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Phase Transition Kinetics of Phosphatidic Acid Bilayers. A Pressure-Jump Relaxation Study†

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ABSTRACT: The phase transition kinetics of unilamellar vesicles of dilauroyl-, dimyristoyl-, and ditetradecylphosphatidic acid were investigated by the pressure-jump technique with optical detection. At neutral pH, phosphatidic acids display three relaxation processes within the temperature range of the phase transition. The time constants of these processes are in the 1–10-, 10–100-, and 100–1000-ms ranges, respectively. They exhibit distinct maxima at the midpoint of the phase transition as determined by differential scanning calorimetry. The temperature dependence of the relaxation times and amplitudes indicates that these processes are of a cooperative nature and thus directly related to the phase transition. The cooperative units calculated from the relaxation amplitudes agree well with those determined from the differential scanning calorimetry curves. Whereas changes in head group structure generally have only small effects on the thermodynamic properties of the transition, the kinetics of the transition are changed drastically. Phosphatidic acids show relaxation times more than 1 order of magnitude slower than those observed for phosphatidylcholines. Increasing the negative charge of the phosphatidic acid head group leads to a decrease in the relaxation times by more than 1 order of magnitude. Addition of 0.1 M NaCl has the opposite effect. Compared with these large changes in the relaxation behavior due to variations in the head group interactions, changes in hydrocarbon chain length have no effect. Thus, the phase transition kinetics of phosphatidic acids are dominated by the strength of the interactions between the polar head groups.

The thermodynamic aspect of the gel to liquid-crystalline phase transition of phospholipid bilayers has been a field of intensive research over the past years. Several theories have been developed that try to describe the equilibrium properties of the transition on the basis of statistical-mechanical or mean-field calculations [for a review, see Nagle (1980)]. Compared with the large amount of data available for the thermodynamic properties of the lipid phase transition, the kinetic aspects have received relatively little attention. Up to now most kinetic investigations focused on the behavior of PC,[1] applying different kinetic methods like ultrasonic and dielectric relaxation or the temperature-jump method (Träuble, 1971a; Tsong, 1974; Tsong & Kanehisa, 1977; Kaatze et al., 1975; Eggers & Funck, 1976; Gamble & Schimmel, 1978; Mitaku et al., 1978; Gruenewald et al., 1981; Gruenewald, 1982; Mitaku & Date, 1982). Recently, we have reported on the application of the pressure-jump method using optical detection in studying the transition kinetics of PC vesicles and liposomes (Gruenewald et al., 1980). This technique has the advantage over the temperature-jump method that no orientation effects on the lipid head groups and no dielectric breakdown of the bilayer due to the large electric fields can occur (Zimmermann et al., 1974; Shepherd & Büldt, 1978). This method is also suitable for the investigation of charged phospholipids as, in contrast to the temperature-jump experiments, there is no need for the addition of salt to obtain sufficient conductivity. This is important because changes in the ionic strength have large effects on the phase transition characteristics of charged lipids (Träuble & Eibl, 1974; Träuble et al., 1976; Jähnig, 1976).

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[1] Abbreviations: DLPA, dilauroylphosphatidic acid; DMPA, dimyristoylphosphatidic acid; DTPA, ditetradecylphosphatidic acid; DPPC, dipalmitoylphosphatidylcholine; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PA, phosphatidic acid; DPH, 1,6-diphenylhexatriene; T_m, transition temperature; DSC, differential scanning calorimetry; NMR, nuclear magnetic resonance.

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Phosphatidylcholines were shown to exhibit at least two relaxation processes as observed in the pressure-jump experiment. Both time constants exhibit a maximum at the phase transition temperature and can thus be ascribed to cooperative processes. The relaxation times showed a dependence on the state of aggregation of the lipids, i.e., whether vesicles or multilamellar liposomes were investigated, and on the length of the fatty acyl chain of PC (Gruenewald et al., 1980).

In this work we report on pressure-jump relaxation studies on vesicles prepared from different phosphatidic acids. Our aim was to determine how changes in head group structure influence the kinetic behavior. Phosphatidic acids are well suited for this purpose as head group interactions can easily be altered by changing the pH or the ionic strength of the solution. In addition, phosphatidic acid vesicles are relatively stable at most pH values due to their negative surface charge, and they can easily be prepared by a simple short sonication procedure (Hauser & Gains, 1982; Elamrani & Blume, 1983). We will show that head group interactions play a dominant role in determining the relaxation kinetics affecting intramolecular dynamics as well as motions within the lattice. Phosphatidic acids display much slower transition kinetics as compared to phosphatidylcholines. The third relaxation process, which is hidden in the instrumental dead time in the case of PC, can now be evaluated. All three relaxations can be altered by changing the pH or the ionic strength. The effects of increasing the fatty acyl chain length are negligible.

Materials and Methods

The synthetic lipids DLPA, DMPA, and DTPA were purchased from Paesel (Frankfurt), FLUKA (Neu-Ulm), and Medmark (Grünwald), respectively. The lipids were chromatographically pure as tested by thin-layer chromatography and used without further purification.

Vesicle suspensions were prepared by dispersing the desired amount of the disodium salt of PA in bidistilled water or 0.1 M NaCl solution by vigorous shaking. The suspension was then sonicated at room temperature for 1 min in a MSE ultrasonic disintegrator with a $^3/_8$-in. titanium tip. After the pH was adjusted to the desired value, the vesicles were annealed at temperatures above their respective phase transition for 1 h. This procedure gives reproducible vesicle suspensions. The vesicle diameters as characterized by freeze–fracture electron microscopy are in the 50–140-nm range for DLPA and in the 50–240-nm range for DMPA, the main DMPA fraction having a diameter of 100–180 nm (Elamrani & Blume, 1983).

For the determination of the transition temperatures, turbidity vs. temperature curves were recorded on a PYE UNICAM SP 1800 spectrophotometer with a variable-temperature cell holder and temperature programmer SP 876. The turbidity was recorded at 360 nm with a scan rate of 1 °C/min. Calorimetric measurements were made on a Privalov differential scanning calorimeter (Privalov et al., 1975). Evaluation of transition enthalpies was done as described before (Blume, 1980).

The pressure-jump method with optical detection has been described before (Knoche & Wiese, 1976; Gruenewald et al., 1980). The pressure-jump autoclave (Fa. Dialog, Düsseldorf) was used in connection with a temperature-jump spectrophotometer (Messanlagen Studiengesellschaft, Göttingen). The 0.08-mm brass membranes used gave a bursting pressure of 110 ± 5 atm. The dead time as given by the decrease of the pressure to ambient after the rupturing of the membrane was determined to be 60 μs. Turbidity vs. time curves after the pressure drop were recorded with a Datalab DL 905 transient recorder. The curves were then stored in a home-built 64K solid-state memory (Messner, 1978) and subsequently read into a Hewlett-Packard 9845A computer for further data processing. Time constants of the relaxation curves and the amplitudes were determined by semilogarithmic linear least-squares fits of the experimental curves.

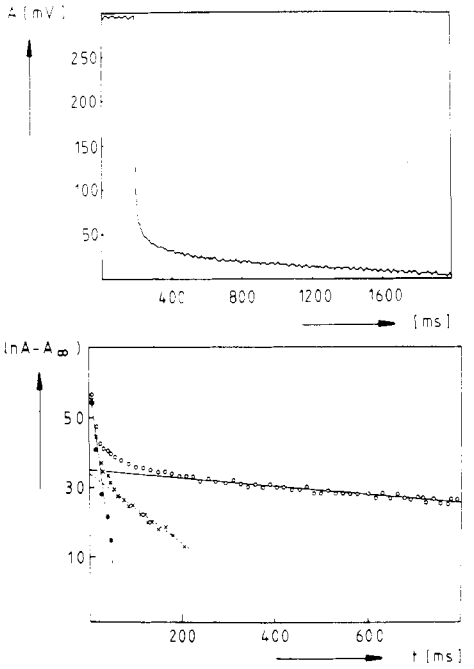

FIGURE 1: (Top) Time dependence of optical density A in millivolts (10 mV = 0.001 OD) at 360 nm for DLPA vesicles after a pressure jump from 110 to 1 atm (c = 0.5 mM, pH 7.0, T = 32.8 °C). The pressure jump was initiated at t = 200 ms. (Bottom) Half-logarithmic plot of experimental data for determination of kinetic parameters τ_i and A: (○) experimental points; (×) difference curve after subtraction of exponential for the slow process; (●) second difference curve after subtraction of exponential for the slow and the intermediate process.

Results

As the gel to liquid-crystal phase transition is connected with an increase in specific volume, increasing pressure shifts the transition temperature to higher values (Srinivasan et al., 1974; Liu & Kay, 1977; Galla & Trudell, 1980). By using a rapid pressure drop from high pressure to ambient, the bilayer phase transition can be triggered at constant temperature. As this is connected with a decrease in turbidity due to a change in refractive index of the bilayer, the kinetics of the transition can easily be followed by recording the optical density as a function of time (Yi & McDonald, 1973; Gruenewald et al., 1980). Figure 1 shows a recording of the turbidity at 360 nm of a DLPA vesicle suspension after a pressure drop from 110 atm to ambient. The pressure drop was initiated at t = 200 ms so that the optical density before the pressure jump can also be observed. The turbidity vs. time curve is complex, three different relaxation times with different amplitudes can be resolved (see Figure 1). Whereas all three relaxation times are concentration independent, the total relaxation amplitude increases linearly with the DLPA concentration (see Figure 2). Therefore, vesicle dissociation as a cause of the turbidity change can be excluded. The transition kinetics of the DLPA vesicles were investigated in the temperature interval between 20 and 40 °C. The transition temperature of DLPA at pH 7 is 32.7 °C as determined from an equilibrium melting curve. All three relaxation times, as well as their amplitudes, have maxima at the midpoint of the transition (see Figure 3). This indicates that the observed processes are of a cooperative

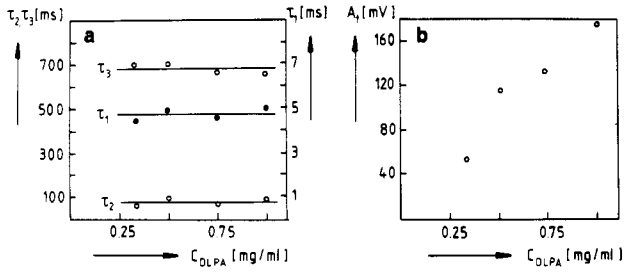

FIGURE 2: Concentration dependence of (a) relaxation times τ_1, τ_2, and τ_3 and (b) total relaxation amplitude A_t in millivolts for DLPA vesicles (pH 7.0, $T = T_m$).

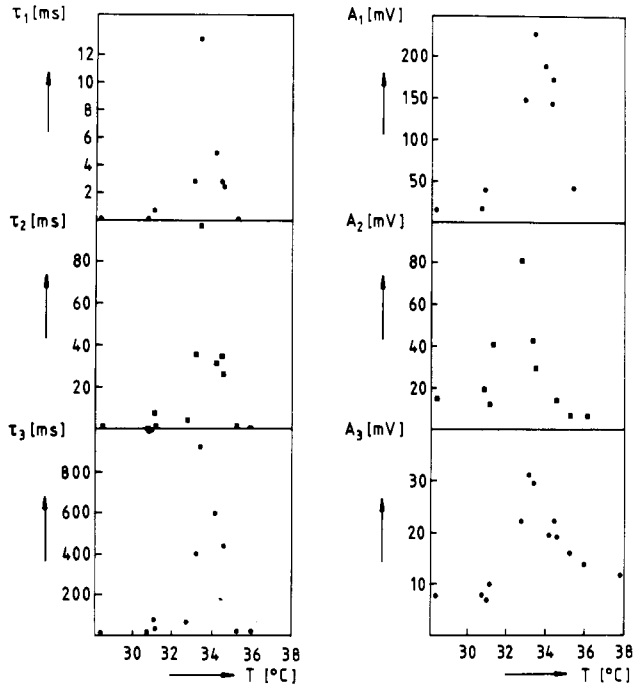

FIGURE 3: DLPA vesicles: c = 0.5 mM; pH 7.0; ΔP = 110 atm. Relaxation times τ_1, τ_2, and τ_3 and relaxation amplitudes A_1, A_2, and A_3 as a function of temperature.

nature and are thus related to the phase transition in the lipid bilayer. The difference between each one of the three relaxation times is roughly 1 order of magnitude. The fastest process displays the largest amplitude. It should be mentioned that outside the transition region, i.e., below 26 and above 38 °C, the total relaxation amplitudes are very small, typically below 40 mV. The time constants become very short compared to the dead time of the instrument. The slowest relaxation process, having a time constant of ca. 1 ms, can still be observed; the others are shorter than 60 μs.

The two relaxations found previously in our investigations of PC were much faster, the third one being observable only at the transition midpoint of DPPC liposomes (Gruenewald et al., 1980). In that study, we had found that the chain length of the acyl chains had an influence on the time constants. To check for this in the PA system, we investigated DMPA vesicles, which have a T_m of 53 °C. Figure 4 shows relaxation times and amplitudes for DMPA vesicles at pH 7.3. Again, three relaxation processes can be observed having their maximum at the transition midpoint. The comparison with the results obtained for DLPA shows that the time constants are almost identical. The reasons for this unexpected behavior will be discussed below.

It is known that phospholipids with an ether linkage of the hydrocarbon chain have slightly different thermodynamic properties compared to the ester phospholipids. In particular,

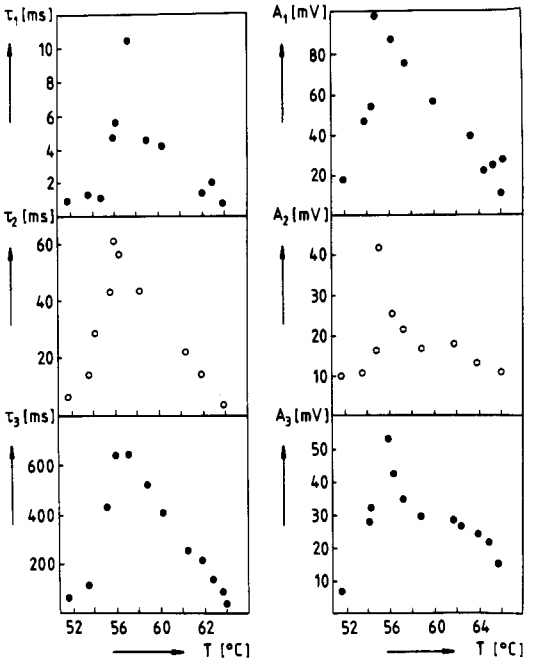

FIGURE 4: DMPA vesicles: c = 0.5 mM; pH 7.3; ΔP = 110 atm. Relaxation times τ_1, τ_2, and τ_3 and relaxation amplitudes A_1, A_2, and A_3 as a function of temperature.

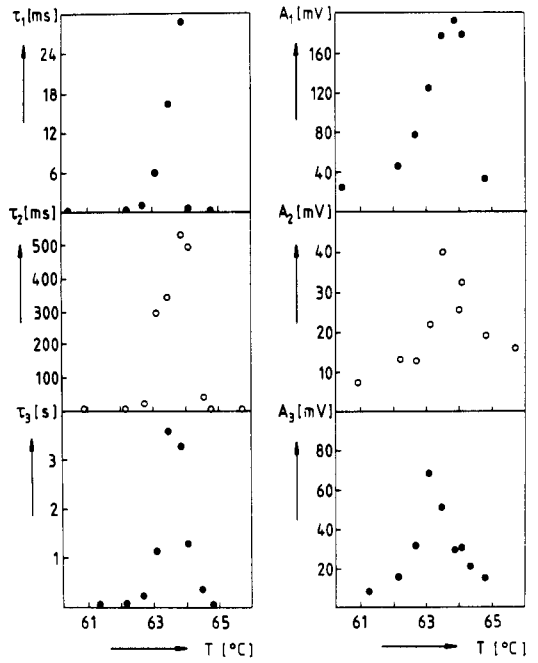

FIGURE 5: DTPA vesicles: c = 0.5 mM; pH 7.0; ΔP = 110 atm. Relaxation times τ_1, τ_2, and τ_3 and relaxation amplitudes A_1, A_2, and A_3 as a function of temperature.

their transition temperatures are slightly higher, and the transition seems to be more cooperative (Vaughan & Keough, 1974; Blume, 1976; Blume & Eibl, 1979). Pressure-jump experiments with the ether analogue DTPA gave the results shown in Figure 5. Again, all three relaxation processes display maximum values at the transition midpoint. In agreement with the known higher cooperativity found under equilibrium conditions, the maximum is much more pronounced. All three relaxation processes are much slower compared to the ester analogue DMPA.

Head group interactions in PA bilayers can be modified in two ways: (a) by adding monovalent salts, which leads to an effective decrease in the surface charge density and a shift in

Table I: Pressure-Jump Experiments with DLPA Vesicles in 0.1 M NaCl Solution[a]

temp (°C)	A_t (mV)	τ_1 (ms)	τ_2 (ms)	τ_3 (s)
27.5	23	0.1	1.2	0.10
28.5	46	0.5	3.2	0.11
29.4	54	2.5	10.6	0.23
30.0	105	9.4	85.3	0.74
30.5	162	71.3	470.0	3.08
31.5	327	37.2	395.0	2.72
32.4	227	13.0	169.0	1.03
33.6	151	10.3	106.0	0.56
35.0	126	7.9	77.0	0.41
36.0	84	4.1	42.0	0.25

[a] Total relaxation amplitude A_t and relaxation times τ_1, τ_2, and τ_3 as a function of temperature. DLPA concentration was 1 mM in 0.1 M NaCl, pH 7.0; ΔP = 110 atm.

the apparent pK; (b) by variation of the external pH. This directly affects the charge of the head group as PAs have two dissociable protons. Addition of 0.1 M NaCl to a neutral DMPA suspension leads to a slight decrease in T_m, an increase in the transition enthalpy, but a decrease in the cooperativity of the transition (Träuble & Eibl, 1974; Blume & Eibl, 1979). Similar results have been obtained with the DLPA vesicles used in this study by DSC (see Table II). The pressure-jump experiments gave the results shown in Table I. In contrast to the effects expected from the equilibrium studies (lower cooperativity, hence, shorter relaxation times), we observed a marked increase in the time constants by a factor of 2–3 for all three relaxation processes. It should be noted that the addition of NaCl to DLPA vesicles leads to an increase in the optical density, indicating a different type of aggregation. The vesicles may be larger or exist in a flocculated form due to the decrease in surface charge density. However, the observed relaxation processes are reproducible and are not due to vesicle dissociation. It could be argued that the difference in the time constants is due to this different type of aggregate, as it was previously found that PC liposomes have longer time constants than those of PC vesicles. However, in these cases, elongation of the time constants was always connected with an increase in the cooperativity of the system, which is clearly not the case for DLPA vesicles. In this system, the cooperativity decreases, but the time constants are longer.

The second method to modify head group interactions is to change the external pH, thus directly altering the head group charge. At high pH values, where phosphatidic acids are doubly charged, the transition temperatures are decreased by as much as 20–30 °C (Träuble & Eibl, 1974). We investigated DLPA vesicles at pH 10, where the transition temperature was found to be 6 °C lower, in agreement with previous results with DMPA vesicles (Eibl & Blume, 1979). Approximately 70–75% of all PA molecules exist in the doubly charged form at this particular pH. Unfortunately, a systematic study of the transition kinetics at high pH could not be carried out because of two reasons: (a) the chemical stability of ester lipids is limited; (b) vesicles of doubly charged PA are unstable to large pressure changes. While the first problem could be overcome by the use of the ether analogue, the second problem cannot be easily solved. Using smaller pressure jumps might give an improvement. However, this results in smaller relaxation amplitudes, which complicates the evaluation of the relaxation times as the signal to noise ratio is very low. In our preliminary studies we always observed only two relaxation processes, one in the 100–300-μs time range and the slower one in the 1–10-ms range. A third faster relaxation seemed to be hidden in the instrumental dead time. Thus, the dissociation of the second proton responsible for intermolecular hydrogen bonding between the head groups leads to much faster transition kinetics. The relaxation times are now in the range observed for PC vesicles, where intermolecular hydrogen bonding between head groups is not possible. It should be mentioned that doubly charged PA resembles PC in another structural aspect, namely, the tilt of the hydrocarbon chains (Jähnig et al., 1979).

The equivalence between pressure-jump and temperature-jump experiments has been demonstrated before (Gruenewald et al., 1980). The application of a simple two-state model for the lipid phase transition allows the determination of the cooperative unit size from the integrated relaxation amplitudes. Table II shows the thermodynamic data of the PA vesicle systems determined by DSC together with the cooperative unit size calculated from the integrated amplitudes of the pressure-jump relaxation curves. The agreement of the data determined by these two different methods is very good. This indicates that the turbidity changes in the pressure-jump experiment run parallel to the heat uptake in the calorimetric experiment and reflect the changes in internal and van der Waals energy of the bilayer system. The ca. 1–2 °C higher T_m values in the pressure-jump experiments reflect a systematic error in the temperature determination of the vesicle suspension in the pressure-jump autoclave. Obviously, there is a temperature gradient from the outside of the autoclave, where the temperature is measured to the inside of the cell. However, this gradient does not affect the accuracy of the kinetic constants.

Discussion

The phase transition kinetics of lipid bilayers are very complex, and different relaxations are observed with different techniques. In our previous pressure-jump study on the kinetics of PC vesicles and liposomes, we observed two relaxation processes in the 1- and 10-ms time range. A third faster relaxation seemed to be present but could not be resolved with the pressure-jump method (Gruenewald et al., 1980). A recent

Table II: Comparison of Transition Temperatures T_m and Cooperative Unit Sizes n Determined by Differential Scanning Calorimetry and by Pressure-Jump Experiments

phospholipid	pH	T_m (°C)[a]	n[b]	T_m (°C)[c]	n[d]	ΔH_{cal} (kcal/mol)
DLPA in water	7.0	32.7	70	31.0	73	3.4
DLPA in 0.1 M NaCl	7.0	31.0	47	29.0	48	3.7
DLPA in water	10.0	*e*	*e*	25.0	56	3.3
DMPA in water	7.3	53.6	65	52.2	60	5.5
DTPA in water	7.0	63.0	108	62.3	106	4.4

[a] Calculated from the integrated pressure-jump amplitudes. [b] Calculated from $\Delta H_{v'Hoff}/\Delta H_{cal} = n$, where $\Delta H_{v'Hoff}$ was determined from the integrated pressure-jump amplitudes. [c] Calculated from the integrated DSC curves. [d] Calculated with $\Delta H_{v'Hoff}$ determined from the integrated DSC curves. [e] Cannot be determined due to vesicle breakdown.

temperature-jump study applying fluorescence detection with the probe DPH confirmed our earlier results (Gruenewald, 1982). Both of these studies also showed that the time constants of the relaxations depend on the structure of the lipid aggregate, i.e., vesicles displaying faster kinetics than multilamellar liposomes. The relaxation times for unilamellar vesicles were found to depend on the vesicle size when the vesicle radius is below 50 nm, indicating an influence of the hydrocarbon chain packing on the transition kinetics (Gruenewald, 1982).

Faster processes with relaxation times in the nanosecond range can be observed by ultrasound absorption (Eggers & Funck, 1976; Gamble & Schimmel, 1978; Harkness & White, 1979; Mitaku et al., 1978; Mitaku & Date, 1982; Sano et al., 1982) and by a laser temperature technique with turbidity detection (Gruenewald et al., 1981). Whereas ultrasound absorption clearly shows that the observed process is of a cooperative nature as the time constant, as well as the absorption, displays a maximum at the transition midpoint, the relaxation time derived from the laser temperature-jump experiment is almost temperature independent. Dielectric relaxation studies of the zwitterionic head groups also revealed relaxation processes in the nanosecond time range, and it was shown that the head group motions depend on the vesicle size and are of a cooperative nature (Kaatze & Henze, 1980). This effect was also found in a recent ultrasound absorption study (Sano et al., 1982). It is possible that the processes observed by ultrasound are actually related to head group reorientation as suggested earlier by Eggers & Funck (1976). The noncooperative nanosecond process observed in the laser temperature-jump experiment could possibly be ascribed to motions within the hydrocarbon chains.

For a better understanding of these various relaxation processes and their relation to particular molecular events, we thought it important to study the influence of changes in phospholipid structure on the slower kinetic processes. Up to now, all kinetic studies focused on the behavior of PC, a lipid that is not ideal for this purpose as the behavior is complicated by the presence of the microscopically heterogeneous $P_{\beta'}$ phase just below T_m, which displays some properties of the "fluid" L_α phase (Janiak et al., 1976, 1979; Wittebort et al., 1981, 1982). PA is a much simpler system. Due to the small head group, the hydrocarbon chains are oriented almost perpendicular to the bilayer surface. No rippled $P_{\beta'}$ phase is observed below the main phase transition (Jähnig et al., 1979; Blume & Eibl, 1979). Intermolecular hydrogen bonds between the phosphate head groups can be formed and lead to higher transition temperatures and smaller changes in molecular area and specific volume at the transition. This can be observed in monolayer experiments, by X-ray diffraction, and indirectly by the pressure dependence of the transition temperature (Albrecht et al., 1978; Blume, 1979; Jähnig et al., 1979; Galla & Trudell, 1980). Because of the tighter packing of the hydrocarbon chains and the additional head group interactions, the kinetic behavior should be different from that of PCs. All three PAs investigated displayed much slower kinetics at neutral pH than PC. In all cases, three relaxations could be resolved. These processes are of a cooperative nature as their amplitudes, as well as time constants, display a distinct maximum at the transition midpoint.

Hydrophobic as well as polar interactions can be increased by substituting the ester bonds with ether linkages. Ether analogues generally have higher transition temperatures, but the effects vary with the different phospholipid classes. The increase in T_m is much larger for PAs than for PEs and PCs (Vaughan & Keough, 1974; Blume & Eibl, 1979). The T_m of DTPA is ca. 10 °C higher than that of DMPA. The missing carbonyl oxygen atom allows for better chain packing but also for increased head group interactions. These changes also influence the kinetic behavior. The relaxation times of DTPA are increased by a factor of ca. 3 for the fastest and by 6–8 for the slower processes. Again, this result shows that generally the kinetics of the transition is much more sensitive to these structural changes than the thermodynamic properties.

Head group interactions can also be varied by changing the ionic strength of the solution. Addition of 0.1 M NaCl to DMPA vesicles shifts the apparent pK of the second dissociable proton of the phosphate group to a lower value due to charge-screening effects at the negatively charged bilayer surface (Träuble & Eibl, 1974). The transition enthalpy increases, but surprisingly, the cooperativity of the transition is reduced (Blume & Eibl, 1979). This was also observed for the DLPA vesicle system used in this study (see Table II). The lower transition temperatures in 0.1 M NaCl are the result of the shift in pK, as the vesicles were compared at the same pH (Träuble et al., 1976). Again, the thermodynamic effects of salt addition are small compared to the much larger changes in the transition kinetics. In 0.1 M NaCl, the time constants increase by a factor of 4–6 (see Table I).

An additional test for the validity of the assumption that head group interactions are dominant in determining the transition kinetics are experiments using DLPA vesicles at high pH. Though a complete temperature dependence of the relaxation times could not be obtained due to the limited mechanical stability of these vesicles, it was evident that no slow relaxation in the second time range was present. All three processes were now faster by 2 orders of magnitude. The fastest could not be resolved, but its presence could be deduced from a comparison of the experimental with the sum of the calculated relaxation amplitudes. Clearly, the removal of the proton responsible for hydrogen bonding between head groups and the generation of the second negative charge drastically reduce the attractive interactions between head groups. The kinetic behavior of PA is now very similar to that of PC.

The variations in the relaxation behavior with pH and ionic strength cannot be ascribed to changes in the cooperativity of the transition. A comparison of cooperative unit sizes of DLPA vesicles at pH 7 in 0.1 M NaCl and at pH 10 shows that NaCl reduces the cooperativity but increases the relaxation times. On the other hand, changing the pH to 10 has only a minor effect on the cooperativity, but now the relaxation times are drastically shorter. Thus there is no simple correspondence between the size of the cooperative unit and the magnitude of the relaxation times. Obviously, changes in the kinetics of the nucleation step dominate the transition kinetics.

Two models have been proposed for the kinetics of the gel to liquid-crystalline phase transition of phospholipid bilayers, both based on the two-dimensional Ising model (Adam, 1973; Kanehisa & Tsong, 1978). Model calculations predict at least two relaxation times of different magnitude having their maximal values at the transition midpoint. Actually, the cluster model proposed by Kanehisa and Tsong gives a whole range of kinetic constants corresponding to the growing and shrinking of clusters of different sizes, the slowest process representing the nucleation step. While both models are adequate for the description of the temperature dependence of the relaxation times and amplitudes, they are too crude to give detailed information about the events on the molecular level, as the lipid molecules can only adopt two macroscopically different conformational states, namely, a "fluid" and a "solid"

state. All intramolecular processes are neglected and polar interactions are not considered. While this approach has been successful for the interpretation of passive permeation of small molecules through bilayers (Kanehisa & Tsong, 1978), it is not precise enough for a detailed description of the transition kinetics in these systems. In order to come to a better kinetic model, we have to gain more insight into the molecular processes responsible for the observed relaxation processes. Turbidity is a rather unspecific method of detection but has the advantage of not having to rely on the potentially perturbing properties of a probe molecule. The specific turbidity of a lipid vesicle suspension depends on the size of the vesicles and on the refractive index of the lipids within the bilayer. As the outer radius of lipid vesicles does not change on passing through the transition (Ceuterick et al., 1979), the observed decrease in optical density at the transition is only due to a change in refractive index, which in turn depends on the specific volume of the lipid (Yi & McDonald, 1973; Nagle, 1973; Peterson & Chan, 1978; Nagle & Wilkinson, 1978). Several types of processes lead to an increase in specific volume. Intramolecular motions like formation of gauche conformations lead to kinks or jogs in the otherwise all-trans chain and create void volumes (Träuble, 1971b; Pace & Chan, 1982). Intermolecular or lattice motions like formation of defects in the hexagonal arrangement of the hydrocarbon chains or the formation of clusters of fluid lipids in surrounding gel-state lipids also lead to an increase in specific volume. If we want to assign these different motions to the observed relaxation processes, we have to consider their time scales in both phases.

In the L_α phase, all motions are very fast; i.e., the correlation times are between 10^{-7} and 10^{-12} s, as determined by ^{13}C and ^2H NMR relaxation time measurements and ^2H NMR line-shape analysis (Lee et al., 1976; Brown et al., 1979; Pace & Chan, 1982; Meier, 1982). Lateral diffusion can be measured by various probe methods or the pulsed gradient NMR technique, and jump frequencies of 10^7–10^8 s^{-1} for the L_α phase can be calculated (Kornberg & McConnell, 1971; Sackmann & Träuble, 1972; Galla & Sackmann, 1974; Rubinstein et al., 1979; Kuo & Wade, 1979). Much less is known about the dynamics in the gel phase. ^2H NMR line-shape simulations have now shown that all motional modes with the exception of vibrations and torsional oscillations are drastically slowed down (Huang et al., 1980; Blume et al., 1982). Trans–gauche isomerizations in the chains, bond rotations in the head group, and rotational and lateral diffusion within the lipid lattice are still observable. In cerebrosides, rotational diffusion is very slow with rates below 10^3 s^{-1}, whereas rates of 10^5–10^6 s^{-1} were observed in PC and PE bilayers. Lateral diffusion is reduced by ca. 3 orders of magnitude in PC (Rubinstein et al., 1979) and probably in other gel-state lipids as well. Trans–gauche isomerization rates range from 10^3 to 10^5 s^{-1} and depend on the chemical nature of the lipid (Huang et al., 1980; Blume et al., 1982; Meier, 1982). Unfortunately, the rate constants determined by NMR experiments are measured under equilibrium conditions, so that no statement can be made about the possible cooperative nature of these motions, which is important if we want to correlate them to the pressure-jump relaxation processes. Because of the inherent differences between the two methods, a simple and direct correlation is therefore not possible. However, some arguments based on the different time scales of the motions can be made. In the gel phase, trans–gauche isomerizations occur in the form of kink formation. This is a relatively slow process. As it is influenced by the packing mode of the chains, it seems likely that this is a cooperative process and could display the same "slowing down" effect at the transition as observed for other motions within the lattice. On the time scale of the pressure-jump experiment, trans–gauche isomerization even below the transition is very fast, as relaxation times of ca. 1 μs can be calculated from the NMR data for PC and PE. However, in the transition region, where all relaxation times increase by ca. 2 orders of magnitude, this process should just be observable. We would tentatively assign the fast process observed in PC bilayers only at the transition midpoint but in PA vesicles through a wider transition range to cooperative trans–gauche isomerizations within the hydrocarbon chains. This requires that in PA vesicles trans–gauche isomerization is slower than in PC bilayers.

The other two slower relaxation processes may be related to the expansion of the lattice and could, in terms of the cluster model, represent nucleation and growth of liquid-crystalline clusters in the surrounding gel-state lipid. Lattice motions are expected to be slower than intramolecular isomerizations. However, we want to emphasize that these assignments can only be tentative, as, solely on the basis of changes in optical density, no distinctions can be made between intramolecular and lattice-type motions.

The results from our kinetic experiments in combination with the NMR data suggest that with the exception of vibrations and torsions all other motional modes are not independent. Their time scales are determined by packing constraints within the lipid lattice and thus by the structural properties of the bilayer. It has been recognized before that models for the thermodynamics of the bilayer phase transition have to include intramolecular terms as well as intermolecular interactions (Nagle, 1980). While electrostatic interactions between head groups and formation of intermolecular hydrogen bonds are only minor corrections for the thermodynamics of the transition, they are very important to describe the kinetics, as the time constants of the relaxations are very sensitive to this type of interaction. Any future model for the kinetics of the bilayer phase transition will have to take these interactions into account as they dominate the kinetic behavior of lipids more than previously considered.

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