The Cubic Phase of Monoglyceride–Water Systems. Arguments for a Structure Based upon Lamellar Bilayer Units

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Abstract: A combination of low-angle X-ray diffraction and NMR diffusion techniques may be used for the elucidation of the internal structure of cubic lyotropic liquid crystalline lipid systems. The advantage of the NMR method for this kind of structure determinations lies in the facts that restricted diffusion can be observed and that the lateral diffusion in lamellar systems can be measured directly. The value of the apparent diffusion coefficient depends strongly on the overall structural geometry. Previous X-ray diffraction findings for the cubic phase occurring in aqueous monoglyceride systems have been reevaluated and supplemented and arguments are presented for an internal structure built up of small lamellar bilayer units. The lamellar units are ideally hexagon-shaped disks connected in such a manner that they form a close-packed system of tetrakaidecahedra in which the quadrilateral faces are missing. Thus is obtained an *open* structure which is optically isotropic and is continuous in three axial directions both with respect to the nonpolar lipid and the polar aqueous regions. This structure concept of two continuous regions where the lipid regions consist of a system of lamellar units obtains support from values for the translational diffusion coefficients of the lipid (and water) measured with the NMR pulsed magnetic field gradient method. This type of cubic phases, which occurs in many monoglyceride systems and which can be mixed with other lipids such as lecithin or cholesterol and also with proteins, may be used as model membrane systems.

Introduction

Several cubic phases have been encountered in lipids and in lipid-water systems (cf. ref 1). The structure of one such phase, proposed to consist of two interpenetrating networks of rod-like aggregates,[2] is considered as conclusively established, as it is based on many observed X-ray diffraction lines, while other structure propositions are still somewhat tentative.

From a theoretical point of view there are two fundamentally different alternatives for cubic lipid-water structures, viz., (1) structures with continuous regions of both water and hydrocarbon chains and (2) structures composed of closed aggregates of "oil-in-water" or "water-in-oil" type. Measurements of the self-diffusion coefficients of both lipid and water using the NMR pulsed field gradient technique provide a possibility of differentiating between these basic alternatives, and may in addition give information about the structural units of a particular phase.

A cubic phase occurs in aqueous monoglyceride-water systems at chain lengths of and above C_{14}.[3,4] Some general arguments have been given in an earlier paper[5] for a lamellar organization of the lipid molecules in this phase, and a structure consisting of closed polyhedra was proposed. However, the water and lipid diffusion coefficients as measured by the NMR technique are not compatible with a concept of closed aggregates. We have therefore considered the possibility of a structure based on bilayer units forming a continuous lipid medium intermixed with a continuous water medium. Such a structure was found to agree with renewed X-ray observations of lattice relations between the lamellar and cubic phases.

Furthermore, the present NMR analysis shows independently that the lipid molecules are arranged in a bilayer type of structure.

Experimental Section

Materials. The 1-monoolein and 1-monostearin were synthesized according to standard methods and their purity was checked by TLC. The sodium diethylhexyl sulfosuccinate (Aerosol OT) and the heavy water were obtained from Fluka AG and Ciba-Geigy AG, respectively. The specimens were weighed out in ampules, flame-sealed, homogenized, and stored as previously described.[6] When ordinary water was used it was doubly distilled.

In order to reduce the influence of water diffusion on the measurement of the spin-echo attenuation of the lipid it was necessary to exchange its free protons with deuterons. This was achieved by treating the lipids three times with excess of heavy water and followed by freeze-drying before making up the samples for the NMR experiments.

Methods. X-ray Diffraction. X-ray diffraction studies were performed (1) in a camera where the diffraction pattern is recorded continuously vs. temperature, a DPT camera,[7] with the sample in a capillary mounted parallel to the X-ray line focus, (2) in a Guinier camera according to Luzzati,[8] and (3) in a point collimated Kiessig camera,[9] where the sample was enclosed in a capillary, which could be rotated along its axis.[1]

NMR. Sample preparation and the macroscopic alignment of the lamellar samples were performed as described in previous papers.[10,11] The studied lamellar 1-monoolein–heavy water sample underwent transition to a cubic structure when the temperature was raised above 40 °C. As it was not possible to obtain a transition from lamellar to cubic phase in the Aerosol OT system simply by raising the temperature, two samples were prepared whose compositions were located on either side of the narrow two-phase zone between lamellar and cubic phases.

The diffusion coefficients were measured at 61 MHz on a Bruker 322s spin-echo NMR spectrometer using the pulsed magnetic field gradient technique developed by Stejskal and Tanner.[12] The spectrometer was equipped with a modified Bruker pulsed magnetic field gradient unit. The diffusion experiment was performed as follows: a spin-echo was produced by the common two-pulse sequence 90°-τ-180° and the magnetic field gradient was also applied as two pulses, one before and one after the 180° rf pulse. The advantage of this technique is that it is possible to use much larger gradients than can be used in the static field gradient method and thus lower diffusion coefficients can be measured. The molecular diffusion will attenuate the spin-echo amplitude E at 2τ according to the equation

$$\ln \frac{E_g}{E_0} = -(\gamma \delta g)^2 D \left(\Delta - \frac{\delta}{3}\right) \qquad (1)$$

where E_g/E_0 is the echo attenuation. The magnitude of the gradient, g, and the time spacing, Δ, between the gradient pulses were kept constant at approximately 2.9 T m^{-1} and about 15 ms, respectively. The gradient width, δ, was varied between 1.0 and 5 ms. δ and Δ were

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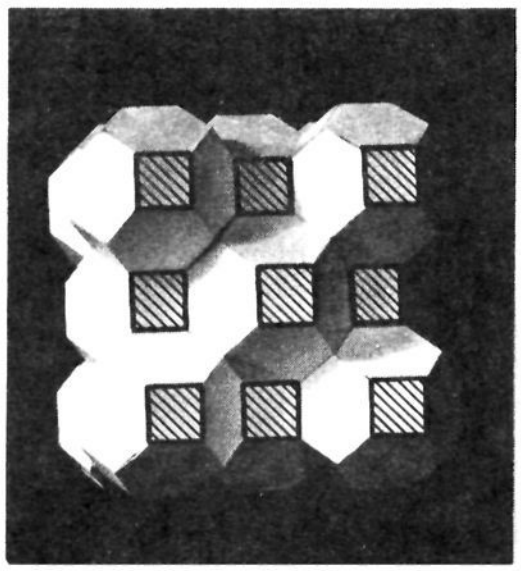

Figure 1. An attempt to visualize the proposed structure composed of "open" tetrakaidecahedra. The hexagon-shaped surfaces represent the gaps between the methyl end groups of the lipid bilayer units, whose molecular organization is shown in Figure 2. The shaded quadrilateral surfaces represent the water channels connecting the polyhedral units (see Figure 3).

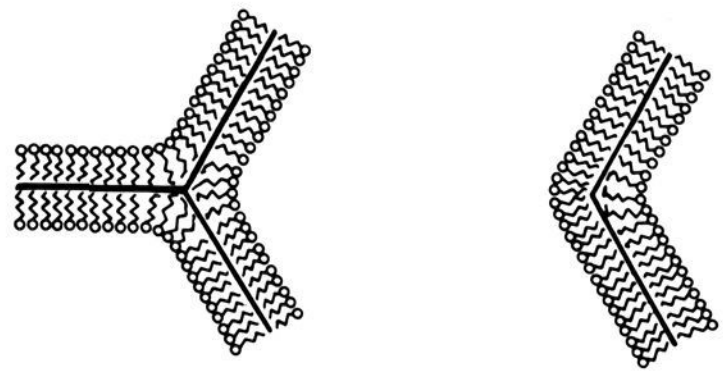

Figure 2. Schematic illustration of the principles for the molecular arrangement at the joints between neighboring lamellar units which build up the cubic phase. Such hexagon-shaped units would meet either three and three (to the left) or two and two (to the right). The angles between the planes are 120°.

set at very exact values obtained with the aid of a home-built pulse timing unit connected to the Bruker field gradient unit. The echo amplitude was measured repeatedly several times on an oscilloscope. The effective diffusion coefficient, D, was calculated from a least-squares determination of the slope in a plot of ln E_g against $\delta^2(\Delta - \delta/3)$ for both the mesophase sample and a glycerol reference. The ratio between diffusion coefficients was obtained from the slopes of the plots and the known diffusion coefficient of glycerol[13] was then utilized to calculate the diffusion coefficient of the lipid.

Results and Discussion

X-ray Diffraction. As already mentioned a cubic phase occurs in monoglyceride–water systems at chain lengths of and above C_{14}. At certain concentrations an originally lamellar phase with a rise in temperature transforms into a cubic phase (i.e., without change in composition). The X-ray diffraction pattern of the cubic phase can be indexed in a body-centered lattice.

Some important features of the X-ray data obtained for cubic phases in monoglyceride–water systems will first be underlined.

(1) The lattice parameters of different samples vary linearly with the reciprocal lipid content at constant temperature as in the neighboring lamellar liquid crystalline phase.

(2) When the temperature is raised the recordings of the X-ray diffraction pattern show a continuous change in dimensions near the transition from the lamellar liquid crystalline phase to the cubic phase and the dominating first-order lamellar spacing persists through the transition (with index (222) after the transition). The *decrease* in the values for the spacings with the *increase* in temperature contradicts an assumption of a structure composed of closed aggregates.

(3) There are not enough diffraction lines observed for a definite determination of the symmetry, and other additional structural evidence must be used in order to make a structural proposal feasible.

In an earlier paper[5] some general arguments were given for the existence of lamellar bilayer structure units in this particular cubic phase. If such lamellar units build up the structure the planar disks must be connected at their edges in order to avoid contact between water and the bilayer hydrocarbon chain core. There are two main possibilities for the construction of the cubic structure. The first alternative is that the disks form closed units, polyhedra, inside which the water is located (cf. ref 5). The second alternative is that the disks form an *open* network, so that there will be a continuous water and a continuous lipid medium. This is a feature that is also characteristic for the cubic structure based on rod systems, found by Luzzati and co-workers.[2] It is important to realize that bilayer units can form continuous structures. The NMR data given below are a strong support for the idea that both the lipid molecules and the water molecules are distributed in continuous regions. The X-ray data summarized above and further discussed below are compatible with a structure of the cubic monoglyceride–water phase as pictured in Figure 1.

The X-ray data, as pointed out above, cannot be used to determine the space group unambiguously. We have therefore started to consider how lamellar units can be arranged in networks. In space group no. 229, $Im3M$, there are 16-fold positions [(000) (½ ½ ½)] + [(xxx) $(x\bar{x}\bar{x})$ $(\bar{x}x\bar{x})$ $(\bar{x}\bar{x}x)$ $(\bar{x}\bar{x}\bar{x})$ $(\bar{x}xx)$ $(xx\bar{x})$ $(x\bar{x}x)$]. The same positions also occur in two other space groups, $I432$ and $Im3$. If the lamellar units are arranged according to this symmetry (the coordinates representing the center of the lamellar unit and the lamellar planes perpendicular to the vector defined by the coordinates) the units will join into a continuous network when they are hexagon shaped as shown in Figure 1.

The two possible manners by which the lamellar hexagons join to each other are shown in Figure 2. At every second edge one hexagon connects to two other hexagons, and at each of the other three edges the hexagon connects to one other hexagon. The former type of joint between lamellar units resembles the geometry that is observed in a foam. The three-edge joints result in a geometry in the bilayers such that the molecules will diverge from the polar head group toward the methyl end group, whereas the tail ends of the molecules at the two-edge joints have to converge in the sides facing the water channels. However, this unfavorable geometry can be compensated for if the water channels are considered to be circular in cross section, not square shaped as in the idealized model. There should be equivalent packing geometries for every molecule, and in such an average packing there will be more space available in the middle of the bilayers so the molecules can diverge from the polar head group toward the chain tail.

An alternative way to describe this structure is to start with the close-packed arrangement of tetrakaidecahedra. This polyhedron with six quadrilateral and eight hexagonal faces represents a minimum of the surface-to-volume ratio and if the lipid is assumed to form the faces of the polyhedra the maximum amount of water can be enclosed in a close-packed structure. This was the structure originally proposed for this cubic phase,[5] when there was no information indicating that the water and lipid formed a continuous matrix. If the quadrilateral faces are taken away as shown in Figure 3 the water regions will be connected. It should be emphasized again that the picture of planar hexagons represents an idealized structure, and that instead there is a smooth change in the curvature of the bilayer units with the result that there are no abrupt changes at the joints between these units.

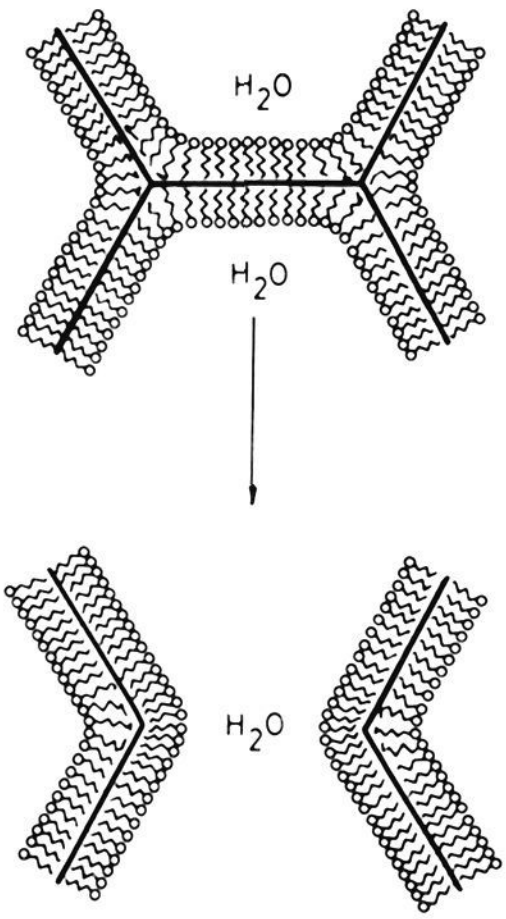

Figure 3. The relation between a cubic structure where the water is located within "closed" tetrakaidecahedra as proposed in ref 5 and one with "open" units having continuous water and continuous bilayer regions as proposed in this paper (Figure 1). If the six quadrilateral faces of the tetrakaidecahedra are taken away there will be a three-dimensional continuity in respect to both water and lipid.

Table I. X-ray Findings for the Cubic Phase in the System 1-Monostearin–Water at Two Different Concentrations and at Different Temperatures[a]

80 °C		75 °C		
d, Å	*I*	*d*, Å	*I*	$h^2 + k^2 + l^2$
		Weight Fraction 0.70		
91.5	m	99.5	m	4
72.7	s	79.6	s	6
61.6	m	70.5	m	8
50.6	m	56.4	m	12
41.6	m			18
		44.2	w	20
36.7	w			24
		Weight Fraction 0.55		
85.7	m	100.2	m	6
61.7	m	70.2	m	12
50.1	m	58.8	m	18

[a] The samples with weight fraction of lipid equal to 0.70 were recorded in a Luzzati camera and those with weight fraction equal to 0.55 were recorded in a DPT camera.

The X-ray data of different samples of cubic phases of 1-monostearin and 1-monoolein are given in Tables I and II. There are differences in the relative intensities of the reflections depending upon composition. Furthermore, pronounced orientation effects were observed, in that the relative intensities between the reflections could vary from one sample preparation to another and, particularly, different sample geometries (Luzzati and Kiessig cameras, respectively) showed different intensity distributions (Figure 4). The following ratios of spacings were observed: $\sqrt{4}/\sqrt{6}/\sqrt{8}/\sqrt{10}/\sqrt{12}/\sqrt{14}/\sqrt{16}/\sqrt{18}/\sqrt{20}/\sqrt{22}/\sqrt{24}/\sqrt{26}/\sqrt{30}/\sqrt{34}/\sqrt{38}/\sqrt{42}/\sqrt{46}/\sqrt{50}/\sqrt{52}/\sqrt{58}/$. The forbidden index in cubic symmetry of $(h^2 + k^2 + l^2)^{1/2}$ equal to $\sqrt{7}$ necessitated the

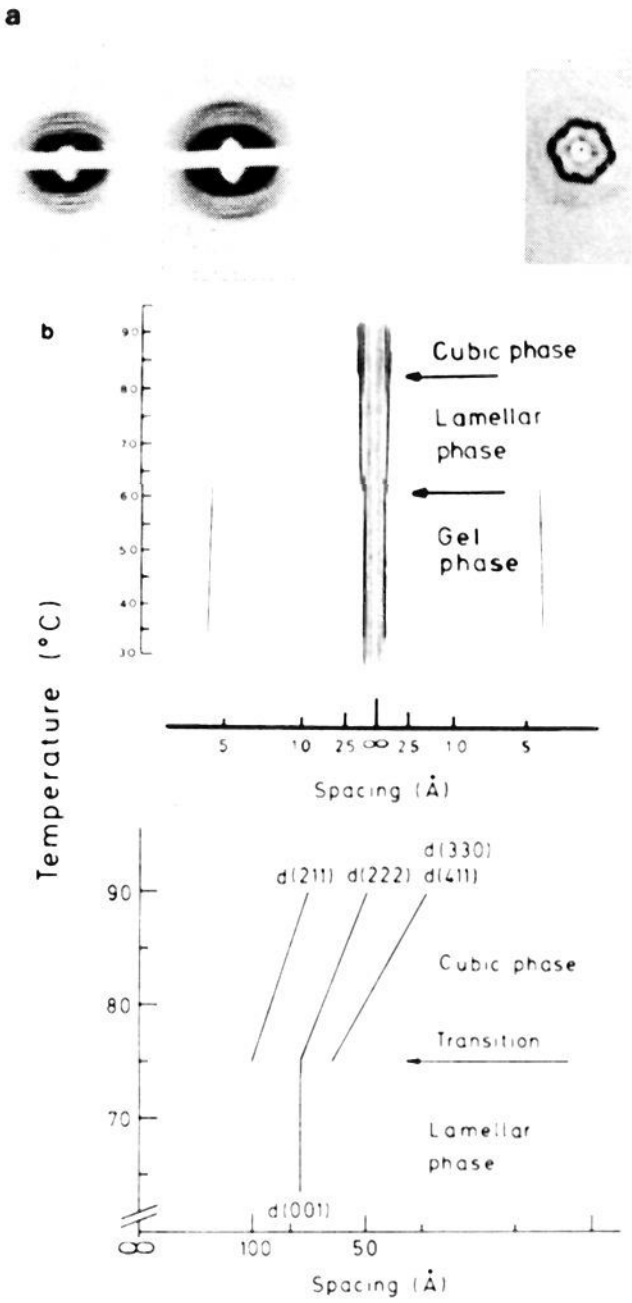

Figure 4. (a) Examples of actual diffractograms from monoglyceride–water systems, 1-monoolein in water, 22 °C. Left: diffractograms obtained with a Guinier camera of Luzzati-type, 65 and 75% (w/w), respectively. Right: a diffractogram obtained with a Kiessig camera where the sample had been rotated during the exposure, 65% (w/w). Note the orientation effect caused by the capillary resulting in a hexagon-shaped pattern. (b) Top: an actual DPT diffractogram showing the transition *gel* to *lamellar* to *cubic* phase in a sample of 80% (w/w) of 1-monostearin in water. Bottom: an idealized version of the DPT recording of the transition *lamellar* to *cubic* phase. Note that the lamellar *d*(001) spacing persists through the transition and forms the (222) spacing of the cubic phase and the 4.5-Å spacing becomes much weaker at the transition *gel* to *lamellar* phase.

choice of these ratios and they are representative for a body-centered lattice.

The planes containing the hexagons are all parallel to the planes ±(111) $(\bar{1}11)$ $(1\bar{1}1)$ and $(11\bar{1})$ of the cubic lattice. The cross-section area of the (111) planes in the unit cell is $a^2\sqrt{3}$. If thus hypothetically the cubic unit cell content, consisting of 16 hexagon-shaped bilayer units, at the transition cubic to lamellar phase forms three continuous bilayers parallel to the (111) plane, there should be a direct connection between the cubic (222) spacing and the lamellar *d*(001) spacing, which we have observed at different water content; see Figure 4. These observed geometrical relations between the cubic and lamellar phases can hardly be explained unless the cubic phase consists of bilayer units.

The transition from lamellar to cubic structure can be explained in the following way. In a sample of fixed composition an increase in temperature will increase the chain mobility toward the chain ends resulting in a tendency to form a structure where the cross-section area per molecule at the methyl end groups is larger than at the polar head groups. This aim is fulfilled by formation of the proposed cubic structure

Table II. X-ray Findings for the Cubic Phase in the System 1-Monoolein-Water Recorded in a Luzzati Camera at 22 °C[a]

water (w/w), %	d, Å	I	a_0, Å	water (w/w), %	d, Å	I	a_0, Å	water (w/w), %	d, Å	I	a_0, Å	water (w/w), %	d, Å	I	a_0, Å	$h^2 + k^2 + l^2$
25.05	48.9	vs	169.4	29.79	54.5	vs	188.8	34.93	59.8	vs	207.2	39.87	64.2	vs	222.3	12
	42.2	s	168.8		47.0	s	188.1									16
									49.3	s	209.2		52.2	s	221.5	18
									43.4	m	203.5					22
													46.0	w	225.2	24
	32.8	w	167.1		36.1	w	183.7									26
	30.8	w	168.7		33.9	w	185.4									30
									35.2	m	205.2					34
	27.3	w	168.0		30.1	m	185.2									38
	26.1	m	169.2		29.1	w	188.3									42
	25.0	w	169.8						30.3	w	205.8		32.6	m	221.1	46
	24.1	vw	170.1													50
									28.7	w	203.8		30.7	w	221.0	52
									27.1	vw	206.7		29.3	w	223.4	58
mean			169.9				187.7				205.9				222.4	

[a] The indexing is made by employing $\sin^2\theta$ ratios, a_0 = unit cell dimension, intensity scale vs = very strong, s = strong, m = medium, w = weak, vw = very weak.

while a part of the bilayers simultaneously remains at its original position, viz., the lamellar bilayers are broken up into small disk-shaped units and the recombination will result in a larger average cross-section area per molecule at the methyl end group compared to the cross section of the polar head group. At constant temperature an increase in the water content of a lamellar sample results in a tighter molecular packing (36 → 32 Å^2) and the samples obtain simultaneously a more solid consistency. The transition to the cubic structure will allow the incorporation of more water while a critical minimum molecular packing is preserved.

In the NMR diffusion studies reported in the next section some parallel measurements to those of the monoolein system were performed on the cubic and lamellar phases, respectively, of the Aerosol OT-water system. For this reason we want to point out that the indexing of the X-ray reflections of the cubic phase in this Aerosol OT system[14] favored the body-centered space group *Ia3d* (no. 224) and that the structure therefore was proposed to be of the same continuous interwoven network type built up from rod aggregates as that described by Luzzati.[2]

NMR Diffusion Technique. It is well-known that NMR diffusion techniques can be used for determinations of restricted diffusion[12,15] in lipid systems. This is possible because in the NMR magnetic field gradient method the experimental diffusion distances are of colloidal dimensions. The diffusion time may vary from milliseconds to seconds for different lipid systems and thus changes in the apparent diffusion coefficient may be detected. For example, a molecule may travel an average distance of about 300 nm when the translational diffusion of a lipid molecule has a diffusion coefficient equal to 10^{-12} m^2/s and is measured for a period of 60 ms. This is molecular diffusion. On the other side, if the lipid molecule is part of a small entity (<300 nm), such a movement is not possible for the molecule and therefore the measured diffusion coefficient will be lower than for a true molecular system. The measured diffusion coefficient represents instead micellar diffusion. About 10 years ago Moll and Baldeschwieler[16] measured the self-diffusion coefficient of micelles of dimethyldodecylamine oxide but they were unable to observe any diffusion *within* the micelles. Since then several NMR studies of diffusion for both micellar solutions and also for lyotropic liquid crystalline phases have been performed for a number of lipid systems.[17-22] While diffusion studies are straightforward in phases with cubic structure, the standard techniques cannot be used directly in anisotropic mesophases.[20] However, the amphiphile diffusion coefficient can be determined when the lamellar samples are macroscopically aligned at the so-called magic angle in the applied magnetic field. When it is possible to measure the diffusion coefficient for both the cubic and the lamellar phases of the same system, a quantitative comparison between the measured diffusion coefficients will give information about the geometrical structure of the building units of the cubic phase. A brief account of such studies has recently been presented.[20]

By several NMR diffusion studies[17-21] it is now well established that two fundamentally different cubic lipid phases exist, namely, those having continuous lipid regions in the directions of the three coordinate axes over macroscopic distances and those with discrete hydrocarbon regions. This conclusion originates from a direct qualitative comparison between the apparent diffusion coefficients of cubic and micellar systems and between cubic and lamellar systems. The amphiphile diffusion as measured with NMR in the closed aggregates of a cubic mesophase is more than an order of magnitude smaller than in a micellar system. Recently we have considered also the restricted diffusion within the aggregates building up a cubic lattice.[20] In the paper we considered a local diffusion tensor, where the component representing restricted diffusion in a certain direction was set to zero. The measured diffusion coefficient, D, is an average value of the local diffusion tensor, $\mathbf{D}$, which is given by the equation

$$D = \tfrac{1}{3}\overline{\mathrm{Tr}\mathbf{D}} \tag{2}$$

where the bar denotes an average over all the sites in a unit cell of the liquid crystal. Depending on how many directions that are effectively available for the amphiphile diffusion within the aggregate three different cases can be distinguished.

(1) For a cubic phase composed of spherical aggregates the lipid diffusion is restricted in all three directions in the local principal coordinate system. Therefore all the diagonal elements of the local diffusion tensor $\mathbf{D}_{\mathrm{sphere}}^{\mathrm{cub}}$ are equal to zero. Thus

$$D = \tfrac{1}{3}\mathrm{Tr}\mathbf{D}_{\mathrm{sphere}}^{\mathrm{cub}} \approx 0 \tag{3}$$

(2) A structure of tetrahedrally arranged rods permits lipid diffusion in one direction, namely, along the rod, and we denote this diffusion coefficient, $D_{\parallel}^{\mathrm{cub}}$. Equation 2 then obtains the form

$$D = \tfrac{1}{3}D_{\parallel}^{\mathrm{cub}} \tag{4}$$

Table III. Experimental and Calculated Values for the Amphiphile and Water Diffusion Coefficients[a]

A. Amphiphile Diffusion Coefficient as Function of Temperature

composition (w/w)	temp, °C	phase structure	$D \times 10^{11}$, m²/s	$D_L \times 10^{11}$, m²/s	$D_L^{cub} \times 10^{11}$, m²/s	$D_{\|}^{cub} \times 10^{11}$, m²/s
monoolein-heavy water	22	lamellar	0.73	1.1		
88/12	29	lamellar	0.85	1.4		
	35	lamellar	1.1	1.6		
	43	cubic	1.5		2.3	4.5
	49	cubic	2.0		3.0	6.0
	57	cubic	2.6		3.9	7.8
Aerosol OT-heavy water	24	lamellar	0.67	1.0		
70.9/29.1	29	lamellar	0.93	1.4		
	35	lamellar	1.1	1.7		
72.4/27.6	24	cubic	0.36		0.54	1.1
	29	cubic	0.50		0.75	1.5
	35	cubic	0.59		0.89	1.8

B. Heavy Water Diffusion Coefficients Measured Relative to the Diffusion of D_2O at 25 °C[b]

composition (w/w)	phase structure	$D_{D_2O} \times 10^{10}$ m²/s
monoolein-heavy water		
75/25	cubic	5.3
70/30	cubic	7.2
58/42	cubic	7.3

[a] D_L represents the lateral diffusion coefficient in a lipid bilayer, obtained by correcting D for the "magic angle". D_L^{cub} and $D_{\|}^{cub}$ are estimated diffusion coefficients for translational motion along the amphiphilic surface at assumed different aggregate structures for the cubic phase (lamellar and rod-like aggregate units, respectively).[20] D is the experimentally determined effective diffusion coefficient (cf. Experimental Section). [b] $D_{D_2O} = 2.5 \times 10^{-9}$ m²/s at 25 °C.

(3) If the amphiphile diffusion within the aggregates can occur in two directions (i.e., on a surface) the lateral diffusion coefficient in the cubic liquid crystal, D_L^{cub}, is given by the equation

$$D = {}^2/_3 D_L^{cub} \qquad (5)$$

As can be inferred from eq 2-5 the aggregate structure will have considerable influence on the magnitude of the measured diffusion coefficient. Conclusions about the geometry of the aggregates can thus be drawn from the apparent diffusion coefficients if the amphiphile diffusion within an aggregate is known. For oriented lamellar phases[20] it is possible to directly measure a local diffusion coefficient, D_L. Several recent NMR studies indicate that the local surroundings are similar for different types of aggregates. Theoretically the order parameter for a methylene segment in a lamellar system should be twice as large as the value for a hexagonal mesophase if the local surroundings are identical.[23] Experimental data on several systems are in excellent agreement with these theoretical predictions.[24,25] However, the fact that the local ordering is the same in the different aggregate structures does not necessarily mean that the local environment of the molecules in the diffusing process is the same. The arguments given above may therefore only be taken as good indications that our assumption about the local environment is correct.

In the present study amphiphile diffusion coefficients were measured at different temperatures for cubic and lamellar samples composed of (a) monoolein and water and (b) Aerosol OT and water. For the monoolein system the composition was kept constant and a change in the temperature was utilized for the transition from one phase to the other (see phase diagram in ref 3 and 4). For the Aerosol OT system it was not possible to obtain the two structures at the same composition by raising the temperature but the diffusion was instead studied for cubic and lamellar samples located on either side of the narrow two-phase region close to the phase boundaries (see phase diagram in ref 13). The experimentally obtained diffusion coefficients are summarized in Table III together with estimated ones under assumption of continuous bilayer or rod structures for the cubic phases.

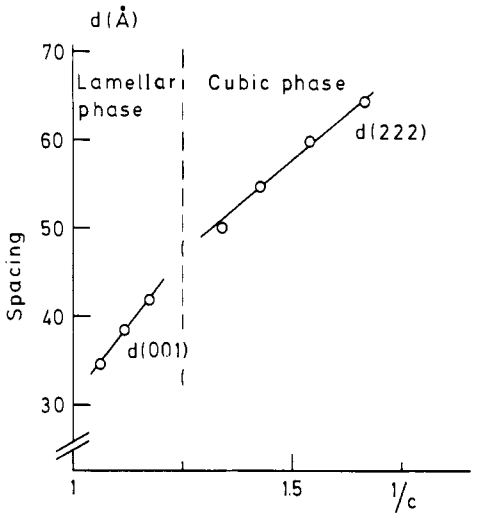

Figure 5. The linear variation of the innermost spacing against the reciprocal concentration in the lamellar and cubic phases, respectively, of 1-monoolein, 22 °C.

Figure 6 shows the temperature dependence of the lateral diffusion coefficient for the monoolein system; the measured coefficient for the lamellar structure was corrected for the "magic angle" and that for the cubic structure was calculated under the assumption of the proposed model of lamellar units building up "open" tetrakaidecahedra.

As can be seen from the figure there is continuity in the local diffusion coefficient at the transition from the cubic to the lamellar structures. If one uses instead values for the local diffusion coefficient calculated for other structure alternatives a discontinuity will be obtained at the transition from one phase to another. Thus it can be concluded that these results support the proposed structure of the cubic phase in the monoolein system, a structure which is based upon continuous bilayer structure units.

As mentioned, in the Aerosol OT system it is not possible to obtain the two-phase structures only by changing the tem-

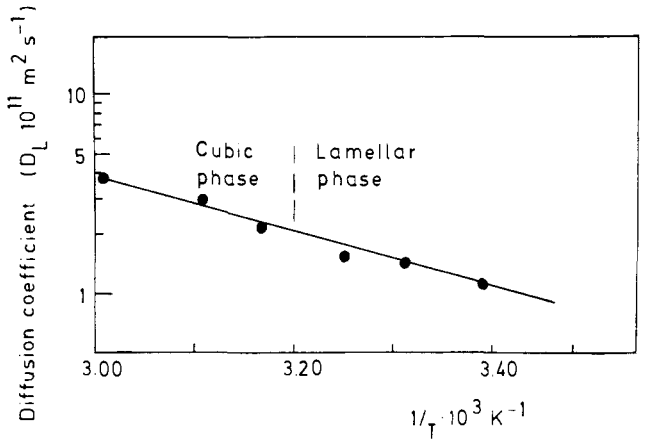

Figure 6. The temperature dependence of the lamellar lipid diffusion coefficient for a sample of 88% (w/w) 1-monoolein in heavy water. Note that the cubic structure is assumed to be open and built up of lamellar units as proposed in this paper. An assumption of a rod structure of the type proposed by Luzzati[2] or a structure composed of closed aggregates would give a discontinuity at the *cubic* to *lamellar* transition; however, the slope would be the same.

perature. Nevertheless, as can be inferred from Table III, the lateral diffusion coefficients, calculated under assumption of a cubic structure of the network type proposed by Luzzati,[2] agree well with those in the lamellar phase.

Another strong argument for the correctness of our basic assumption of the same local environment for all lipid molecules, irrespective of the phase structure, is the fact that the apparent energies of activation of diffusion are the same for the different phases of the same system. These activities were obtained from an Arrhenius plot and were found to be 19 and 32 kJ/mol for the monoolein system and the Aerosol OT system, respectively, for both the lamellar and cubic phases (see, for example, the slope in Figure 6).

Final Remarks

The X-ray and NMR data presented in this paper taken together provide strong arguments for the proposed structure of the cubic phase in the system 1-monoolein-water: a three-dimensional structure of *lamellar lipid bilayer units* in a water medium which is also continuous in three directions. The edges of each bilayer disk are connected to equivalent units in a manner resembling that of the lamellae in a foam. It may rather safely be assumed that the structure of the cubic phases in other monoglyceride-water systems is the same.

This cubic 1-monoolein-water system possesses the capability to solubilize various amphiphile substances such as diglycerides,[4] cholesterol (up to a molar ratio of 1:3 between cholesterol and 1-monoolein), and protein. The lamellar structure of such isotropic lipid phases makes them interesting model systems for studies with the NMR technique.

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