The Effect of Increasing Membrane Curvature on the Phase Transition and Mixing Behavior of a Dimyristoyl-*sn*-glycero-3-phosphatidylcholine/ Distearoyl-*sn*-glycero-3-phosphatidylcholine Lipid Mixture as Studied by Fourier Transform Infrared Spectroscopy and Differential Scanning Calorimetry

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ABSTRACT The phase transition behavior of a lipid bilayer of dimyristoyl-*sn*-glycero-3-phosphatidylcholine/distearoyl-*sn*-glycero-3-phosphatidylcholine (DMPC-d54/DSPC) (1:1) on a solid support with varying curvatures was investigated with differential scanning calorimetry, infrared spectroscopy, and model calculations. With increasing curvature the temperatures of the liquidus and solidus points are shifted to lower values by up to 7°C and 15°C, and the mixing of the two lipid species in the two phase region is altered. With increasing curvature the DSPC dominates the gel phase, whereas the DMPC-d54 is expelled to the fluid phase. Whereas the planar system shows a nearly simultaneous phase transition of DSPC and DMPC-d54, the spherical system with the highest curvature exhibits an almost complete separation of the phase transitions of the two lipids. Model calculations suggest that the shift of the liquidus point can be understood as a reduction of the lateral pressure in the bilayer with increasing curvature. The shift of the solidus line is interpreted as a result of the increased demixing of the two components in the two-phase region with increasing curvature due to lowering of the lateral pressure.

INTRODUCTION

Curvature is a general feature of all biological membranes. Among the highest membrane curvatures known in eucaryotic cells is that of the synaptic vesicles in axons, whereas very low curvatures are observed in the membranes of cell walls and in stacks of certain plasma membranes. All other membrane curvatures are between these extremes. The implications of curvature for membrane function and integrity are not well understood. However, it is well known that the various lipid species that constitute a biological membrane prefer differently curved environments, owing to their various effective molecular shapes (Israelachvili, 1992; Epand and Epand, 1994). Hence, a membrane that exhibits a heterogeneous curvature (as most cells do) may in addition exhibit a heterogeneous distribution of its lipids which, in turn, may impart different functional properties to different areas of the membrane surface. This might be one of the reasons why nature offers such a great variety of different lipids in cells, although most of their specific functions are not yet known. Understanding membrane function therefore requires a better knowledge of the effects of membrane curvature on lateral and transversal lipid distribution.

One of the major factors that determine the distribution of lipids in a membrane at a certain temperature is their mixing behavior, which often can be adequately described by regular solution theory (Lee, 1978). There exists a wealth of knowledge about the mixing behavior of many binary and even some ternary lipid mixtures, mostly for saturated lipid species. These studies were done mainly for multilamellar dispersions of lipids (mostly vesicles) where the membrane curvature is an ill-defined parameter. However, very limited experimental results exist on how the mixing behavior is modulated by the membrane curvature. Only for pure lipid phases has it been demonstrated by microcalorimetry that high membrane curvature may drastically reduce the phase transition temperature of saturated phospholipids and may even render this transition undetectable (Lentz et al., 1987). The reason for the lack of experimental data is mainly technical in nature. Until recently, no bilayer systems were available which exhibit a uniform curvature that can be varied by the experimenter and which are suitable for spectroscopic and thermodynamic measurements. The introduction of single bilayers on a spherical support of silica (spherical supported vesicles, SSVs) to the field of membrane spectroscopy and the exploration of the physical properties of this membrane model system paved the way for systematic studies of the curvature effects in bilayers (Bayerl and Bloom, 1990; Naumann et al., 1992).

Using this type of model system in combination with planar bilayers and multilamellar vesicles, we wish to demonstrate in this work how drastically the mixing behavior of a binary phospholipid mixture is changed with the curvature of the bilayer in a range of curvature diameters that relate to those occurring in biological systems.

Another motivation for this work is the high number of papers published over recent years about changes of the thermodynamical behavior of lipid vesicles after association with other molecules (e.g., cholesterol, peptides, proteins). Our results indicate that great care should be taken in

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interpreting these results without consideration of the effect that foreign molecules may have on the average curvature of the systems.

MATERIALS AND METHODS

The lipids distearoyl-*sn*-glycero-3-phosphatidylcholine (DSPC) and chain perdeuterated dimyristoyl-*sn*-glycero-3-phosphatidylcholine (DMPC-d54) were purchased from Avanti Polar Lipids (Alabaster, AL) and used without further purification. To distinguish the lipids in the Fourier transform infrared spectroscopy (FTIR) measurements, chain-perdeuterated DMPC-d54 was used for all experiments.

As spherical solid support, silica beads of 640 ± 40 nm and 60 ± 6 nm diameter were used. They were obtained from Degussa AG, Anorganic Chemistry (Hanau, Germany) and from Professor Erwin Killmann (Institute for Physical Chemistry, TU-Munich). As a planar support we used the large face of a polished silicon ATR crystal.

The coating of the solid supports with a single bilayer was done by vesicle condensation technique, as described in detail previously for silica beads (Bayerl and Bloom, 1990; Naumann et al., 1992) and for planar ATR crystals (Reinl et al., 1992). The coating temperature was adjusted to values 2–3°C above the corresponding main phase transition temperature (for one-component bilayers) or the liquidus point (for binary mixtures). This ensures that the supported bilayers exhibit a minimum of lateral stress at this temperature.

The differential scanning calorimetry (DSC) measurements were performed using a Microcal MC-2 (Microcal, Northampton, MA) and a Hart Calorimeter (Hart Scientific, Provo, UT) at a scan rate of 20°C/h for all experiments. The temperature values of the phase transition were extrapolated from the steep sides of the transition endotherms according to criteria discussed previously by Bayerl et al. (1988). The phase diagram of the DMPC/DSPC mixture is characterized by a broad miscibility gap in the gel phase and an extended two-phase region (Knoll et al., 1981). To avoid long equilibration times all temperature scans shown in this work were started from the homogeneous fluid phase in the descendent temperature mode. These cooling scans were repeated at slower scan rates (10 and 5°C/h) but did not show any changes compared to the 20°C/h scans. Heating scans were also recorded, but these endotherms were extremely dependent on the incubation time of the sample at low temperature, and even an incubation at 2°C for 36 h did not provide reproducible signals. The likely reason for these rather ill-defined heating scans is that the the gel state of supported bilayers is not characterized by an L_β or L_c phase as in multilamellar vesicles (MLVs), but by an L_β* phase, owing to the lateral stress in the bilayer. We demonstrated this for a one-component DMPC supported bilayer (Johnson et al., 1991; Naumann et al., 1992), but this feature will certainly affect also the gel phase formation of a binary mixture on a solid support. This stress effect might result in very long solid phase relaxation times of the latter, thus rendering their heating scans even more sample history dependent, as is known for MLV systems.

The FT-IR measurements were performed in the ATR mode using a Nicolet 60-SXR spectrometer (Nicolet Analytical Instruments Co., WI). We used a horizontal ATR setup with a 80 × 10 × 3 mm^3 silicon crystal thermostated by a water bath connected to the bottom of the crystal and well insulated to avoid temperature gradients. The computer-controlled thermostat (Julabo F-10 HC; Julabo Labortechnik, Seelbach, Germany) made possible a temperature reproducibility of ±0.2°C, with the temperature of the water bath controlled directly at the crystal. For the measurements of the silica beads the sample was layered in excess water on top of the ATR crystal, whereas for planar support measurements the top of the crystal itself was coated with a single bilayer. For a temperature scan the sample was heated to the starting temperature in the fluid phase and successively cooled in 1°C steps. Each temperature step included a 10-min waiting period for equilibration. For each measurement 4000 scans were accumulated at a resolution of 2 cm^{-1} and apodized by a Happ-Genzel function before Fourier transform. At each temperature a water background was measured, against which the sample spectrum was normalized. The effect of the silica beads on the reference spectra was considered by doing a temperature scan with plain beads in water as reference and subtracting it at the corresponding temperature from the sample spectra.

Solidus and liquidus points of the phase transition were determined by the plot of the CD_2 and CH_2 symmetric stretching vibration frequencies versus temperature as that temperature that showed a 10% deviation from the linear dependence.

The microscopic model used in the simulations is based on the ten-state Pink model originally developed to describe the chain melting transition of one-component lipid bilayers (Pink et al., 1980). The one-component bilayer model has in a recent paper been extended to binary lipid mixtures composed of saturated phospholipids (Jørgensen et al., 1993) by explicitly incorporating a mismatch term that takes account of the incompatibility between acyl chains of different hydrophobic lengths. The model, which is a multistate lattice model, is primarily based on the acyl chain conformational statistics of the chain melting transition and includes several terms that in a detailed manner take care of, for example, the interaction between the different acyl conformational states and their internal energy. The model also contains an intrinsic lateral pressure term, π, which is incorporated in order to ensure bilayer stability. For a complete description of the microscopic interaction model and the extension to nonideally behaving binary lipid mixtures composed of nondeuterated lipid species like DMPC and DSPC, see Jørgensen et al. (1993) and Mouritsen (1990). The microscopic interaction model for the nondeuterated DMPC-DSPC lipid mixture has been applied without further modifications because it is expected that the phase behavior for the mixture containing the deuterium-labeled DMPC-d54 lipid would be very similar to the nondeuterated DMPC-DSPC system.

Within the model, the intrinsic lateral pressure π represents in an approximate manner part of the stabilizing forces in bilayer formation. Specifically, π covers the delicate balance between the polar headgroup and the acyl chain intrinsic pressure. On a curved substrate the outmost monolayer of the bilayer would experience a shift of this balance toward a lower interfacial pressure in the headgroup region. Therefore, to a first approximation the effect of increasing the curvature of the solid support could be mimicked within the model as a corresponding decrease in π. We have therefore studied in our Monte Carlo computer simulation calculations the effects of lowering the lateral pressure on the equilibrium mixing phase behavior in an equimolar DMPC-DSPC lipid mixture.

RESULTS

Four bilayer systems of DMPC-d54/DSPC (1:1) that exhibit different curvature were studied by DSC and FT-IR in the descending temperature mode: 1) MLVs, 2) single supported bilayer silica beads (640 nm diameter), 3) same as 2) but on beads of 60 nm diameter, 4) single bilayer on a planar support.

A distinct feature of the DSC endotherms obtained for systems 1)–3) is the existence of two peaks in the C_p versus temperature representation superimposed by a broader feature (Fig. 1). Because the high temperature end of the endotherm defines the liquidus point of the mixture in the phase diagram and the low temperature end defines the solidus point, we will denote in the following the two peaks as the L region (high temperature peak) and the S region (low temperature peak).

The DSC endotherms in Fig. 1 exhibit significant changes with increasing curvature. Whereas at the lowest curvature (MLV sample) the L and S regions rather overlap, increasing curvature (640 and 60 nm systems) not only reduces the intensity in the overlap region but causes a drastic downward shift in temperature of the S region. In contrast, the L region of the supported systems does not

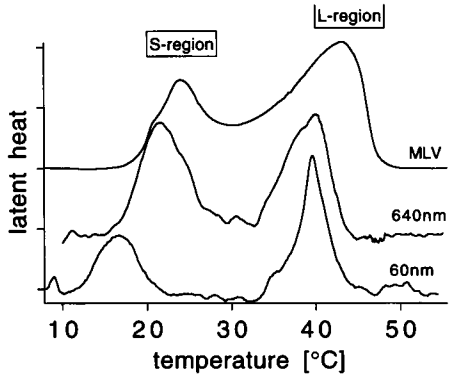

FIGURE 1 DSC endotherms (cooling scans) of DMPC-d54/DSPC (1:1) obtained for differently curved bilayer systems: multilamellar vesicles (upper trace) and spherical supported vesicles of 640 nm (mid-trace) and 60 nm (lower trace) diameter. L and S regions (cf. text) are indicated.

shift significantly in temperature but becomes remarkably narrower at high curvature.

More information about the contribution of the two lipid components in the mixture to the L and S regions is obtained by FT-IR. This method enables a separate but simultaneous measurement of the phase transition for both DMPC-d54 and DSPC owing to the isotopic shift of the DMPC-d54 absorption signal. FT-IR measurements were performed for a planar bilayer (i.e., zero curvature) and for the two spherical systems (640 and 60 nm diameter). Fig. 2 shows the frequency of the asymmetric methylene stretching vibration as a function of temperature for the three supported bilayer model systems and for both components of the mixture. The comparison with the DSC results (Fig. 1) clearly indicates that the L region peak arises mainly from the transition of DSPC, whereas the S region is dominated by the DMPC-d54 transition. A similar behavior is observed for the MLV system, which is omitted from Fig. 2 for the sake of clarity. Moreover, the FT-IR results confirm the DSC data regarding the downward shift in temperature of the S region (mainly DMPC-d54) and the narrowing of the L region (mainly DSPC) with increasing curvature. Fig. 2 shows that the narrowing of the L region in the DSC measurements with increasing curvature is due to the shift of the starting temperature of the DSPC transition (cf. Table 1, TS), whereas the end temperature of the phase transition (cf. Table 1, TE) remains nearly constant. Thus, the DSPC transition is also shifted to lower temperatures.

A comparison of the FT-IR data for the planar system with those for the curved systems provides a remarkable demonstration of the curvature effects on the phase transition (Fig. 2): the planar bilayer exhibits the highest temperatures for the L and S regions. The L region is 3°C higher than in the MLV measurement, and the beginning of the S region is 12°C above that of the MLV. The differences become even larger when compared with the 640 and 60 nm systems. Moreover, the two transition regions become increasingly separated with increasing curvature. Whereas the planar system (zero curvature) shows a rather simultaneous

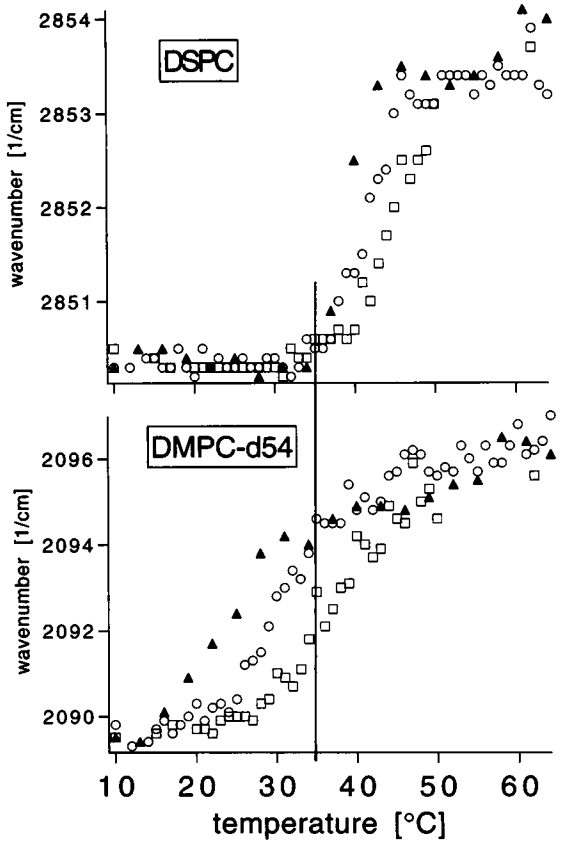

FIGURE 2 Plot of the CH_2 (DSPC) and CD_2 (DMPC-d54) symmetric stretching vibration frequency as a function of temperature for a planar bilayer (□) and for 640-nm (○) and 60-nm (▲) spherical supported vesicles. The vertical line indicates the completion of the DSPC transition (see text). The uncertainty is 0.5 cm^{-1} in wave number and 0.2°C in temperature.

and broad transition of DMPC-d54 and DSPC, the highest curvature system (60 nm) exhibits two almost completely separated transitions owing to the S region downward shift and the L region narrowing. The limiting temperatures of the transitions of both regions for the different curvatures as obtained by DSC and FT-IR are listed in Table 1.

Further information about the phase transition behavior of the mixture as a function of curvature is obtained

TABLE 1 Phase transition temperatures of the equimolar DMPC-d54/DSPC lipid mixture for the different lipid systems

	DSC		FTIR			
			DSPC		DMPC-d54	
	TS	TE	TS	TE	TS	TE
MLV	47	18.5	47	30	33	21
SPB	—	—	50	33	45	27
600 nm	45	16.5	46	30	35	27
60 nm	43	12	43	31	30	13

The values were determined with DSC and FT-IR (cf. Figs. 1 and 2). TS and TE denote starting and ending temperature of the DSC endotherm or of the individual transition of the two components measured by FT-IR. The uncertainty is ±1°C in the DSC values and ±2°C in the FT-IR values.

by analyzing the FWHM of the asymmetric methylene stretching vibration signals. We calculated the FWHM as the frequency difference between high frequency point (HF) and the low frequency point (LF) at half signal magnitude at the peak frequency (PF) (as indicated in Fig. 4). Previous FT-IR work on lipid phase transitions established that both PF and FWHM of this signal change at the transition in the same way for pure (i.e., single component) MLV systems (Mantsch and McElhaney, 1991; Casal and Mantsch, 1984).

Interestingly, this does not hold for the DSPC signal of the mixture in the L region, as shown in Fig. 3. The FWHM shift is lagging behind the PF shift by almost 5°C. In fact, one can distinguish two domains. The first domain of the L region transition (denoted the L1 region) is characterized by a drastic change of PF by 4 cm^{-1} (asymmetric stretching vibration) but only a slight change in FWHM. For the second domain (L2 region), PF changes are much less significant (about 1–2 cm^{-1}), but the FWHM is drastically reduced. This distinction becomes more obvious by the variation of the LF values in these regions: LF shifts to lower frequencies in the L1 region but is constant in the L2 region. In contrast, the HF value undergoes changes in both the L1 and L2 regions. We find that the subdivision of the L region into L1 and L2 can be made for all curvatures studied and that the temperature which marks the boundary between both regions is in the 39–43°C range (indicated by the solid line in Fig. 3). This behavior is also demonstrated in Fig. 4. The derivative spectra (*right column*) clearly show that the change in FWHM dominates the L2 region and that the change in LF is limited to the L1 region. For the S region, which is dominated by the DMPC-d54 transition, no such distinction can be made because both PF and FWHM change simultaneously in a manner analogous to those measured for single-component lipid phases.

Fig. 5 shows computer simulation results for the temperature-dependent acyl chain order parameter S of the indi-

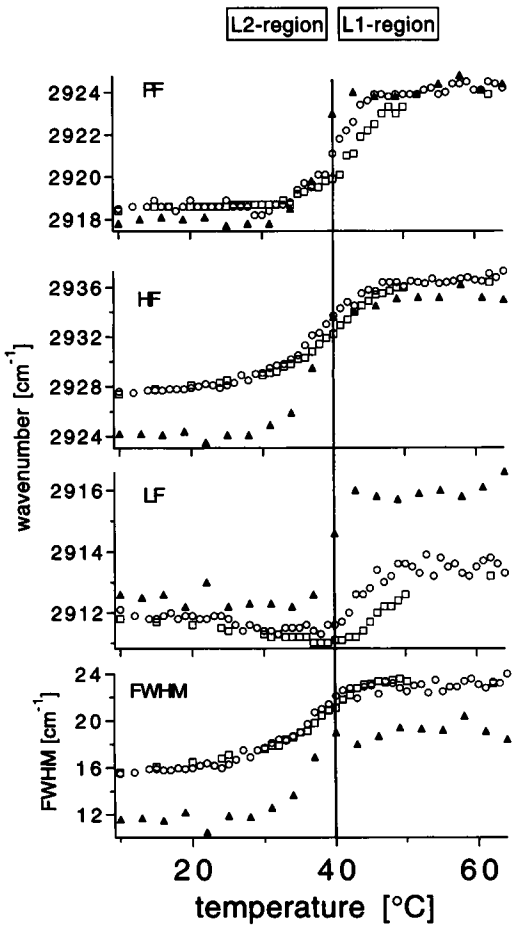

FIGURE 3 Plot of the temperature dependence of PF, HF, LF, and FWHM for the asymmetric methylene stretching vibration frequency of DSPC in an equimolar mixture with DMPC-d54 for a planar supported bilayer (□) and for a spherical supported bilayer of 640 nm (○) and 60 nm (▲) diameter. The definition of the notations PF, HF, and LF is given in Fig. 4. The vertical line indicates the position of the boundary between the L1 and L2 regions.

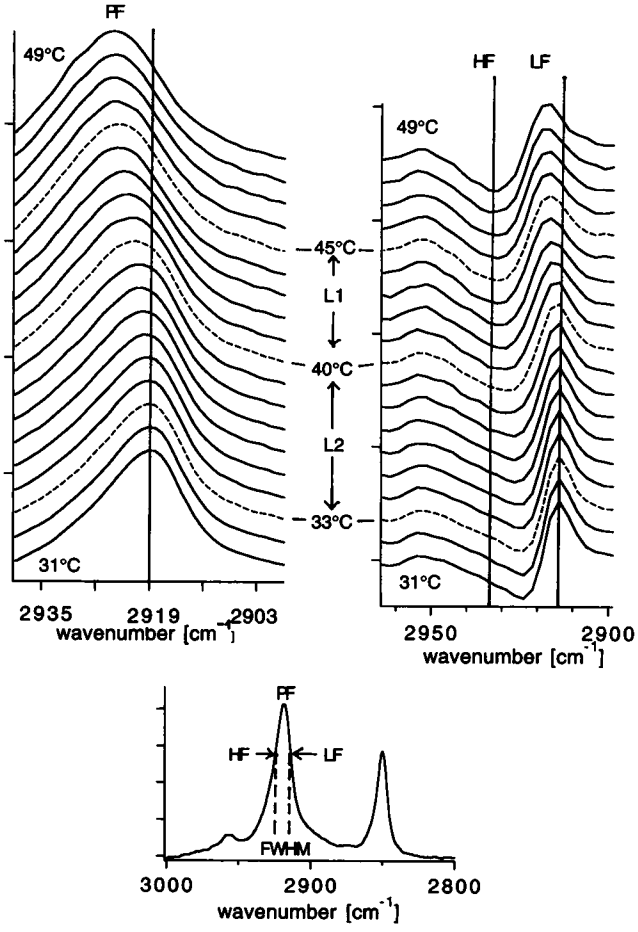

FIGURE 4 Temperature dependence of the DSPC asymmetric stretching vibration signal (*left column*) and of its first derivative of the equimolar mixture on 640 nm solid support. The position of PF and LF for the spectrum at 31°C and of HF for the spectrum at 49°C is indicated by the vertical lines. The dotted lines represent signals obtained at the boundaries of the L1 and L2 regions. The frequency axis of the absorption signal is scaled with a tanh function to highlight the variation of the signals with temperature. The absorption signal below indicates the definitions of PF, LF, and HF.

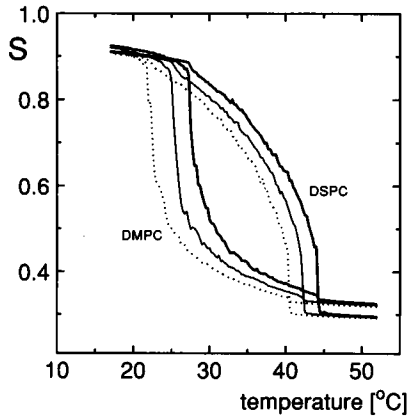

FIGURE 5 Equilibrium acyl chain order parameter S for each lipid species as a function of temperature for an equimolar mixture of DSPC-DMPC as obtained from computer simulation calculations for three different lateral pressures, π = 30 dyn/cm (*heavy line*), π = 27.5 dyn/cm (*thin line*), π = 25 dyn/cm (*dotted line*). The simulated system consists of a total of 1800 lipid molecules.

vidual species in an equimolar DMPC-DSPC lipid bilayer for three different lateral pressures, π = 30, 27.5, 25 dyn/cm. S is given by

$$S = \frac{1}{2(n-1)} \sum_{i=2}^{n} (3\cos^2\theta_i - 1) \qquad (1)$$

where the summation extends over all n CH_2 segments of the acyl chain of the lipid species in question, and θ_i is the angle between the bilayer normal and the normal to the plane spanned by the ith CH_2 group of the chain. The value of S, obtained from the computer simulations, can be related to the average quadrupolar splitting as measured in ^{2}H-NMR for a chain perdeuterated lipid and thus is a measure of the molecular order in the bilayer. The results in Fig. 5 show clearly that both liquidus and solidus points decrease in temperature with decreasing π. However, the decrease of the solidus point in temperature is more pronounced. This behavior is similar to the results of the measurements with increasing curvature.

To separate the effect of curvature on the mixing behavior from its effect on the single lipid components we measured their phase transition for the various curvatures. Fig. 6 shows DSC endotherms of pure DMPC and of DSPC for the model systems studied. For DMPC the maximum downward shift of the peak temperature by 3°C compared to MLV is already reached for the 640 nm system. Further curvature increase (60 nm) does not cause a further reduction of this temperature. In contrast, DSPC clearly shows not only a reduction of the peak temperature between the MLV and the 640 nm system by 2°C but also a further reduction by 1°C for the 60 nm system (i.e., a total reduction of 3° compared to MLV).

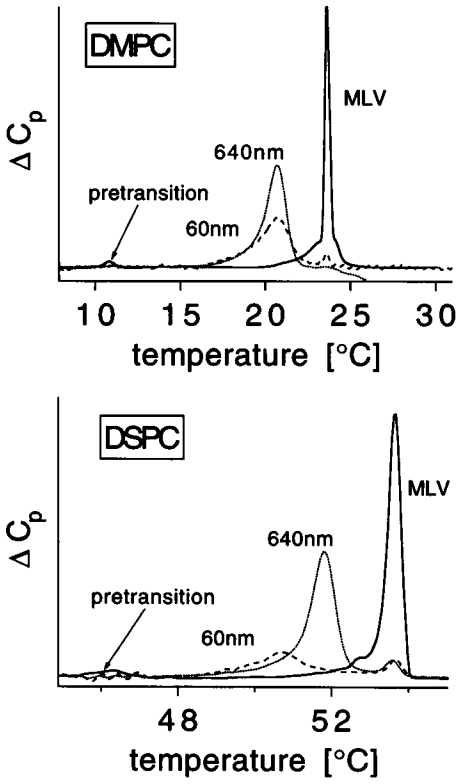

FIGURE 6 DSC endotherms (cooling scans) of multilamellar vesicles (MLVs) and of spherical supported vesicles of 640 nm and 60 nm diameter prepared with either pure DMPC (*upper plot*) or pure DSPC (*lower plot*). Peak intensities are not normalized to the lipid concentration because the latter is not exactly known.

DISCUSSION

The results can be summarized as follows:

1) For the phase transition behavior of the equimolar DMPC-d54/DSPC mixture a drastic effect due to curvature is observed. Whereas planar single bilayers of the mixture on a solid support exhibit a rather broad but simultaneous transition of both components, the spherical supported systems show a strong tendency to separate the transitions of the two components with increasing curvature. In contrast to the results for the pure components (cf. point 2, below), the separation is essentially caused by a lowering of the DMPC-d54 transition temperature in the mixture with increasing curvature, whereas the transition region of the DSPC component mainly becomes narrower.

2) The phase transition behavior of pure DMPC-d54 and of pure DSPC on a spherical solid support exhibits significant differences with respect to the curvature of the solid support. For DMPC, a reduction of T_m by 3°C can be observed already for the 640 nm support (and is not changed for 60 nm). In contrast, DSPC gives a T_m reduction of 2°C for 640-nm support and a further reduction by 1°C for the 60-nm support.

3) The FT-IR indicates that the DSPC transition of the mixture is a two-stage process with respect to the changes in frequency and in FWHM.

The effect of curvature on the equimolar mixture

In principle, one may distinguish three different mechanisms that can change the physical properties of the bilayer with increasing curvature:

1) The expansion of the fluid bilayer with increasing curvature may cause a reduction of its lateral pressure and a concomitant change in area density that can explain both the reduction in phase transition temperature and the demixing of the system (see below).

2) The bending of the bilayer. By bending the outer bilayer, the leaflet gets expanded while the inner leaflet comes under compression with increasing curvature leading to a change in the free energy of the system, and this may give rise to the observed phenomena.

3) The lateral stress creation in the gel phase due to the $\sim$10% area reduction of the lipids at the transition from the fluid to the solid state. This shrinking will exert a stress on the remaining fluid lipid due to the area mismatch between the bilayer and the solid support. However, the latter effect is essentially independent on curvature and will give rise to a large number of defects at temperatures below the solidus point.

4) Furthermore, the formation of excess bilayer area in the fluid phase of the supported systems characterized by bilayer foldouts, blebs, and theters, as suggested by Dolainsky et al. (1993), must be considered regarding its potential to modify the phase behavior. However, it should be noted that excess bilayer formation may occur only at the transition from the solid to the fluid state, whereas our measurements were done in the descendent temperature mode. Moreover, the supported samples were prepared at temperatures slightly above the liquidus point and thus no excess bilayer should be formed. We tested this by measuring samples at this temperature using ^{2}H-NMR, where the presence of excess bilayer is revealed by an isotropic peak (cf. Bayerl and Bloom, 1990), but no such peak was observed for the supported binary mixture samples used in this work. Finally, the extent of possible excess area formation is to a good approximation independent on the curvature of the solid support. All of these reasons let us conlude that any significant contributions of excess bilayer to the observed phenomena are unlikely.

We interpret the curvature effects in terms of a bilayer expansion for two reasons. First, the change in free energy due to expansion is two orders of magnitude larger than that due to bending and the presence of the solid support must give rise to an expansion with increasing curvature. Second, the width of the transition may enable the remaining fluid lipid to fill the gaps between the gel domains over most of the transition range.

Assuming that increasing curvature reduces the lateral pressure and that the MLV system can be used as a reference system owing to the absence of geometrical restrictions, the data in Table 1 can be explained as follows.

The planar system experiences an increased lateral pressure, so that the transition temperatures of both components shift to higher values compared to the MLV. With increasing curvature the bilayer undergoes a reduction of the lateral pressure and consequently the transition temperature decreases. For DSPC this is accompanied by a narrowing of the transition, indicating that with increasing reduction of the lateral pressure the gel phase domains tend to segregate the DSPC component by expulsion of DMPC-d54 to the fluid phase. This "forced" demixing in the gel phase is also indicated by the DMPC-d54 transition, which shows a drastic shift to lower values with increasing curvature. The broad overlap between the S and L regions for the planar system, indicating a tendency toward mixing in the gel phase, must be understood as a consequence of the higher lateral pressure in this system. The relief of this pressure due to curvature causes the separation between the two regions that is highest for the 60 nm system.

The presence of gel phase domains seems to be a prerequisite for this demixing phenomenon: assuming that the demixing occurred already in the fluid phase, there should be a shift of the transition temperature of the DSPC toward its T_m as a pure lipid at higher temperatures, which is clearly not the case.

To exclude the possibility that an asymmetry in the lipid composition between both monolayer leaflets of the SUV (small unilamellar vesicle) used for coating of the beads is transferred onto the solid support, we did the following experiment. An equimolar SUV solution of choline group deuterated DMPC-d9 with DSPC in D_2O was measured with high-resolution ^{1}H-NMR. Here the $N(CH_3)_3$ group signal arising from the DSPC was compared with that obtained after the addition of 20 mM $PrNO_3$ to the sample. This addition caused a downward shift of 50 $\pm$ 8% of this signal. This indicates that such SUV exhibit no significant compositional asymmetry between the two monolayers.

Regular solution theory can provide the theoretical basis for the observed phenomena. This is achieved by introducing a chemical potential that accounts for the elastic energy of the bilayer, analogous to the approach of Ipsen and Mouritsen (1988), where the chemical potential is expressed as a function of the lipid area density and hence is sensitive to the bilayer expansion. Suppose further that this change of chemical potential mainly modifies the free energy of the gel phase because the lipids are packed closer together and increasing their mean distance will drastically change the free energy of the bilayer. Then it is possible to show that in the coexistence region the concentration of the high melting lipid is increased in the gel phase with increasing expansion, i.e., curvature.

The results of the computer simulations according to this theory shown in Fig. 5 demonstrate that both the solidus and the liquidus phase boundaries are shifted to lower temperatures when the lateral pressure is decreased from 30 to 25 dyn/cm. Moreover, the simulation results clearly reveal that lowering of the lateral pressure has the stronger effect on the short-chain DMPC-lipid, whereas the influence on the long-chain DSPC-lipid is less pronounced. Consequently, lowering of the lateral pressure gives rise to more nonideal behavior of the mixture. This is manifested as a demixing of the two lipid species in the coexistence region leading to a fluid phase, which becomes enriched in the short-chain DMPC-lipid and a gel phase containing a higher amount of the long-chain DSPC-lipid. A remarkable similarity is ob-

served by comparison of the computer simulation results of the individual conformational acyl chain order parameter presented in Fig. 5 and the FT-IR experimental results shown in Fig. 2 of the symmetric methylene stretching vibration, which is closely related to the conformational states of the lipid species. The results presented in the two figures reveal that the overall effect on the short-chain DMPC-lipid is more pronounced than the effect on the long-chain DSPC-lipid caused by either decreasing lateral pressure or increasing the curvature of the solid-supported DMPC-DSPC bilayer.

Another theoretical approach in terms of free energy of the bilayer as a function of curvature comes from the liquid crystal theory by Chandrasekhar (1977). Here a change in the van der Waals interaction due to an inclination of adjacent molecular directors modifies the free energy per unit volume of the system according to

$$\delta F \approx \frac{1}{2} C V^{-7/2} S^2 \frac{1}{R^4} \qquad (2)$$

where V is the molar volume, S is the order parameter, R is the radius of the solid support, and C is a constant.

In contrast to the bilayer expansion model, no explicit change of density is required to modify the free energy. Moreover, the explicit dependence on the order parameter supports the above assumption that the curvature mainly modifies the free energy of the gel phase, leading to a demixing of the lipids.

The observed two-stage characteristics of the DSPC component of the mixture (Figs. 3 and 4) for all supported systems must be a result of the mixing behavior, because they are not observed for pure DSPC. A likely explanation for this two-stage behavior is the area mismatch between the bilayer and the solid support. It is certainly conceivable that the crystalline domains that are formed upon cooling the mixture, which are rich in high-melting-point DSPC, come under considerable lateral stress owing to the 10% shrinkage of the molecular area. This stress is partly relieved by the expulsion of DMPC from the domains, which might be a prerequisite for the formation of stable L_β-gel phase. This process of DMPC expulsion could be accompanied by the observed reduction of FWHM that is characteristic for the L2 region.

CONCLUSION

Our results demonstrate that the bilayer curvature strongly influences the phase transition behavior of the lipid system. The computer simulations suggest that an increase in curvature and a concomitant increase of the bilayer lateral stress have a similar effect on the mixing properties of the lipid components as a decrease in the lateral pressure of the bilayer. The combined experimental and simulation studies propose that an intimate relationship and coupling may exist between curvature-induced stress of the solid-supported bilayer and the effect of lateral pressure in the bilayer. Such results might open up a deeper understanding of curvature-induced effects on microscopic molecular properties like conformational acyl chain states and associated effects on mixing properties of the lipid species, which in turn might influence the composition of coexisting phases in bilayers and vesicles characterized by different curvatures.

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