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THE INFLUENCE OF DIOXAN ON THE BILAYER AND MONOLAYER PHASE TRANSITION OF PHOSPHOLIPIDS

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The influence of 1.4.-dioxan on the bilayer phase transition of various phospholipids was studied by differential scanning calorimetry and turbidity measurements. The addition of 1.4.-dioxan to lipid bilayers decreases the transition temperature T_m but increases the transition enthalpy of the transition. The cooperativity of the transition is unaffected. The phospholipid monolayer transition from the liquid-condensed to the liquid-expanded phase was measured by recording area versus temperature curves at constant surface pressure (isobars). The monolayer transition temperature at constant surface pressure is increased when 1.4.-dioxan is added to the subphase. The change in molecular area becomes larger. A comparison of monolayer isobars on water and water/dioxan as subphase at constant surface tension rather than surface pressure leads to a decrease of the transition temperature on water/dioxan as subphase. This decrease as well as the larger change in molecular area at the monolayer transition can be correlated to the decrease in T_m and the increase in the transition enthalpy of the corresponding bilayer system. 1.4.-Dioxan seems to accumulate at the lipid head group/water interface, thus lowering the tension of the bilayer membrane. This cyclic ether can be used for altering the characteristics of bilayer membranes without disturbing the lipid chain organization.

1. Introduction

The influence of organic molecules on the properties of phospholipid bilayers is a subject of increasing interest [1–11]. Especially the interaction of anaesthetics and drugs acting on the central nervous system with phospholipid bilayers has been investigated by several workers [2–5,8]. The effects observed with these molecules are due to the fact that they are incorporated into the phospholipid bilayer, thus perturbing the organization of the lipid acyl chains. The effects observed after the addition of alkanols show a strong dependence on the chain length of the alcohol [1,5,6,8]. Whereas short chain alcohols tend to lower the transition temperature of the lipid phase transition, the effect is reversed when the length of the alcohol approaches the chain length of the fatty acid chains of the phospholipid under investigation. Similar effects can be observed with long chain n-monocarboxylic acids [6]. The concentration of alcohol needed to produce a certain degree of shift of the phase transition temperature T_m depends on membrane/water partition coefficient of the alcohol [10]. These partition coefficients, on the other hand, depend on the state of the lipid, whether it is in the gel of the liquid-crystalline state, and on the chain length of the respective alcohol. Short alcohols like propanol or butanol have very low partition coefficients, so that high alcohol concentrations are needed to shift T_m to lower values [11]. The phospholipid/water partition coefficients are usually lower than those measured for the partitioning between bulk organic liquids and water, due to the anisotropic nature of the lipid bilayer. In summary, one can say, that the effects exerted by these molecules are related to their degree of hydrophobicity.

A different class of organic molecules are those which are essentially hydrophilic, like ethylene glycol, glycerol or sugar molecules. These substances are not incorporated into the bilayer. Therefore they usually have only very little effect on the phase transition of lipids. Thus, ethylene glycol/water mixtures can be used to record the phase transition of phospholipids which have T_m-values below 0°C without changing the transition characteristics. The hydrophilic character of these molecules manifests itself in the small decrease of the surface tension and of the dielectric constant in mixtures with water [12].

Organic molecules which show an intermediate behaviour between "hydrophobic" and "hydrophilic" molecules are cyclic ethers like ethylene oxide, tetrahydrofuran or dioxan. These compounds are completely miscible with water due to their polar nature. Various studies showed, that among cyclic ethers 1.4.-dioxan is quite unique in nature. It could be shown by nmr-spectroscopy, calorimetry and dilatometry, that 1.4.-dioxan has a structure promoting and stabilizing effect on the water molecules [13–16]. In this study we want to describe the effect of 1.4.-dioxan on the monolayer and bilayer phase transition of several phospholipids with different polar head groups.

2. Materials and methods

1.2.-Dipalmitoyl-sn-glycero-3-phosphorylcholine (DPPC), 1.2.-dimyristoyl-sn-glycero-3-phosphorylethanolamine (DMPE), and 1.2.-dipalmitoyl-sn-glycero-3-phosphorylethanolamine (DPPE) were purchased from FLUKA (Neu-Ulm). The purity of these compounds was checked by thin layer chromatography. 1.2.-dimyristoyl-sn-glycero-3-phosphatidic acid was a gift from Dr. H. Eibl (Max-Planck-Institut für biophysikalische Chemie, Göttingen). 1.4.-dioxan (p.A. grade) was purchased from Merck (Darmstadt).

Differential scanning calorimetry was performed as described before using the differential scanning calorimeter developed by Grubert [17,18]. Some of the calorimetric measurements were performed with the Privalov calorimeter [19], which has a sample volume of 1 ml. The lipid concentration for calorimetry was in the range from 0.3 to 1.5 mM. The transition heats were determined by measuring the area under the excess heat versus temperature curve by paper weighing.

Turbidity measurements were made using a Hitachi-Perkin-Elmer Model 124 UV-spectrophotometer equipped with a thermostatted cell holder. Temperature in the sample was varied by means of a water thermostat (Lauda K2R kryothermostat, Lauda). Turbidity at 400 nm versus temperature curves were recorded with a Hewlett-Packard 2FAM x-y-recorder. The temperature inside the sample was measured with a digital thermometer with a diode as temperature sensor (developed by M. Grubert). The thermometer has an analogue output for driving the x-axis of the recorder.

Isobars of phospholipid monolayers were recorded using a commercial Langmuir film balance (Messgerätewerk Dr. Wobser, Lauda) equipped with an automatic pressure control device for keeping the film pressure at a constant value. The isobars were recorded at increasing temperature. The heating rate was approx. 2 degrees per minute. The surface pressure of the monolayer during each run was kept constant to ca. ±2 dyne/cm. Each experiment was performed with a freshly prepared film. At least three different measurements were made for each selected value of the surface pressure. The monolayers were prepared by applying a solution of the desired phospholipid in dichloromethane (p.A. grade, Merck, Darmstadt, distilled three times before use) to the subphase using a microliter syringe equipped with a micrometer.

3. Results and discussion

3.1. Bilayers

The dependence of the transition temperature T_m upon the dioxan content of the dispersion was measured by recording the turbidity and by differential

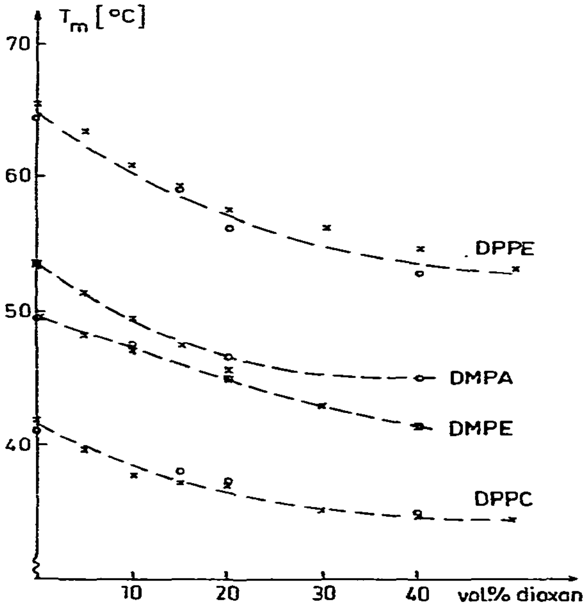

Fig. 1. Transition temperature T_m of the bilayer transition as a function of 1.4.-dioxan content; determined by: o: differential scanning calorimetry; X: turbidity measurements.

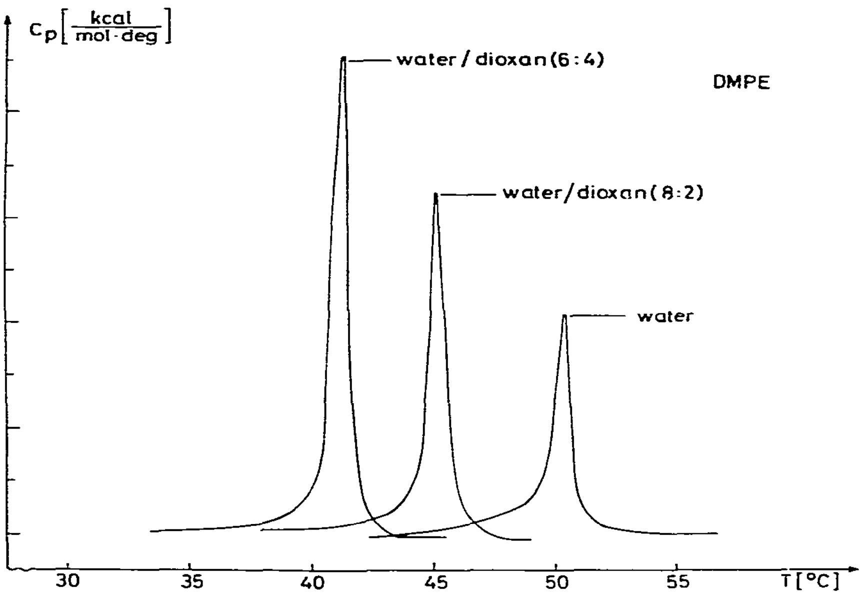

Fig. 2. Differential scanning calorimetry curves of DMPE dispersions in water and water/dioxan mixtures (v : v).

scanning calorimetry. Both methods gave similar results for the decrease of the transition temperature T_m with increasing dioxan content. The results of these measurements are shown in fig. 1, where the transition temperature T_m of four different phospholipids is plotted versus the dioxan content of the dispersion. Fig. 2 shows calorimetric scans of DMPE dispersions in water and in water/dioxan mixtures. The peak heights in the excess heat versus temperature curves increase with increasing dioxan content and the temperature interval of the transition becomes smaller. From the normalized transition curves, which were evaluated by integration of the excess heat versus temperature curves, the van't Hoff transition enthalpy $\Delta H_{\text{van't Hoff}}$ can be determined according to the equation $\Delta H_{\text{van't Hoff}} = 4RT_m^2(\mathrm{d}\theta/\mathrm{d}T)_{\theta=1/2}$. The quotient $\Delta H_{\text{van't Hoff}}/\Delta H_{\text{cal}} = n$ gives an estimate for the size of the cooperative unit involved in the transition. With increasing dioxan content n decreases only very slightly, which means that the cooperativity of the transition is almost unaffected by the addition of dioxan up to a content of 50 vol.%. ΔH_{cal} increases in the same manner as $\Delta H_{\text{van't Hoff}}$, thus leading to the constancy of the size of the cooperative unit n. Table 1 summarizes the values for T_m, ΔH_{cal}, and $\Delta S_{\text{cal}} = \Delta H_{\text{cal}}/T_m$ as determined by calorimetry.

3.2. Monolayers

To find out whether the increase in ΔH_{cal} with higher dioxan content is due to larger changes in molecular area at the transition, we investigated the monolayer phase transition of two phospholipids on water and water/dioxan mixtures as subphase by continuously recording isobars at different surface pressures. From these isobars the area change Δf at the monolayer phase transition from the liquid-condensed to the liquid-expanded phase can easily be evaluated. Figs. 3a and 3b show isobars for DPPC and DMPE monolayers at different surface pressures with water and water/dioxan (9 : 1) as subphase. From these curves the transition temperature T_m and the values for the area change Δf were determined and summarized in table 2. For a constant surface pressure the transition is shifted to higher temperature when the subphase contains dioxan. The values for the temperature dependence of the equilibrium pressure depend on the nature of the polar head group of the phospholipid. For DPPC $\mathrm{d}\pi/\mathrm{d}T_m$ amounts to 1.89 dyne cm^{-1} degree^{-1} and for DMPE a value of 1.20 dyne cm^{-1} degree^{-1} can be evaluated when the subphase is pure water. Addition of dioxan to the subphase increases this value to 2.6 dyne cm^{-1} degree^{-1}

Table 1
Transition temperature T_m, transition enthalpy ΔH, and transition entropy ΔS for the bilayer phase transition of phospholipids in water and water/dioxan mixtures.

	water/dioxan ratio (v : v)	T_m (°C)	ΔH (kcal mol^{-1})	ΔS (cal mol^{-1} deg^{-1})
DPPC	10 : 0	41.0	8.3	26.4
	8 : 2	37.4	11.4	36.7
	6 : 4	35.0	12.0	38.9
DMPE	10 : 0	49.6	6.4	19.8
	8 : 2	45.0	9.1	28.6
	6 : 4	41.4	11.0	35.0
DPPE	10 : 0	64.3	8.2	24.3
	8 : 2	56.0	9.4	28.4
	6 : 4	52.7	10.9	33.5
DMPA	10 : 0	53.5	5.6	17.2
	8 : 2	46.5	8.8	27.5
	6 : 4	44.9	10.4	32.7

for DPPC, whereas for DMPE it remains unchanged. The area change at the monolayer phase transition depends on the surface pressure and on the nature of the polar head group (see table 2). For DMPE with its intermolecular linkages via hydrogen bonds between the head groups the dependence of Δf on the surface pressure is less than for DPPC with its bulkier head group. For both lipids addition of dioxan to the subphase leads to a stronger dependence of Δf on the surface pressure, so that at lower values for π the area change Δf at the transition is considerably larger on water/dioxan as subphase (see figs. 3a and 3b and table 2).

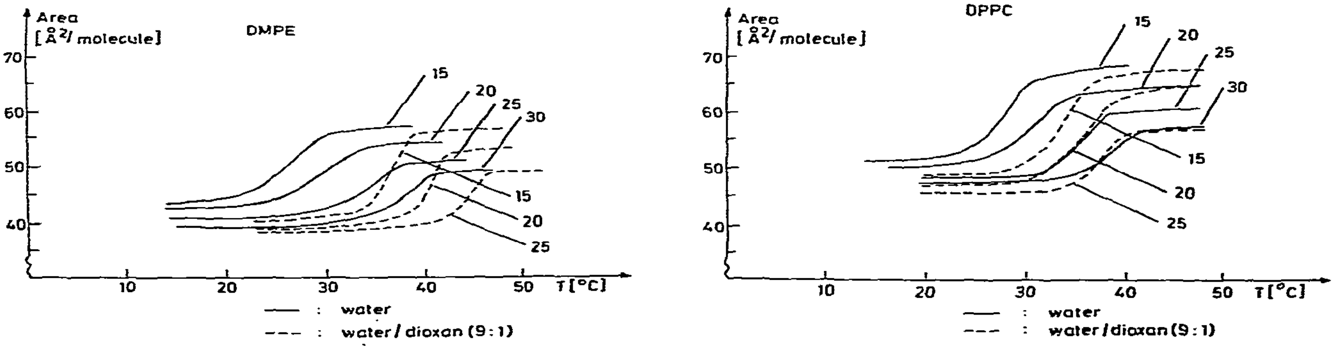

Fig. 3. Continuously recorded isobars on water (——) or water/dioxan (9 : 1) (— — — —) as subphase for a) DPPC-monolayers, b) DMPE-monolayers. The numbers designate the surface pressure of the monolayer in dyne/cm.

Table 2
Transition temperature T_m and area change Δf for the monolayer phase transition of phospholipids on water and water/dioxan (9 : 1) as subphase

	π (dyne/cm)	γ (dyne/cm)		T_m (°C)		Δf (Å^2/molecule)	
		water (25°C)	water/dioxan (25°C)	water	water/dioxan (9 : 1)	water	water/dioxan (9 : 1)
DPPC	15	57	42.5	28.0	33.5	15.0	17.6
	20	52	37.5	30.8	35.5	13.0	16.3
	25	47	32.5	35.2	37.3	11.5	11.2
	30	42	27.5	37.5		9.0	
DMPE	15	57	42.5	26.5	36.4	12.1	15.7
	20	52	37.5	29.5	40.5	10.3	13.3
	25	47	32.5	34.4	44.4	9.1	9.4
	30	42	27.5	37.0		8.6	

4. Discussion

4.1. Bilayers

The lowering of the bilayer phase transition temperature T_m upon addition of dioxan to the subphase cannot be explained by the assumption that the dioxan molecules are incorporated into the bilayer, thus disturbing the organization of the lipid chains and lowering T_m. As judged from the almost unchanged cooperativity of the phase transition the dioxan molecules are mainly restricted to the aqueous phase. It can be assumed that the bilayer/water partition coefficient for dioxan is very low, so that only at very high dioxan concentrations these molecules are incorporated into the bilayer to a notable extent. Similar effects, i.e., that T_m is only slightly lowered without affecting the width of the transition, are observed for short chain alcohols, which also have very low bilayer/water partition coefficients, like propanol and butanol [8,10]. These more polar molecules probably act on the head group/water interface of the lipid bilayer, i.e. are situated between the polar head groups, whereas longer chain alcohols or larger hydrophobic molecules like local anaesthetics which increase the width of the phase transition, are incorporated between the lipid chains, thus perturbing the chain organization.

Several possibilities of the action of short chain alcohols or dioxan have to be discussed, when the effect of the molecules on the transition temperature are to be explained. One possibility is that these substances act on the specific intermolecular organization of the polar groups of the respective phospholipid. The transition temperatures of phospholipids do not only depend on the chain length of the fatty acid chains but also on the chemical structure of the head group and its ability to form intermolecular hydrogen bond networks as it was shown for phosphatidyl-ethanolamines and is probably also true for phosphatidic acids [20–22]. One would expect now the solute molecules to have a pronounced influence on the extent of polar head group hydration and intermolecular interactions via hydrogen bonds. Thus phospholipids with different polar groups should be influenced according to their specific hydration and ability to form hydrogen bonds. It was found that the transition temperature of all three classes of phospholipids was decreased by the addition of dioxan. However, the phase transition temperature of DPPC with its strongly hydrated head group and no possibility for hydrogen bonding was decreased to a lesser extent ($\Delta T = 5$°C) than the transition temperatures of DPPE ($\Delta T = 11.7$°C), DMPE ($\Delta T = 8.2$°C), and DMPA ($\Delta T = 8.6$°C), i.e. those lipids with strong intermolecular hydrogen bonding. Thus changes in head group hydration and hydrogen bonding may indeed play a role in the observed phenomena. There are alternative possibilities which might be responsible for the lowering of the transition temperature. These are the decrease of the dielectric constant of the solution by the addition of dioxan, the lowering of the surface tension at the air/water interface, which would in turn influence the interfacial tension at the lipid head group/water interface, or a combination of both effects.

When the physical properties of the three compounds glycerol, ethanol, and dioxan are compared in this respect, one finds that glycerol decreases the dielectric constant as well as the surface tension only to a very small extent. Ethanol, on the other hand, decreases the surface tension very strongly, but has only minimal effects on the dielectric constant of the solution. Dioxan, however, decreases the dielectric constant as well as the surface tension very strongly [12]. This is shown in figs. 4a and 4b. We would assume that the lowering of the dielectric constant together with the changes in hydration and hydrogen bonding is the main reason for the decrease of the transition temperature. This seems to be confirmed by the observation that ethanol or glycerol, which change the dielectric constant and the water structure only slightly, have only little influence on the transition temperature [23]. The increase in the transition enthalpy ΔH, on the other hand, which is observed after the addition of dioxan and likewise ethanol (A. Blume, unpublished observations), may be due to a change in membrane tension as a consequence of the decrease of the surface tension at the air/water interface, because these molecules probably accumulate at the lipid head group/water interface. The increase in the transition enthalpy ΔH should in some way run parallel to a larger change in area at the phase transition, because the enthalpic effect at the phase transition arises from the increase in the distance between the hydrocarbon chains and the larger number of gauche conformations possible in the liquid-crystalline state. To test our hypothesis we measured the monolayer phase transition on water/dioxan as subphase. The monolayer transition should be influenced by dioxan in a similar manner as the bilayer transition, the advantage being that the area change Δf at the monolayer transition is a directly measurable quantity.

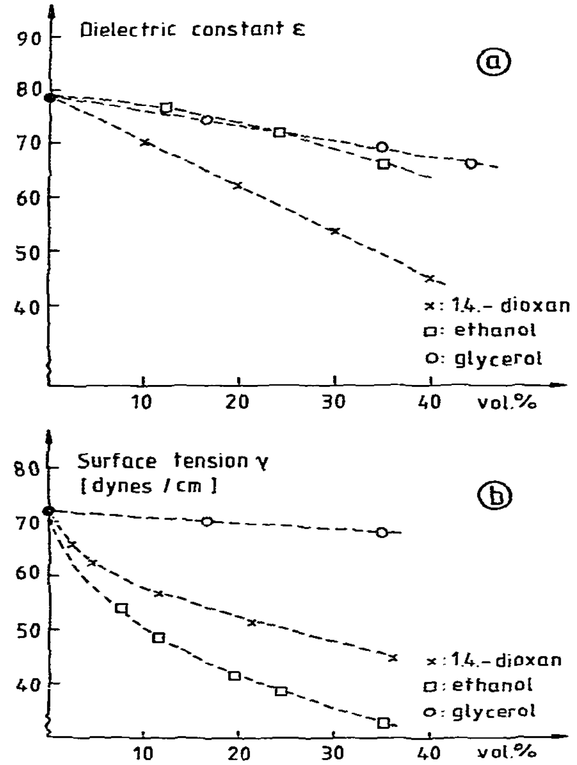

Fig. 4. The dependence of a) the dielectric constant and b) the surface tension at the air/water interface on the dioxan content ($T = 25°C$). Data taken from ref. [12].

4.2. Monolayers

When isobars for DPPC and DMPE on water and water/dioxan mixtures are compared at a constant value of the surface pressure π we find that the monolayer transition temperature is higher on water/dioxan as subphase. This result is in contrast to the finding for the bilayer system, where T_m decreases upon addition of dioxan. This seems to be a contradiction, but one has to take into account the following point. As already mentioned above, the surface tension at the air/water interface of the pure subphase without monolayer is drastically reduced when dioxan is added to the subphase. The surface tension γ of the subphase covered with a monolayer is defined by the expression $\gamma = \gamma_0 - \pi$, where γ_0 denotes the surface tension of the subphase without monolayer and π is the lateral surface pressure of the monolayer. When we compare monolayer isobars at a constant value of π, we in fact make a comparison at different surface tensions γ, because γ_0 is lowered due to the addition of dioxan. At 25°C, for instance, γ_0 changes from 72.14 dyne/cm for water to 57.5 dyne/cm for a water/dioxan mixture with 10 vol.% dioxan content [12]. In our opinion it seems more justified to compare both monolayers at approx. the same surface tension γ, or in other words surface free energy. Therefore, we have to compare monolayer isobars on water as subphase at a surface pressure π, which is ca. 15 dyne/cm higher than the surface pressure of the respective monolayer on water/dioxan (9 : 1) as subphase. For DPPC, for instance, we have to compare a monolayer isobar on water at a surface pressure of 30 dyne/cm with an isobar on water/dioxan at a surface pressure of 15 dyne/cm. This comparison indeed leads to a lower T_m-value for the monolayer transition on water/dioxan as subphase as would be expected from our results for the bilayer system (see fig. 3a and table 2). The same result is obtained for DMPE, only that in this case the shift in T_m is somewhat smaller than for DPPC (see table 2).

When this method of comparison is used the area change Δf at the monolayer phase transition is appreciably larger on water/dioxan as subphase than on pure water. For DPPC, for instance, we found for Δf a value of 9 Å^2/molecule on water as subphase when the surface pressure is adjusted to 30 dyne/cm ($\gamma = 42$ dyne/cm). On water/dioxan as subphase Δf increases to 17.6 Å^2/molecule at $\pi = 15$ dyne/cm, which is equivalent to $\gamma = 42$ dyne/cm. The area change at the transition thus becomes almost twice as large. Addition of dioxan, however, does not only increase the area change at the transition but also the absolute value of the molecular area in the liquid-condensed phase below the transition. The area for the liquid-condensed phase for a DPPC monolayer is ca. 1.5 Å^2/molecule larger on water/dioxan than on pure water as subphase when isobars at a surface tension γ of 42 dyne/cm are compared. For DMPE monolayers the effect of dioxan is similar, though not quite as large. Δf increases from 8.6 to 15.7 Å^2/molecule on water/dioxan and the area of the liquid-condensed phase is ca. 0.6 Å^2/molecule larger on water/dioxan as subphase (see figs. 3a and 3b and table 2).

Principally, the transition entropy or enthalpy for the monolayer transition can be calculated from the Clausius-Clapeyron-equation, because the values for $d\pi/dT_m$, T_m, and Δf are known from the experiments. A comparison with the calorimetrically determined bilayer transition enthalpies cannot be made, however, since for the calculation of the monolayer transition enthalpy only the area change Δf enters the Clausius-Clapeyron-equation, whereas for the bilayer system the change in volume is the relevant parameter. During the bilayer phase transition the change in area is partly compensated by a decrease in thickness of the bilayer, however, thus leading to lower transition entropies than would be calculated for the monolayer transition.

5. Conclusions

The cyclic ether 1.4.-dioxan has an intermediate behaviour between being purely "hydrophilic" or "hydrophobic". 1.4.-dioxan influences the phase transition of phospholipids in two ways: it decreases the phase transition temperature, while the phase transition enthalpy is increased. The cooperativity of the transition remains unaffected. The increase in transition enthalpy can be attributed to a larger change in molecular area at the phase transition. This could be verified by the investigation of the monolayer phase transition of the same phospholipids. When lipid monolayers at constant surface tension on water and on water/dioxan as subphase were compared, the change in molecular area at the monolayers transition was found to be almost twice as large on water/dioxan as subphase than on pure water. The decrease in the transition temperature T_m is probably due to a change of the hydration of the polar head groups of the phospholipids and to the decrease of the dielectric constant of the solution after the addition of dioxan. Dioxan and probably other cyclic ethers, too, tend to accumulate at the lipid head group-water interface, thus reducing the membrane tension. This in turn leads to the observed increase in the transition enthalpy. Therefore, these molecules can be useful for mediating the characteristic properties of bilayer membranes by increasing the molecular area of the phospholipid in the gel phase and to an even larger extent in the liquid-crystalline phase. The advantage of these substances is, that they are not incorporated into the lipid bilayers to a notable extent, so that the hydrocarbon chain interactions are not disturbed and the cooperativity of the phase transition remains high.

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