Srisiri, W., Benedicto, A. & O'Brien, D. F. Stabilization of a bicontinuous cubic phase from polymerizable monoacylglycerol and diacylglycerol. Langmuir 14, 1921–1926

ARTICLE *in* LANGMUIR · MARCH 1998

Impact Factor: 4.46 · DOI: 10.1021/la970897m

Stabilization of a Bicontinuous Cubic Phase from Polymerizable Monoacylglycerol and Diacylglycerol

Warunee Srisiri, Alto Benedicto, and David F. O'Brien*

C.S. Marvel Laboratories, Department of Chemistry, University of Arizona, Tucson, Arizona 85721

Theodore P. Trouard

Department of Radiology, University of Arizona, Tucson, Arizona 85721

Greger Orädd, Stefan Persson, and Göran Lindblom

Department of Physical Chemistry, Umeå University, Umeå S-90187, Sweden

Received August 11, 1997. In Final Form: January 21, 1998

Technological applications of lipids may be possible through stabilization of various liquid-crystalline phases. One important approach to stabilized self-assembling materials utilizes polymerization of liquid-crystalline phases composed of reactive lipids. Polymerization of lipids has been utilized to modify the chemical and physical properties of lamellar assemblies (e.g., lipid monolayers, multilayers, and bilayer vesicles). In addition, polymerization of the lipid region of three-dimensional nonlamellar lipid-phase structures has recently been reported, including the reversed bicontinuous cubic (Q_{II}) phase, belonging to the space group $Pn3m$ and the reversed hexagonal (H_{II}) phase. Here we show that an easily prepared polymerizable monoacylglycerol combined in a 9/1 molar ratio with the corresponding polymerizable 1,2-diacylglycerol forms nonlamellar phases upon hydration at room temperature. Phase investigation using cross-polarized light, ^{2}H NMR spectroscopy, and X-ray diffraction showed that the lipid mixture formed a well-defined cubic phase from at least 5 to 45 °C. The X-ray diffraction pattern corresponded to a cubic phase with $Ia3d$ symmetry and a unit cell size of 131 Å at 25 °C. Polymerization to high conversion of this cubic phase was accomplished via the thermal decomposition of H_2O_2. The resultant polymers dissolved in organic solvent, indicating they were not cross-linked. The visual clear character, cross-polarized light test, and X-ray diffraction showed that isotropic architecture was maintained up to at least 70 °C after sample polymerization. The diffusion coefficient of water (23 °C) within the polymerized cubic phase, determined by pulsed field gradient NMR spectroscopy, was $1.2 \pm 0.2 \times 10^{-10}$ m^2/s, a value consistent with retention of the cubic phase during and after the polymerization. The biocompatible and mesoporous nature of the polymerized cubic phase suggests it could be used as the host for incorporation of synthetic or biological molecules in a manner that has already proven especially useful in microporous solids.

Introduction

Amphiphile/water systems exhibit a rich polymorphism to yield several different liquid-crystalline phases. Several reviews of the extensive studies of the phase behavior of hydrated amphiphiles have been published.[1−8] These liquid crystalline phases are of great interest in biological sciences and for the preparation of new materials. Lipids are of course major components of cellular membranes and are active participants in the functioning of the living cell. It is now clear that lipids play a much more important role in biological membranes than previously thought.[9−11] Support for the view that lipids are functionally relevant for membrane proteins is steadily being published.[3, 12−14]

Interesting technological applications of lipids are possible through stabilization of various liquid-crystalline phases. One important approach to stabilized self-assembling materials utilizes polymerization of liquid-crystalline phases composed of reactive lipids.[15−19] Such stabilized lipid microstructures could serve as templates for the preparation of composites.[20] There is also considerable interest in the prospect of using lipid phases as biocompatible encapsulating and controlled release media.[21−23] The great interest in this area of lyotropic liquid crystals is demonstrated not only by an increase in

(1) Ekwall, P. *Adv. Liq. Cryst.* **1975**, *1*, 1−142.
(2) Fontell, K. *Mol. Cryst. Liq. Cryst.* **1981**, *63*, 59−82.
(3) Lindblom, G.; Rilfors, L. *Biochim. Biophys. Acta* **1989**, *988*, 221−256.
(4) Lindblom, G.; Rilfors, L. In *Structural and Dynamic Properties of Lipids and Membranes*; Portland Press: London, U.K., 1992; pp 51−76.
(5) Laughlin, R. G. *Adv. Liq. Cryst.* **1978**, *3*, 41−148.
(6) Seddon, J. M. *Biochim. Biophys. Acta* **1990**, *1031*, 1−69.
(7) Tate, M. W.; Eikenberry, E. F.; Turner, D. C.; Shyamsunder, E.; Gruner, S. M. *Chem. Phys. Lipids* **1991**, *57*, 147−164.
(8) Lindblom, G. In *Advances in Lipid Methodology*; Christie, W. W., Ed.; Oily Press, Ltd: Dundee, Scotland, 1996; pp 133−209.
(9) de Kruijff, B. *Nature (London)* **1997**, *286*, 129−130.
(10) Andersson, A.-S.; Rilfors, L.; Bergqvist, M.; Persson, S.; Lindblom, G. *Biochemistry* **1996**, *35*, 11119−11130.
(11) Morein, S.; Andersson, A.-S.; Rilfors, L.; Lindblom, G. *J. Biol. Chem.* **1996**, *271*, 6801−6809.
(12) Jensen, J. W.; Schutzbach, J. S. *Biochemistry* **1988**, *27*, 6315−6320.
(13) Keller, S. L.; Besrukov, S. M.; Gruner, S. M.; Tate, M. W.; Vodyanoy, I.; Parsegian, V. A. *Biophys. J.* **1993**, *65*, 23−27.
(14) Brown, M. F. *Chem. Phys. Lipids* **1994**, *73*, 159−180.
(15) O'Brien, D. F. *Trends Polym. Sci.* **1994**, *2*, 183−188.
(16) Ringsdorf, H.; Schlarb, B.; Venzmer, J. *Angew. Chem., Int. Ed. Engl.* **1988**, *27*, 113−158.
(17) Lee, Y.-S.; Yang, J.; Sisson, T. M.; Frankel, D. A.; Gleeson, J. T.; Aksay, E.; Keller, S. L.; Gruner, S. M.; O'Brien, D. F. *J. Am. Chem. Soc.* **1995**, *117*, 5573−5578.
(18) Srisiri, W.; Sisson, T. M.; O'Brien, D. F.; McGrath, K. M.; Han, Y.; Gruner, S. M. *J. Am. Chem. Soc.* **1997**, *119*, 4866−4873.
(19) Sisson, T. M.; Lamparski, H. G.; Kölchens, S.; Elayadi, A.; O'Brien, D. F. *Macromolecules* **1996**, *29*, 8321−8329.
(20) Schnur, J. M. *Science* **1993**, *262*, 1669−1676.

S0743-7463(97)00897-4 CCC: $15.00
Published on Web 03/12/1998

the number of publications but also by the emergence of commercial enterprises aiming to capitalize on the pharmaceutical and cosmetic applicability of the more tractable materials.

During the past decade, polymerization has been utilized to modify the chemical and physical properties of lamellar assemblies (e.g., lipid monolayers, multilayers, and single bilayer vesicles).[15,16,19] The polymerization of reactive amphiphiles in the lipid region of three-dimensional nonlamellar lipid-phase structures has recently been achieved.[17,18] The goal of this research is to stabilize nonlamellar phases through polymerization, therefore expanding their useful temperature and concentration range. Examples of nonlamellar phases that have been successfully stabilized by polymerization are the reversed bicontinuous cubic liquid-crystalline (Q_{II}) phase, belonging to the space group $Pn3m$,[17] and the reversed hexagonal liquid-crystalline (H_{II}) phase.[18] The success of this strategy relies on the design of suitable polymerizable lipids, which form the nonlamellar phase upon hydration.

This study reports the phase behavior of a polymerizable monoacylglycerol, a relatively simple lipid to prepare in quantity. The temperature−composition phase diagrams of several monoacylglycerols (MAG) in water have been described, and it is clear that they are able to form different nonlamellar phases.[24−27] The phase behavior of monooleoylglycerol (monoolein) alone or mixed with other lipids (e.g., diacylglycerols and dioleoylPC) has been particularly well-investigated. Moreover, it has served as a model membrane system for the physicochemical investigation of lipid structures and dynamics.[25,27−30] In the present case, a polymerizable monoacylglycerol combined with the corresponding diacylglycerol was found to form a Q_{II} phase with $Ia3d$ symmetry, which could be stabilized by polymerization of the reactive amphiphiles.

Experimental Section

Syntheses. Glycerol-d_5 was obtained from Isotech, Inc. Hydrogen peroxide (30% solution) was ordered from Aldrich Chemical. Trimethyl 4-phosphonocrotonate was purchased from Lancaster Synthesis Inc. D_2O was from Cambridge Isotope Laboratories. Acetone was distilled from CaH_2 and stored over molecular sieves. THF was distilled from sodium benzophenone ketyl and chloroform was distilled from CaH_2. Compounds containing a UV-sensitive group were handled under yellow light. The reactions were monitored by TLC visualized by UV light. NMR spectra were recorded on a 250 MHz magnetic resonance spectrometer in chloroform-*d* with TMS as an internal reference. UV absorption spectra were recorded on a Hewlett-Packard 8452A diode array spectrophotometer. Melting points were not corrected. Elemental analyses were performed by Desert Analytics. The FAB mass spectra were obtained from the Nebraska Center for Mass Spectrometry.

*1,2-O-Isopropylidene-sn-glycerol-d_5 (**3**).* Glycerol-d_5 (1 g) was dissolved in excess dry acetone solvent. A catalytic amount of *p*-toluenesulfonic acid was added into the mixture, and a reaction was allowed to take place overnight at room temperature. After evaporation of the acetone, the residue was extracted many times with ether and water. The organic layer was combined and concentrated, giving exclusively protected glycerol-d_5 **3** without furthur purification. (100% yield) ^{1}H NMR ($CDCl_3$): 1.91 (s, 1H), 1.45 (s, 3H), 1.38 (s, 3H).

*1,2-O-Isopropylidene-3-(2,4-(E,E)-tetradecadienoyl)-sn-glycerol-d_5 (**5**).* Protected glycerol-d_5 **3** (0.7 g, 4.8 mmol), 2,4-(*E*,*E*)-tetradecadienoic acid (0.9 g, 4.0 mmol), and 4-(dimethylamino)-pyridine (0.5 g, 4.0 mmol) were dissolved in 20 mL of chloroform and then 0.8 g (4.0 mmol) of DCC in 10 mL of chloroform was added. After stirring at room temperature overnight, the urea was filtered and the filtrate was concentrated. The crude product was purified by column chromatography using hexane/EtOAc (9/1), to give the protected glyceride **5** in 96% yield. ^{1}H NMR ($CDCl_3$): 7.31−7.21 (m, 1H), 6.14−6.11 (m, 2H), 5.82−5.67 (d, J = 15.29 Hz, 1H), 2.17−2.09 (m, 2H), 1.41 (s, 6H), 1.39−1.23 (br, 14H), 0.87−0.82 (t, J = 6.85 Hz, 3H). Elem. Anal. Calcd.: C, 71.00; H, 10.06. Found: C, 71.42; H, 10.43.

*1-(2,4-(E,E)-Tetradecadienoyl)-sn-glycerol-d_5 (**1**).* A solution of protected glyceride **5** (1.3 g) in 20 mL of methanol was treated with 5 mL of 1 N HCl solution. The solution was stirred at room temperature for 2 h and then diluted with 50 mL of ether. The ether solution was washed with saturated $NaHCO_3$ and brine. After being dried with anhydrous $MgSO_4$, the organic layer was concentrated. The crude product was purified by column chromatography using hexane/EtOAc (1/1), affording the monoacylglycerol **1** as a white solid in 81% yield, mp 52−53 °C. IR (NaCl): 3279, 2920, 1705 cm^{-1}. ^{1}H NMR ($CDCl_3$): 7.25−7.19 (m, 1H), 6.13−6.09 (m, 2H), 5.77−5.71 (d, J = 15.27 Hz, 1H), 2.23 (br, 2H), 2.12−2.07 (m, 2H), 1.36−1.20 (br, 14H), 0.84−0.79 (t, J = 6.85 Hz, 3H) ppm. FABMS calcd.: 303 (M+), 207 (fatty acid − OH). FABMS found: 303 (M+), 207 (fatty acid − OH). HRMS found: 304 (M + 1), 207 (fatty acid − OH).

Phase Study of the Lipid Mixture Before Polymerization. *^{2}H NMR Spectroscopy.* Deionized water was added to a 9/1 lipid mixture of **1** and **2** at the concentration of 25% water by weight. The sample was centrifuged and incubated at room temperature for a week: a clear isotropic sample was obtained and confirmed by viewing the sample through cross-polarized light. The deuterium NMR spectra were recorded at 38.397 MHz on a Bruker ACP-250 spectrometer. The NMR signal was collected with the quadupole echo sequence with a 90° pulse length of 13 μs, interpulse delay of 60 μs, and a relaxation delay of 0.5 s.[31] Temperature was controlled by a heated air flow and monitored by means of a thermocouple close to the sample.

X-ray Diffraction. The X-ray measurements were performed at station 8.2 at the Daresbury Laboratory, England, using a monochromatic beam of wavelength 1.5 Å. SAXS data were calibrated against a sample of wet rat tail collagen. Immediately before the diffraction experiment, the samples were placed between mica sheets held by copper spacers. The temperature was thermostatically controlled by mounting the samples on a modified microscope cryostage (Linkam, U.K.) and monitored by a thermocouple embedded in the sample adjacent to the beam.

Pulsed Field Gradient NMR Spectroscopy. A 9:1 molar ratio of **1** and **2** was placed in a cylindrical quartz cuvette with a flat bottom, centrifuged at 1000 rpm for 20 min, and heated to 40 °C until the mixture was clear. A sonicated 6.67 wt % solution of ANTS (8-aminonaphthalene-1,3,6-trisulfonic acid, disodium salt) in D_2O heated to 40 °C was slowly laid on top of the lipid mixture to a final concentration of 26 wt % D_2O. The resultant mixture was capped with a septum and left overnight at 40 °C until the layers merged into one. The mixture was cooled to room temperature, sonicated, and brought back to 40 °C, and the process was repeated until most of the sample turned to a clear pinkish visually isotropic solution. A cylindrical quartz rod (outer diameter slightly smaller than the inner diameter of the quartz cuvette) was slowly inserted into the cuvette such that the lipid mixture flowed up the sides of the rod. The sample was sealed with Parafilm.

Water diffusion in the lipid mixture was measured at a field strength of 4.7 T using a Bruker Biospec imager/spectrometer.

(21) Lasic, D. D. *Liposomes: From Physics to Applications*; Elsevier: Amsterdam, 1993.
(22) Leslie, S. B.; Puvvada, S.; Ratna, B. R.; Rudolph, A. S. *Biochim. Biophys. Acta* **1996**, *1285*, 246−254
(23) Engström, S. *Lipid Technol.* **1990**, *31*, 157−158.
(24) Lutton, E. S. *J. Am. Oil Chem. Soc.* **1965**, *42*, 1068−1070.
(25) Gutman, H.; Arvidson, G.; Fontell, K.; Lindblom, G. In *Surfactants in Solutions*; Plenum Press: New York. 1984; pp 143−152.
(26) Lindblom, G.; Larsson, K.; Johansson, L.; Fontell, K.; Forsén, S. *J. Am. Chem. Soc.* **1979**, *101*, 5465−5470.
(27) Orädd, G.; Wikander, G.; Lindblom, G.; Johansson, L. B.-×fbA. *J. Chem. Soc., Faraday Trans. 1.* **1994**, *90*, 305−309.
(28) Eriksson, P. O.; Lindblom, G. *Biophys. J.* **1993**, *64*, 129−136.
(29) Nilsson, A.; Holmgren, A.; Lindblom, G. *Chem. Phys. Lipids* **1994**, *71*, 119−131.
(30) Nilsson, A.; Holmgren, A.; Lindblom, G. *Biochemistry* **1991**, *30*, 2126−2133.
(31) Davis, J. H.; Jeffrey, K. R.; Bloom, M.; Valic, M. I.; Higgs, T. P. *Chem. Phys. Lett.* **1976**, *44*, 390−394.

A stimulated echo pulse sequence was employed with 25 μs 90° pulse lengths, 26 ms echo delays, and 41 ms "storage" delay (time between the second and third 90° pulses).[32,33] Balanced Stejskal-Tanner-type diffusion gradient pulses of duration δ = 15 ms, separation Δ = 67 ms, and strength g were applied during the echo delays. Gradient strengths of 4.2, 21.2, 42.4, 63.6, 84.8, 106.0, and 127.3 mT/m were employed giving diffusion weighting "b" factors of 18.0, 450, 1799, 4048, 7197, 11 245, and 16 193 s/mm^2, respectively, where $b = (\gamma\delta g)^2(\Delta - \delta/3)$. The magnitude of the acquired signal I is given by $I = I_0 \exp(-bD)$ where γ is the gyromagnetic ratio and D is the molecular self-diffusion coefficient.[32,33] The sample was kept either at yellow light or in the dark during the preparation and measurement. The sample temperature was ca. 23 °C during the NMR experiment.

The sample was subsequently polymerized for 1 h at 254 nm light in a Rayonet Photochemical Reactor equipped with eight UV tubes (RPR 2537, Southern NE Ultraviolet Co.). The chamber temperature climbed from 22 to 36 °C at the end of polymerization. Water diffusion in the lipid mixture was measured again by pulsed field gradient NMR.

Polymerization of a Bicontinuous Cubic Phase. A 9/1 molar ratio lipid mixture of **1** and **2** was hydrated with deoxygenated H_2O_2 solution (30% H_2O_2 in water) at a concentration of 25% aqueous H_2O_2 by weight. The sample was centrifuged at room temperature and kept in the dark at 5 °C for 1 day to ensure the equilibrated formation of a cubic phase. Polymerization was performed at 45 °C under $Ar_{(g)}$ for 2 days. The water was removed by lyophilization, and the methanol extract of a known weight lipid was characterized using UV spectroscopy. The absorbance of the dienoyl peak at 265 nm was measured for the calculation of percent conversion to polymer.

The molecular weight of the lipid polymer was estimated by the use of size exclusion chromatography (SEC) on a Ultrastyragel linear column calibrated with poly(methyl methacrylate) standards. SEC chromatograms were obtained from a Waters Maxima 820 chromatography workstation equipped with a Waters R401 differential refractometer detector. The mobile-phase CH_2Cl_2 was filtered through 0.45 μm Waters nylon filters and purged with helium. Each chromatogram was obtained from a 100 μL injection of a polymer sample with a concentration of 2−3 mg/mL. The reported molecular weights are the average of at least two samples.

Results and Discussion

1-(2,4-(*E*,*E*)-Tetradecadienoyl)-*sn*-glycerol-d_5 (**1**) containing a polymerizable diene moiety conjugated to the acyl carbonyl group in the chain was designed and synthesized. Compound **1** was deuterated to facilitate the phase characterization by ^{2}H NMR spectroscopy. Our previous studies of the polymerization of nonlamellar phases suggest that polymer formation near the glycerol backbone did not significantly perturb the motions of the hydrophobic lipid tail.[17,18] Polymerization of the diene reactive group can be accomplished with the aid of either thermal or redox radical initiators or by direct photopolymerization.[34,35]

Diacylglycerols (DAG) are known to affect membrane structure in a variety of ways, including modification of membrane curvature,[36] lateral phase separation,[37] formation of ripple phase,[38] promotion of membrane fusion,[39] and production of nonlamellar phases.[40,41] A polymerizable 1,2-bis(2,4-(*E*,*E*)-tetradecadienoyl)-3-*sn*-glycerol **2** was prepared to aid in the formation of a Q_{II} phase.

Syntheses. Lipid **1** was obtained in 80% yield from the acylation between 2,4-(*E*,*E*)-tetradecadienoic acid and 1,2-*O*-isopropylidene-*sn*-glycerol-d_5 **4**.[42] The protected glycerol was obtained in quantitative yield from glycerol-d_5 and dry acetone. The acylation in the presence of 4-(dimethylamino)pyridine and dicyclohexylcarbodiimide afforded the desired product in high yield. The final step is the deprotection of the isopropylidene group using dilute HCl solution, giving deuterated **1** in quantitative yield as a white solid after purification by column chromatography. The synthesis of lipid **2** was previously published.[42] The purity of the polymerizable lipids **1** and **2** was established by elemental analysis, ^{1}H NMR, and high-resolution mass spectroscopy. Lipid **1** has a λ_{max} at 260 nm (ϵ 1.24 × 10^4 L/mol cm) in water. The extinction coefficient of **2** was approximately twice that of **1** (both in methanol).

Characterization of Lipid Phase before Polymerization. The lipid phase behavior was investigated with cross-polarized light, ^{2}H NMR spectroscopy, and X-ray diffraction. A sample of lipid **1** with 25 wt % water remained opaque after incubation at room temperature for a week, and the ^{2}H NMR spectrum showed a relatively broad quadrupole splitting up to a temperature of 70 °C. This is indicative of an anisotropic phase, most probably a lamellar liquid crystalline phase. The characteristic physical properties of the reversed bicontinuous cubic phase include a high viscosity and a clear appearance in transmitted light, whereas the sample is totally dark when viewed between crossed polarizers. In this work we were interested in the polymerization of a cubic phase. Since pure lipid **1** in water does not form such an isotropic phase below 70 °C, this problem was solved by the addition of a second lipid, of the diacylglycerol class known to induce the formation of nonlamellar phases. Therefore, increasing amounts of lipid **2** was added to lipid **1** and the phase behavior of the mixtures studied. A hydrated 9/1 molar ratio lipid mixture of **1** and **2** at the concentration of 25 wt % water became clear after incubation at room temperature for 1 day. This isotropic character of the sample was confirmed by examination with cross-polarized light.

The phase behavior of lipid **1** with 5, 10, and 15 mol % of **2** was determined through measurements of the quadrupole splitting obtained in the ^{2}H NMR spectrum and X-ray diffraction.

Figure 3 shows the ^{2}H NMR spectra of three sample compositions of **1**/**2**/H_2O at various temperatures. The NMR spectra exhibit a typical line shape, generally observed for isotropic samples, which is present at all temperatures and compositions studied. In addition, there are also line shapes arising from anisotropic phases in some of the spectra recorded for samples containing 5 and 15 mol % of **2**. Provided the local environment of the lipid does not change, the lamellar phase should theoretically exhibit twice as large a quadrupole splitting as that of the hexagonal phase, while a cubic phase, since it is isotropic, will give rise to a characteristic sharp singlet peak.[8] At 5 mol % of **2** at 25 °C (Figure 3, top, left), the NMR spectrum consists of a superposition of an isotropic peak and an anisotropic powder pattern characteristic for a lamellar phase. As the temperature is raised, the isotropic

(32) Stejskal, E. O.; Tanner, J. E. *J. Chem. Phys.* **1965**, *42*, 288−292.
(33) Tanner, J. E. *J. Chem. Phys.* **1970**, *52*, 2523−2526.
(34) Tsuchida, E.; Hasegawa, E.; Kimura, N.; Hatashita, M.; Makino, E. *Macromolecules* **1992**, *25*, 207−212.
(35) Srisiri, W.; Sisson, T. M.; O'Brien, D. F. *J. Am. Chem. Soc.* **1996**, *118*, 11327−11328.
(36) Ohki, T.; Sekiya, T.; Yamauchi, T.; Nozawa, T. *Biochim. Biophys. Acta* **1982**, *693*, 341−350.
(37) Ortiz, A.; Villalain, J.; Gomez-Fernandez, J. C. *Biochemistry* **1988**, *27*, 9030−9036.
(38) Lopez-Garcia, F.; Villalain, J.; Gomez-Fernandez, J. C.; Quinn, P. J. *Biophys. J.* **1995**, *68*, 558−566.
(39) Siegel, D. P.; Banschbach, J.; Alford, D.; Ellens, H.; Lis, L. J.; Quinn, P. J.; Yeagle, P. L.; Bentz, J. *Biochemistry* **1989**, *28*, 3703−3709.
(40) Siegel, D. P.; Banschbach, J.; Yeagle, P. L. *Biochemistry* **1989**, *28*, 5010−5019.
(41) Orädd, G.; Lindblom, G.; Fontell, K.; Ljusberg-Wahren, H. *Biophys. J.* **1995**, *68*, 1856−1863.
(42) Srisiri, W.; Lamparski, H. G.; O'Brien, D. F. *J. Org. Chem.* **1996**, *61*, 5911−5915.

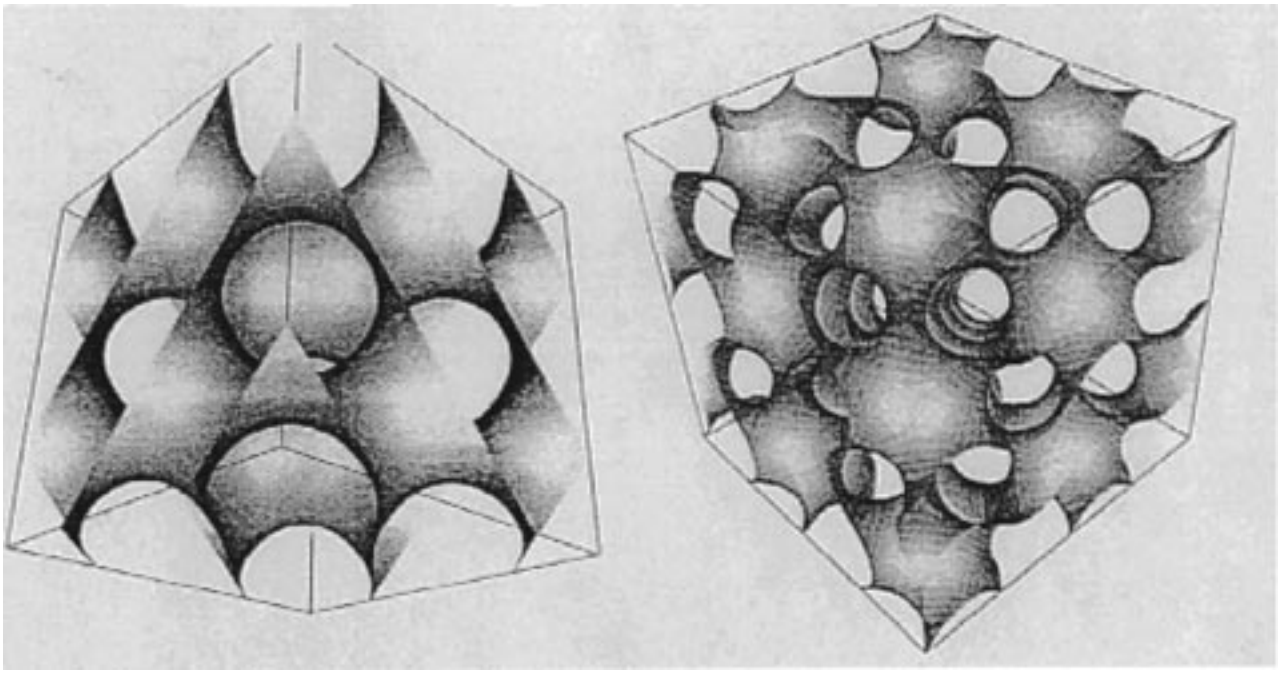
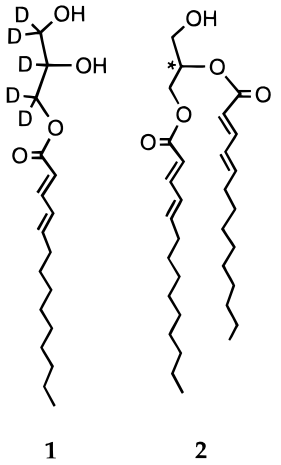


Figure 1. A minimum surface graphic illustration of a bicontinuous cubic assembly of lipids with *Pn*3*m* (left) and *Ia*3*d* (right) symmetry (Benedicto, A. D.; O'Brien, D. F. *Macromolecules* **1997**, *30*, 3395−3402). The two interpenetrating aqueous channels are separated from each other by a continuous lipid bilayer surface. These cubic architectures are bicontinuous with respect to the polar (aqueous) and nonpolar (lipid) regions.



1 **2**

Figure 2. Polymerizable monoacylglycerol **1** and 1,2-diacylglycerol **2**.

contribution in the NMR spectrum grows and at 55 °C only a small trace of the anisotropic line shape remains (Figure 3, top, right). The ^{2}H NMR spectra from the sample with 10 mol % of **2** give an isotropic peak at all temperatures studied (Figure 3, middle). At 15 mol % of **2** and 25 °C (Figure 3, bottom, left), the NMR line shape is also a superposition of isotropic and anisotropic parts, the anisotropic powder pattern being about half as wide as for the 5 mol % sample, indicating the presence of a hexagonal phase.[8] The anisotropic line shape gradually decreases and finally disappears between 35 and 45 °C.

Measurements of the X-ray diffraction were performed on samples corresponding to the same compositions used in the NMR measurements above. The X-ray diffraction pattern for the sample with 5 mol % of **2** at 25 °C gives reflections with repeat distances in the ratio 1:1/2:1/3:1, typical for a lamellar phase,[6] and the *d* spacings were 48.3 and 44.6 Å at 25 and 45 °C, respectively. As the temperature is increased to 55 °C, additional weak reflections appear that most probably originate from a cubic phase. Figure 4a shows an X-ray diffraction pattern of a cubic phase for the sample with 10 mol % of **2** at 25 °C, which is typical for the sample down to 5 °C. It can be inferred from this figure that several reflections are observed that can be indexed to a cubic phase of space group *Ia*3*d* (see also Figure 5).[43] At temperatures above 45 °C, the diffractogram changes to a broad featureless line shape. The X-ray diffractogram for the sample with 15 mol % of **2** at 25 °C shows weak reflections which are also characteristic for this cubic phase. As the temperature is increased to about 35 °C, the structural features in the diffractogram disappear and only a broad featureless reflection remains. In Figure 4b, the inverse spacing (1/*d*, 1/Å) is plotted against $\sqrt{h^2+k^2+l^2}$ for a sample with 10 mol % of **2** at 25 °C. The straight line obtained, passing through the origin, is strong support for the space group *Ia*3*d*. The unit cell length calculated from the line slope was 131 Å. A similar analysis showed that 15 mol % of **2** in **1** induces a cubic phase of the same space group, but with a unit cell length of 123 Å at 25 °C. Thus, there is strong agreement between the phase behavior observed by the ^{2}H NMR and the phase structures determined by X-ray diffraction.

Taken together, the results for the unpolymerized samples show that a sample composed of 9/1 of **1** and **2** in 25 wt % water is preferable for polymerization of the cubic phase. This phase, located between the lamellar and the reversed hexagonal phases, has also been shown by NMR diffusion measurements to consist of bicontinuous lipid and water regions. As measured through pulsed field gradient NMR spectroscopy, the water diffusion coefficient prior to polymerization of the lipid mixtures **1** and **2** is $1.0 \pm 0.4 \times 10^{-10}$ m^2/s (Figure 6), an order of magnitude smaller than the diffusion of pure water (2×10^{-9} m^2/s). This apparent increase in viscosity is attributed to the non-Newtonian flow of water molecules in the 3D network of water channels in the cubic phase

(43) *International Tables for X-ray Crystallography*; Kynoch Press: Birmingham, U.K., 1952.

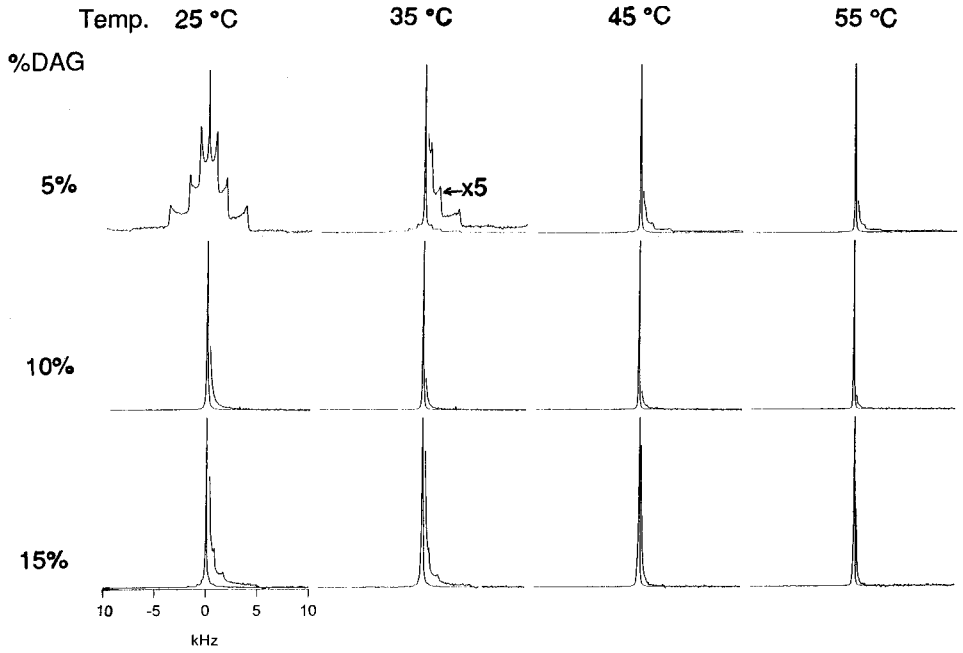


Figure 3. ^{2}H NMR spectra of a lipid mixture of **1** and **2** at different sample compositions and temperatures. The water content is 25% w/w. To the right of each spectrum (except the top left), a 5 times magnification is inserted.

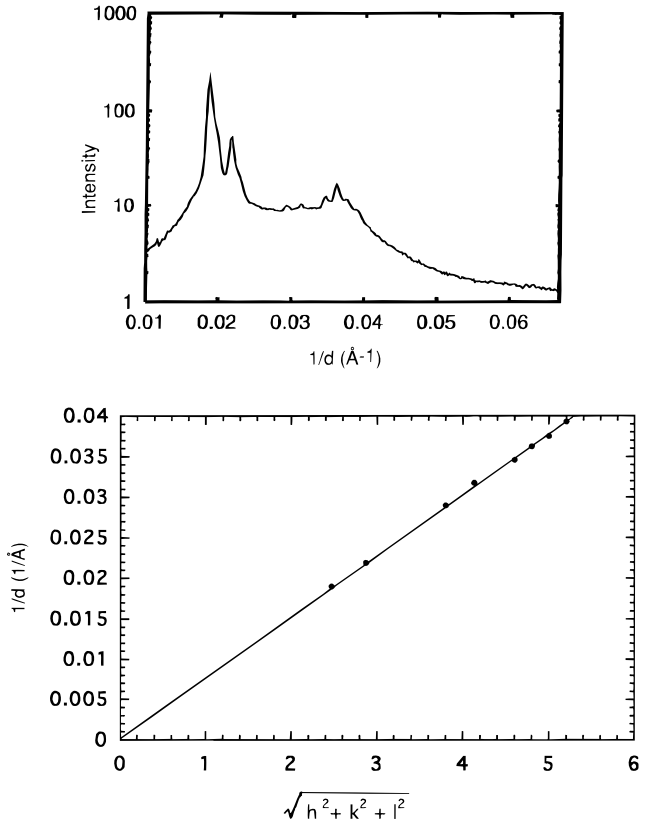


Figure 4. (a) X-ray diffraction of a 9/1 molar lipid mixture of (**1**) and (**2**) at 25 °C. (b) A plot of the inverse spacing vs $\sqrt{h^2+k^2+l^2}$. The line fitted to the data points is consistent with the *Ia*3*d* space group.

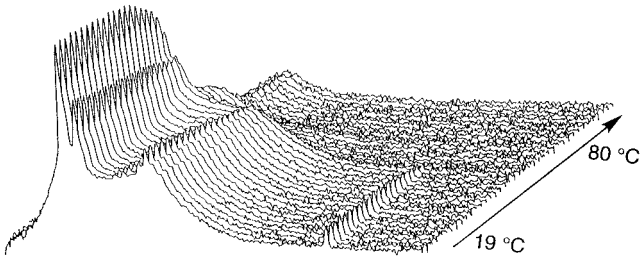


Figure 5. X-ray diffraction of a 9/1 molar lipid mixture of **1** and **2** at different temperatures. The axis are the same as Figure 4a.

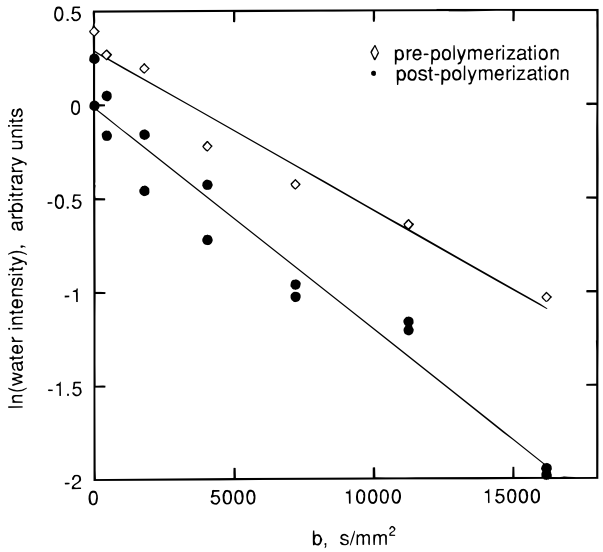


Figure 6. Water intensity as function of b ($=(\gamma\delta g)^2(\Delta - \delta/3)$) before and after UV polymerization of aqueous lipid mixtures **1** and **2**. Water diffusion coefficient (before polymerization) = $1.0 \pm 0.4 \times 10^{-10}$ m^2/s. Water diffusion coefficient (after polymerization) = $1.2 \pm 0.2 \times 10^{-10}$ m^2/s.

because of the interaction of the water molecules with the hydrophilic lipid headgroups. This is supported by the fact that there are only 7.3 water molecules/lipid molecule in the sample. The diffusion of water inside the 3D channel network for lipids that form cubic phases such as monoolein is similarly retarded.[26] The diffusion coefficient of water molecules after lipid polymerization was $1.2 \pm 0.2 \times 10^{-10}$ m^2/s, the same as before polymerization suggesting that there is no change in cubic phase structure (Figure 6).

Phase diagrams have been published previously for several monoacylglycerols (MAG), and detailed analyses of some unsaturated MAG have appeared.[44−46] Cubic phases were present in most of these binary lipid−water systems. MAG with saturated acyl chains of fourteen carbons (C_{14}) or more exhibited cubic phase(s) located between the lamellar and the reversed hexagonal phase on the water-rich side of the lamellar phase. However, the cubic phase formed for the C_{14} MAG occurs only well-above 100 °C, whereas the lamellar phase is present over a relatively wide temperature and concentration region. This is consistent with the findings in this study of lipid **1**, which also contains 14 carbon atoms in its acyl chain. The trend for the formation of the different phases follows the molecular shape concept introduced several years ago.[47−49] These results and analyses prompted us to introduce a diacylglycerol into the MAG bilayer in order to favor the formation of a cubic phase (i.e., lipid **2** alters the effective molecular shape of the combined lipids to a more wedge-shaped structure) which is more prone to form the curved aggregates necessary for the formation of nonlamellar phases.

Polymerization of the Cubic Phase. A 9/1 lipid mixture of **1** and **2** was hydrated with deoxygenated H_2O_2 solution at a concentration of 25% H_2O_2/H_2O by weight and incubated at 5 °C for 1 day to ensure the equilibrated formation of an isotropic sample, which was confirmed by cross-polarized light. The sample was isotropic at temperatures up to 45 °C. The lack of polymerization during this incubation period was indicated by the absence of a change in the absorbance of the polymerizable group at 265 nm. Polymerization of the sample was then performed at 45 °C under $Ar_{(g)}$ for 2 days. Water was removed by lyophilization and the methanol extract of a known weight lipid was characterized using UV spectroscopy. The decrease in absorbance of the dienoate at 265 nm was used to calculate the conversion to a polymer. The almost complete loss of absorbance at 265 nm indicated that the reaction proceeded with high conversion. The polymerized sample was soluble in common organic solvents, indicating that the polymer formed was linear in nature.[19] The number-average molecular weight (M_n) was estimated by size exclusion chromatography to be $5 \pm 0.2 \times 10^4$ which corresponds to a degree of polymerization (X_n) of 200 ± 20.

A polymerized sample of the 9/1 mixture of **1** and **2** was completely dark when viewed with cross-polarized light, indicating that the sample maintained its isotropic character during and after the polymerization process. The isotropic nature of the polymerized sample was observed up to at least 70 °C, indicating the increased stability of the polymerized cubic phase compared to the unpolymerized hydrated lipids. As expected, the ^{2}H NMR spectrum of the polymerized sample showed a relatively broad quadrupole splitting superimposed by a broad central peak, indicating that the lipid molecules are unable to move in an unrestricted translational manner in the polymerized cubic phase. Preliminary X-ray diffraction data for the polymerized phase indicated that the structure still belongs to the *Ia*3*d* space group. There is a qualitative similarity between the diffractograms before and after the polymerization; however, there is a degradation of the resolution upon polymerization. This probably arises from local disordering of the cubic symmetry resulting in poor line shapes. Although the poor resolution makes it difficult to obtain a plot similar to the one shown in Figure 5, the general form of the line shape permits an estimate of the cubic cell size (135 Å) which is slightly larger than that of the unpolymerized sample.

Conclusions

The polymerizable monoacylglycerol **1** alone did not form an identifiable nonlamellar phase upon hydration. The inclusion of the diacylglycerol **2** facilitated the formation of a nonlamellar phase. Phase investigation using cross-polarized light, ^{2}H NMR spectroscopy, and X-ray diffraction showed that the lipid mixture formed a well-defined cubic phase from at least 5 to 45 °C. The X-ray diffraction pattern corresponded to a cubic phase with *Ia*3*d* symmetry and a unit cell size of 131 Å at 25 °C. Water diffusion inside the aqueous 3D channel network remained the same both before and after UV polymerization, suggesting that polymerization did not alter the structure of the water channel in the cubic phase. The experimentally determined water diffusion coefficient is 1×10^{-10} m^2/s, an order of magnitude smaller than diffusion of pure water, suggesting a tortuous path by water molecules along the water channel and a considerable interaction between water molecules and the hydrophilic lipid headgroup. The polymerization to high conversion of this cubic phase was accomplished via the thermal decomposition of H_2O_2. The resultant polymers dissolved in an organic solvent, indicating that they were not cross-linked. The visual clear character and cross-polarized light test showed that an isotropic architecture was maintained during and after sample polymerization. The biocompatible and mesoporous nature of the polymerized cubic phase suggests it could be used as the host for the incorporation of synthetic or biological molecules in a manner that has already proven especially useful in microporous solids.

Acknowledgment. The authors thank the National Science Foundation (D.F.O.) and the Swedish Natural Science Research Council (G.L.) for financial support of this research.

LA970897M

(44) Hyde, S.; Andersson, S.; Ericsson, B.; Larsson, K. *Z. Kristallografiya.* **1984**, *168*, 213−219.
(45) Briggs, J.; Caffrey, M. *Biophys. J.* **1994**, *66*, 573−587.
(46) Briggs, J.; Caffrey, M. *Biophys. J.* **1994**, *67*, 1594−1602.
(47) Tartar, H. V. *J. Phys. Chem.* **1955**, *59*, 1195−1199.
(48) Tanford, C. *J. Phys. Chem.* **1972**, *76*, 3020−3024.
(49) Israelachvili, J. N.; Mitchell, D. J.; Ninham, B. W. *J. Chem. Soc., Faraday Trans. 2* **1976**, *72*, 125−1568.