HYDROPHOBIC MOLECULES IN LECITHIN–WATER SYSTEMS

I. Formation of Reversed Hexagonal Phases at High and Low Water Contents

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ABSTRACT The system dioleoylphosphatidylcholine (DOPC)–*n*-dodecane–^2H_2O was investigated with different nuclear magnetic resonance (NMR) techniques: (*a*) a tentative phase diagram was determined by ^2H- and ^{31}P-NMR, (*b*) translational diffusion coefficients were determined for the three components with the pulsed magnetic field gradient NMR technique, and (*c*) order parameters for perdeuterated *n*-dodecane were obtained by ^2H-NMR. *n*-Dodecane induces the formation of reversed hexagonal (H_{II}) phases at low and high water concentrations, and cubic phases at low water contents. The translational diffusion coefficients of *n*-dodecane in a cubic phase with 6 mol water per mol DOPC, and in an H_{II} phase with 48 mol water per mol DOPC, were just ~2.5 times lower than in pure dodecane. Perdeuterated dodecane gave large quadrupole splittings in a lamellar phase, much smaller in an H_{II} phase at low water contents, and a narrow single peak in an H_{II} phase at high water contents. This latter observation indicates that a large fraction of the dodecane molecules is located in separate regions between the water cylinders. Our results support the model given by Gruner concerning the aggregation of membrane lipids in the presence of hydrophobic molecules (Gruner, S. M., 1985, *Proc. Natl. Acad. Sci. USA*, 82:3665–3669).

INTRODUCTION

The effect of hydrophobic molecules like *n*-alkanes on lipid bilayers is of great biological interest for several reasons. It gives information about the nature of the bilayer as a solvent for hydrophobic molecules. Such knowledge is important since intrinsic membrane proteins seem to be anchored with transmembrane segments consisting of ~20 hydrophobic amino acid residues (von Heijne, 1985). Signal peptides of proteins that are inserted into a membrane, or translocated through one or several membranes, contain a segment of ~8–12 hydrophobic amino acid residues (von Heijne, 1985). Furthermore, ionophores like gramicidin and valinomycin are also very hydrophobic. The electron-transporting ubiquinones in the membranes of bacteria and mammalian mitochondria, and plastoquinones in the thylakoid membranes, also belong to this group of hydrophobic molecules. Recently, the possible role of dolichols, long-chain isoprenoid alcohols, in membranes was discussed (Valtersson et al., 1985; Gruner, 1985). Further, the study of alkanes in bilayer systems can be viewed from an environmental health aspect since many solvents used in daily life are hydrocarbons. Alkanes are also of interest because of the anesthetic effect of the short chain species (Janoff and Miller, 1982). Several proposals have been put forward to explain the mechanism of general anesthesia: perturbations of the lipid bilayer (Janoff and Miller, 1982) and binding of anesthetic molecules to amphiphilic pockets on proteins (Franks and Lieb, 1985, 1986). The membrane lipid composition of the bacterium *Acholeplasma laidlawii* is drastically altered when *n*-dodecane is incorporated into the membrane (Wieslander et al., 1986). Finally, it is of general interest in surface chemistry to study amphiphile–hydrocarbon–water systems, and such systems have been the object of various theoretical treatments (Kirk et al., 1984; Gruner, 1985; Siegel, 1986).

Several studies have been performed on lipid–hydrocarbon–water systems using a range of techniques including x-ray diffraction, neutron diffraction, differential scanning calorimetry, and nuclear magnetic resonance (NMR). The organization of *n*-alkanes in lipid bilayers depends on the length of the hydrocarbon. Short alkanes ($<C_{12}$) are probably located mainly in the center of the bilayer and are disordered relative to the lipid molecules, while the longer alkanes (C_{12}–C_{16}) seem to align parallel to the lipid acyl chains (McIntosh et al., 1980; White et al., 1981; Pope et al., 1984).

The addition of *n*-alkanes to phosphatidylethanolamine (PE)–water and PE–phosphatidylcholine (PC)–water mixtures promotes the formation of a reversed hexagonal (H_{II}) phase (*a*) hexane, octane, decane,and dodecane lower the temperature for the transition from a lamellar phase to an H_{II} phase (T_{LH}) for egg PE (Hornby and Cullis, 1981); (*b*) dodecane and tetradecane lower the T_{LH} for dioleoyl–PE (DOPE) and mixtures of DOPE and dioleoyl–PC

(DOPC) (Kirk and Gruner, 1985); and (*c*) eicosane lowers the T_{LH} for dielaidoyl–PE and 1-palmitoyl-2-oleoyl–PE (Epand, 1985). The above mentioned investigations have almost exclusively been performed with samples containing excess water.

Here the phase equilibria of the system DOPC–*n*-dodecane–^2H_2O were studied in the temperature range 20°–60°C and at water concentrations ranging from 3 to 95% (wt/wt). DOPC forms a lamellar phase up to at least 100°C in excess water (Kirk and Gruner, 1985). However, cubic phases were formed at low water concentrations and H_{II} phases were formed at both low and high water concentrations by adding *n*-dodecane to DOPC–^2H_2O mixtures. This unusual phase behavior is discussed in terms of molecular interactions.

MATERIALS AND METHODS

Sample Preparation

DOPC was prepared according to Gupta et al. (1977). The lipid was purified by column chromatography on silica gel followed by treatment with decolorizing charcoal. Only trace amounts of contaminants could be detected by thin-layer chromatography analysis of the lipids. Dioleoyldiglucosyldiglyceride (DODGDG) was isolated from the membranes of the bacterium *A. laidlawii* (Lindblom et al., 1986). Perdeuterated *n*-dodecane was obtained from ICN K&K Laboratories Inc., Plainview, NY. Deuterium oxide was purchased from Ciba-Geigy, Basel, Switzerland, and the *n*-alkane solvents from Sigma Chemical Co., St. Louis, MO. The purity of the alkanes was given as 99% and no further purification was done.

Samples were prepared in test tubes from lipids dried to constant weight in vacuum, a procedure that most likely gives the monohydrate form of phosphatidylcholine. Heavy water and alkane were then added and the tubes were sealed. The samples were mixed by centrifugation and left at room temperature for several days until they were homogeneous. To promote the growth of microcrystallites in the mixtures, the samples were repeatedly freezed and thawed. ^{31}P-NMR spectra were recorded repeatedly from all samples during a period of one or two months, and the spectra were in all cases reproducible. During the NMR measurements the test tubes were placed in 10 mm NMR tubes and a small amount of glycerol was added between the tubes to improve thermal contact.

^{31}P-NMR and ^{2}H-NMR Spectroscopy

^{31}P-NMR spectra were obtained with a Bruker WM-250 Fourier transform spectrometer at 101.3 MHz and with a Bruker MSL-100 Fourier transform spectrometer at 40.5 MHz. A phase-cycled Hahn echo sequence (Rance and Byrd, 1983) with high power proton decoupling was used. This pulse sequence largely eliminates the spectral distortion, often seen in spectra obtained with a single pulse experiment, that is caused by the inability to record the first part of the free-induction decay. The pulse lengths were 25 and 7 μs, respectively. A relaxation delay of 0.5–8 s was used dependent on the amount of hydrocarbon in the sample. The spectral width was 30 kHz. For a typical spectrum 500–5000 transients were accumulated and an exponential multiplication corresponding to 20-Hz line broadening was applied before Fourier transformation.

^{2}H-NMR spectra were obtained with a Bruker WM-250 Fourier transform spectrometer at 38.4 MHz. A quadrupole echo sequence was used with 50 μs between both of the radio frequency pulses. The pulse length was 22 μs (90° flip angle) and the relaxation delay 0.3 s. The spectral width was 20 kHz and before Fourier transformation an exponential multiplication corresponding to 20-Hz line broadening was applied. A typical spectrum was obtained from 500–10,000 transients depending on the ^2H_2O content. The samples were thermally equilibrated for ~1 h before the measurements. It has been shown in many previously published works that ^{2}H- and ^{31}P-NMR can be conveniently used to determine phase diagrams of lipid–water systems (Ulmius et al., 1977; Brentel et al., 1985; Eriksson et al., 1985; Lindblom et al., 1986). The NMR method is a rapid and nondestructive way to determine a phase diagram without the problem of separating the individual phases. By systematic variations of sample composition and temperature the phase diagram was established.

NMR Diffusion Measurements

Translational diffusion coefficients were measured with the NMR pulsed magnetic field gradient technique (Stejskal and Tanner, 1965; Lindblom and Wennerström, 1977), using a Bruker MSL 100 FT NMR spectrometer equipped with a 2.35 T superconducting magnet. The magnetic field gradients were generated with a slightly modified Bruker B-Z 18 B gradient unit. At each side of the 180° radio frequency pulse in a 90°-τ-180° Hahn spin echo sequence a gradient pulse of constant magnitude, g, separation, Δ, and varying width, δ, was applied. Due to diffusion the echo amplitude, E, at 2τ will be attenuated according to

$$E/E_o = \exp\left[-(\gamma g\delta)^2 D(\Delta - \delta/3)\right], \quad (1)$$

where τ is the magnetogyric ratio, E/E_o the echo attenuation, and D the diffusion coefficient. For samples with several diffusing components differing greatly in the magnitude of D, the diffusion coefficient of the most slowly diffusing component can be obtained from a fit of Eq. 1 to the latter part of the echo attenuation curve in the time domain. The individual diffusion coefficients in a sample with several species with small differences in the magnitude of D may be obtained after Fourier transformation of one-half of the spin echo followed by a fit of Eq. 1 to the signal amplitudes in the frequency domain spectra as a function of δ. The magnitude of the field gradient pulses was determined by measurements on doubly distilled water, the diffusion coefficient of which is known from the literature (Mills, 1973). The gradient amplitudes were 1.24 Tm^{-1} and 0.18 Tm^{-1} for slow and fast diffusion, respectively. Typical settings for τ was 30 ms and for Δ 40 ms. δ was varied from 1 up to at most 16 ms depending on the value of the diffusion coefficient.

Polarized Light Microscopy

Different lipid phase structures have textures that can be recognized by examination in a light microscope equipped with polarization optics (Rosevear, 1954). The samples were prepared in two different ways: (*a*) DOPC–*n*-dodecane–^2H_2O. A few milligrams of samples that had been studied by NMR were sealed under a coverslip on an object glass; (*b*) dioleoyldiglucosyldiglyceride (DODGDG)–*n*-dodecane–^2H_2O. DODGDG–H_2O mixtures with a certain amount of H_2O, or DODGDG–dodecane mixtures with a certain amount of dodecane, were deposited on an object glass with a coverslip. The void space between the object glass and the coverslip was then filled with dodecane and H_2O, respectively, and the sample texture was studied for one or several days.

RESULTS

Phase Equilibria in the System DOPC–*n*-Dodecane–^2H_2O

^{31}P- and ^{2}H-NMR measurements were performed in the temperature range 20°–60°C on ~40 different samples. These NMR spectra constitute the basis for the construction of the tentative phase diagram for the system DOPC–*n*-dodecane–^2H_2O, shown in Fig. 1.

At 25°C all DOPC–^2H_2O mixtures investigated (4–95 wt% ^2H_2O) gave ^{31}P-NMR spectra with a low-field shoulder and a high-field peak (Fig. 2 *a*); such spectra are

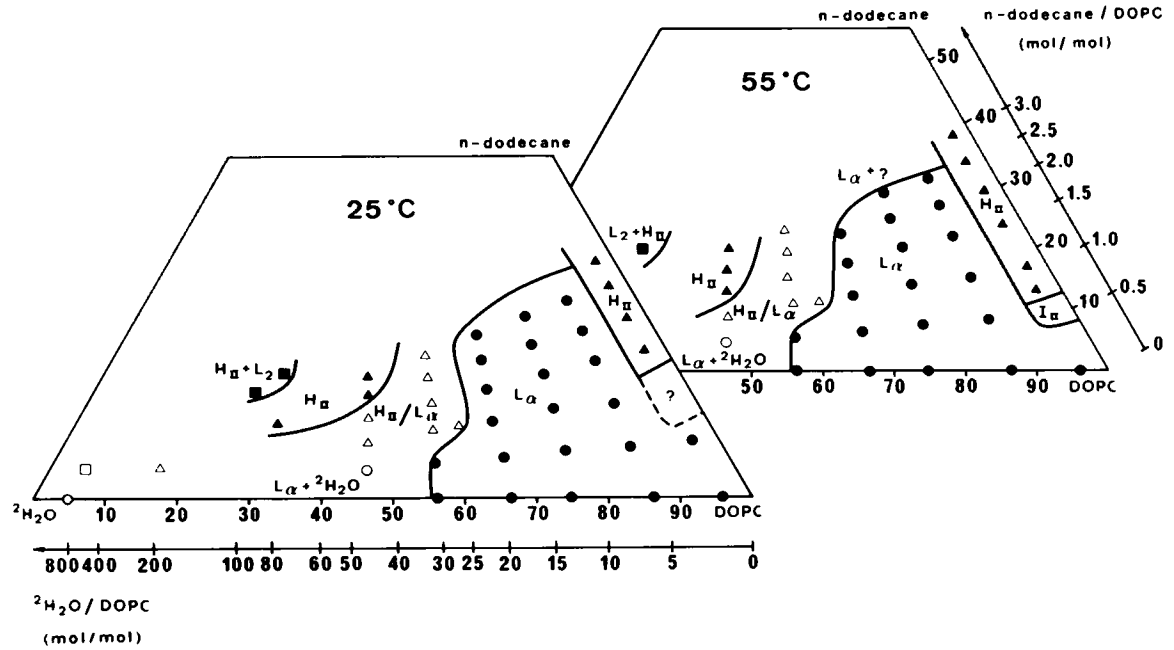

FIGURE 1 Part of a ternary phase diagram for the system DOPC–*n*-dodecane–^2H_2O at 25° and 55°C. ●, L_α (lamellar liquid-crystalline phase); ▲, H_{II} (reversed hexagonal phase); △, $H_{II} + L_\alpha$; ○, $L_\alpha + {}^2H_2O$; ■, L_2 (phase giving rise to an isotropic ^{31}P-NMR signal) + H_{II}; □, $L_\alpha + L_2$; I_{II} (reversed cubic phase). The phase border lines drawn are tentative and should be considered as an aid for the eye. Compositions are given in two ways, wt% and molar ratios.

characteristic for a lamellar liquid-crystalline phase (McLaughlin et al., 1975). However, at very low water contents the NMR line shape deviates from that obtained from ideally uniaxial lamellar systems. Samples with water contents up to ~44 wt% ^2H_2O (31 mol ^2H_2O/ mol DOPC) gave ^{2}H-NMR spectra with a quadrupole splitting (Ulmius et al., 1977). At higher water contents a sharp singlet was superimposed on the quadrupole splitting, which indicates that the lamellar liquid-crystalline phase stands in equilibrium with free water (Gutman et al., 1984; Fig. 1).

A DOPC–^2H_2O sample with 1.6 mol ^2H_2O per mol DOPC transformed into a cubic phase between 65° and 70°C; a micellar phase is ruled out by the high viscosity and the low water content of the sample. The addition of 0.5 mol dodecane per mol lipid lowered the transition temperature interval to ~50°–60°C (Fig. 2 *b*; Table I). At temperatures between 25° and 40°C samples with 1.6 mol ^2H_2O and 0.75 or 1.0 mol dodecane per mol DOPC gave rise to ^{31}P-NMR spectra that were difficult to interpret, and the spectra probably represent mixtures of lamellar, cubic, and H_{II} phases. These samples formed an H_{II} phase at temperatures above 40°C. This is seen from ^{31}P-NMR spectra (Fig. 2 *c*) exhibiting a low-field peak and a high-field shoulder (McLaughlin et al., 1975). The H_{II} phase was formed at all temperatures investigated when the dodecane content was between 1.5 and 3.0 mol per mol DOPC. At this low water content the phase transitions were found to be very sensitive to small variations in the water concentration.

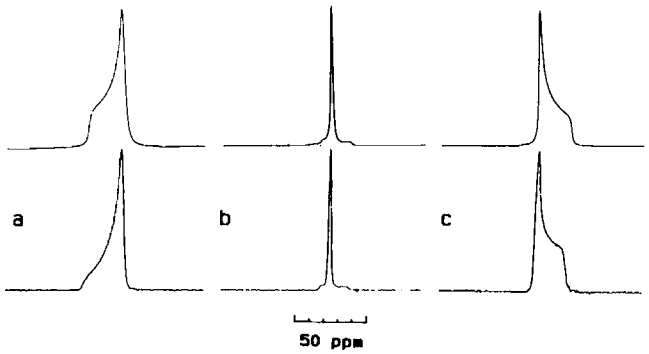

FIGURE 2 Experimental (*top*) and simulated (*bottom*) ^{31}P-NMR spectra recorded at 101.3 and 40.5 MHz and 60°C from samples containing 1.6 mol ^2H_2O per mol DOPC and different amounts of *n*-dodecane: (*a*) 0; (*b*) 0.5; and (*c*) 1 mol per mol DOPC. See Table I for the parameters used in the simulations.

Samples with 6, 13, and 20 mol ^2H_2O per mol DOPC formed a lamellar liquid-crystalline phase with dodecane concentrations up to at least 2.5 mol per mol lipid in the temperature interval 25°–55°C (Fig. 1). However, non-lamellar phases were formed at higher temperatures with 2.5 mol dodecane. The sample with 6 mol ^2H_2O formed a mixture of lamellar and cubic phases between 60° and 70°C. These phases were gradually replaced by an H_{II} phase by further raising the temperature, and an H_{II} phase only was obtained at 80°C. The ^{31}P-NMR spectrum from the sample with 13 mol ^2H_2O contained a small isotropic component at 60°C, and the sample with 20 mol ^2H_2O formed a mixture of lamellar and H_{II} phases at temperatures between 65° and 75°C.

TABLE I

CHEMICAL SHIFT ANISOTROPY IN ppm AND RELATIVE COMPOSITION OF LIQUID CRYSTALLINE PHASES USED IN SIMULATIONS OF ^{31}P-NMR SPECTRA RECORDED FROM DOPC–*n*-DODECANE–$^{2}H_2O$ MIXTURES

Figure	*n*-Dodecane/DOPC	$^{2}H_2O$ content	Temp.	Lamellar phase		Hexagonal phase		Isotropic phase	$\nu_{1/2}$
				Chemical shift anisotropy	DOPC	Chemical shift anisotropy	DOPC	DOPC	
	mol/mol	*wt%*	*°C*	*ppm*	*mol%*	*ppm*	*mol%*	*mol%*	*Hz*
2 *a*	0:1	3.9	60	−24.7	100	—	—	—	200
2 *b*	0.5:1	3.8	60	−41.5	8	20.7	8	84	150
2 *c*	1:1	3.5	60	—	—	22.7	100	—	150
3 *a*	1:1	38.8	25	−40.5	70	20.2	30	—	350
3 *b*	1:1	38.3	55	−36.5	55	18.3	45	—	400
4 *a*	2:1	2.9	25	—	—	21.7	100	—	200
4 *b*	2:1	10.2	25	−30.6	100	—	—	—	200
4 *c*	2:1	35.4	25	−41.0	48	20.5	52	—	300
4 *d*	2:1	60.0	25	—	—	18.8	100	—	300
4 *e*	2:1	93.0	25	−45.4	79.5	22.7	6	14.5	200

Spectra shown in Figs. 2 *a*, 3, *a* and *b*, and 4 *c* were recorded at 40.5 MHz and the rest were recorded at 101.3 MHz.

Additional increases of the water concentration gave rise to the most remarkable feature of the phase diagram: an H_{II} phase was formed again at dodecane/DOPC molar ratios between 1.0 and 2.5 (Figs. 1 and 4). A lamellar phase in equilibrium with an H_{II} phase was observed already at 25°C in a sample with 25 mol $^{2}H_2O$ and 1.0 mol dodecane per mol DOPC (Fig. 1). This phase equilibrium is displaced towards the H_{II} phase by three different manipulations: (*a*) By increasing the temperature (Fig. 3). The relative amounts of the lipid in the lamellar and H_{II} phases were obtained by simulation of the ^{31}P-NMR spectra (Table I). The influence of the temperature on this phase equilibrium was more pronounced at higher dodecane/DOPC ratios. (*b*) By increasing the water concentration up to at least 85 mol $^{2}H_2O$ per mol DOPC (Figs. 1 and 4, *c* and *d*; Table I). At this water concentration an H_{II} phase only was formed at 25°C. Moreover, an H_{II} phase and a very small fraction of a lamellar phase was obtained at 25°C in a sample with 207 mol $^{2}H_2O$ and 1.5 mol dodecane per mol DOPC (Fig. 1). (*c*) By increasing the dodecane concentration (compare Figs. 3 [25°C] and 4 *c*; Table I).

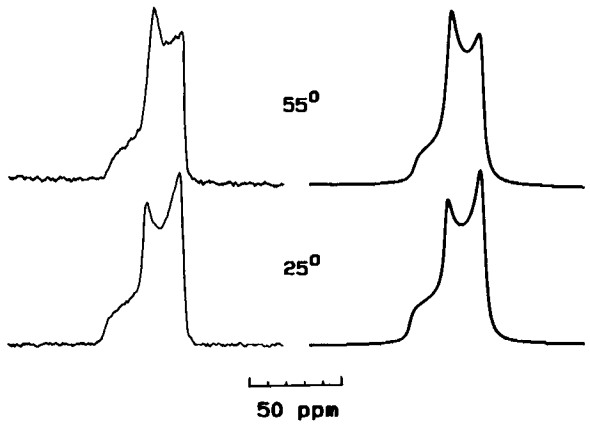

FIGURE 3 Experimental (*left*) and simulated (*right*) ^{31}P-NMR spectra recorded at 40.5 MHz and at two temperatures from a sample containing DOPC–*n*-dodecane–$^{2}H_2O$. The *n*-dodecane to DOPC molar ratio is 1.0 and the sample contains 30 mol $^{2}H_2O$ per mol DOPC (38.8 wt%). See Table I for the parameters used in the simulations.

The samples giving rise to the spectra shown in Fig. 4 *b* and *d* were investigated by polarized light microscopy. The sample with 6 mol $^{2}H_2O$ per mol DOPC exhibited a mosaic texture typical for lamellar phases, whereas the sample with 85 mol $^{2}H_2O$ per mol DOPC showed a striated pattern typical for hexagonal phases (Rosevear, 1954). This supports the statement made above that the new phase appearing at water concentrations above 25 mol $^{2}H_2O$ per mol DOPC, and at dodecane/DOPC molar ratios between 1.0 and 2.5, is a hexagonal phase. This phase is most probably of the reversed type (H_{II}) since (*a*) the relative amount of this phase increases when the temperature and dodecane concentration is increased (Israelachvili et al., 1980; Rilfors et al., 1984); (*b*) an H_{II} phase is obtained at high alkane concentrations even in some soap–alkane–water systems, i.e., with amphiphiles that together with water form micellar solutions and normal hexagonal phases (Ekwall, 1975); and (*c*) normal hexagonal phases cannot be in equilibrium with a diluted micellar solution or free water (Israelachvili et al., 1976; Wennerström, 1979).

Location of *n*-Dodecane–d_{26} in DOPC–*n*-Dodecane–d_{26}–H_2O Mixtures

^{2}H-NMR spectra were recorded at 35°C from three samples with 2.5 mol dodecane–d_{26} per mol DOPC and with 1.7, 31, and 46 mol H_2O per mol DOPC, respectively. The sample with the lowest water content, forming an H_{II} phase, gave a spectrum with two observable quadrupole splittings of 75 and 285 Hz. The smaller one originates from the two methyl groups, whereas the larger splitting originates from the ten methylene groups. These splittings correspond to order parameters of 0.001 and 0.004, respec-

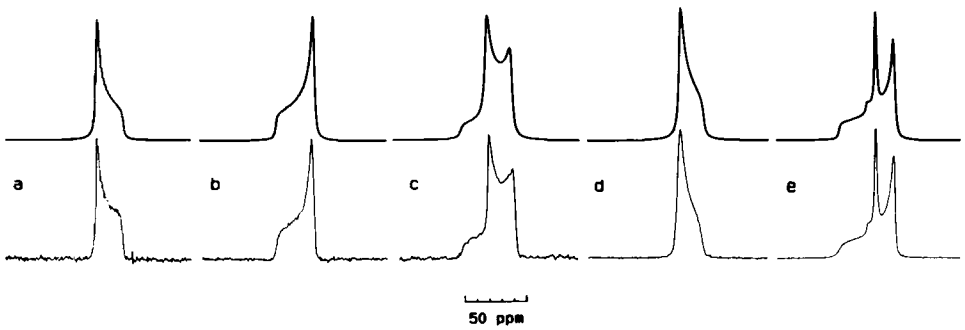

FIGURE 4 Experimental (*top*) and simulated (*bottom*) ^{31}P-NMR spectra recorded at 101.3 and 40.5 MHz at 25°C from samples containing 2.0 mol *n*-dodecane per mol DOPC and different amounts of ^2H_2O (mol per mol DOPC): (*a*) 1.7 (2.9 wt%); (*b*) 6.4 (10.2 wt%); (*c*) 31 (35.4 wt%); (*d*) 84 (60.0 wt%); and (*e*) 748 (93.0 wt%) mol ^2H_2O per mol DOPC. See Table I for the parameters used in the simulations.

tively. The spectrum from the sample with 31 mol H_2O per mol DOPC, forming an H_{II} phase together with a small amount of a lamellar phase, exhibited a singlet peak with a half-height width of 50 Hz. A similar spectrum was obtained from the sample with 46 mol H_2O per mol DOPC, forming an H_{II} phase. The order parameter for dodecane is thus close to zero in the latter two mixtures. A lamellar phase with 0.12 mol dodecane and 13.6 mol H_2O per mol DOPC gave three quadrupole splittings of 1.36, 4.61, and 5.43 kHz, corresponding to order parameters of 0.01, 0.03, and 0.04, respectively. These results indicate that dodecane molecules have low order in the H_{II} phase formed at higher water contents; dodecane is probably located between the cylinders building up the phase. However, dodecane is slightly oriented in the H_{II} phase formed at low water contents, probably along the acyl chains of DOPC. The molecular order of dodecane in the lamellar phase is much larger. This is schematically shown in Fig. 5.

Diffusion in Cubic Phases of DOPC–*n*-Dodecane–Water

The translational diffusion coefficients for the different components were determined at ~65°C in DOPC–*n*-dodecane–water samples forming cubic phases (Table II). The diffusion was very slow for both DOPC and water in a DOPC–H_2O sample with 3.3 wt% H_2O. When 0.5 mol dodecane per mol DOPC was added to such a mixture the diffusion coefficient for DOPC increased. The diffusion coefficient for dodecane was about 200 times greater than for DOPC. In the sample with 9.6 wt% ^2H_2O and 2.5 mol dodecane per mol lipid, the diffusion coefficient of DOPC further increased, and the diffusion of dodecane was approximately 130 times faster than for DOPC.

Phase Equilibria in the System DODGDG–*n*-Dodecane–H_2O

It has previously been determined by polarized light microscopy that a lamellar phase of the *A. laidlawii* membrane lipids dioleoylmonoglucosyldiglyceride (DOMGDG)–DODGDG (1.2:1, mol/mol) is transformed to an H_{II} phase at room temperature by the addition of *n*-dodecane (Wieslander et al., 1986). DOMGDG and DODGDG in excess water form H_{II} and lamellar phases, respectively (Lindblom et al., 1986). In this study it was shown by polarized light microscopy that a sample of DODGDG–H_2O with 10 mol H_2O per mol lipid remained lamellar after the addition of dodecane. When excess water was added to a DODGDG–dodecane mixture with 2.0 mol dodecane per mol lipid, the central part of the sample formed a lamellar phase, while smaller areas at the edge of the sample formed a hexagonal phase.

DISCUSSION

Phase Equilibria in the DOPC–*n*-Dodecane–^2H_2O System

This work is part of a systematic investigation of the effect of hydration and alkane concentration on the phase equilibria, structure, and dynamics for a model membrane system. Here the investigation was performed with DOPC, a lipid that together with water forms a lamellar phase except at very low water contents and high temperatures (Gutman et al., 1984). However, the addition of *n*-dodecane to the system DOPC–water induces the formation of H_{II} phases at low as well as high water contents (Fig. 1). How can we understand this rather remarkable behavior in terms of molecular interactions? There are several theoretical treatments of phase diagrams and aggregate shapes formed by amphiphiles (Tanford, 1980; Israelachvili et al., 1976; Jönsson and Wennerström, 1981, 1987; Kirk et al., 1984; Gruner, 1985).

In the theory of self-assembly of amphiphiles developed by Israelachvili et al. (1976, 1977, 1980), it is concluded that the effective geometry of the amphiphilic molecules in the system determines the lipid aggregate shape. The different aggregate structures, such as spheres, rods, and lamellae, then build up different liquid-crystalline phase structures. Here only a qualitative discussion of the factors determining the various phase structures can be performed. In several previous communications (Wieslander et al., 1980, 1986; Rilfors et al., 1984; Lindblom et al., 1986) we have used a very simple approach considering changes in the molecular shape at phase transitions to describe processes in the membrane of *Acholeplasma laidlawii*. For example, it was shown that alterations in the lipid molecular shape were sufficient to explain the regulation of lipid composition in *A. laidlawii* membranes. Thus it has been found that this qualitative and simplified theory

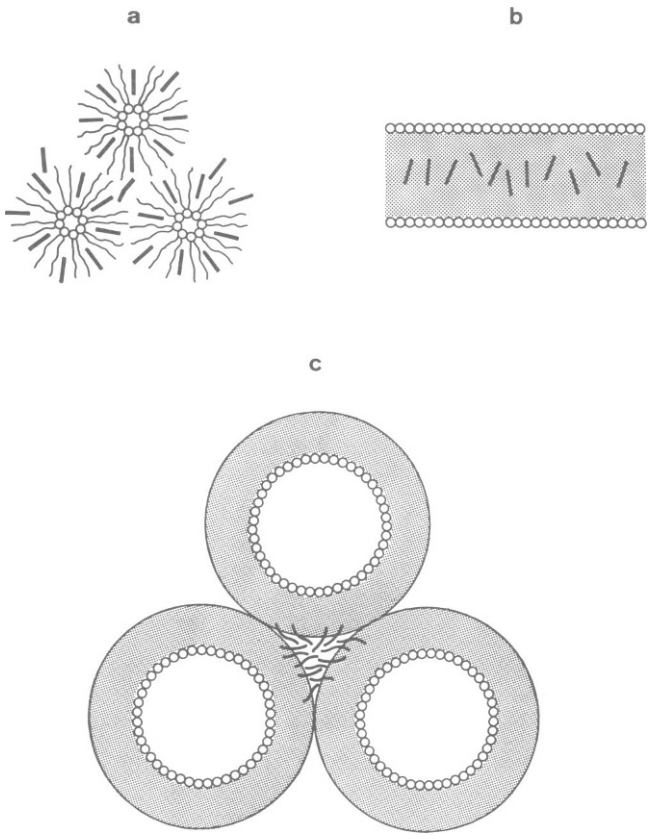

FIGURE 5 Schematic representation showing the location of *n*-dodecane (*black rods*) solubilized in (*a*) a reversed hexagonal phase with low water content, (*b*) a lamellar phase, and (*c*) a reversed hexagonal phase with high water content.

can lead to rather far reaching conclusions, and it will be used also here, although modified in particular according to the work by Gruner (1985). Let us start to consider the corner of the three-component triangle with low water contents (Fig. 1), where probably a lamellar phase is present at room temperature. At low water contents the effective size of the not fully hydrated polar head group is relatively small, and by raising the temperature the hydrophobic part of the molecule is shortened and broadened due to the introduction of *trans-gauche* isomerizations into the acyl chains (Rilfors et al., 1984). This leads to the formation of a wedge-shaped molecule, and a cubic phase of the reversed type is formed (see Results).

Incorporation of *n*-dodecane in DOPC at low water content induces the formation of reversed cubic and H_{II} phases (Fig. 2). Since the alkane partitions into the hydrophobic part of the DOPC molecules and is slightly oriented along the acyl chains, the alkane can be viewed as creating a more wedge-like effective molecular shape. Hence, nonlamellar phases of the reversed type will form even at lower temperatures. An H_{II} phase thus forms at 25°C with an *n*-dodecane/DOPC molar ratio larger than 1.0 (Fig. 1).

At higher water contents DOPC, even in the presence of dodecane, forms a lamellar phase up to ~25–31 mol ^2H_2O per mol DOPC depending on the alkane concentration (Fig. 1). This result is reasonable since a higher degree of hydration of the polar head group increases the interfacial area of DOPC and thus produces a more cylindrical-like molecule. Nonlamellar phases are not formed until the temperature is raised above 60°C.

A lamellar phase in equilibrium with free water is formed at water contents above 31 mol ^2H_2O per mol DOPC in the absence of dodecane. Also more unsaturated PC species like dilinoleoyl–PC (Dekker et al., 1983) as well as more saturated PC species like 1-palmitoyl-2-oleoyl–PC (Dekker et al., 1983) and dipalmitoyl–PC (Ulmius et al., 1977) form only lamellar phases in excess water at room temperature. The maximum hydration of the lamellar phases and the area per polar head group increases with the unsaturation, where the largest step is taken by going from saturated to monounsaturated acyl chains (Demel et al., 1972; Ulmius et al., 1977; Lis et al., 1982; Gutman et al., 1984). Thus, despite the fact that the molecular shape should change from cylindrical-like to a more wedge-like one with increasing degrees of unsaturation, only lamellar phases are formed. Obviously, for some reason, the system is forced to maintain a bilayer structure even at high degrees of unsaturation, and the area per polar head group therefore has to increase with increasing hydration to keep the effective molecular shape cylindrical-like. This behavior might cost extra free energy and should be unfavorable at high degrees of unsaturation because the water molecules cannot be in contact with the hydrophobic part of the bilayer. This poses at once the question why not, from an energetic point of view, more favorable structures are formed, like reversed hexagonal or cubic phases, where the water molecules are effectively hindered to reach the hydrophobic region. This problem was recently very nicely solved by Gruner (Gruner, 1985; Gruner et al., 1987). He realized that although the lipid monolayers would prefer to curl they cannot do so because a bending of the bilayers, to form an H_{II} structure, will create hydrocarbon "empty" regions that will highly increase the free energy and prevent the formation of the H_{II} phase. Thus, there is a natural tendency for the lipid monolayers to bend or assume an intrinsic radius of curvature, R_o, which cannot be expressed by the lipids themselves for geometric reasons. The expression of large radii of curvature can consequently be prevented by hydrocarbon packing constraints and the amount of available water. If large water cylinders should be able to form there must be enough water to fill the cylinders. Packing of these large cylinders into an H_{II} phase will give rise to "holes" that cannot be filled out by the lipid acyl chains since they cannot be sufficiently stretched. However, the addition of a hydrophobic substance, such as an alkane, will remove the hydrocarbon packing constraint since the alkane can fill out the volumes between the cylinders (Fig. 5).

The formation of an H_{II} phase at water contents between 25 and 207 mol ^2H_2O per mol DOPC and at *n*-dodecane/

DOPC molar ratios between 1.0 and 2.5 supports Gruner's suggestions. DOPC must have a large value of R_o since the lamellar phase is the dominating one for the system DOPC–water. If an H_{II} phase should be able to form at all, except at very low water contents, this should be at high dodecane and water contents, and this is actually found. Addition of dodecane to a lamellar phase of DOPC–water allows the formation of an H_{II} phase by filling out the volumes between the lipid cylinders. This location of the alkane was supported by experiments with perdeuterated dodecane (see Results). By increasing the water content at a dodecane/DOPC molar ratio above 1.0 the amount of water will eventually be large enough to permit all the lipids to form an H_{II} phase (Fig. 4, *c* and *d*).

Preliminary results from two other lipid–alkane–water systems further support the above mentioned interpretation (Sjölund, M., et al., manuscript to be submitted for publication). If the radius of the water cylinders is increased there will be larger volumes to fill with water and dodecane (Fig. 5). There are two ways to increase the value of R_o; incorporation of either saturated acyl chains or charged lipids into the system (Gruner, 1985). Thus, it was found that larger amounts of water and dodecane were needed to form an H_{II} phase with 1-palmitoyl-2-oleoyl–PC. Furthermore, by incorporating the anionic lipid dioleoylphoshatidylglycerol into the DOPC–*n*-dodecane–^2H_2O system, the phase equilibrium was shifted towards the lamellar phase.

Phase Equilibria in DOPC–Gramicidin–^2H_2O Systems

Interesting comparisons can be made between the systems DOPC–*n*-dodecane–^2H_2O and DOPC–gramicidin–^2H_2O (Killian and de Kruijff, 1985). In the latter system an increased fraction of DOPC forms an H_{II} phase when the gramicidin/DOPC molar ratio is increased from 1:50 to 1:10, and when the water content is increased from 7 to 30 mol ^2H_2O per mol DOPC. It was suggested that the driving force for this phase transition is the hydration of gramicidin (Killian and de Kruijff, 1985). In the case with DOPC–*n*-dodecane–^2H_2O the hydration of dodecane cannot be the reason for the lamellar–H_{II} phase transition at high water contents (Fig. 4). It is therefore less likely that the hydration of the very hydrophobic gramicidin molecule is a prerequisite for the formation of an H_{II} phase in the DOPC–gramicidin–^2H_2O system. Moreover, if the hydration of gramicidin is important for the lamellar–H_{II} phase transition, the equilibrium ought to be displaced towards the H_{II} phase when ionic analogues of gramicidin are incorporated into the DOPC–^2H_2O system. However, this is not the case (Killian et al., 1986). Hence, the properties of the DOPC molecule per se are most likely equally important for this phase transition; partition of gramicidin into the hydrophobic part of DOPC can reduce the hydrocarbon constraints and allow the intrinsic radius of curvature of the lipid to be expressed at high water contents.

Regulation of Lipid Composition in *A. laidlawii*

The membrane lipid composition of *A. laidlawii* is influenced by hydrocarbons incorporated into the membrane (Wieslander et al., 1986). Monoglucosyldiglyceride (MGDG) and diglucosyldiglyceride (DGDG) are the dominating lipids in the *A. laidlawii* membrane and account for between 55 and 75 mol%. MGDG forms an H_{II} phase in excess water at physiological temperatures and the other lipids form a lamellar phase under the same conditions (Wieslander et al., 1978; Lindblom et al., 1986). A very important effect on the polar head group composition, caused by changes of the environmental conditions, is the variation of the molar ratio MGDG/DGDG (Wieslander et al., 1980). Incorporation of *n*-dodecane into the *A. laidlawii* membrane lowered the glucolipid ratio from 0.83 to 0.13 (Wieslander et al., 1986). Addition of *n*-dodecane to a lamellar phase of DOMGDG–DODGDG–H_2O (molar ratio MGDG/DGDG 1.2:1, excess water) induced the formation of an H_{II} phase (Wieslander et al., 1986). However, a sample with 2 mol *n*-dodecane per mol DODGDG essentially formed a lamellar phase in excess water. Thus, by lowering the value of the ratio MGDG/DGDG, *A. laidlawii* counteracts the nonbilayer-promoting ability of the alkane and the stability of the bilayer is maintained.

Diffusion in Cubic Phases of DOPC–*n*-Dodecane–Water

The translational diffusion of DOPC and water in a sample with 3.3 wt% H_2O is about two orders of magnitude slower as compared with the diffusion of DOPC in a lamellar phase containing 20 wt% ^2H_2O (Lindblom et al., 1981) and the diffusion of water in pure water (Mills, 1973), respectively. The low diffusion coefficients are probably due to the very low water content in this cubic phase. When dodecane is incorporated into the phase and the water content is raised to 9.6 wt% the diffusion of DOPC is just four times slower than the diffusion in the lamellar phase. The small difference indicates that the lipid molecules can diffuse over macroscopical distances in the cubic phase. This phase is thus bicontinuous, i.e., both the hydrocarbon and the water regions are continuous (Lindblom et al., 1979). The translational diffusion of dodecane in the cubic phase is more than two orders of magnitude faster than that of DOPC (Table II) and just 2.5 times slower than in pure dodecane. The hydrocarbon region of a model membrane lipid system therefore have about the same dynamical properties as simple hydrocarbons. In the H_{II} phase the translational diffusion coefficient of dodecane is also just 2.5 times lower than for pure dodecane. This, together with the very low molecular order of dodecane, supports the suggested location of dodecane in the H_{II} phase formed at high water contents (Fig. 5).

TABLE II

MEASURED VALUES OF TRANSLATIONAL DIFFUSION COEFFICIENTS IN LIQUID CRYSTALS FORMED IN THE DOPC–*n*-DODECANE–WATER SYSTEM

Sample	Composition	Phase	Temperature	Diffusion coefficient
	wt%		*°C*	$10^{-12} m^2 s^{-1}$
DOPC	96.7			0.2
^1H_2O	3.3	Cubic	66.2	40
Dodecane	—			—
DOPC	86.9			0.9
^2H_2O	3.6	Cubic	63.6	—
Dodecane	9.5			180‡
DOPC	58.6			5.2
^2H_2O	9.6	Cubic	67.5	200*
Dodecane	31.8			670‡
DOPC	36.1			—
^2H_2O	44.5	Rev. hex	25.0	300*
Dodecane	19.4			340‡

*In the samples prepared with ^2H_2O the diffusion of trace amounts of ^1H_2O was measured. The diffusion coefficient of ^1H_2O in ^2H_2O is 2.0×10^{-9} m^2s^{-1} at 25°C and 5×10^{-9} m^2s^{-1} at 65°C.

‡The diffusion coefficient of neat *n*-dodecane is 8.6×10^{-10} m^2s^{-1} at 25°C and 16.6×10^{-10} m^2s^{-1} at 65°C.

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Note Added in Proof: The spectrum shown in Fig. 4 *e* was recorded 2 mo after the sample preparation. However, a true equilibrium was not obtained until after ~6 mo. The sample then formed a reversed hexagonal phase. This shows that extreme care must be taken when samples with very high water contents (excess water) are investigated.

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