Thermodynamics of Interdigitated Phases of Phosphatidylcholine in Glycerol

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ABSTRACT Comparison of the electron spin resonance spectra of phosphatidylcholines spin-labeled in the *sn*-2 chain at a position close to the polar region and close to the methyl terminus indicate that symmetrical saturated diacyl phosphatidylcholines with odd and even chain lengths from 13 to 20 C-atoms (and probably also 12 C-atoms) have gel phases in which the chains are interdigitated when dispersed in glycerol. The chain-length dependences of the chain-melting transition enthalpies and entropies are similar for phosphatidylcholines dispersed in glycerol and in water, but the negative end contributions are smaller for phosphatidylcholines dispersed in glycerol than for those dispersed in water: $d\Delta H_t/d\mathrm{CH_2} = 1.48$ (1.43) kcal·mol^{-1}, $d\Delta S_t/d\mathrm{CH_2} = 3.9$ (4.0) cal·mol^{-1}K^{-1}, and $\Delta H_o = -12.9$ (-15.0) kcal·mol^{-1}, $\Delta S_o = -29$ (-40) cal·mol^{-1}K^{-1}, respectively, for dispersions in glycerol (water). These differences reflect the interfacial energetics in glycerol and in water, and the different structure of the interdigitated gel phase.

INTRODUCTION

As is well known, the phospholipid dipalmitoyl phosphatidylcholine dispersed in glycerol forms a lamellar gel phase in which the acyl chains are fully interdigitated (McDaniel et al., 1983). This is in contrast to dispersions in aqueous buffer for which a normal noninterdigitated bilayer gel phase is obtained. The behavior in glycerol may be relevant to the use of glycerol for cryostabilization, but more generally from the biophysical point of view it raises interesting implications for the interfacial energetics of lipid membranes. These are also the primary factors controlling the stability of biological membranes.

One approach to the thermodynamics of the interdigitated gel phase is via DSC of the gel-to-fluid chain-melting transition. It has already been demonstrated that the transition enthalpy of diacyl phosphatidylcholines in glycerol differs significantly from that of aqueous dispersions, although the transition occurs in a similar temperature range (Boggs and Rangaraj, 1985). Most informative is a systematic investigation of the chain-length dependence of the calorimetric properties because effects of the chain packing in the interdigitated phase can then be differentiated from interfacial, i.e., end, effects (cf. Cevc and Marsh, 1987).

Whether diacyl phosphatidylcholines of chain lengths other than that of dipalmitoyl phosphatidylcholine form interdigitated gel phases in glycerol is not known with certainty, or it is firmly established in only a few cases. Therefore, we have performed ESR experiments with phosphatidylcholine spin-labeled in the *sn*-2 chain close to the polar headgroup and close to the terminal methyl group in order to test this (cf. Boggs and Rangaraj, 1985; Bartucci et al., 1993), and we have made systematic measurements of the chain-length dependence of the chain-melting thermodynamics by using high resolution DSC.

MATERIALS AND METHODS

Materials

Saturated diacyl phosphatidylcholines, diCn:0-PC, of all odd and even chain lengths from C12:0 to C20:0 were obtained from Avanti Polar Lipids (Alabaster, AL). DiC20:0-PC was also obtained from Larodan (Malmö, Sweden). Spin-labeled phosphatidylcholines were synthesized as described in Marsh and Watts (1982). Glycerol (99%) was from Baker (Deventer, Holland).

Sample preparation

For ESR spectroscopy, ~2 mg of the lipid and 1 mol % of the appropriate spin label were co-dissolved in CH_2Cl_2, and a thin film was formed by evaporation of the solvent under a stream of dry nitrogen gas. The samples were then vacuum-dried overnight and hydrated with ~100 μl of glycerol or water at ≈10° above the phase transition temperature of the lipid. The hydrated lipid sample was transferred to a 100-μl glass capillary and pelleted by centrifugation in a bench-top microcentrifuge. The excess supernatant was removed, and the capillaries were flame-sealed before ESR measurements.

For DSC, 3–10 mg of the lipid was weighed into the DSC ampoule, and 500 μl of water or ≈200 μl of glycerol was added. The ampoules were closed with screw caps. Samples were subjected to two cycles of heating and cooling at fast scan rates (≈60°/h) to a temperature well above the phase transition temperature of the lipid in order to ensure complete hydration. After this, the samples were incubated at the lower end of the scan range for ~20 min before the DSC experiments were performed, as described below.

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Abbreviations used: DSC, differential scanning calorimetry; diCn:0-PC, 1,2-diacyl-*sn*-glycero-3-phosphocholine, where *n* is the number of C-atoms in the (saturated) acyl chain; *n*-PCSL, 1-acyl-2-[*n*-(4,4-dimethyloxazolidine-*N*-oxyl)stearoyl]-*sn*-glycero-3-phosphocholine; ESR, electron spin resonance.

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ESR spectroscopy

ESR spectra were recorded on a Varian Century Line 9 GHz spectrometer (Varian, Palo Alto, CA) equipped with nitrogen gas flow temperature regulation. Samples in sealed 1-mm i.d. glass capillaries were accommodated in a standard quartz ESR tube containing light silicone oil for thermal stability. Temperature was measured with a fine-wire thermocouple located in the silicone oil just above the top of the microwave cavity.

DSC

DSC was performed with a Model 4207 heat-flow calorimeter from Hart Scientific (Pleasant Grove, UT). Samples were contained in 1-ml Hastelloy ampoules, with an empty reference ampoule. The baseline correction was made with a blank glycerol or water sample. Scan rates used for heating and cooling were 5°/h or 10°/h. Transition enthalpies were determined with use of the peak integration software provided with the instrument, which employs the instantaneous scan rate measured at the point of the transition.

RESULTS

ESR spectroscopy

The ESR spectra of phosphatidylcholine spin-labeled at the 5- and at the 12- or 14-C atoms of the *sn*-2 chain are given in Fig. 1 for diacyl phosphatidylcholines of different chain lengths dispersed in water and in glycerol, at temperatures in the gel phase. It is seen that the spectra from the two different positional isomers are similar for all the phosphatidylcholines dispersed in glycerol. In contrast, for the phosphatidylcholine dispersions in water (which are known to form noninterdigitated gel phases), the spectra of 12- or 14-PCSL evidence a higher degree of mobility and a smaller outer hyperfine splitting, $2A_{max}$, than those of 5-PCSL. The spectra of the lipid spin labels in diC19:0-PC exhibit pronounced broadening from spin-spin interactions, indicating that part of the spin-label population is excluded from the gel phase of the host lipid. Nevertheless, the hyperfine splittings of the spin labels that are incorporated within the gel phase can be readily resolved. The same is true for ESR spectra from the longer chain diC20:0-PC host lipid (not shown). The differences in outer hyperfine splitting, $\Delta 2A_{max}$, between the 5-PCSL and 12-/14-PCSL and between 5-PCSL and 16-PCSL labels in the phosphatidylcholines of different chain lengths are given in Table 1. From comparison of these data for dispersions in water and in glycerol (cf. Boggs and Rangaraj, 1985; Bartucci et al., 1993), it can be concluded that the lipid chains of phosphatidylcholines with chain lengths from C13:0 to C20:0 are all interdigitated in the gel phase when the lipids are dispersed in glycerol. The situation with regard to diC12:0-PC is less clear-cut, probably because the aqueous (solvent) phase is also frozen at temperatures corresponding to the gel phase in water for this lipid. Another reason for the lack of sensitivity could be that the length of the spin-labeled chain is appreciably greater than that for this particular lipid.

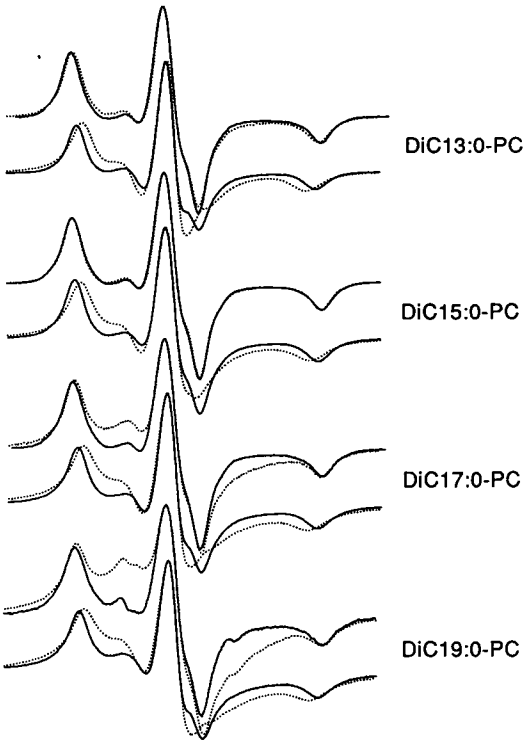

FIGURE 1 ESR spectra of the 5-PCSL (——) and 12- or 14-PCSL (· · · ·) phosphatidylcholine spin label positional isomers in saturated diacyl phosphatidylcholines, of the different chain lengths (Cn:0) indicated, dispersed in glycerol or in water, recorded at 0°C in the gel phase. The upper spectra of each pair are from samples in glycerol and the lower from samples in water. From upper to lower, the sets of spectra refer to di-C13:0, C15:0, C17:0, and C19:0 phosphatidylcholines. Total spectral width: 100 gauss.

Calorimetry

Representative DSC thermograms for phosphatidylcholines of various chain lengths dispersed in glycerol are given in Fig. 2. The scan rates used were 5°/h. The heating scans are mostly characterized by a single sharp endothermic chain-melting transition, whereas the cooling scans are characterized by a double exotherm. The latter has been observed previously for diacyl phosphatidylcholines in glycerol and was ascribed to a metastable behavior in which a noninterdigitated gel phase is formed first on cooling, followed by an exothermic conversion to the interdigitated state (Boggs and Rangaraj, 1985). The relative enthalpies contributed by these two exothermic peaks observed on cooling, and their separation in temperature, are highly variable with chain length. For the shorter chain lengths, diC12:0-PC to diC14:0-PC, an additional small endotherm is observed at higher temperature. The intensity of this additional endotherm was variable, not always being as pronounced as the examples given in Fig. 1. The enthalpy of the major endotherm did not vary appreciably, however, with varying proportions of the minor endotherm. It is possible, therefore, that the

TABLE 1 Difference in the outer hyperfine splitting values, $\Delta 2A_{max}$, for phosphatidylcholine spin labels bearing the nitroxide probe near the headgroup and near the end of the acyl chains in the dispersions of DiCn:0-PCs in glycerol and in water

		In glycerol Δ $2A_{max}$ (gauss)		In water Δ $2A_{max}$ (gauss)	
Lipid	Temperature (°C)	5-PCSL-12-/14-PCSL*	5-PCSL-16-PCSL	5-PCSL-12-/14-PCSL*	5-PCSL-16-PCSL
DiC12:0-PC	−10	2.6		2.6	
	0	5.2		5.8	
	5	5.1		7.8	
DiC13:0-PC	−10	0.9		3.8	
	0	1.1		4.1	
	5	1.3			
	10	2.4		6.0	
DiC14:0-PC	0	0.4		4.2	
	10	0.4		4.4	
	15			4.2	
	20	1.0			
DiC15:0-PC	0	0.3		3.8	
	10	0.5		4.5	
	20	0.4		2.0	
	30	1.3		18.1	
DiC16:0-PC	0	0.5	−0.1	3.5	4.8
	10	0.6	0.1	4.2	11.7
	20	0.7	0.9	6.5	19.1
	30	1.1	1.6	18.6	19.8
	40	1.0	2.9	22.0	17.0
DiC17:0-PC	0	0.7	−0.2	4.1	4.7
	10		0.3	6.0	3.1
	20	1.5	1.0	10.5	20.6
	30	1.9	1.7	17.0	20.0
	40	2.4	3.0	20.6	22.6
DiC18:0-PC	0	0.8	−0.3	4.4	7.0
	10	1.1	0.4	6.6	19.0
	20	1.9	0.8	11.8	22.0
	30	2.2	1.3	15.4	20.9
	40	3.1	2.5	17.6	20.6
DiC19:0-PC	0	0.4	0.4	3.3	6.6
	10	1.0	0.9	5.2	5.8
	20	1.8	1.5	8.4	25.1
	30	2.1	1.8	14.2	17.7
	40	3.2	3.3	13.5	13.7
DiC20:0-PC	0	0.3	0.0	4.3	8.9
	10	2.3	1.5	5.4	18.8
	20	3.4	1.4	8.4	21.5
	30		1.9	11.6	20.3
	40	6.9	2.1	20.4	22.4
	50	9.8	4.0	16.3	18.7

*The spin label used is 12-PCSL for DiC12:0 PC and DiC13:0 PC and 14-PCSL for all other lipids.

minor endotherm may correspond to the chain-melting of a small proportion of the sample that is in a crystalline lamellar state and is strongly overrepresented in the calorigrams because of its much higher intrinsic transition enthalpy. The total enthalpy on cooling is found to be equal to that of the main endotherm observed on heating.

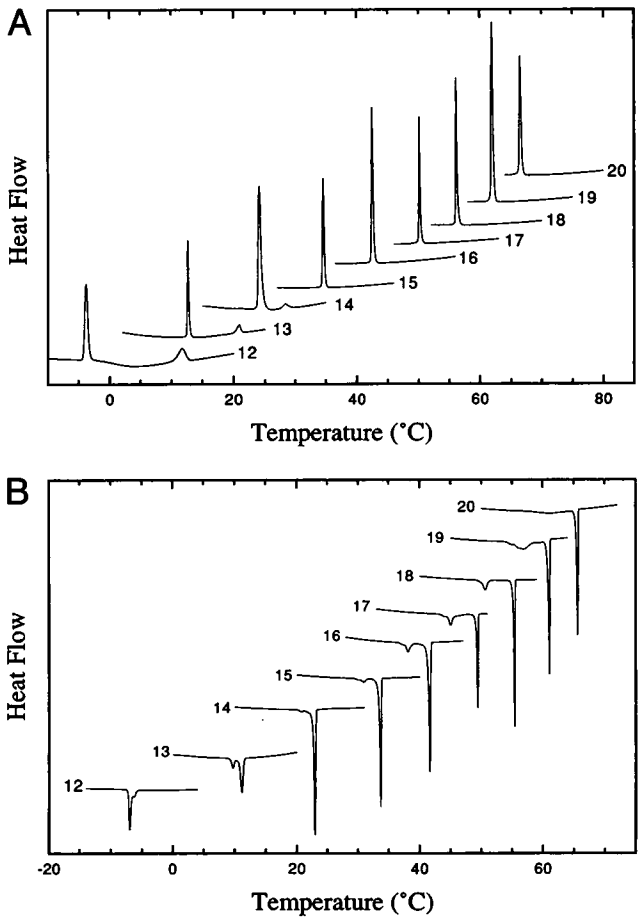

FIGURE 2 DSC of saturated diacyl phosphatidylcholines of the chain lengths Cn:0 indicated, dispersed in glycerol. Scan rate = 5°/h. A, endotherms from heating scans; B, exotherms from cooling scans.

The chain-length dependence of the calorimetric enthalpy, $\Delta H_{t,i}$, and entropy, $\Delta S_{t,i}$, for diacyl phosphatidylcholines dispersed in glycerol is given in Fig. 3. (Here the subscript t is taken to indicate the transition between fluid and (stable) gel phases, and the additional subscript i = w (water) or G (glycerol) indicates the medium in which the lipids are dispersed.) In calculating the transition entropy, a first-order transition is assumed (i.e., $\Delta G_{t,i} = 0$) so that $\Delta S_{t,i} = \Delta H_{t,i}/T_{t,i}$, where $T_{t,i}$ is the transition temperature. No systematic or significant difference in these quantities was found between heating and cooling scans when the total enthalpy (i.e., of all exotherms) was taken for the cooling scan. The values reported are the means of those for heating (taken from the single main endotherm) and cooling (taken from the multiple exotherms). It is seen from Fig. 3 that with the exception of diC19:0-PC and diC20:0-PC, the transition enthalpy and entropy depend linearly on lipid chain length, n, of the diCn:0-PCs:

$$\Delta H_{t,i} = \Delta H_{inc,i} \cdot n + \Delta H_{o,i} \quad (1)$$

$$\Delta S_{t,i} = \Delta S_{inc,i} \cdot n + \Delta S_{o,i} \quad (2)$$

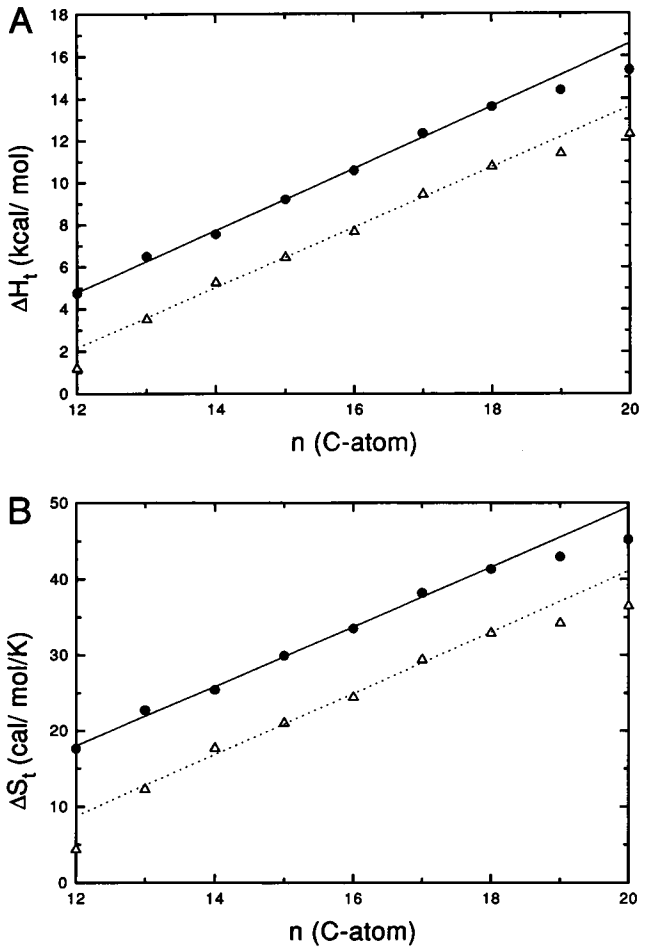

FIGURE 3 Chain-length dependence of (A) the transition enthalpy, ΔH_t, (*upper*) and (B) the transition entropy, ΔS_t, (*lower*) for saturated diacyl phosphatidylcholines dispersed in glycerol (●); corresponding data are given for dispersions in water (△). The straight lines are linear regressions, omitting the data points from diC19:0-PC and diC20:0-PC, and also that from diC12:0-PC for aqueous dispersions.

The fact that the transition enthalpy and transition entropy for diC12:0-PC are consistent with this linear dependence suggests strongly that the gel phase for this lipid is also interdigitated, although the spin label ESR results were not decisive on this point. From a linear regression omitting diC19:0-PC and diC20:0-PC, the incremental enthalpy and entropy are $\Delta H_{inc,G} = 1.48 \pm 0.03$ kcal·mol^{-1}CH$_2$$^{-1}$ and $\Delta S_{inc,G} = 3.93 \pm 0.10$ cal·mol^{-1}·K^{-1}·CH$_2$$^{-1}$, respectively, and the end contributions are $\Delta H_{o,G} = -12.9 \pm 0.5$ kcal·mol^{-1} and $\Delta S_{o,G} = -29.2 \pm 1.5$ cal·mol^{-1}K^{-1}. For comparison, the corresponding data for the phosphatidylcholines dispersed in water are also given in Fig. 3. It is seen that the transition enthalpies and entropies are all consistently higher for the lipids dispersed in glycerol than for those dispersed in water (cf. Boggs and Rangaraj, 1985). More significantly, the incremental values with increasing chain length are similar in the two cases, implying that the (negative) end contributions are smaller for the dispersions in glycerol. The corresponding values in water obtained by linear regression over the range n = 13–18 are $\Delta H_{inc,w} = 1.43 \pm 0.04$ kcal·mol^{-1}CH$_2$$^{-1}$, $\Delta S_{inc,w} = 4.04 \pm 0.14$ cal·mol^{-1}CH$_2$$^{-1}$ and $\Delta H_{o,w} = -15.0 \pm 0.6$ kcal·mol^{-1}, $\Delta S_{o,w} = -39.8 \pm 2.2$ cal·mol^{-1}CH$_2$$^{-1}$.

DISCUSSION

A comparative discussion of the data for dispersions in glycerol with those for dispersions in water can be divided into a comparison of the incremental calorimetric values and a comparison of the end contributions to the calorimetric values. These two aspects are considered separately below (where the main interest centers on the different end contributions), followed by a treatment of the transition temperatures. In principle, the incremental as well as the end contributions can be influenced both by the different structures of the two gel phases, interdigitated and noninterdigitated, and by the different interfacial interactions with water and with glycerol. It must be emphasized, however, that the net calorimetric observables are determined by the differences in properties between the gel and fluid phases and not solely by the properties of one particular phase.

Incremental calorimetric contributions

The incremental value of the entropy, $\Delta S_{inc,i}$, is determined primarily by the increase in chain rotational isomerism at the chain-melting transition, whereas that of the enthalpy, $\Delta H_{inc,i}$, is determined by both chain isomerism and the van der Waals and steric interactions between the chains (e.g., Marsh, 1974). The latter are determined in turn by the chain- packing density, which will be influenced by both the structure of the phase and the interfacial interactions with the solvent. The comparable incremental enthalpy and entropy for dispersions in glycerol relative to those in water, i.e., $\Delta H_{inc,G} \approx \Delta H_{inc,w}$ and $\Delta S_{inc,G} \approx \Delta S_{inc,w}$, suggest therefore that the decreases in chain flexibility (and increases in chain packing) in the gel and fluid phases of the glycerol dispersions (cf. Bartucci et al., 1993) approximately balance one another. The net change on chain-melting is therefore similar to that for dispersions in water. Significantly, the incremental transition enthalpy and entropy of biotinylated phosphatidylethanolamines that form interdigitated gel phases have also been found to be similar to those of the parent phosphatidylethanolamines that form noninterdigitated gel phases (Swamy et al., 1994).

Calorimetric end contributions

In principle, there are several possible end contributions to the entropy and enthalpy of the chain-melting transition other than trivial effects involving the origin of counting the chain segments (cf. e.g., Meraldi and Schlitter, 1981). As mentioned above, these can be divided into influences arising primarily from differences in the structures of the two

gel phases and from differences in the interfacial interactions with the two suspension media, glycerol and water. For concreteness and to aid the discussion, a simple mathematical model is introduced in which the interfacial effects are given by a term of the form $\gamma_i^{\alpha}\Delta A_{t,i}$, which is linear in the interfacial area and in particular is directly proportional to the number of molecules solvating the surface. Here $\Delta A_{t,i}$ is the increase in area per lipid molecule at the chain-melting transition in medium i, and γ_i^{α} is the surface density of the interfacial contribution to the transition enthalpy ($\alpha \equiv H$) and to the transition entropy ($\alpha \equiv S$) in medium i. The difference in structure of the two gel phases is therefore incorporated in the values of the area change, $\Delta A_{t,i}$, and the difference in the interfacial interactions with the medium is incorporated in the enthalpic and entropic surface densities, γ_i^{α}, contributing to the interfacial and solvation free energy. The end contributions to the transition enthalpy and entropy are then given respectively by

$$\Delta H_{o,i} = \Delta H^{o}_{o,i} + \gamma_i^{H}\Delta A_{t,i} \quad (3)$$

$$\Delta S_{o,i} = \Delta S^{o}_{o,i} + \gamma_i^{S}\Delta A_{t,i} \quad (4)$$

where $\Delta H_{o,i}{}^{o}$ and $\Delta S_{o,i}{}^{o}$ are the end contributions to the transition enthalpy and entropy, respectively, that are not dependent on the interfacial area (e.g., to a first approximation from the lipid headgroups). The net differences in these two end contributions for dispersions in glycerol and water are then given by

$$\delta\Delta H_o = \Delta H^{o}_{o,G} - \Delta H^{o}_{o,w} + \gamma_G^{H}\Delta A_{t,G} - \gamma_w^{H}\Delta A_{t,w} \quad (5)$$

$$\delta\Delta S_o = \Delta S^{0}_{o,G} - \Delta S^{o}_{o,w} + \gamma_G^{S}\Delta A_{t,G} - \gamma_w^{S}\Delta A_{t,w} \quad (6)$$

with the definitions: $\delta\Delta H_o \equiv \Delta H_{o,G} - \Delta H_{o,w}$ and $\delta\Delta S_o \equiv \Delta S_{o,G} - \Delta S_{o,w}$.

Contributions to the area-independent term, $\Delta S_{o,i}{}^{o}$, in Eq. 4 for the transition entropy could come from a decrease in ordering of the lipid headgroups and also of the chain ends, if the latter is different from the incremental value established for the main part of the chain. Solid state nuclear magnetic resonance experiments have established that the motional freedom of the lipid headgroups is still high in the gel phase. Therefore this is unlikely to make a large contribution, at least for the dependence on glycerol concentration. In considering the incremental entropies, it was concluded that the decreases in chain flexibility in the gel and fluid phases of dispersions in glycerol approximately compensate each other. It is therefore likely that the same is true for the contributions from flexibility of the chain ends to the values of $\Delta S_{o,i}{}^{o}$ for glycerol dispersions, compared with those in water. However, a most important contribution to the transition entropy $\Delta S_{o,i}$ comes from the ordering of the solvent molecules by the lipid headgroups and interfacial regions. It has been pointed out that this alone could account for the strongly negative value of $\Delta S_{o,w}$ for bilayers in water (Seelig, 1981), in agreement with the finding that the transition entropy decreases with increasing hydration (Cevc and Marsh, 1985). This ordering of the solvent will contribute both to the area-independent term, $\Delta S_{o,i}{}^{o}$, and to the area-dependent term, $\gamma_i^{S}\Delta A_{t,i}$, in Eq. 4. For the reasons advanced in discussion of the transition entropy, it is likely that conformational changes in the lipid headgroups and in the chain ends also do not give sizeable contributions to the area-independent differences in transition enthalpy $\Delta H_{o,i}{}^{o}$ in Eq. 3 for dispersions in glycerol relative to those in water. Steric interactions between the chain ends probably are also not significant because of the freedom in adjustment of packing allowed at the bilayer center and will make at most only small contributions to $\Delta H_{o,i}{}^{o}$. Again, however, headgroup and interfacial solvation would be expected to contribute strongly to the values of $\Delta H_{o,i}{}^{o}$ and $\gamma_i^{H}\Delta A_{t,i}$, respectively, in Eq. 3. In addition, interactions between the lipid headgroups may also make significant contributions to the transition enthalpy, primarily to the area-dependent term $\gamma_i^{H}\Delta A_{t,i}$, in Eq. 3.

The smaller (negative) end contributions to the calorimetric enthalpy and entropy for the dispersions in glycerol can therefore be attributed to the different interfacial structure of the interdigitated gel phase and to the different interfacial solvation in glycerol from that in water. From Eqs. 5 and 6 it can be seen that the resulting values of $\delta\Delta H_o$ and $\delta\Delta S_o$ will depend on the relative sizes of the terms $\Delta H_{o,G}{}^{o}$ and $\Delta H_{o,w}{}^{o}$, and correspondingly $\Delta S_{o,G}{}^{o}$ and $\Delta S_{o,w}{}^{o}$, as well as especially on the relative sizes of $\gamma_G{}^{\alpha}$ and $\gamma_w{}^{\alpha}$ and of $\Delta A_{t,G}$ and $\Delta A_{t,w}$. The phosphatidylcholine headgroups are more closely packed in the fluid phase than in the interdigitated gel phase (McDaniel et al., 1983), which is the opposite situation from that which obtains with a normal gel phase. From this it follows immediately that $\Delta A_{t,w} > 0 > \Delta A_{t,G}$ and therefore that the area-dependent contributions to both the transition enthalpy and the transition entropy will tend to be smaller in glycerol than in water because of the difference in gel-phase structures (cf. Eqs. 5 and 6). Most importantly, the number of interfacial solvating molecules will tend to decrease at the chain-melting transition for dispersions in glycerol, whereas it will increase for dispersions in water. Also, the repulsive interactions between the lipid headgroups probably will increase at the chain-melting transition in glycerol but will decrease in water. As regards the transition enthalpy, it is likely that the (negative) solvation energies will be greater in water than in glycerol, which means that both $\Delta H_{o,}{}^{o} < \Delta H_{o,G}{}^{o} < 0$ and $\gamma_w{}^{H} < \gamma_G{}^{H} < 0$ (when allowance is made for the negative sign). The net result is that the transition enthalpy is increased positively in glycerol relative to water (cf. Eq. 5), consistent with the experimentally observed value of $\delta\Delta H_o = +2.1$ kcal·mol^{-1}. As regards the transition entropy, the degree of ordering of the individual solvent molecules at the bilayer surface is expected to be intrinsically smaller in glycerol than in water, i.e., $\Delta S_{o,w}{}^{o} < \Delta S_{o,G}{}^{o} < 0$ (allowing for the negative sign associated with ordering). In particular, the hydrophobic effect at the exposed lipid chains will be weaker in glycerol than in water (i.e., $\gamma_w{}^{S} < \gamma_G{}^{S} < 0$), as evidenced, for instance, by the greater solubility of glycerol than of water in oil. This means that the net entropy will be

decreased less, i.e., that the transition entropy will be greater for dispersions in glycerol than in water (cf. Eq. 6), as is observed, leading to a net difference of $\delta\Delta S_o = +11$ cal·mol^{-1}·K^{-1}. Indeed, the weaker, energetically positive hydrophobic interaction (i.e., free energy: $G_{phob,i} = -T\gamma_i^S A_i$, with $0 > \gamma_G^S > \gamma_w^S$) is the basis for the stability of the interdigitated gel phase in glycerol, the formation of which is then driven by the steric repulsions between the bulky phosphatidylcholine headgroups. This therefore constitutes a direct connection between the present measurements and the energetics of assembly of phospholipids in biological membranes generally.

Transition temperatures

Although the chain-melting transition temperatures for dispersions in glycerol are comparable with those in water (rather surprisingly for such different gel-phase structures), it might be expected that the differences found in the enthalpies and entropies could be expressed in the dependence of transition temperature on chain length. The chain-length dependences of the transition temperatures measured from the heat capacity maxima in the DSC heating scans are given in Fig. 4. These have been fitted to the expression (Marsh, 1991)

$$T_t = T_t^\infty[1 - (n_o - n_o')/(n - n_o')] \quad (7)$$

where n_o and n_o' are the chain lengths at which the transition enthalpy and entropy, respectively, extrapolate to zero, and T_t^∞ ($=\Delta H_{inc}/\Delta S_{inc}$) is the transition temperature extrapolated to infinite chain length. The values obtained by nonlinear least-squares fitting to Eq. 3 are $n_o = 6.56$ (6.43), $n_o' = 3.67$ (3.62), and $T_t^\infty = 412.5$ (407.4 K) with χ^2 of 0.2 K (0.4 K) for dispersions in glycerol (water). To within the sensitivity of the fits, these values do not differ significantly between dispersions in glycerol and in water, and the direct calorimetric data must be relied on for a sensitive comparison.

Because the transition temperatures are almost equal in glycerol and in water on the absolute temperature scale and because the incremental calorimetric quantities, $\Delta H_{inc,i}$ and $\Delta S_{inc,i}$, are also approximately equal in glycerol and in water, it is possible to derive a relationship between the quantities in Eqs. 3 and 4. Assuming a first-order transition, the transition temperature is given by (cf. Cevc and Marsh, 1987)

$$T_{t,i} = \frac{n \cdot \Delta H_{inc,i} + \Delta H_{o,i}}{n \cdot \Delta S_{inc,i} + \Delta S_{o,i}} \quad (8)$$

where for bilayers in glycerol and in water: $\Delta H_{inc,G} \approx \Delta H_{inc,w}$ and $\Delta S_{inc,G} \approx \Delta S_{inc,w}$, but $\Delta H_{o,G} \neq \Delta H_{o,w}$ and $\Delta S_{o,G} \neq \Delta S_{o,w}$. Therefore, from the experimental finding that $T_{t,G} \approx T_{t,w}$, the following relation ensues:

$$\delta\Delta H_o \approx T_t^\infty \delta\Delta S_o \quad (9)$$

where $T_t^\infty \equiv \Delta H_{inc}/\Delta S_{inc}$ is a constant ($\approx$365 K) and $\delta\Delta H_o$ and $\delta\Delta S_o$ are defined in Eqs. 5 and 6, respectively. This implies an approximate entropy-enthalpy compensation for the differences between dispersions in glycerol and water at a temperature $T \approx T_t^\infty$ and more importantly that both entropy and enthalpy differences should have the same sign, as is found. Expanding Eq. 9 by using Eqs. 5 and 6 yields the further condition:

$$\delta\Delta G_o^o(T_t^\infty) \approx -\gamma_G(T_t^\infty) \cdot \Delta A_{t,G} + \gamma_w(T_t^\infty) \cdot \Delta A_{t,w} \quad (10)$$

where $\Delta G_{o,i}^o(T) \equiv \Delta H_{o,i}^o - T \cdot \Delta S_{o,i}^o$ and $\gamma_i(T) \equiv \gamma_i^H - T \cdot \gamma_i^S$. This is the relation between the structural differences ($\Delta A_{t,i}$) and the interfacial free energy differences ($\delta\Delta G_o^o$, γ_i) that must be fulfilled if the dispersions with normal and interdigitated gel phases are to have approximately the same chain-melting transition temperature.

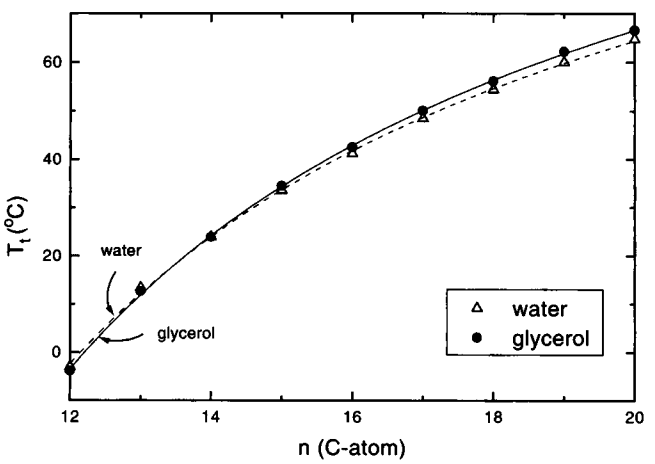

FIGURE 4 Chain-length dependence of the chain-melting transition temperature for saturated diacyl phosphatidylcholines dispersed in glycerol (●) and in water (Δ). Lines are nonlinear least-squares fits of the data from dispersions in glycerol (——) and in water (- - - -) to Eq. 7.

Conclusions

The present data on the calorimetric enthalpies and entropies therefore provide information on the stability of the interdigitated gel phase in glycerol and the parameters that are necessary for any detailed description of its thermodynamics. This relates directly to the interfacial solvation and hydrophobic interaction that are the controlling features of biomembrane assembly.

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