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A COMPARATIVE STUDY OF THE PHASE TRANSITIONS OF PHOSPHOLIPID BILAYERS AND MONOLAYERS

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Summary

Phase transitions in bilayers and monolayers of various synthetic phospholipids with different chain lengths as well as different polar head groups were studied by differential scanning calorimetry or with the film balance technique, respectively. With the film balance, area versus temperature curves (isobars) were recorded at different surface pressures. The monolayer phase transition from the fluid-condensed to the fluid-expanded phase is shifted towards higher temperature when the lateral pressure in the monolayer is increased. The temperature dependence of the equilibrium pressure as well as the magnitude of the area change at the transition depends only on the nature of the phospholipid head group and not on the chain length of the hydrocarbon chains of the lipid. Phospholipids with strong intermolecular attractive interactions between the head groups show low values for $d\pi/dT_m$ and for the area change, Δf, whereas phospholipids with negatively charged head groups without intermolecular attractive forces exhibit higher values for $d\pi/dT_m$ and Δf. The shift of the monolayer phase transition temperature when increasing the chain length of the lipid is almost identical to the shift in T_m observed for the bilayer system of the same phospholipids. A comparison of monolayer and bilayer systems on the basis of the absolute value of the molecular area of the phospholipid in the bilayer gel phase and the change in area at the bilayer and monolayer transition leads to the following conclusions. The behaviour of the bilayer system is very similar to that of the respective monolayer system at a lateral pressure of approx. 30 dyne/cm, because at this pressure the absolute area and the area change in both systems are the same. Further support for this conclusion comes from the experimental finding that at a lateral pressure of

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Abbreviations: DLPE, 1,2-dilauroyl-*sn*-glycero-3-phosphorylethanolamine; DMPE, 1,2-dimyristoyl-*sn*-glycero-3-phosphorylethanolamine; DMPC, 1,2-dimyristoyl-*sn*-glycero-3-phosphorylcholine; DPPC, 1,2-dipalmitoyl-*sn*-glycero-3-phosphorylcholine; DMPA, 1,2-dimyristoyl-*sn*-glycero-3-phosphatidic acid; DMPM, 1,2-dimyristoyl-*sn*-glycero-3-phosphorylmethanol.

30 dyne/cm the shift in T_m due to the increase in charge when the methyl ester of phosphatidic acid is investigated is the same for the bilayer and the monolayer system.

Introduction

The properties of phospholipid bilayers have been analyzed by numerous investigators in order to gain more information about the behaviour of biological membranes [1—5]. (For reviews, see Refs. 4 and 5 and references cited therein.) The phase transition of lipid bilayers is an example of a two-dimensional cooperative transition. It is normally induced by raising the temperature, but can also be caused by changing the ionic strength or the pH of the surrounding aqueous medium [6—8]. When the pressure of the dispersion is increased the transition is shifted to higher temperatures, indicating an increase in molecular volume during the transition [9,10]. The phase transition characteristics, such as temperature and enthalpy of the transition, are dependent on the chain length of the hydrocarbon chains, on the nature of the phospholipid head group and on the ionic strength of the surrounding medium.

Due to their amphiphilic structure, phospholipids also form two-dimensional monolayers on water surfaces. The isothermal properties of phospholipid monolayers have been studied by various groups during the past years [11—18]. Phospholipid monolayers are of special interest because they resemble half of the lipid bilayer of the lipid membrane model system. Experiments with phospholipid monolayers have the advantage that the molecular arrangement of the molecules can be controlled by changing the temperature and the molecular area or the surface pressure of the monolayer. The data obtained by the variation of these parameters give more information about lipid-lipid and lipid-subphase interactions than can be obtained from experiments with bilayer vesicles or liposomes. In order to transfer the information won by monolayer experiments to the bilayer system, it is important to establish those experimental conditions where bilayer and monolayer behaviour are similar.

The pressure vs. area curves (isotherms) of phospholipid monolayers at the air/water interface have slope discontinuities which are the beginning of a phase transition between a 'liquid-expanded' and to 'liquid-condensed' phase [12,16, 18]. This phase transition can be compared to the well-known bilayer phase transition of phospholipids from the liquid-crystalline to the so-called crystalline or gel phase at lower temperatures. In principle, the change in molecular area at the bilayer phase transition can be determined from X-ray data if the partial molar volume of the phospholipid in both phases is known. The determination of this parameter, however, is of low accuracy and hence values of the molecular area change calculated from X-ray data have large errors. Nevertheless, the change in molecular area can be estimated from the X-ray data available for the phospholipids DMPC and DPPC.

In the monolayer system molecular area and change in area at the transition are directly measurable quantities. For the determination of these area changes isotherms have to be recorded at different temperatures. It is more convenient, though, to measure area vs. temperature curves (isobars) at different surface

pressures, because not only can the area change be evaluated from these curves very easily, but also the temperature and width of the transition can be determined directly.

A comparison of the area change at the monolayer and the bilayer phase transition should lead to a value for the monolayer pressure, at which both systems have similar behaviour. From this value an estimation of the surface tension of the lipid-water interface of the bilayer system can be made.

In this paper we wish to present the results of monolayer experiments with various phospholipids with different polar head groups and chain lengths, for which the thermodynamic parameters of the bilayer phase transition were determined by calorimetric measurements. An attempt is made to establish similarities and differences between phospholipid monolayers and bilayers.

Materials and Methods

The calorimetric measurements were made using the adiabatic scanning calorimeter developed by M. Grubert, as already described [19,20], or the adiabatic scanning calorimeter designed by Privalov [21]. With the Privalov calorimeter, a heating rate of 1 deg. C per min was used. The transition enthalpies were determined by measuring the area under the excess heat vs. temperature curve by paper weighing. The molar transition enthalpies were calculated by determining the phospholipid concentration of the dispersions by phosphorus analysis.

The lipid dispersions for the measurements with the calorimeter developed by Grubert were prepared by sonication of approx. 25 mg of the respective phospholipid for 2 min in 20 ml of bidistilled water above the transition temperature of the lipid. The dispersion was then transferred to the calorimeter vessel and filled up to 25 ml with bidistilled water.

The dispersions for the measurements with the Privalov calorimeter were prepared similarly, except that the concentration was somewhat lower (0.5 mg/ml). The calorimeter vessel of this calorimeter holds 1 ml of dispersion. The values for the transition enthalpies obtained with these two different calorimeters deviated by only 5%, well within the normal deviations from the mean value.

Monolayer isotherms and isobars were measured using a commercial Langmuir film balance (Messgerätewerk Dr. Wobser, Lauda) equipped with a continuous measuring system. To minimize drifts of the zero position of the measuring system during the recording of the isobars due to temperature changes of the measuring bar, this bar had to be thermostatically controlled and therefore was equipped with a heating system. The drift in the zero position of the pressure measuring system could be kept below 2 dyne/cm in the temperature range 5—50°C.

To avoid evaporation of large amounts of water from the subphase, especially at temperatures above 40°C, the temperature of lid of the film balance was also thermostatically maintained, using a water thermostat (K2R, Messgerätewerk Dr. Wobser, Lauda). During each monolayer experiment the film balance was purged with ultrapure nitrogen.

For the recording of the isotherms the monolayers were compressed at a rate of approx. 3 $Å^2$/molecule per min. The temperature was kept constant

with the help of a water thermostate to within about ±0.5 deg. C. The temperature was measured using a digital thermometer with a diode as temperature-sensitive element, developed and built in our laboratory by M. Grubert. The thermometer has an analogue output for driving the x-axis of the x-y recorder (Hewlett-Packard 2FAM) when measuring isobars.

Isobars were always recorded with increasing temperature. The heating rate was 2 deg. C/min. The preselected surface pressure was kept constant to about ±2 dyne/cm using the automatic pressure control system of the film balance. Each experiment was performed with a freshly prepared film. At least three measurements were made for each selected value of the surface pressure. At low surface pressures the isobars were completely reproducible. Only at high surface pressures, when high temperatures had to be achieved for the recording of the phase transition, did the isobars vary due to film loss and surface pressure oscillations. In these cases mean values for the isobars were calculated from at least three to five different experiments.

DLPE, DMPE, DMPC, and DPPC were purchased from Fluka, Neu-Ulm. The purity of these phospholipids was checked by thin-layer chromatography. Batches which gave only one spot on the TLC plate were used without further purification. DPMA and DMPM were gifts from Dr. H. Eibl, Max-Planck-Institut für biophysikalische Chemie, Göttingen. These lipids were chromatographically pure.

The water used as subphase for the monolayer experiments was freshly distilled twice from a quartz still before use. Before applying the lipid solution to the subphase, spurious amounts of surface active contaminations were sucked off after moving the compression bar to a distance of approx. 2 mm from the measuring float. The pH of the subphase was adjusted with HCl or NaOH, respectively (grade 'Suprapur', Merck, Darmstadt). The phospholipids were applied to the subphase using a solution in dichloromethane with a lipid concentration of 0.2 mg/ml. Dichloromethane (p.A. grade, Merck, Darmstadt) was distilled three times before use.

Results

Bilayers. The bilayer phase transitions of the phospholipids used in this study have been measured by various workers, the exception being the lipid DMPM. Our phase transition data agree with those reported in the literature [4,47]. They are summarized in Table I.

Monolayers. The isotherms of the phospholipids DPPC and DMPE measured during our study were identical to those reported by other workers [12,13,17]. Recently, Albrecht et al. [18] reported isotherms and isobars for DMPA. Our results for the isotherms of DMPA on pure water (pH 5—6) as subphase are shown in Fig. 1a. At pH 5—6 the head group of DMPA has one negative charge [7,18,22]. The isotherms shown in Fig. 1a are nearly identical to those obtained by Albrecht et al. Fig. 1b shows the isotherms of another charged lipid, the methyl ester DMPM. At pH values above 8.5 this lipid has one negative charge per polar group, whereas at pH 5—6 it is half protonated (Eibl, H., personal communication). The isotherms for DMPM are smeared out to larger areas as compared to the isotherms of DMPA.

TABLE I

TRANSITION TEMPERATURE, TRANSITION ENTHALPY AND TRANSITION ENTROPY FOR THE BILAYER PHASE TRANSITION OF PHOSPHOLIPIDS

	DMPC	DPPC	DLPE	DMPE	DMPA (pH 5)	DMPM (pH 5)	DMPM (pH 10)
T_m (°C)	24.0	41.5	30.5	50.2	54.0	43.0	30.5
ΔH (kcal · mol^{-1})	6.3	8.3	4.0	6.1	5.5	5.6	5.7
ΔS (cal · mol^{-1} · deg^{-1})	21.1	26.4	13.0	18.9	16.8	17.7	18.8

TABLE II

TRANSITION ENTROPIES FOR PHOSPHOLIPID BILAYERS AND PHOSPHOLIPID MONOLAYERS AT DIFFERENT SURFACE PRESSURES OF THE MONOLAYER, INCLUDING VALUES FOR TEMPERATURE DEPENDENCE OF THE EQUILIBRIUM PRESSURE $d\pi/dT_m$

	DMPC	DPPC	DLPE	DMPE	DMPA (pH 5)	DMPM (pH 5)	DMPM (pH 10)	
$d\pi/dT_m$ (dynes · cm^{-1} · deg^{-1})	1.89	1.89	1.18	1.20	1.08	1.08	3.03	Surface pressure π (dynes · cm^{-1})
$\Delta S_{monolayer}$ (cal · mol^{-1} · deg^{-1})	37.0	38.6		19.8	17.6	28.8	98.6	15
	32.5	34.0	18.9	18.2	15.3	22.6	76.3	20
	28.8	30.4	16.5	16.1	11.7	17.9	63.6	25
	24.5	24.2	14.5	14.7	9.2	14.3	46.6	30
	21.7	20.4	12.4	13.3	8.6		36.1	35
$\Delta S_{bilayer}$ (cal · mol^{-1} · deg^{-1})	21.1	26.4	13.0	18.9	16.8	17.7	18.8	

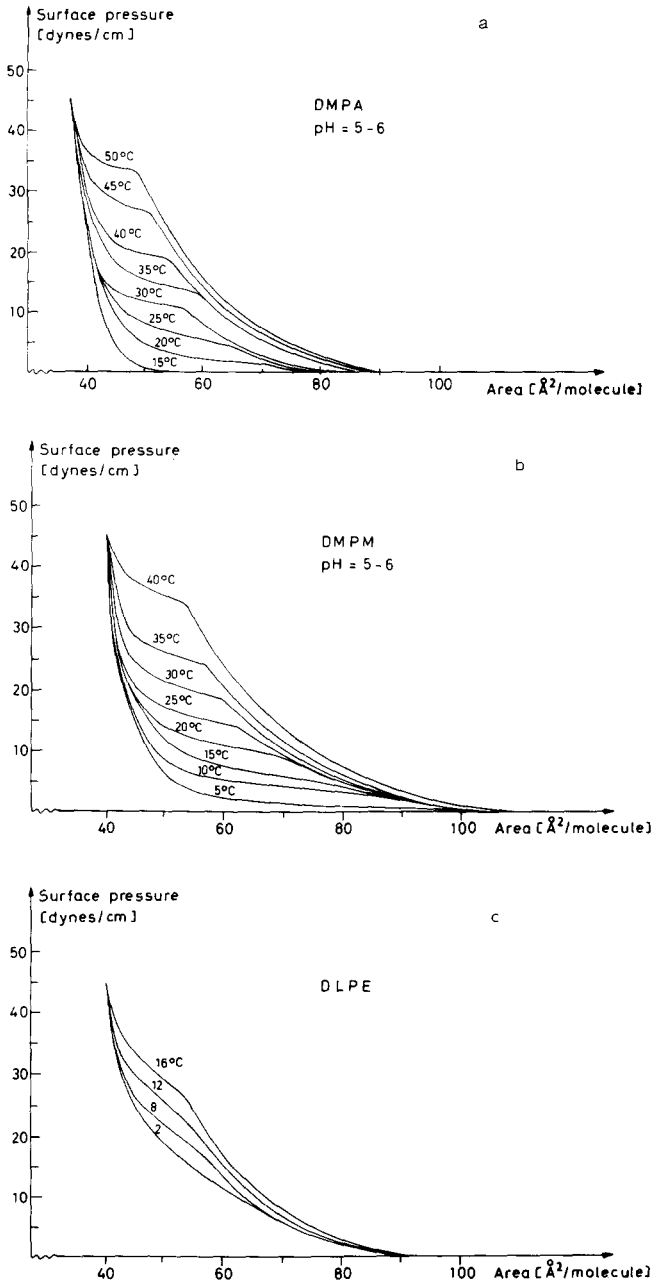

Fig. 1. Monolayer isotherms at different temperatures for (a) DMPA, (b) DMPM, (c) DLPE, on pure water (pH 5—6) as subphase.

Isotherms for a phospholipid with rather short chains, i.e., the lipid DLPE, are shown in Fig. 1c. The isotherms in the phase transition region are much more inclined than those of lipids with longer chains. The loss of hydrophobic interaction energy due to the shorter chains obviously leads to a decrease in cooperativity of the phase transition.

The same effect could be observed for the bilayer phase transition [25]. Isotherms at temperatures above 16°C could not be recorded with this lipid due to a decrease in film stability at high surface pressures.

As already mentioned above, the recording of isobars has the advantage that the change in molecular area and in the transition temperature can be determined very easily. Fig. 2 shows as an example isobars for DMPC recorded at different surface pressures. Similar isobars were recorded for the other phospholipids. However, head group and chain length of the fatty acid chains of the lipid have pronounced effects on the transition temperature, molecular area and change in area at the transition. Increasing surface pressure shifts the transition temperature to higher values, while the molecular areas and the change in area at the transition become smaller. The dependence of the transition temperature on the surface pressure ($d\pi/dT_m$) depends mainly on the nature of the phospholipid head group. Fig. 3 shows T_m (taken at the midpoint of the monolayer transition of the isobars) as a function of the surface pressure. Phospholipids with strong intermolecular interactions between the head groups (DLPE, DMPE, DMPA, DMPM) have low values for $d\pi/dT_m$. DMPC and DPPC with the bulkier head groups have values which are almost twice as large. The highest value for $d\pi/dT_m$ was found for DMPM at pH 10, when the head group of this lipid has one negative charge [8]. The values for $d\pi/dT_m$ are also shown in Table II.

Fig. 4 shows the dependence of the change in molecular area at the transition on surface pressure. Again, the change in area, Δf, as well as the value for $d\Delta f/d\pi$ depends only on the nature of the lipid head group. However, at high surface pressure the differences between the Δf values for lipids with different head groups become smaller.

The entropy for the monolayer phase transition can be calculated from the Clausius-Clapeyron equation:

$$\frac{d\pi}{dT_m} = \frac{S'' - S'}{f'' - f'} = \frac{\Delta S}{\Delta f}$$

with S'' and f'' being the entropy and area for the lipid-expanded phase and S' and f' for the liquid-condensed phase, respectively. Table II summarizes the

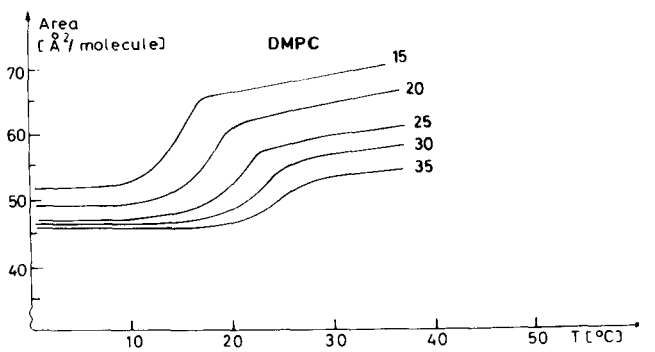

Fig. 2. Monolayer isobars for DMPC on pure water as subphase. Numbers designate the surface pressure in dyne/cm.

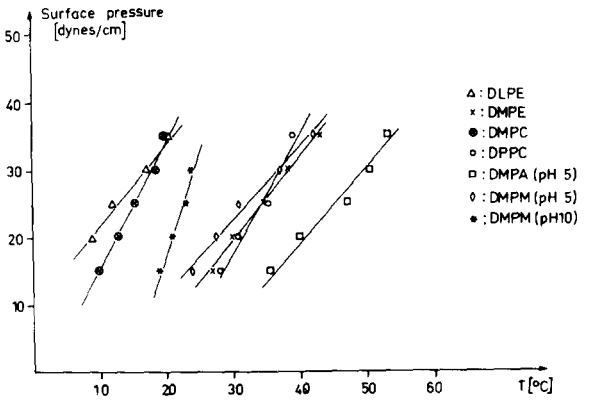

Fig. 3. Diagram of monolayer surface pressure π vs. transition temperature T_m of the monolayer transition. T_m was taken from the isobars at the midpoint of the transition.

data calculated for the transition entropy, ΔS, of all phospholipids investigated. The last line of this table shows for comparison the values of the bilayer transition entropy, ΔS_{cal}. The transition entropies of the monolayer transition depend strongly on the nature of the polar head group but are almost independent of the chain length. The calorimetrically determined transition entropies for the bilayer system, on the other hand, depend more strongly on the fatty acid chain length, and not so much on the structure of the polar head group. The complications arising for the comparison of bilayer and monolayer transition entropies will be discussed in the next section of this paper.

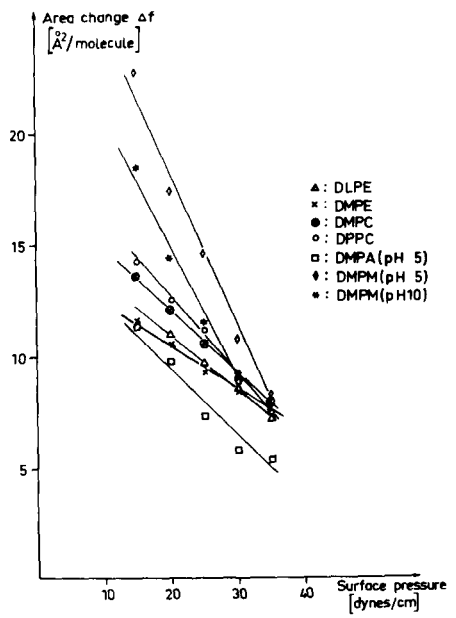

Fig. 4. Area change, Δf, of the monolayer transition as a function of monolayer surface pressure.

Discussion

Bilayers. The results of our calorimetric investigations are in good agreement with those reported in the literature [4,5,47]. The T_m values seem to be determined by the extent of the intermolecular attractive interactions between the lipid head groups. Intermolecular hydrogen bonding leads to higher transition temperatures, as is the case with phosphatidylethanolamines [23] and probably with phosphatidic acids [7,22,24,25,47]. However, the transition enthalpies exhibit only small variations when the head group is changed, because the enthalpic effect at the transition arises mainly from the increase in the number of *gauche* conformations and the increase in distance between the hydrocarbon chains (see Table I).

Monolayers. Similar effects of a change of the head group or chain length on the transition temperature are observed for the monolayer phase transition of the same phospholipids investigated by differential scanning calorimetry. In Fig. 3 the effect of changes in head group structure are clearly demonstrated. For lipids with identical head groups but different chain lengths, the differences in T_m for the monolayer phase transition can be compared to the differences in T_m of the bilayer phase transition. Because the slope $d\pi/dT_m$ is the same for compounds with identical head groups, the shift in T_m due to the elongation of the chains does not depend on the surface pressure of the monolayer. The differences in T_m, i.e. the values of ΔT_m, for the monolayer system are 19°C for phosphatidylcholines and 21.5°C for phosphatidylethanolamines. These values correspond very closely to the differences observed for the bilayer phase transition where the ΔT_m values are 17.5°C and 19.7°C, respectively (see Table I). Another analogy of monolayer and bilayer systems is found in the shift of T_m induced by the partial protonation of DMPM by changing the pH from 10.0 to 5. For the bilayer system a value of ΔT_m of 12.5°C is observed. In the monolayer system ΔT_m is not a constant value, because the slopes $d\pi/dT_m$ are different at pH 5 and pH 10. In this case ΔT_m ranges from 4.0°C at a surface pressure of 15 dyne/cm to 13.3°C at 30 dyne/cm. Comparing the T_m-values of the bilayer and the monolayer system, the closest agreement between the two systems appears to be at a monolayer surface pressure of approx. 30 dyne/cm.

We wish to compare now our experimental data with those gained from model calculations for the bilayer and monolayer phase transition which were recently proposed by several authors [27—33]. The model reported by Scott et al. [32] describes the observed surface pressure as the sum of the hydrocarbon chain pressure and the surface pressure of the phospholipid head groups. A consequence of this assumption that the chains are effectively isolated from the head groups on the water surface would be that the phase transition in the monolayer is mediated by the hydrocarbon chains alone, and that at a given temperature the molecular area at which the isotherm kink at the beginning of the phase transition appears depends exclusively on the chain length and not on the chemical structure of the head group. A plot of the area of the liquid-expanded phase, f_1, at the onset of the phase transition (taken from the isobars) vs. the temperature of the onset of the transition at the high temperature side of the isobars should then coincide for phospholipids with the same

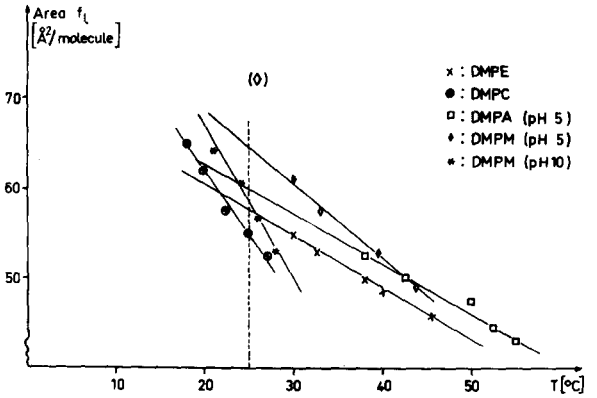

Fig. 5. Plot of the area f_1 of the liquid-expanded phase versus T_{onset}. Both values were taken from the isobars at the onset of the monolayer transition to the liquid-condensed phase.

chain length, regardless of their type of head group. Fig. 5 shows that almost linear plots of f_1 vs. T_{onset} are obtained. When we compare the f_1 values of all phospholipids at 25°C, for instance, the measured areas at the onset of the transition for DMPC, DMPE and DMPM (pH 10) and the extrapolated f_1 value for DMPA (pH 5) are very similar (55—60 Å²/molecule). Only for DMPM (pH 5) is the extrapolated value slightly larger (65 Å²/molecule). At a higher temperature, however, the extrapolated f_1 values for DMPC and DMPM (pH 10) become considerably smaller than those of the other lipids, so that the approximation that head groups and chains are effectively isolated from each other seems to be justified only for a limited temperature interval. Despite the fact that at 25°C the molecular area f_1 is similar for lipids with identical chain lengths, the area changes, Δf, at this respective temperature are quite different, because transitions recorded at different surface pressures are compared. This is illustrated in Fig. 6, where Δf is plotted versus T_{onset}. At 25°C the Δf values range from 8 to almost 20 Å²/molecule. The different slopes of the lines again indicate that the head groups do have marked influence on the monolayer

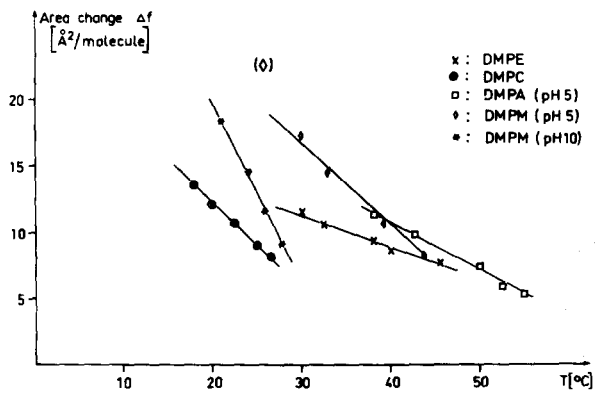

Fig. 6. Area change, Δf, of the monolayer transition as a function of T_{onset}. T_{onset} was taken from the isobars at the onset of the transition to the liquid-condensed phase.

transition. This head group effect is also obvious in the Δf vs. π diagram in Fig. 4. Only at high surface pressures do the Δf values for all phospholipids become very similar, with the exception of DMPA, which has a smaller Δf value. This is probably due to the fact that DMPA has the smallest head group and that the phosphate groups are connected via intermolecular hydrogen bonds [22,24].

The aim of our work was to find the surface pressure at which the phase transition characteristics of lipid monolayers are similar to those of the corresponding bilayers. Phillips et al. [26], Hui et al. [34], and Nagle [30] proposed the equalization of the transition temperatures of monolayer and bilayer which leads to a surface pressure of 50 dyne/cm. Albrecht et al. [18] pointed out that this pressure is close to the critical pressure of the monolayer at which the transition is vanishing, and made the proposal of equalizing the area changes and the transition entropies of monolayer and bilayer. We agree with the suggestion of comparing the area changes but the equalization of the transition entropies of monolayer and bilayer is not possible in our opinion, because the bilayer transition entropies depend on the volume change, i.e., the change in area and the change in thickness of the bilayer, while the monolayer transition entropies are calculated solely from the area changes and thus do not take into account the third dimension. Therefore, the equalization of monolayer and bilayer transition entropies can lead to erroneous results. For the equalization of the area changes the molecular areas in both bilayer phases have to be determined from X-ray data and the partial specific volumes. However, the calculations based on the data reported by various workers for the lipid bilayer thickness, the partial specific volumes, and the volume change at the transition [35—41] lead to Δf values which vary between approx. 6.3 and 17 $Å^2$/molecule. A comparison of the Δf values thus leads only to a very rough estimate for the appropriate surface pressure. However, the approximation can be improved when the absolute value of the molecular area in the gel phase determined from X-ray data (48 $Å^2$/molecule for DPPC [36,37]) is taken into account. One finds that in the monolayer system this value is reached at π = 30 dyne/cm. Δf at this pressure amounts to 9.5 $Å^2$/molecule, thus being in the range of the Δf values calculated for the bilayer transition. Recently, Jähnig et al. [42] determined the area change for dihexadecyl phosphatidic acid from X-ray data to be 5.8 $Å^2$/molecule (pH 7) with an area for the gel phase of 41.3 $Å^2$/molecule. When we compare this with our monolayer data for DMPA (pH 5) we again find the closest agreement when the surface pressure is 30 dyne/cm (see Fig. 4). Another experimental finding leading to the same approximation for π is that the shift in T_m upon protonation of DMPM is only equivalent in both systems when the monolayer pressure is 30 dyne/cm. Comparing the action of phospholipases on erythrocyte membranes and lipid monolayers, Demel et al. [43] concluded that the pressure in the outer monolayer of the erythrocyte membrane is equivalent to a monolayer surface pressure of 31—34.8 dyne/cm, which is additional support for our results.

Finally, we wish to approximate the interfacial tension of the lipid head group/water interface and compare it with the reported values for the bilayer tension. From the expression $\pi = \gamma_o - \gamma$, where γ_o is the surface tension of pure water (71.4 dyne/cm at 30°C), we can calculate the surface tension, γ, of

the subphase covered with the monolayer. γ is approx. 40 dyne/cm when a value of 30 dyne/cm is taken for the surface pressure, π. Following the assumption made by Scott et al. [29,32], γ would consist of two terms, the surface tension, γ_h of the hydrocarbon/air interface and the interfacial tension, γ_w, of the head group/water interface. Values for γ_h have been reported by Girifalco and Good [44] for long-chain alkanes and various oils. In the case of tetradecane, for example, γ_h is 25.6 dyne/cm and for white oil 28.9 dyne/cm. Because of the more ordered hydrocarbon chains in the monolayer, γ_h in this system may be even higher. The resulting interfacial tension, γ_w, for the head group/water interface is then lower than 10 dyne/cm. Because the hydrocarbon/air interface does not exist in a bilayer, the membrane tension is determined solely by the interfacial tension, γ_w, of the head group/water interface. Bilayer membrane tensions using the bubble pressure method adapted to black lipid membranes were measured by Neher and Eibl [45]. The γ_w values for saturated phospholipids with different head groups are in the range 0.5 to 4.5 dyne/cm and thus agree with our approximation when we use a value of 30 dyne/cm for the surface pressure.

Having obtained the appropriate value for the surface pressure where monolayer and bilayer system can be compared, it is now possible to study the influence of ions or organic molecules on the monolayer behaviour [46] and to transfer the information won by these experiments to the corresponding bilayer system. The technique of measuring isobars greatly facilitates the evaluation of the relevant transition parameters: transition temperature, T_m, and area change, Δf.

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