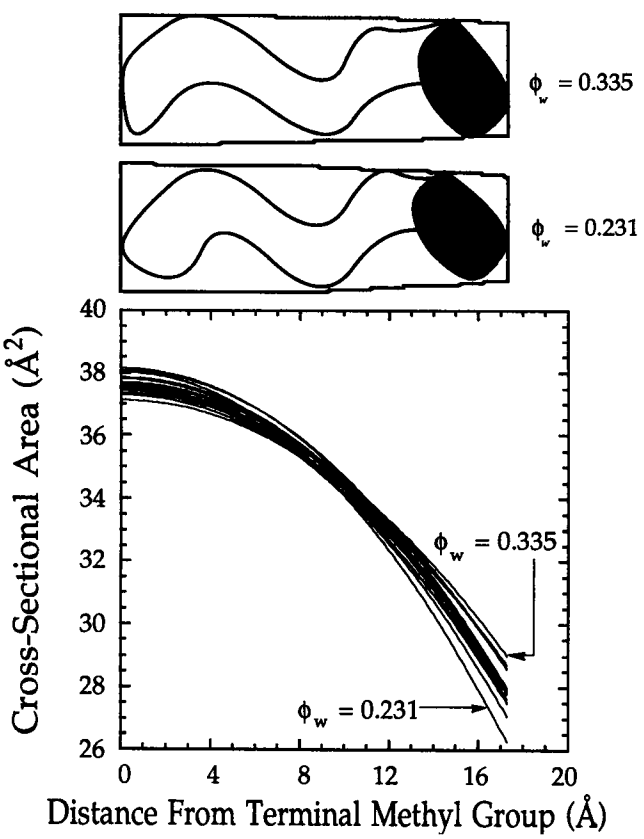

FIGURE 2 Hydration-dependence of the cross-sectional area of a monoolein molecule in the Ia3d cubic phase at 25°C as a function of the distance from the terminal methyl group. The hydration-dependence profiles of all samples used in the experiment are included in the figure to give the reader a sense of the spread in the cross-sectional area data along the length of the molecule. A quantitative measure of the spread is reported in Fig. 3. Sample composition in weight fraction of water is shown for the driest and wettest samples. Assuming that the lipid molecules are circular in cross-section, the side-view of two lipid molecules drawn to scale is shown at the top of the figure. The upper and lower drawings correspond to 0.34 and 0.23 weight fraction of water, respectively. We are careful to note that these drawings do not imply that individual molecules adopt such shapes. Rather, we wish to convey how space in the unit cell is divided up when averaged over all lipid volume and the degree to which the "wedge-shape" changes with hydration.

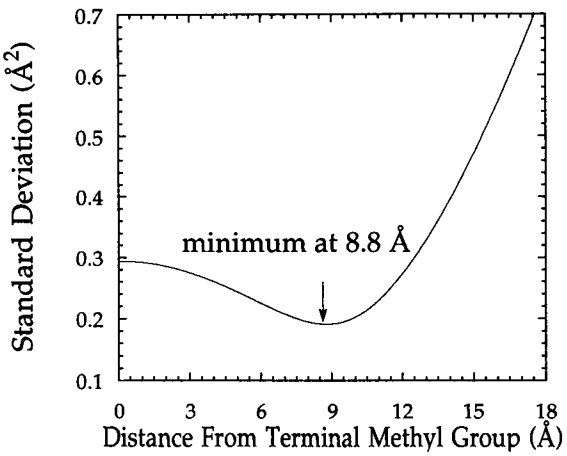

FIGURE 3 The standard deviation of the hydration-dependent cross-sectional area in the Ia3d cubic phase at 25°C as a function of distance from the terminal methyl group. The standard deviation was calculated as follows:

$$S(d) = \sqrt{\frac{\sum (A_{cs}(d) - \bar{A}_{cs}(d))^2}{n - 1}} \quad (1)$$

where $\bar{A}_{cs}(d)$ is the average cross-sectional area at a distance d for all samples, and n (=16) is the number of the data points used in the summation. $S(d)$ reaches a minimum at d = 8.8 Å from the terminal methyl group that identifies the location of the neutral surface in the corresponding lipid monolayer.

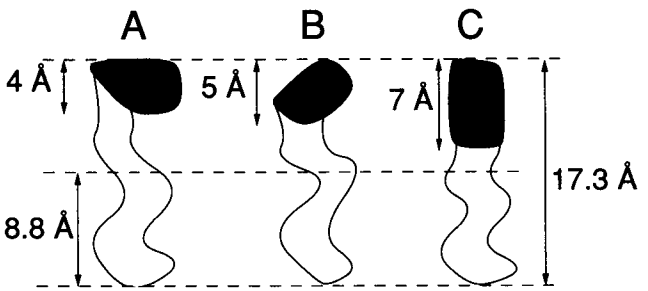

FIGURE 4 A schematic representation of monoolein in the Ia3d cubic phase at 25°C displaying different headgroup orientations. The total lipid length is fixed at 17.3 Å. The filled and open areas represent the lipid headgroup and hydrocarbon chain, respectively. The location of the neutral point at a distance of 8.8 Å from the minimal surface is indicated.

TABLE 3 The hydration-dependence of the lattice parameter of monoolein in the L_α phase at 25°C

Weight fraction of water	Lattice parameter (Å)*
0.0575	34.43
0.1198	39.13
0.1288	39.60
0.1626	41.44

* The lattice parameter, d_{L_α} includes the thickness of the water layer, d_w, and that of the lipid bilayer (= $2l_{L_\alpha}$).

d = 8.8 ± 1.0 Å, which points to the position of the neutral surface. To detail further the location of this surface, the standard deviation of the cross-sectional area per lipid molecule at each point along the length of the lipid was calculated for all samples in the hydration series and plotted as a function of d in Fig. 3. Where the standard deviation reaches a minimum identifies the neutral surface. Such an analysis places the neutral surface at 8.8 Å from the minimal surface where the cross-sectional area per lipid molecule is 35.1 ± 0.2 Å².

In the case of the H_{II} phase formed by hydrated phospholipids, Rand et al. (1990) found that their so-called pivotal surface is located at a distance of approximately one-third of the hydrocarbon chain length from the surface containing the ester bond. To compare this result with ours for the Ia3d phase, we consider several possible headgroup orientations for monoolein because this is not known for the current system. Based on measurements with Corey-Pauling-Koltun (CPK) space-filling atomic models (Harvard Apparatus, South Natick, MA), the dimensions of the monoolein headgroup, which is taken to include the carbonyl carbon of the

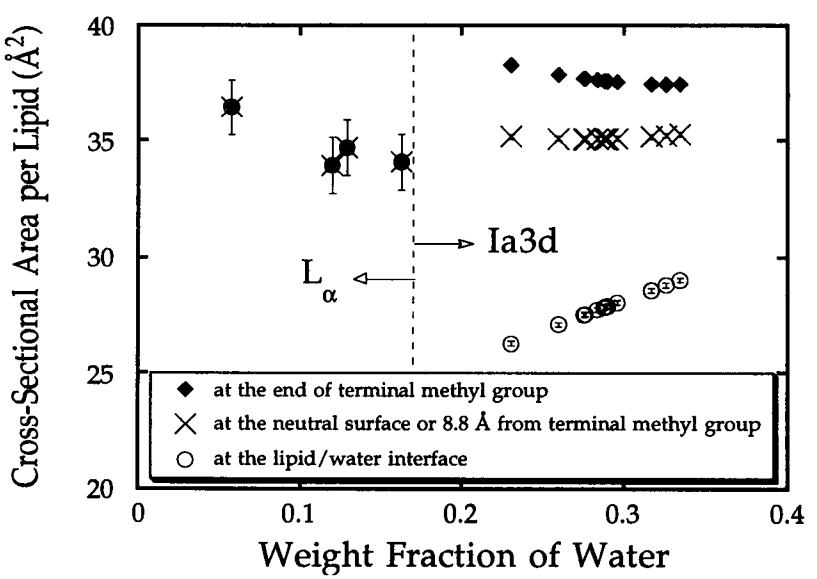

FIGURE 5 The cross-sectional area per lipid molecule calculated at various points along the length of the lipid molecule as a function of hydration in the L_α and Ia3d phases of monoolein at 25°C. The L_α-to-Ia3d phase boundary is located at ~0.17 weight fraction of water. The average cross-sectional area of the lipid is assumed to be circular in shape and constant along the lipid molecule in the L_α phase. The error bars shown correspond to ~±1.2 and ±0.1 Å for the L_α and Ia3d phases, respectively. At the neutral surface in the Ia3d phase, the cross-sectional area is essentially constant and independent of hydration in the range studied.

oleoyl moiety, are ~7 × 4 × 4 Å^3. Accordingly, the neutral area surface is located at ~one-half, one-seventh, or one-third of the hydrocarbon chain length from the surface containing the ester bond when the long axis of the headgroup is oriented either normal, parallel, or at an angle of 45° to that of the acyl chain (Fig. 4). The latter matches the result found with the H_{II} phase in the phospholipid system. However, the significance of this agreement cannot be fully appreciated until the molecular details of the cubic mesophase have been established.

In a related diffraction measurement, we have determined that monoolein at 25°C in the hydration range from 5 to 17 w/w% water exists in the lamellar liquid crystalline (L_α) phase. The corresponding bilayer lattice parameters d_{L_α} are listed in Table 3. The lipid length in the L_α phase is given by Luzzati (1968) and Cevc and Marsh (1987).

$$l_{L_\alpha} = \frac{d_{L_\alpha}}{2\,[1 + (\bar{v}_w/\bar{v}_l)(\phi_w/\phi_l)]} \tag{4}$$

where $\bar{v}_w$ and ϕ_w are the specific volume and the volume fraction of water, respectively. We assume that $\bar{v}_w$ and $\bar{v}_l$ are 1 cm^3/g. Thus, Eq. 4 reduces to $l_{L_\alpha} = 0.5\, d_{L_\alpha}\, \phi_l$. Assuming individual molecules adopt a dynamically averaged cylindrical shape, the cross-sectional area per lipid molecule in the L_α phase can be calculated as follows:

$$A_{cs,L_\alpha} = \frac{M\bar{v}_l}{N_A l_{L_\alpha}}. \tag{5}$$

The results of calculations concerning the L_α phase provide an average molecular cross-sectional area of 34.7 ± 1.2 Å^2 in the water weight fraction range 0.05–0.17 at 25°C. This is remarkably close to the cross-sectional area per lipid molecule at the neutral surface in the Ia3d phase determined above (35.1 ± 0.2 Å^2, Fig. 5). In the context of phase connectivity and transition mechanisms, it will be interesting to compare these values with that for the H_{II} phase in this and related systems.

CONCLUSION

A means for locating and characterizing the neutral surface in the Ia3d cubic phase has been established. The approach has general applicability and can be used with the other bicontinuous mesophases. In calculations concerning bending parameters and curvature energy, the neutral surface now can be used directly. The need to make these calculations at arbitrary surfaces such as the polar-apolar interface or the midplane of the lipid monolayer is obviated.

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