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Phase equilibria and formation of vesicles of dioleoylphosphatidylcholine in glycerol/water mixtures

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The lipid 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC) forms a lamellar liquid crystalline phase (L_α) in arbitrary mixtures of glycerol and water. The phase has been characterized by means of X-ray diffraction, ^{31}P-NMR spectroscopy and differential scanning calorimetry (DSC). In the L_α state, and for DOPC concentrations greater than 50% (w/w), the thickness of the lipid bilayer decreases, while the area of the polar head group increases with increasing glycerol concentration. The phase transition from gel to L_α state occurs in the range of 240 to 260 K. Contrary to a previous (McDaniel, R.V., McIntosh, T.J. and Simon, S.A. (1983) Biochim. Biophys. Acta 731, 97) study of 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) we find that in the gel state, the thickness of the DOPC lipid bilayer is greater than that in the L_α state. This suggests that in the gel state, the lipid acyl chains of DOPC are in extended configuration. The lamellar phase reaches its maximum swelling at about 50% (w/w) of DOPC. At lower DOPC concentrations a two-phase system is formed where the lamellar phase exists in equilibrium with excess of solvent. Unilamellar vesicles can be prepared from a diluted suspension of the lamellar phase either by using the sonicator or extruder technique. We show this by means of ^{31}P-NMR, EPR and fluorescence spectroscopy. The mean radius of the vesicles, prepared by a sonicator, has been determined at different glycerol/water mixtures. It is found to decrease continously from 100 Å at 100% water to a minimum of 75 Å at about 50% water in the solvent mixture. By further decreasing the water content in the solution, the radius rapidly increases, and a mean radius of 450 Å is estimated at a water content of 10%. The rotational relaxation times of a fluorescent probe and two EPR spin probes, solubilized in DOPC vesicles, have been measured at different glycerol/water mixtures. It is found that the rotational rates are always much slower in the systems containing glycerol.

Introduction

In recent years, questions concerning the role of polar solvents, other than water, in lipid bilayer systems have been raised [1–8]. Typically, glycerol, formamid and ethylene glycol have been used to replace water [1–4]. In biological systems, glycerol is often used as a cryoprotectant and it substitutes very well for water [5–8]. Moreover, glycerol and ethylene glycol have been shown to act as fusogens [9]. Recently, it was demonstrated that lipid vesicles and a lamellar liquid crystalline phase (L_α, formed with 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC) and glycerol or water are useful as model systems for investigating and modelling electronic energy transfer in anisotropic systems [10,11,22]. From the point of view of theoretical modelling and the development of artifical light antennae, it is important to explore how anisotropic orientation, as well as slow or negligible rotational motions influence energy migration among fluorescent molecules. This is experimentally feasible by using vesicles and lamellar liquid crystals containing high concentrations of glycerol. Thus, there are several reasons for studying how a polar solvent like glycerol affects the physico-chemical properties of lipid bilayers. The present work aims at characterizing the phase behaviour, the packing properties and the average size of vesicles formed by DOPC at different concentrations of water and glycerol. For this purpose, X-ray diffraction, differ-

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ential scanning calorimetry, EPR, ^{31}P-NMR, fluorescence spectroscopy and refractometry were used.

Materials and Methods

2,5,8,11-Tetra-*tert*-butylperylene (TBPe) was synthesized by Friedel-Crafts alkylation of perylene. Further details concerning the synthesis and the purification are given in Refs. 10, 22 and papers cited therein. 1,2-Dioleoyl-*sn*-glycero-3-phosphocholine (DOPC) was purchased from Avanti Polar Lipids. The purity was better than 99% as checked by thin layer and gas chromatography in our laboratory. Glycerol (Omnisolv, BDH, spectroscopic grade) was used after checking the fluorescence background. 4-oxo-2,2,6,6-tetramethyl-piperidine-1-oxyl (TEMPONE) and *n*-(4,4-dimethyl-3-oxa-zolidinyloxy) stearic acid (*n*-doxyl stearic acid, $n = 5$ and 16 (denoted by 5-DS and 16-DS, respectively)) were purchased from Molecular Probes and they were used without any further purification. Potassium-tri-oxalato-chromate ($K_3Cr(C_2O_4)_3$), was synthesized according to the procedure outlined by Malati and Azim [12]. The purity of the product obtained was checked by UV-VIS measurements. The spectrum and the calculated molar absorptivity were in agreement with those previously reported.

The lamellar liquid crystalline phases were prepared by mixing weighted amounts of DOPC and the appropriate solvent The samples were equilibrated in sealed ampullae at room temperature during at least one week before measurements. Equilibrated samples were stored at about 250 K.

The vesicles were prepared by sonication as follows. Solutions of DOPC dissolved in chloroform and a fluorophore or a spin label dissolved in ethanol were mixed. The solvents were evaporated and dried at 320 K and 0.1 torr during 2 h. Then, 3 ml of the glycerol/water mixture was added and the suspension was sonicated 8 times in intervals of 5 min. During the sonication the sample was cooled at about 283 K. The sonicator was a Soniprep 150 (MSE Scientific Instruments) supplemented with an exponential microprobe. The level of amplitude used was 10–14 mm. After sonication the sample was centrifuged at $5000 \times g$ during 15 min. Vesicles were also prepared by using an extruder (Lipex Biomembranes). Nucleopore polycarbonate filters with a pore diameter of 100 nm were used after removal of the PVP layer with 0.5 M acetic acid solution. All solvents used in the vesicle preparation were separately filtered through 100 nm filters. The lipid suspensions were passed through the extruder 12 times at a pressure of 10–50 psi.

The X-ray data were collected with two different equipments as described below. Most of the data were obtained by using a low-angle Kiessig camera equipped with a position-sensitive electronic detector PSD 100 (Tennelec). The X-ray source was Ni-filtered Cu K_α ($l = 1.542$ Å) radiation. Exposure times of approx. 1 min were required for a good diffraction pattern. The detector was readily calibrated with crystalline sodium octanoate (repeat distance of 23 Å). The samples were contained in freshly prepared flame-sealed capillaries. The samples were investigated in the temperature range of 210 to 300 K. For cooling to low temperatures a stream of chilled dry nitrogen was used.

X-ray diffraction was also studied by using the film-technique. The samples were mounted between thin mica-windows in customized metal holders. The construction of the powder small-angle camera with pin-hole collimation was based upon the Philips Universal flat-plate camera PW 1030, which has been modified by L. Hernquist (PhD thesis Polymorphism of Fats, University of Lund, 1984). The specimen-to-film distance was 200 mm and the X-ray radiation used was Ni-filtered Cu K_α. The exposure time was 20 h. The temperature control was obtained by pumping a coolant from a thermostat through the specimen holder. The temperature of the specimen was recorded with a thermocouple.

Differential scanning calorimetry (DSC) was studied by means of a Perkin-Elmer DSC-2 equipment. The system has been described previously together with the techniques for measuring specific heat capacity and relaxation of enthalpy [13,23,24]. In this work the calorimeter was cooled by a freon-cooler (Perkin-Elmer Intracooler II) which allows for cooling to 210 K. The temperature scale was calibrated versus the melting points of cyclohexane (279.7 K), indium (429.78 K), tin (505.06 K) and lead (600.65 K). A separate calibration function was determined for every rate of scanning. The samples (< 6.5 mg) were enclosed in gas tight capsules of aluminium in order to reduce thermal gradients.

A Bruker WM-250 NMR spectrometer equipped with a superconducting magnet was used as described in [14] for recording the ^{31}P-NMR spectra.

The steady-state fluorescence spectra and anisotropies were obtained using a SPEX Fluorolog 112 instrument (SPEX), equipped with Olan-Thompson polarizers. The spectral bandwidths were 5.6 and 2.7 nm for the excitation and emission monochromators, respectively.

A PRA 3000 system (Photophysical Research Ass.) was used for single-photon-counting measurements of the fluorescence decay. The excitation source is a thyratron-gated flash lamp (Model 510C, PRA) filled with deuterium gas and operated at about 30 kHz. The excitation wavelengths were selected by interference filters (Omega/Saven AB) centered at 409.4 nm (HBW = 13.0 nm). The fluorescence emission was observed above 470 nm through a long pass filter Schott KV 470 (Schott). The maximum absorbance of all

samples was kept below 0.08 which corresponds to a total concentration of less than 10^{-6} M. The time-resolved polarized fluorescence decay curves were collected and analyzed as described in Refs. 10 and 22.

The refractive indices were measured at 439 and 589 nm with an Abbe refractometer (Model A, Zeiss). In measurements of the birefringence the ocular was supplemented with a sheet polarizer.

The solvent volume enclosed by the lipid vesicles was determined by EPR spin label measurements. Spectra were recorded at 298 K with a Varian model E-109 X-band spectrometer (9 GHz). The spectrometer was interfaced to a Zenith-111-32 personal computer [15]. The spin label, which can permeate the lipid membranes, is effectively quenched in the exterior vesicular solution by the addition of paramagnetic ions. This method is based on the fact that the chemical exchange over the membrane is slow on the EPR time scale and that the paramagnetic ions do not penetrate the vesicles. From the enclosed volume determined experimentally, the length of the lipid molecule and its polar head group area it is possible to calculate the mean-radius of the vesicles [16–19]. In a first run small aliquots of a glycerol – H_2O solution containing the spin probe TEMPONE, and sonicated vesicles of DOPC were thoroughly mixed and diluted with an equal amount of glycerol/H_2O without the spin probe. The EPR spectrum recorded for this system consists of three hyperfine lines (Fig. 1A). In a second run, sonicated vesicles of DOPC were thoroughly mixed with a glycerol – H_2O solution containing a suitable concentration of the spin broadening agent, chromium oxalate (Fig. 1B).

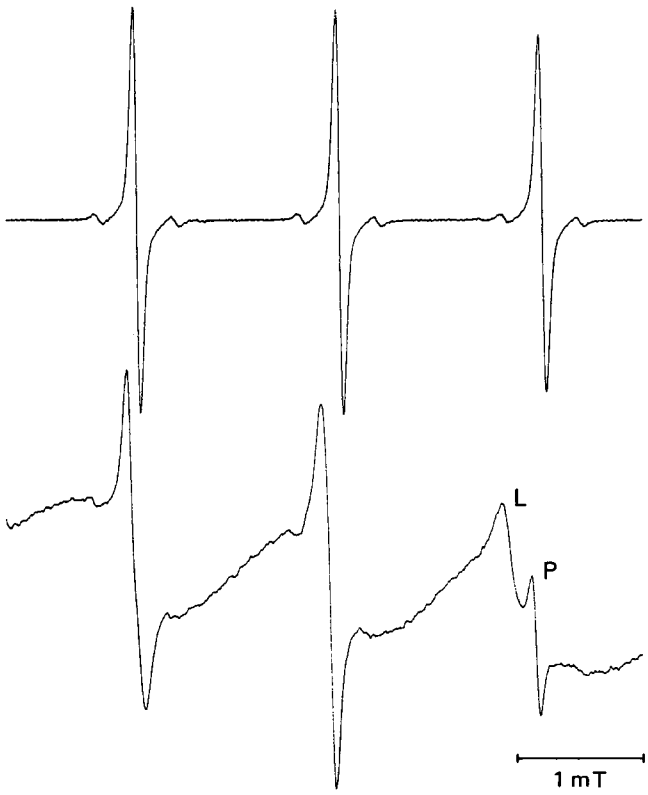

Fig. 1. (A) The EPR spectrum of TEMPONE in a glycerol/water (10/90% (w/w)) vesicle suspension of DOPC (5 mg/ml solvent). (B) The corresponding spectrum (amplified 160-times) upon addition of chromium oxalate. P denotes the signal of TEMPONE which is solubilized in the interior volume of the vesicles, while L denotes the signal from that solubilized in the lipid bilayer.

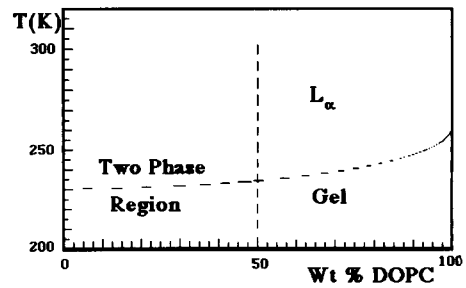

Fig. 2. Schematic phase diagram of DOPC in glycerol/water mixtures. The phase transitions are illustrated by dotted lines.

Except for a tremendous decrease in the signal intensity due to the effective spin-spin broadening, the spectrum now consists of four lines. Since the hyperfine coupling constant of the spin label is affected by the polarity of the medium, the high field line is split into a doublet due to a distribution of TEMPONE between the interior of the vesicles and the lipid bilayer. The line at the highest field (denoted by P in Fig. 1B) arises from the spin probes, which are located in the interior of the vesicles and the other line (denoted by L in Fig. 1B) is due to those solubilized in the lipid bilayer. The intravesicular volume, V_{in} (μl/g lipid), is calculated from

$$V_{in} = c^{-1}10^3[I'_p I^{-1}_p] \quad (1)$$

where c is the concentration of the lipid (g/cm^3), I'_p, is the normalized intensity of the highfield line of the quenched system (i.e., vesicles with addition of chromium oxalate) and I_p is the normalized intensity of the highfield line in the unquenched system.

Results and Discussion

The liquid crystalline phase composed of DOPC and different mixtures of water and glycerol has been examined by means of X-ray diffraction, EPR, NMR, optical spectroscopy and DSC. The results obtained from these studies are summarized as the phase diagram given in Fig. 2. Depending on temperature either an L_α or a gel phase is found at DOPC concentrations between about 50 and 90% (w/w). A gel–liquid crystal transition occurs at about 250 K. At DOPC contents of less than 50%, the L_α or the gel phase is in equilibrium with excess of solvent. The phase behaviour at these

concentrations was not investigated. In the following we first present the results obtained at high DOPC concentrations, and thereafter we proceed with the diluted systems, which were studied by means of ^{31}P-NMR, EPR and fluorescence spectroscopy.

X-ray diffraction

X-ray data are consistent with the formation of an L_α phase of DOPC at arbitrary ratios of glycerol and water. In many samples, especially those at high DOPC contents, two reflexes at a ratio of 1:2 were found.

The Bragg distances (d) measured for L_α phases containing 35, 50 and 80% DOPC, at varying glycerol fractions in a water-glycerol mixture are summarized in Fig. 3. There is a significant decrease in d as the DOPC concentration increases from 50 to 80% DOPC, while d from 35 and 50% DOPC are almost constant. This means that a maximum swelling of the L_α phase is reached at 50% of DOPC at all glycerol/water ratios. It is also evident that d changes little with the solvent composition. Only a slightly smaller value of d is found in pure glycerol, as compared to pure water. In Fig. 4 the swelling of the L_α phase at different temperatures is illustrated. The solvent mixture of glycerol and water was 91:9 (w/w). The d values increase with decreasing lipid concentration and at less than about 50% DOPC a plateau value is reached, clearly indicating the two phase boundary. The Bragg spacing, and thereby also the thickness of the lipid bilayer (d_l), increases with decreasing temperature. The gel phase has a larger bilayer thickness than the L_α phase. We obtain a value of about 40 Å, as compared to d_l 33 Å for the L_α phase. Normally, this is also observed for aqueous systems. However, our finding is contrary to

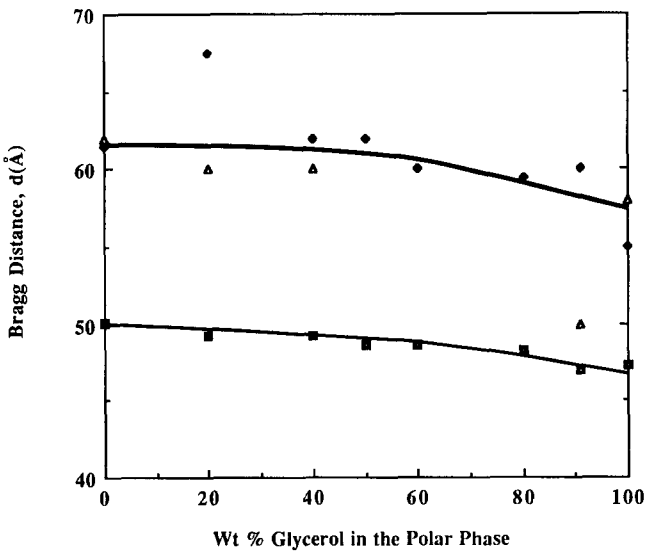

Fig. 3. Bragg spacing (d) obtained for lamellar phases of DOPC at different fractions (% (w/w)) of glycerol in the polar phase. The DOPC concentrations are 35 (□), 50 (△) and 80% (w/w) (♦). The temperature was 298 K.

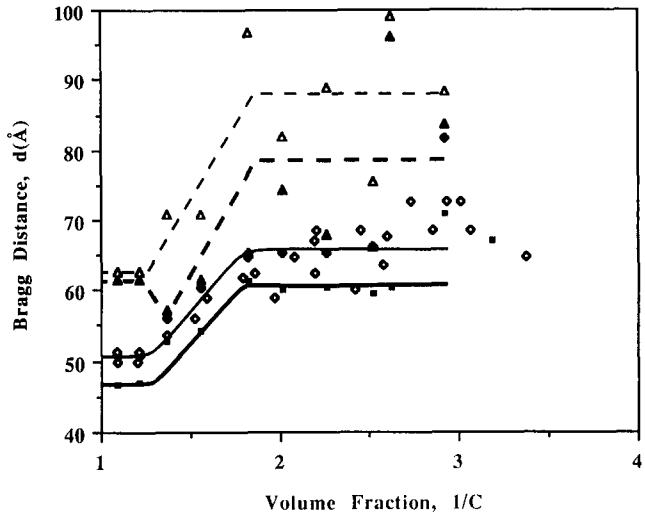

Fig. 4. Bragg spacing d as a function of the inverse volume fraction of DOPC (C^{-1}) at 298 K (■), 256 K (◇), by PS detection and (♦), by photographic detection), 243 K (▲) and 213 K (△).

the behaviour of 1,2-dipalmitoyl-*sn*-glycerol-3-phosphocholine (DPPC) in glycerol, previously reported by McDaniel et al. [1]. They found that $d_l = 37$ Å in the liquid crystalline state, and $d_l = 30$ Å in the gel state which, according to McDaniel et al., means that the acyl chains of DPPC are fully interdigitated in the gel state. We estimate, that in the gel state of DOPC, the thickness of the lipid bilayer increases by about 3 Å, compared with the liquid crystalline state at 298 K. This suggests, that the lipid acyl chains are more extended in the gel state. The area per polar head group of DOPC was calculated from the Bragg distances presented in Fig. 4. The area corresponding to a maximum swelling of the phase is 82 Å^2 at 298 K and 76 Å^2 at 256 K. At lower temperatures the quality of the data does not allow an estimate of the area. The polar head group area of 82 Å^2, for a molar glycerol/water ratio of 2, is larger than that obtained for DOPC in water (66 Å^2) [4]. This is expected, if the hydration water molecules are replaced by glycerol in the head group region of DOPC. Previously, it has been found that for other non-aqueuos solvents, formamid and methylforamid, the polar head group area was 73 and 74 Å^2, respectively [4]. Furthermore, El Nokaly et al. [3] observed that the area of soybean phosphatidylcholine expanded from 64 to 70 Å^2 upon replacing water with different alcohols. We have also calculated the thickness of the lipid bilayer at concentrations of maximum swelling of the L_α phase. We obtain a value of $d_l = 33$ Å in the solvent mixture of glycerol and water (91:9) at 298 K, which is smaller than the value of 38 Å reported for the corresponding system in water [4]. It has been reported that lecithins may show an indefinite swelling in some non-aqueous solvents [3,4]. No such behaviour (see also Fig. 3) is found in the swelling of DOPC for different glycerol/water mixtures.

Differential scanning calorimetry

The transition temperature between the gel and the L_α phase of DOPC with glycerol and water was studied by means of DSC. Typical melting curves showing the heat capacity as a function of temperature are given in Fig. 5. The corresponding graphs obtained upon freezing the systems are in general shifted towards lower temperatures. In all experiments, where the samples are heated, the phase transitions occur between approx. 240 and 260 K.

^{31}P-NMR spectroscopy

The ^{31}P-NMR spectrum was recorded (see Fig. 6A) for a sample composed of 80% DOPC in glycerol and water (91:9% (w/w)). The spectrum has the typical shape of an L_α phase of a phospholipid. This shape is a result of an anisotropic orientational averaging of the chemical shift tensor in a uniaxial system [14].

In order to get evidence for the formation of vesicles in glycerol, a suspension of DOPC was sonicated. The ^{31}P-NMR spectrum shows one peak (lower spectrum of Fig. 6B) as is expected for molecules undergoing rapid rotational motion on the time-scale of the NMR experiment. By adding Pr^{3+}, a well-known chemical shift agent, another peak appears in the ^{31}P-NMR spectrum (see upper spectrum of Fig. 6B) implying, that all phosphate groups are not accessible. This means that Pr^{3+} ions do not penetrate the vesicles. We have also used the extruder technique for preparing unilamellar vesicles with a radius of approx. 1000 Å. A similar chemical shift pattern was observed for the sonicated samples. Moreover, the shape of the ^{31}P-NMR spectrum was sensitive to the temperature as is illustrated in Fig. 6C. At 343 K, the ^{31}P-NMR spectrum very much resembles that observed for the sonicated systems but by lowering the temperature to 298 K, the spectrum broadens and the lineshape is the same as that observed for an L_α phase (Fig. 6A). The observed changes of the ^{31}P-NMR spectra in Fig. 6C can thus be ascribed to a slower rotational rate of the vesicles with decreasing temperature.

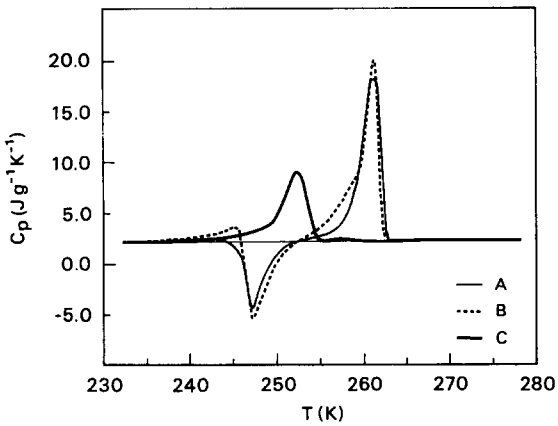

Fig. 5. Heat capacity C_p (J/g per K) obtained for (A) 48.9%, (B) 63.6% and (C) 78.2% DOPC (w/w) in a mixture of glycerol and water (91:9) by using DSC. The curves represent melting curves and were recorded at a rate of 1.25 K/min.

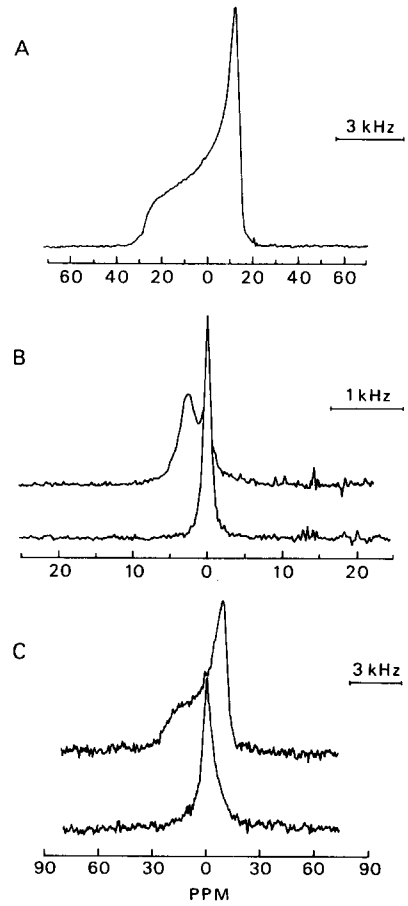

Fig. 6. The ^{31}P-NMR spectra recorded of (A) a lamellar phase of DOPC (80 wt%) in glycerol and water (91:9% (w/w)) at 298 K, (B) DOPC in excess of glycerol and water (91:9 (w/w)) after sonication. The upper and lower spectra were recorded at 313 K in the presence and absence of Pr^{3+}, respectively. (C) DOPC in excess of glycerol and water (91:9 (w/w)) prepared by using the extruder technique. The upper and lower spectra were recorded at 298 K and 333 K, respectively.

Optical spectroscopy

The refractive index of an L_α phase composed of 80% DOPC in a 91:9% mixture of glycerol and water has been determined as a function of temperature. The results obtained for the ordinary (n_o) and extraordinary (n_{eo}) refractive indices at 439 and 589 nm are summarized in Table I, together with the calculated mean refractive index n. By increasing the temperature in the region of 240 to 260 K, n decreases rapidly. At higher temperatures a small and linear decrease is observed. The strongly changing value of n, between 240 and 260 K, correlates with the phase transition observed with DSC.

Vesicles containing a hydrophobic fluorophore 2,5,8,11-tetra-*tert*-butylperylene (TBPe) were studied by using steady-state and time-resolved fluorescence spectroscopy. The rotational motions of TBPe in DOPC

vesicles formed in water and in a glycerol/water mixture (91:9% (w/w)) were compared. The steady state anisotropies are 0.21 and 0.32 in the water and the solvent mixture, respectively. Since the fluorescence lifetime of TBPe in these systems are very similar (about 4.8 ns) the different values on the fluorescence anisotropy imply that the rotational rates are considerably slower in the DOPC vesicles formed in the glycerol/water mixture. In these studies it should be noted that the influence of rotational motion of the vesicles can safely be neglected. Direct evidence for slower rotational motions is given from the time-resolved fluorescence anisotropies. For both kinds of systems the decay of the fluorescence anisotropy is biexponential with the rotational correlation times of about four times smaller in the vesicles suspended in water, as compared to those in the solvent mixture. On a nanosecond time-scale, the actual anisotropy decays are given by $r(t) = 0.11 \exp(-t/3.5) + 0.21 \exp(-t/25)$ and $r(t) = 0.1 \exp(-t/10.8) + 0.266 \exp(-t/124)$, respectively, at 274 K. These results are conspicuous, but show that the molecular motions in the bilayers depend on the glycerol concentration. These findings are compatible with EPR experiments where the rotational motions of a spin label was investigated under similar conditions (see below).

EPR spectroscopy

The mean radius of vesicles formed by sonication in different glycerol/water mixtures has been determined by using the EPR method described in Materials and Methods. The result are summarized in Fig. 7. It appears that at high water concentrations the radius is approx. 100 Å. By increasing the glycerol content the radius decreases, reaching a minimum of about 75 Å at 50% (w/w) glycerol. A further addition of glycerol yields a rapid increase of the vesicle radius. One might suspect, that this variation is due to a change in lipid–solvent interaction. However, the X-ray data (see Fig. 3) give no support for any dramatic change in the lipid-solvent interactions. At present, we are not able to give a detailed explanation of the observed dependence of vesicle size on solvent composition. Possibly, the kinetics of the vesicle formation upon sonication may play an important role on the vesicle size.

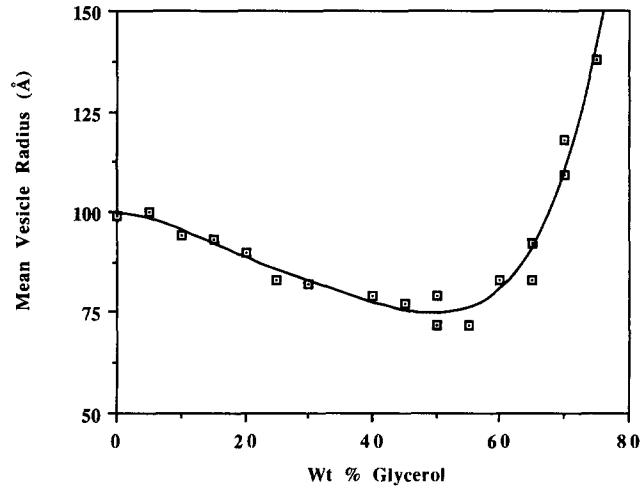

Fig. 7. The average radius of DOPC vesicles obtained for different glycerol/water mixtures (% glycerol (w/w)) at 298 K. The average radius was calculated from EPR spectra as described in Materials and Methods.

DOPC vesicles were doped with the spin labels 5-doxylstearic acid (5-DS) as well as 16-doxylstearic acid (16-DS) and the EPR spectra were recorded at different mixtures of glycerol with water. From an analysis of the EPR spectral lineshape (Ref. 21, and unpublished data) a rotational correlation time and an order parameter (S) of the doxyl group can be estimated. The 16-DS probe, which penetrates the interior of the bilayer, show a low value of the order parameter (S approx. 0) at all contents of glycerol investigated (i.e., 0–90% in the mixture with water). The rotational correlation time increases from about 1.5 ns at 0% glycerol to about 2.2 ns at 90% glycerol in the solvent mixture. For 5-DS, which is located more close to the lipid-solvent interface, S increases continuously from approx. 0.34 at 0% glycerol to approx. 0.44 at 90% glycerol. The effect on the rotational correlation time is also more pronounced with a value of 2.4 ns at 0% glycerol and a continuous increase to 7.3 ns at 90% glycerol. This is compatible with the finding of much slower rotational motions of the fluorescent probe

TABLE I

The ordinary (n_o) and extraordinary (n_{eo}) refractive indices of a lamellar phase of DOPC (20 wt%) in a glycerol/water (91:9% (w/w)) mixture at different temperatures (T) and measured at the wavelengths of 439 and 589 nm

The mean refractive index $n = [n_{eo} + 2n_o]/3$.

T (K)	$n_o(439)$	$n_{eo}(439)$	$n(439)$	$n_o(589)$	$n_{eo}(589)$	$n(439)$
244	1.512	1.538	1.520	1.504	1.525	1.511
250	1.511	1.535	1.519	1.502	1.523	1.509
252	1.515	1.524	1.518	1.507	1.512	1.509
254	1.513	1.523	1.517	1.505	1.510	1.507
258	1.504	1.521	1.510	1.494	1.509	1.499
262	1.491	1.509	1.497	1.484	1.498	1.489
267	1.485	1.508	1.492	1.475	1.495	1.482
271	1.484	1.502	1.490	1.473	1.490	1.478
276	1.481	1.499	1.487	1.471	1.488	1.477
281	1.480	1.498	1.486	1.470	1.486	1.475
285	1.479	1.497	1.485	1.469	1.484	1.474
290	1.477	1.494	1.483	1.467	1.482	1.472
295	1.475	1.492	1.481	1.465	1.481	1.470
299	1.472	1.490	1.478	1.464	1.479	1.469
304	1.471	1.488	1.476	1.463	1.477	1.468
308	1.471	1.485	1.476	1.466	1.476	1.466

TBPe upon increasing the glycerol concentration. It would have been preferable to correlate the changes of rotational rates with those of the translational diffusion of the lipid DOPC. Studies of the lipid translational diffusion using the NMR pulsed field gradient technique are underway and we intend to publish in a forthcoming paper (unpublished data).

Conclusions

DOPC forms a lamellar liquid crystalline phase in arbitrary mixtures of glycerol and water. The maximum swelling of the L_α phase is reached at about 50% (w/w) of DOPC (see Fig. 2). At this concentration the bilayer thickness and the area per the polar head group is essentially constant with solvent composition as seen by X-ray diffraction. A phase transition in the lipid bilayers occurs in the region of 240 to 260 K.

In excess of solvent DOPC can form unilamellar vesicles. These can be prepared either by using the sonication or the extruder technique. The radius of the vesicles prepared by sonication varies with the ratio of glycerol to water. The mean-radius increases rapidly at weight fractions larger than 50% glycerol. Vesicles prepared with glycerol and water at the ratio of 91:9 (w/w) are stable for months, when stored in a refrigerator. These preparations scatter light to a much lesser extent (typically 100-times) than the corresponding vesicles in water. This is important in studies where optical techniques are used. Fluorescent and EPR probes, residing in the lipid bilayer, report much slower rotational rates in vesicles dispersed in glycerol. In a forthcoming paper investigate the effects of glycerol on the dynamics in the lipid bilayer by means of EPR, time-resolved fluorescence and ^{1}H-NMR spectroscopy. For this we use a cubic and lamellar liquid crystals of monoolein (unpublished data).

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