

Lipid and water diffusion in bicontinuous cubic phases measured by NMR

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ABSTRACT Lipid and water diffusion coefficients in bicontinuous cubic liquid crystalline phases have been determined with the NMR pulsed magnetic field gradient technique. In the monoolein-water system, a discontinuity in the variation of the water diffusion coefficient with water content is observed, which coincides with the two-phase region between the two cubic phases in this system. The degree of water association to the lipid has been determined, considering the obstruction factor for diffusion in the cubic phases. The lipid diffusion coefficient increases with increased unsaturation of the lipid, and decreases when larger amphiphile molecules like cholesterol, gramicidin-A, and lyso-oleoyl-phosphatidylcholine are solubilized in the cubic phase. In a cubic liquid crystal of monoolein (MO), dioleoylphosphatidylcholine (DOPC), and water, the individual lipid diffusion coefficients have been determined simultaneously in the same sample. The diffusion coefficients of MO and DOPC differ by a factor of two, and both decrease with increasing DOPC content. The results are discussed in relation to probe techniques for measurements of lipid diffusion.

INTRODUCTION

A large number of amphiphiles and biological lipids form cubic liquid crystalline phases with water (for a review see Lindblom and Rilfors, 1989). It has been proposed that cubic phases may play an important role in many different biological systems, as for example membrane fusion, fat digestion, and as a structural element of so-called prolamellar bodies, developed in plants growing in the dark (Lindblom and Rilfors, 1989). Their possible applications as pharmacological delivery systems has also been discussed (Ericsson et al., 1991), and it can be expected that the cubic phases will have other future industrial applications.

Cubic liquid crystals were first investigated with x-ray crystallographic techniques by Luzzati and co-workers, and several structure models were proposed (Luzzati et al., 1968). Lipid and water diffusion coefficients were early measured with the NMR-diffusion technique (Bull and Lindman, 1974; Lindblom et al., 1976; Lindblom and Wennerström, 1977), which detects translational motion on a millisecond timescale. Based on the NMR results, two categories of cubic phases were shown to exist, viz., bicontinuous phases, with continuous regions of both lipid and water, and cubic phases built from closed aggregates. In bicontinuous phases, the measured diffusion coefficients of the lipid and of the water are of the same order of magnitude as in lamellar phases and in pure water, respectively. On the other hand, in cubic phases built from closed aggregates, the apparent lipid diffusion coefficient is one or two orders of magnitude smaller than in the lamellar phase (Lindblom et al., 1976; Lindblom and Wennerström, 1977; Lindblom et al., 1979; Eriksson et al., 1982; Rilfors et al., 1986; Eriksson et al., 1987; Lindblom and Rilfors, 1989; Lindblom et al., 1992).

Monooleoyl-glycerol ("monoolein", MO) forms a bicontinuous cubic phase together with water (Lutton, 1965; Lindblom et al., 1979). It was found that the lipid diffusion coefficient in the cubic phase was very close to two-thirds of the lipid diffusion in the lamellar phase (Lindblom et al., 1979), a relationship which is ex-

pected, to a first approximation, for a cubic phase built from lamellar units (Lindblom and Wennerström, 1977; Lindblom et al., 1979; Anderson and Wennerström, 1990). Consequently, a structure based on lamellar units was proposed (Lindblom et al., 1979), and it was also pointed out (Larsson et al., 1980) that the cubic structure can be represented by an infinite periodic minimum surface (IPMS) (Scriven, 1976). Later it was shown (Longley and McIntosh, 1983; Larsson, 1983; Hyde et al., 1984) that the cubic phase region consists of two different cubic phases with a body-centered structure at low water content (space group $Ia3d$, gyroid (G) type IPMS) and a primitive structure at higher water content (space group $Pn3m$, diamond (D) type IPMS). Recently, Anderson and Wennerström (1990) calculated the obstruction effects on diffusion in bicontinuous cubic phases, and experimental diffusion data have been interpreted within this framework (Balinov et al., 1991; Ström and Anderson, 1992).

The cubic phases of monoacylglycerides and water exist over an extended water-content and temperature region (Lutton, 1965), and can dissolve a substantial amount of other hydrophobic and amphiphilic molecules (Larsson et al., 1978; Ericsson et al., 1983; Gutman et al., 1984; Ericsson et al., 1991). Here, we have used cubic phases of monoglycerides as model systems for membrane lipid diffusion, to investigate the effect of temperature, water content, degree of acyl chain unsaturation, and solubilized molecules. The NMR diffusion data of the MO-water system lend further support to the existence of two cubic phases in the MO-water system. Finally, in a three-component cubic phase of two different lipids and water, separate determinations of the individual lipid diffusion coefficients have been made.

MATERIALS AND METHODS

Sample preparation

Monoglycerides (1-oleoyl-glycerol and 1-linoleoyl-glycerol), gramicidin-A, and cholesterol were obtained from Sigma Chemical Co. (St.

Louis, MO), and 1,2-dioleoyl-*sn*-phosphatidylcholine from NuCheck Chemical Co. (Elysian, MN). After control of purity by thin-layer chromatography the lipids were used without further purification. 1-oleoyllysophosphatidylcholine was prepared as described previously (Eriksson et al., 1987). Deuteriumoxide was obtained from Ciba Geigy (Basel, Switzerland). Mixing of the amphiphilic molecules was accomplished by dissolution of the lipids in chloroform/methanol 4:1 (vol/vol). The organic solvent was removed by dry nitrogen and finally under high vacuum. For samples intended for measurements of lipid diffusion the labile protons of the lipid(s) were exchanged for deuterium by hydrating the lipid or lipid mixtures in deuteriumoxide, followed by freeze drying. Before preparation of the samples the lipid or lipid mixtures were dried in high vacuum to constant weight. The samples were prepared by weighing appropriate amounts of vacuum dried lipid or lipid mixtures and water ($^2\text{H}_2\text{O}$ or $^1\text{H}_2\text{O}$) into 7-mm OD glass tubes followed by flame sealing. The samples were centrifuged back and forth several times until the liquid crystalline phase was homogeneous and equilibrium was reached, as deemed by inspection with polarized light. To maintain control of the water content at elevated temperatures, the sample tubes for high temperature studies were kept smaller than the region of constant temperature in the NMR probe. The liquid crystalline sample volume was 100 μl in all measurements, which was small enough to ensure that the sample experienced a uniform magnetic field gradient. Care was taken to position the sample tube identically in the diffusion probe at every measurement.

NMR measurements

The self diffusion coefficient was measured with the pulsed magnetic field gradient spin echo (PMFGSE) technique (Stejskal and Tanner, 1964), using a Bruker MSL-100 spectrometer, equipped with a 2.35 T wide-bore (79 mm) superconducting magnet. A Bruker ^1H diffusion probe with two oppositely wound Helmholtz coils, was used. The magnetic field gradient pulses were generated with a Bruker B-Z 18 B gradient unit with two series-coupled 12 V batteries as current source. The gradient unit was modified for digital setting of the gradient amplitude using a set of fixed resistances, and the gradient pulse length was controlled by a train of trigger pulses from the computer.

In the PMFGSE technique two identical magnetic field gradient pulses are generated, one at each side of the π -pulse, in a Hahn spin-echo sequence: $(\pi/2)_x - \tau - (\pi)_{xy} - \tau - [\text{acquisition}]$. The free induction decay is collected from the echo maximum at time 2τ and Fourier transformed. In this study the duration of the gradient pulses, δ , was varied while the magnitude, g , of the gradient pulses and the time, Δ , between the center of the two gradient pulses were kept constant. The spin echo time, τ , between the r.f. pulses was kept constant within a diffusion experiment, eliminating any variation in the echo amplitude due to J -modulation and/or transverse relaxation. The experiment was performed for a series of τ values between 40 and 120 ms with $\Delta = \tau + 10$ ms, corresponding to a root mean square displacement of 1–4 μm for lipids and 10–20 μm for water. The signal amplitude in the spin echo spectrum of a diffusing specie is given by the Stejskal-Tanner equation:

$$A = A_0 \exp[-\gamma^2 g^2 \delta^2 (\Delta - \delta/3) D] \exp(-2\tau/T_2), \quad (1)$$

where γ is the magnetogyric ratio of the nucleus, T_2 is the transverse spin relaxation time, and D is the diffusion coefficient (Stejskal and Tanner, 1964). The diffusion coefficient was obtained from a two-parameter nonlinear least-squares fit of Eq. 1 to the signal amplitude as a function of δ . The gradient strength, g , was calibrated with pure water as a reference for which reliable self-diffusion data exists (Mills, 1974). At the strongest gradient setting (1.19 T m^{-1}) a two-step calibration procedure was used, as suggested by Stilbs (1987). Thus, the amphiphile diffusion coefficient in the cubic liquid crystalline phase of 65 wt% potassium octanoate in $^2\text{H}_2\text{O}$ ($4.8 \cdot 10^{-11} \text{ m}^2 \text{ s}^{-1}$ at 25°C) was first measured at a gradient setting which had been calibrated with water as a reference. Secondly, the gradient strength was increased and calibrated using the cubic liquid crystal as a reference.

RESULTS AND DISCUSSION

Lipid and water diffusion in monoglyceride cubic phases

In the two-component system of MO and water, the cubic phase region extends between ~ 20 and 38 wt% $^1\text{H}_2\text{O}$ at 25°C (Fig. 1). There is a moderate and continuous increase in the measured lipid diffusion coefficient with water content (Fig. 2a), whereas for the variation in the water diffusion with water content there is a discontinuous increase around 34% $^1\text{H}_2\text{O}$ (Fig. 2b) (see also Fig. 12 in Lindblom and Rilfors, 1989). The discontinuity coincides with the two-phase region between the G-phase and the D-phase (cf. Introduction), which occurs between approximately 33% and 35% water (Hyde et al., 1984), thus corroborating the existence of two cubic phases in the MO-water system (Longley and McIntosh, 1983; Larsson, 1983; Hyde et al., 1984). In a recent study, Ström and Anderson (1992) observed discontinuous decreases in the water diffusion coefficient at the transitions between the G- and D-phases and between the D- and P-phases in the system didodecyldimethylammonium bromide-water-styrene.

The obstruction factor on lipid and water diffusion in a bicontinuous cubic phase, as described by an IPMS, was recently calculated by Anderson and Wennerström (1990) for the diamond (D) type IPMS and the Schwartz' surface (P). However, for the gyroid (G) type IPMS the obstruction factor was not possible to calculate. The calculated obstruction factor for the water diffusion was different for the D and P structures, indicating that the obstruction factor generally depends on symmetry if the cubic phase structure is described by an IPMS. As put forward by Luzzati et al. (1968), an alternative description of the cubic phase structures can be based on a network of interconnected rod-like aggregates. The calculations by Anderson and Wennerström (1990) show that the obstruction factor for the water diffusion is generally independent of the symmetry group of the cubic phase if the structure is described by the interconnected rod model. Thus, since we observe a discontinuity in the measured water diffusion over the G \rightarrow D phase boundary, our results point in favor of an IPMS-description of the cubic phase structure.

Assuming that the bound water diffuses with the lipid molecules, then, by applying a simple two-site model (see for example Stilbs, 1987; Anderson and Wennerström, 1990), the measured water diffusion coefficient will be given by:

$$D_{\text{cub}}^{\text{water}} = p_B D_{\text{cub}}^{\text{lipid}} + (1 - p_B) \beta D_0, \quad (2)$$

where p_B is the fraction of water bound to the lipid head-groups, $D_{\text{cub}}^{\text{lipid}}$ is the measured lipid diffusion coefficient in the cubic phase, β is the obstruction factor for the diffusion of unbound water in the cubic phase, and D_0 is the diffusion of bulk water. In the absence of theoretical

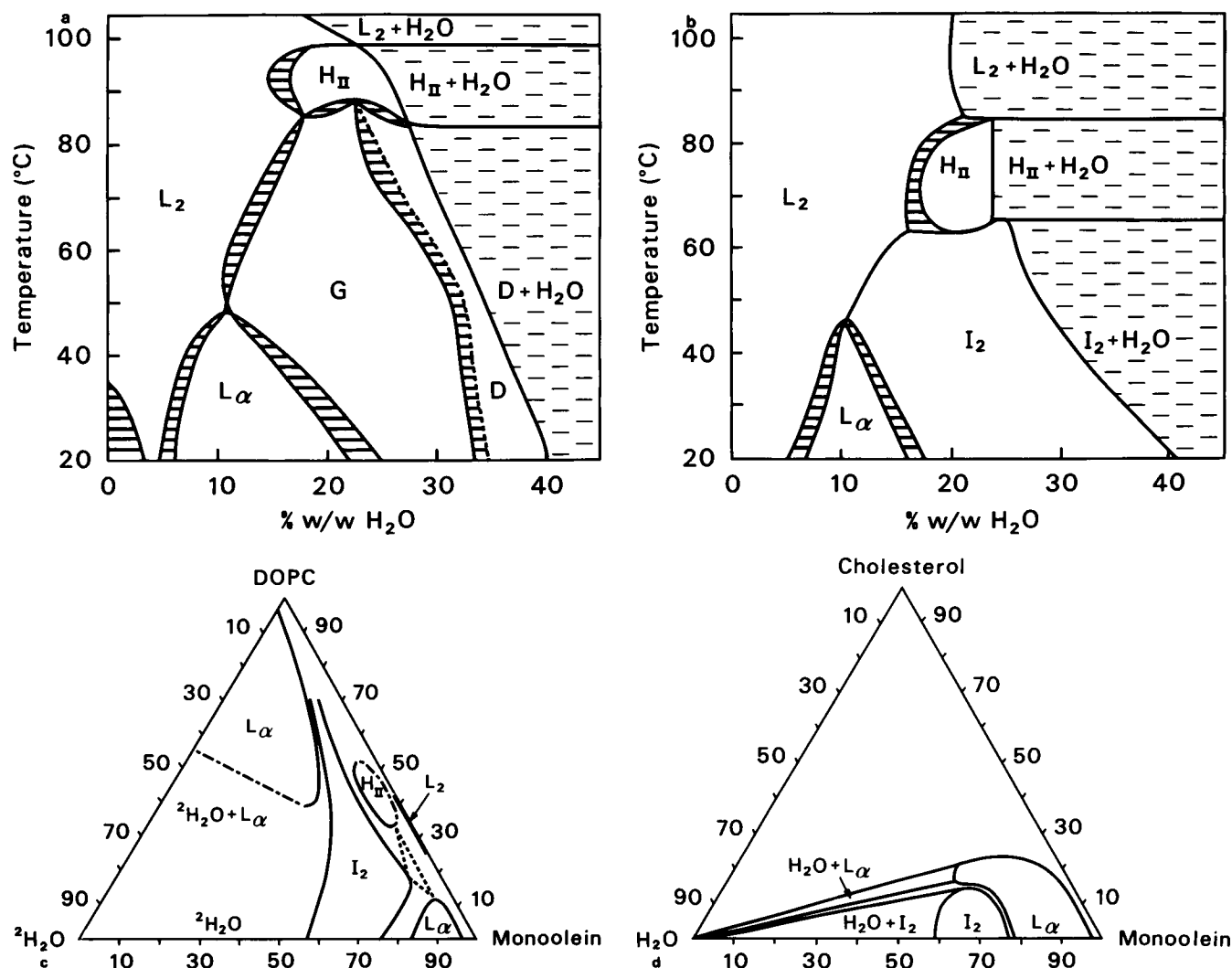


FIGURE 1 Phase diagrams of lipid-water systems. (a) The MO-¹H₂O system, redrawn after Hyde et al. (1984); (b) the ML-¹H₂O system, redrawn after Lutton (1965); (c) the MO-dioleoylphosphatidylcholine-²H₂O system at 28°C (Gutman et al., 1984); (d) the MO-cholesterol-¹H₂O system at 25°C, redrawn after Larsson et al. (1978). The labels denote the following phases: L_α, lamellar phase; I₂, a bicontinuous cubic phase; G, a bicontinuous cubic phase of the gyroid type; D, a bicontinuous cubic phase of the diamond type; H_{II}, reversed hexagonal phase; L₂, reversed micellar phase.

calculations for the G-type IPMS, we will take β from the calculated obstruction factor in the interconnected-rod model, as given by (Anderson and Wennerström, 1990):

$$\beta = \begin{cases} 0.25(\phi + 0.008)^{1/2} + 0.311 & \text{for } \phi < 0.2 \\ 0.26\phi + 0.372 & \text{for } \phi \geq 0.2, \end{cases} \quad (3)$$

where ϕ is the volume fraction of water in the cubic phase, calculated by assuming a specific volume of the lipid of $v = 0.93 \text{ cm}^3 \text{ g}^{-1}$ (Reiss-Husson, 1967). For the MO-water cubic phase, the number of bound water molecules, n , per lipid headgroup increases slightly with water content (from 2.8 to 5.0 at 34°C) (Table 1). However, the results from an application of this simple two-site model should merely be taken as a quantification of the extent to which the amphiphile surface affects the dynamic properties of the water, and not as predicting a

specific number of bound water molecules per lipid headgroup.

In the two-component system of monolinolein (ML) and water the cubic phase region extends from 16 to 34% ¹H₂O at room temperature. As for the MO system there is a moderate and continuous increase in the lipid diffusion coefficient with increasing water content (Fig. 3). The lipid diffusion coefficient of ML is between 30 and 45% larger than the lipid diffusion coefficient of MO at the same water content. Thus, increasing the degree of acyl chain unsaturation (MO → ML) gives a significant increase in the lipid diffusion coefficient. The degree of water binding to the lipid in the cubic phase, calculated from the measured water diffusion coefficient, is somewhat larger for ML than for MO (Table 2).

A tentative rationalization of the faster lipid diffusion of ML than of MO can be based on the difference in lipid

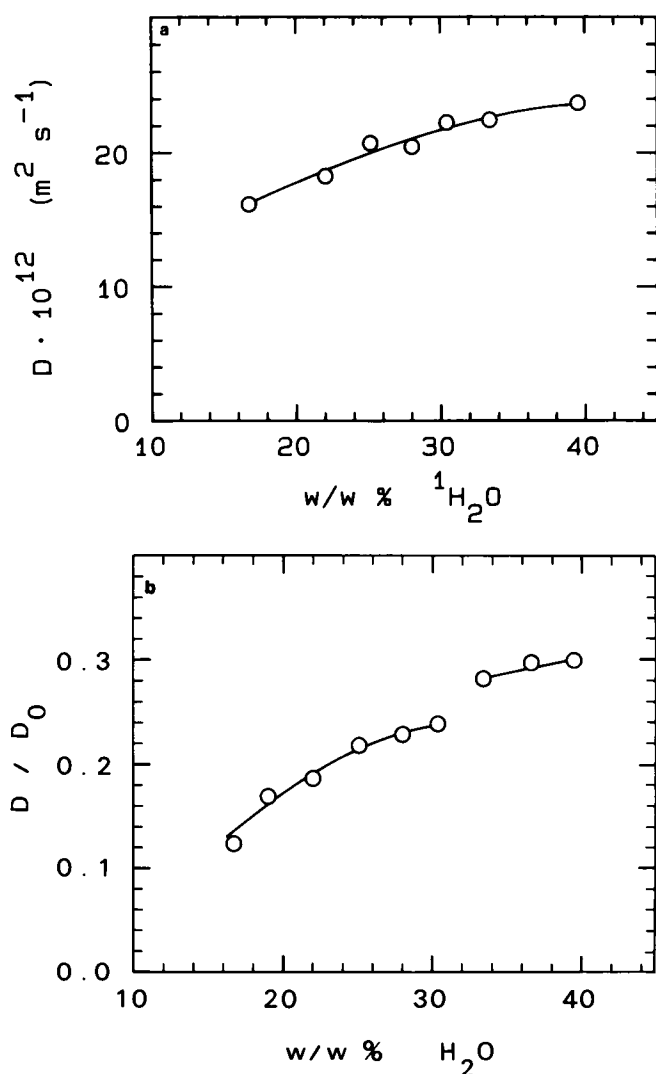


FIGURE 2 Lipid (a) and water (b) diffusion in cubic phases of monoolein and water as a function of water content at 34°C. The water diffusion coefficient is given relative to the diffusion of free water, D_0 , at 34°C.

packing in the cubic phase. MO is probably more tightly packed than ML, leading to faster diffusion for ML. The difference in packing is indicated by the lower temperature stability of the cubic phase of ML than of MO (Fig. 1, *a* and *b*). Also, a looser packing of ML could be expected from the steric constraints on the acyl chains. Introducing an extra double bond in the acyl chain will generally loosen the packing and increase the area per lipid headgroup. The larger water binding to ML than to MO can also be rationalized from the presumed larger area per polar headgroup.

At a water content of 27 wt% the cubic phase of MO and water is stable from below room temperature up to 80°C (Fig. 1). The lipid diffusion coefficient has been measured over this temperature interval and the result is presented in Fig. 4. The Arrhenius plot is slightly non-linear (Fig. 4) and the lipid diffusion coefficient

TABLE 1 Water and lipid diffusion in cubic phases of monoolein and water*

wt% $^1\text{H}_2\text{O}$	Phase	$D_{\text{water}}^{\text{cub}}/D_0$	$D_{\text{lipid}}^{\text{cub}}/10^{-12}$ $\text{m}^2 \text{s}^{-1}$	p_B	n
Temperature 25°C					
28.0	G	0.201	14.4	0.56	4.3
30.4	G	0.237	15.1	0.49	4.2
33.4	G/D	0.276	15.3	0.41	4.1
36.6	D	0.292	15.8	0.39	4.4
39.5	D	0.300	16.9	0.38	5.0
Temperature 34°C					
19.0	G	0.169		0.61	2.8
22.0	G	0.186	18.3	0.58	3.2
25.1	G	0.218	20.7	0.52	3.4
28.0	G	0.228	20.5	0.50	3.9
30.4	G	0.239	22.2	0.48	4.2
33.4	G/D	0.282	22.4	0.40	4.0
36.6	D	0.298		0.38	4.3
39.5	D	0.300	23.7	0.38	5.0

* $D_{\text{water}}^{\text{cub}}/D_0$, the measured water diffusion coefficient in the cubic phase, relative to the diffusion of pure water at the same temperature ($2.30 \cdot 10^{-9} \text{ m}^2 \text{s}^{-1}$ at 25°C and $2.83 \cdot 10^{-9} \text{ m}^2 \text{s}^{-1}$ at 34°C); $D_{\text{lipid}}^{\text{cub}}$, the measured lipid diffusion coefficient in the cubic phase; p_B , fraction of water bound to lipid, calculated from Eqs. 2 and 3; n , number of bound water molecules per lipid molecule. The phases are designed as G (gyroid type IPMS, space group $Ia3d$), or D (diamond type IPMS, space group $Pn3m$). The volume fraction of water ϕ was calculated assuming a specific volume for the lipid of $v = 0.93 \text{ cm}^3 \text{g}^{-1}$ (Reiss-Husson, 1967).

can be represented by the polynom $\log(D/\text{m}^2 \text{s}^{-1}) = -10.930 + 1.826 \cdot 10^3 T^{-1} - 5.44 \cdot 10^5 T^{-2}$, giving an apparent activation energy of 35 kJ mol^{-1} at 25°C and 25 kJ mol^{-1} at 75°C. This can be compared with previous measurements of lipid diffusion in lamellar phases. Thus, for example, NMR measurements of the lipid diffusion in the lamellar phase of dioleoylphosphatidylcholine (DOPC) and 20 wt% $^2\text{H}_2\text{O}$ yielded an activation energy of 38 kJ mol^{-1} (Lindblom et al., 1981)

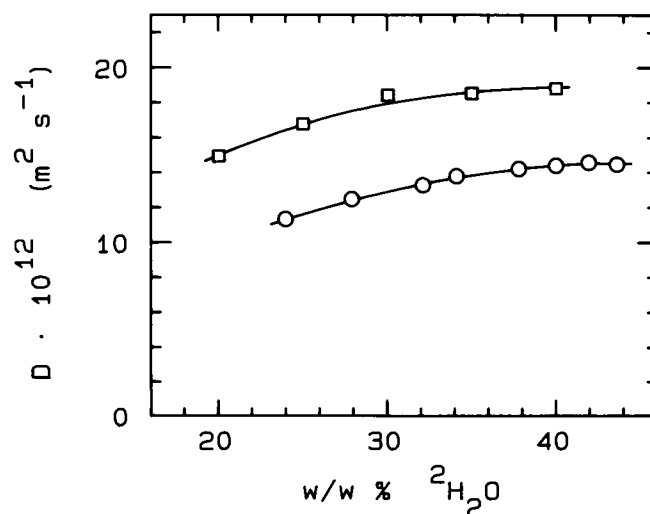


FIGURE 3 Lipid diffusion in cubic phases of monoolein (○) and monoolein (□) at 25°C, as a function of water content.

TABLE 2 Water and lipid diffusion in the cubic phase of monolinolein and water*

wt% $^2\text{H}_2\text{O}$	$D_{\text{water}}^{\text{water}}/D_0$	$D_{\text{lipid}}^{\text{lipid}}/10^{-12}$ $\text{m}^2 \text{s}^{-1}$	p_B	n
Temperature 25°C				
18.0	0.115	14.8	0.74	3.2
22.0	0.153	16.7	0.66	3.7
26.2	0.173	17.9	0.62	4.4
30.1	0.188	18.5	0.60	5.1
34.0	0.221	18.7	0.54	5.4
36.1	0.230	19.0	0.53	6.4

* For explanations of symbols, cf. Table 1.

and measurements in the lamellar phase of MO and 12 wt% $^2\text{H}_2\text{O}$ yielded an activation energy of 19 kJ mol $^{-1}$ (Lindblom et al., 1979). Finally, we note that a nonlinear Arrhenius plot has previously been interpreted as an indication for a "free-volume model" for lipid lateral diffusion (MacCarthy and Kozak, 1982; Vaz et al., 1985; King and Marsh, 1986).

Lipid diffusion in cubic phases of lipid mixtures

In the three-component system monoolein (MO)—dioleoylphosphatidylcholine (DOPC)— $^2\text{H}_2\text{O}$, at a water content of 15 wt%, a cubic phase exists over a large interval in the MO/DOPC ratio (Fig. 1 c) (Gutman et al., 1984). The ^1H -NMR spin echo spectrum consists of two major peaks, located at ~ 1.3 and 3.6 ppm (Fig. 6 b), arising from methylene protons in MO and DOPC (1.3 ppm) and the choline methyl protons of DOPC (3.6 ppm), respectively. The attenuation of the 3.6 ppm peak is significantly greater than the attenuation of the 3.6

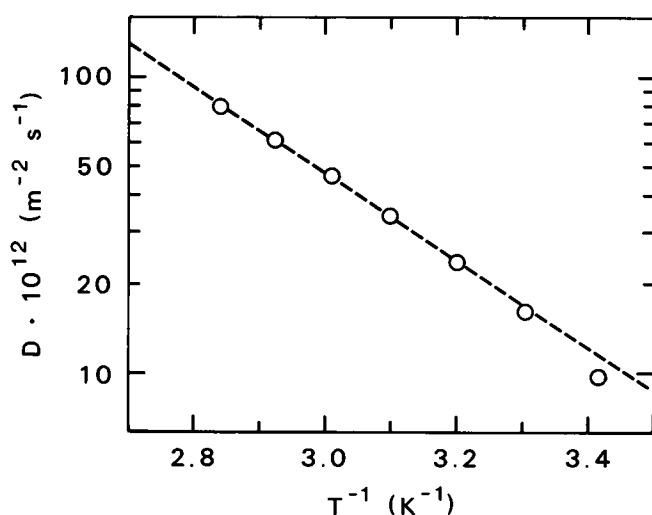


FIGURE 4 The temperature dependence of the lipid diffusion coefficient in a cubic phase of monoolein and 27 wt% $^2\text{H}_2\text{O}$. The dashed line represents a fit of an Arrhenius expression.

TABLE 3 Lipid diffusion in the cubic phase of monoolein (MO), dioleoyl-phosphatidylcholine (DOPC) and $^2\text{H}_2\text{O}$ *

DOPC/MO wt/wt ratio	$D/10^{-12} \text{m}^2 \text{s}^{-1}$	
	DOPC	MO
15/85	4.5 ± 0.1	8.8 ± 0.4
30/70	4.0 ± 0.1	7.5 ± 0.5
40/60	3.9 ± 0.1	7.2 ± 0.2
50/50	2.9 ± 0.1	5.9 ± 0.5

* Water content, 15 wt%; temperature, 25°C.

ppm peak (Fig. 6 b). Thus, the diffusion of MO is faster than the diffusion of DOPC in this cubic phase.

The decay of the 3.6 ppm (choline) peak with increasing δ can be fitted with a single term (Eq. 1), while the decay of the 1.3 ppm (methylene) peak requires a linear combination of two terms. This is evident from the systematic variations of the residuals in a single-term fit (not shown). The (single-term) analysis of the 3.6 ppm peak gives the diffusion coefficient of DOPC. The methylene protons of both MO and DOPC contribute to the 1.3 ppm peak. Therefore, in the two-term fit of the 1.3 ppm peak the slower component was fixed to the value obtained from the analysis of the 3.6 ppm (choline) peak. The faster decaying component of the 1.3 ppm peak then gave the diffusion coefficient of MO. The experiment was performed for at least five τ -values, between 40 and 120 ms, with $\Delta = \tau + 10$ ms, all giving consistent results. We have thus been able to determine the lipid diffusion coefficients of two lipid species simultaneously in the same sample. The diffusion coefficient of MO is approximately a factor of two larger than the diffusion coefficient of DOPC, and both diffusion coefficients decrease with increasing DOPC content (Table 3). Note that the amplitude factors in the two-component fits cannot be directly related to the lipid composition, since differential transverse spin relaxation can be expected to exist.

The observed difference between the diffusion of MO and DOPC, is of relevance for techniques which use probe molecules for the determination of lipid lateral diffusion, as for example fluorescence (Axelrod et al., 1976; Vaz et al., 1985) and electron spin resonance techniques (Moscicki et al., 1989; Shin and Freed, 1989). Generally, a probe molecule is chosen which, as far as possible, resembles the host molecule. The results presented here provide direct experimental evidence for the expectation that if the "intrinsic" diffusion properties of the added molecule (the probe) differs from that of the host, the measured diffusion coefficient of the probe will not reflect the diffusion properties of the host. On the other hand, the results indicate that variations in host molecule diffusion will be reflected in similar variations in the probe diffusion.

The cubic phases of monoglycerides and water are

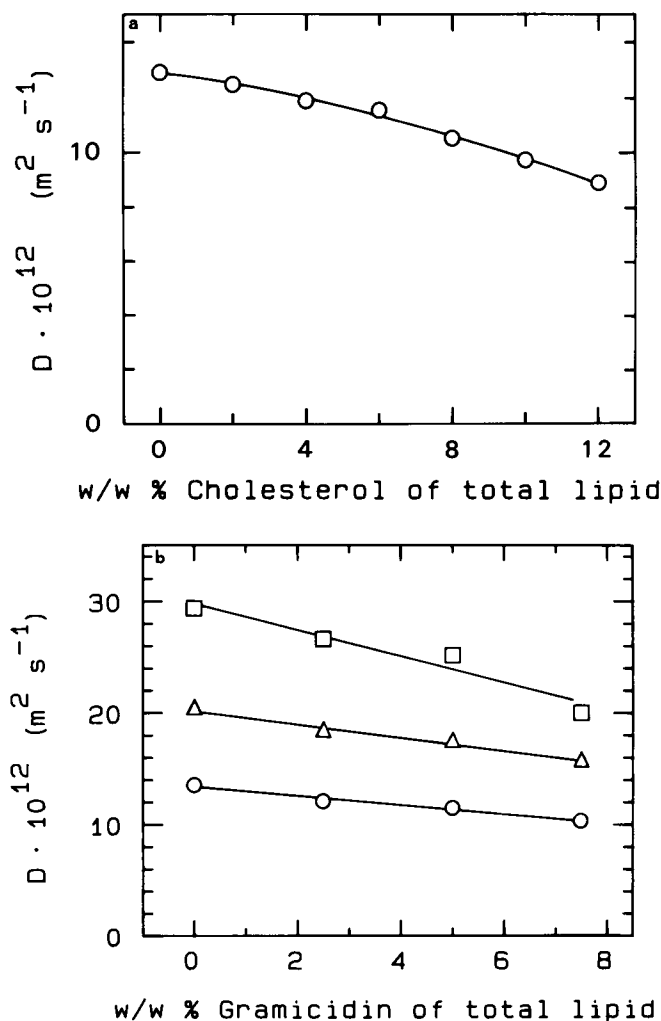


FIGURE 5 (a) Lipid diffusion coefficient at 25°C in the cubic phase of MO and cholesterol as a function of wt% cholesterol (of total lipid content). (b) Lipid diffusion coefficient in the cubic phase of MO, gramicidin-A and $^2\text{H}_2\text{O}$ as a function of wt% gramicidin-A (of total lipid content). Temperatures: 25°C (○), 35°C (△), and 45°C (□).

stable against the addition of various amphiphilic molecules. For example, at 30 wt% $^2\text{H}_2\text{O}$, the cubic phase of MO and water is stable up to a cholesterol content of 12 wt% of total lipid content (Fig. 1 *d*) (Larsson et al., 1978). The measured lipid diffusion coefficient decreases with increasing cholesterol content (Fig. 5 *a*). The spectral lineshape is independent of the gradient pulse length, apart from an attenuation due to diffusion, indicating that the transverse relaxation rate of the cholesterol protons is so fast that they do not contribute to the echo signal at this τ -value (30 ms), which is in line with previous results (Kroon et al., 1975). An alternative interpretation could be that the diffusion coefficients of MO and cholesterol are very similar, which however seems less probable.

At 30 wt% $^2\text{H}_2\text{O}$, the cubic phase of MO and water was found to be stable towards addition of gramicidin-A (GRA), up to a GRA content of ~ 8 wt% of total lipid

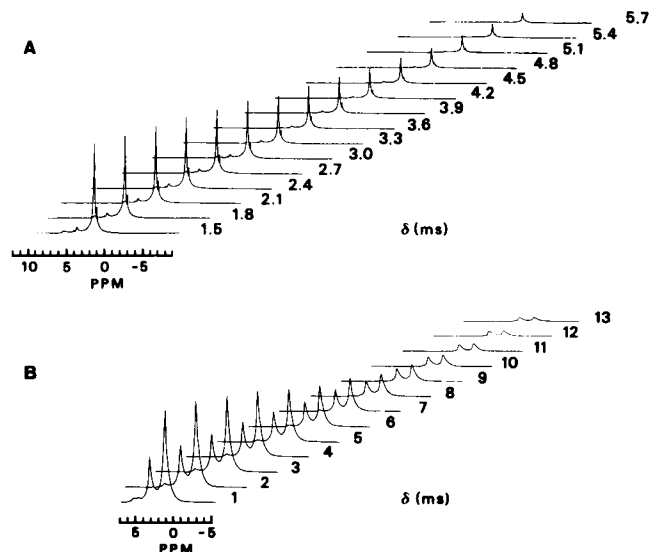


FIGURE 6 NMR-diffusion experiment in cubic phases. Spin echo spectra at increasing δ , with $\tau = 40$ ms, $\Delta = 50$ ms and $g = 119$ Gauss cm^{-1} . Temperature 25°C. Sample compositions: (a) MO/ $^2\text{H}_2\text{O}$ 70:30 wt/wt; (b) MO/DOPC/ $^2\text{H}_2\text{O}$ 42.5:42.5:15 wt/wt.

content. The lipid diffusion decreases with increasing amount of added GRA in the cubic phase (Fig. 5 *b*). The relative decrease is the same at 25°, 35°, and 45°C.

At 30 wt% $^2\text{H}_2\text{O}$, where pure MO forms a cubic phase at 25°C, replacement of 10 wt% of the lipid by 3-oleoyl-lyso-phosphatidylcholine (OILPC) gives an optically anisotropic (presumably lamellar) phase at 25°C. However, at 35°C the cubic phase is reformed, and the lipid diffusion coefficients was measured at this temperature. The diffusion coefficient of OILPC was obtained from the attenuation of the choline methyl peak at 3.6 ppm. The methylene peak at 1.3 ppm mainly arises from the methylene protons in MO and the attenuation of this peak gives the diffusion coefficient of MO. The diffusion coefficients of MO and OILPC are slightly different (Table 4) and the diffusion of MO decreases when OILPC is added to the cubic phase. Finally, the diffusion of MO has been measured as a function of added amount monostearin (MS) at 50°C and 30 wt% $^2\text{H}_2\text{O}$. No significant variation in the measured lipid diffusion was observed, ($D_{\text{lipid}}^{\text{cub}} = (30.5 \pm 0.5) \cdot 10^{-12} \text{ m}^2 \text{ s}^{-1}$) up to a MS/MO lipid weight ratio of 30/70.

TABLE 4 Lipid diffusion in the cubic phase of monoolein (MO) and oleoyl-lysophosphatidylcholine (OI-LPC)*

OI-LPC/MO wt/wt ratio	$D/10^{-12} \text{ m}^2 \text{ s}^{-1}$	
	MO	OI-LPC
0/100	18.5	—
10/90	17.8	15.6

* Water content, 30 wt%; temperature, 35°C.

In summary, additions of cholesterol, GRA, OILPC, and DOPC all lower the MO diffusion in the cubic phase, while the addition of MS does not alter the diffusion of MO. Thus, addition of an amphiphilic molecule, alters the lipid diffusion of the host in such a way that it will increase or decrease depending on whether the "guest" molecule has a substantially larger or smaller translational diffusion in itself. The translational diffusion coefficients are mutually affected by the motion of the molecules building up the lipid aggregates. The reduction of the MO diffusion on solubilization of gramicidin could possibly be considered as a result of an (two-dimensional) obstruction effect from the peptide molecules (Saxton, 1982). It is interesting to note that in previous NMR diffusion measurements on macroscopically aligned lamellar phosphatidylcholine (PC)/water phases it was found (Lindblom et al., 1981) that the addition of cholesterol up to ~33 wt% had a very small effect on the diffusion coefficient of the different PCs studied. If anything, a slight increase in the PC translational diffusion was observed with increasing cholesterol content in the bilayers. In the light of this investigation, it might be expected that the cholesterol diffusion would be very similar to, or slightly higher than, the PC diffusion.

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