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PHASE TRANSITION KINETICS OF PHOSPHATIDIC ACID BILAYERS

A STOPPED-FLOW STUDY OF THE ELECTROSTATICALLY INDUCED TRANSITION

KHALID ELAMRANI and ALFRED BLUME *

Institut fuer physikalische Chemie, Albertstrasse 23a, D-7800 Freiburg (F.R.G.)

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The kinetics of the electrostatically induced phase transition of dimyristoyl phosphatidic acid bilayers was followed using the stopped-flow technique. The phase transition was triggered by a fast change in the pH or the magnesium ion concentration and followed by recording the time dependence of the absorbance. When the phase transition was induced by a pH jump the time course of the absorbance could be described by two exponentials, their time constants displaying the for cooperative processes characteristic maximum at the transition midpoint. The time constants are in the 10 and 100 ms range for the H^+ triggered transition from the fluid to the ordered state. A third slower process shows no appreciable temperature dependence and is probably caused by vesicle aggregation. For the OH^--induced transition from the ordered to the fluid state the time constants are in the 100 and 1000 ms range. The fluid-ordered transition could also be triggered by addition of magnesium ions. Of the several observed processes only the fastest in the 10–100 ms time range could definitely be assigned to the fluid-ordered transition while the others are due to aggregation phenomena. The experimental data were compared with results obtained from pressure jump experiments and could be interpreted on the basis of theories for non-equilibrium relaxation.

Introduction

The thermodynamics of the lipid phase transition has been widely studied in recent years. Much less is known about the kinetic aspects of this transition. We have recently reported on the transition kinetics of phosphatidylcholine liposomes and vesicles and of dimyristoyl phosphatidic acid (DMPA) vesicles applying the pressure jump relaxation technique with optical detection [1,2]. DMPA is an attractive system because the structural properties of DMPA bilayers can be altered by variations of external parameters like pH, ionic strength of monovalent cations and addition of divalent cations. The influence of electrostatic effects on the bilayer phase transition has been studied by several groups [3–8] and compared to theoretical calculations based on the Gouy-Chapman theory for the diffuse electrical double layer [9,10]. In our recent pressure jump relaxation study of phosphatidic acids we found a strong influence of the state of the head group on the kinetic behavior [2]. Strong headgroup interactions lead to slow transition kinetics. Three relaxation times could be distinguished, the time constants displaying the characteristic maximum at the transition midpoint. As the state of the phosphatidic acid headgroup can be altered by a change of the pH or the addition of cations, it offers the possibility to trigger the transition at constant temperature and pressure by mixing the vesicle suspension with

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* To whom correspondence should be addressed.

dilute HCl or NaOH, or by adding a salt solution. The first experiment of this type was reported by Strehlow and Jähnig [11]. These workers mixed a suspension of the methyl ester of dimyristoyl phosphatidic acid with dilute HCl or $CaCl_2$ solution to trigger the transition from the fluid to the ordered state. The kinetics was followed by measuring the time dependence of the fluorescence anisotropy of 1,6-diphenylhexatriene. One relaxation process in the 10 to 200 ms time range was observed.

The aim of this study was to compare the results gained from our previous pressure jump experiments with DMPA vesicles with those from an electrostatically induced transition. In particular we were interested, whether we would find several relaxations as in the pressure jump experiment, or only one as reported by Strehlow and Jähnig [11]. In the case of DMPA we induced the ordered-fluid transition by the addition of dilute NaOH and the fluid-ordered transition by mixing with an HCl or $MgCl_2$ solution. We measured the kinetics in a temperature range covering the transitions of both the initial and the final state. In all cases we found several relaxation processes, their time constants passing through maximum values at temperatures characteristic for the transition of the initial state before mixing. In accordance with the results obtained by Strehlow and Jähnig [11] for methyl phosphatidic acid the kinetics can be described by classical non-equilibrium relaxation with nucleation as the rate-determining step.

Materials and Methods

DMPA was purchased from Fluka (Neu-Ulm, F.R.G.) and used without further purification. Unilamellar vesicles were prepared as described before [12]. Turbidity vs. temperature curves were recorded using a Perkin-Elmer Model 124 spectrophotometer, The variable temperature cell holder was heated with a MGW Lauda K2R thermostat. Heating and cooling rates were approx. 2 K/min. For the kinetic experiments a Durrum Model 110 Stopped-flow spectrophotometer was used. The phase transition of DMPA was triggered by mixing a 0.5 mM vesicle suspension with dilute HCl, NaOH or $MgCl_2$ solution, respectively. The time course of the absorbance at 360 nm was recorded with a Datalab DL 905 transient recorder. Evaluation of time constants was done as described before [13].

Results

The transition temperature T_m of our DMPA vesicle suspensions as a function of pH was determined by recording the absorbance as a function of temperature. Our results for the pH dependence of T_m agree with those published by Träuble and Eibl [3] and Eibl and Blume [6]. In the kinetic measurements we triggered the transition by a pH jump. In the case of a pH jump into the alkaline pH region the change in the degree of transition θ is schematically shown in Fig. 1. As T_m is lower at pH 10 we induce in this type of experiment a transition from the gel to the liquid-crystalline state in the temperature range between 46 and 54°C. Above 56°C the bilayer always remains in the liquid-crystalline state and below 46°C in the gel state, though the gel state bilayer structures for singly and doubly charged DMPA differ in the tilt angle of the hydrocarbon chains [8]. The time-course of the absorbance at 360 nm after a rapid pH jump from 6 to 10 is shown in Fig. 2. We observe a decrease of the absorbance corresponding to a change from the gel to the liquid-crystalline state. The curve can be fitted by two exponentials (see dashed line in Fig. 2).

For the kinetic analysis of the stopped-flow data three processes have to be considered: (1) diffusion of OH^- through the bilayer; (2) dissociation of $DMPA^-$ (ordered) to $DMPA^{2-}$ (ordered); (3) transition of $DMPA^{2-}$ (ordered) to $DMPA^{2-}$ (fluid). As proton transfer reaction are generally

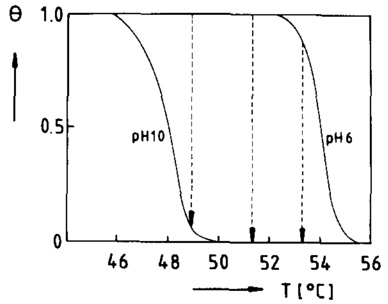

Fig. 1. Scheme of the change of the degree of transition θ for a pH jump from 6 to 10. $\theta = 1$ for the ordered and $\theta = 0$ for the fluid state.

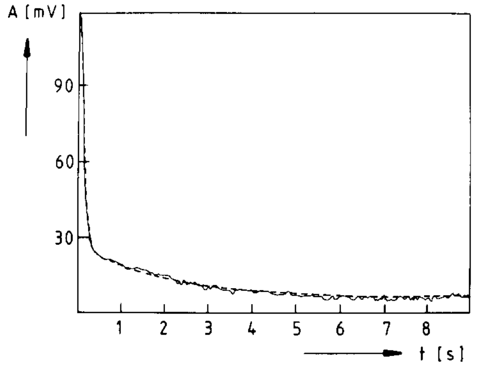

Fig. 2. Time-course of the absorbance at 360 nm of a DMPA vesicle suspension after a pH jump from 6 to 10 (10 mV = 0.001 *A* units). Temperature: 52.8°C, DMPA concentration: 0.5 mM, NaOH concentration: 1 mM. The dashed line is a simulation using two exponentials with time constants of 1.82 s and 61.7 ms, respectively.

diffusion controlled [14], reaction (1) will not be the rate-limiting step. To verify, that the diffusion of OH^- or H^+ through the bilayer is not rate limiting we measured the permeation of H^+/OH^- using an absorbance indicator entrapped in the vesicles [12]. We could show, that the H^+/OH^- permeation in the temperature range above 50°C is faster by at least one order of magnitude and can therefore not be rate limiting. In addition the rate constants for permeation do not display the characteristic maximum at the transition midpoint as usually found for cooperative processes [12]. Both time constants evaluated from the relaxation curves pass through maximum values at T_m (see Fig. 3). This proves that the observed processes are related to the cooperative phase transition and not to permeation. In contrast to the results obtained in the pressure jump relaxation experiments [2] the slowing down of the transition is not observed at the transition temperature of the final state at pH 10, i.e. 48°c, but more at the T_m of the initial state, i.e. at 52–53°C. This apparently contradicting result can explained by the assumption that the ordered-fluid transition proceeds from a bilayer state which is still characteristic for the singly charged form of DMPA. As the dissociation reaction (2) to $DMPA^{2-}$ is very fast, an ordered bilayer state is produced which has not the equilibrium gel state structure of doubly charged DMPA, i.e. tilted chains, but remains in a state where the chains are still almost perpendicular to the bilayer surface [8]. It is from this bilayer structure that the reaction proceeds directly to the liquid-crystalline state. We thus observe a transition from a state which is still structurally related to singly charged DMPA. This is in accordance with the finding that the time constants are much slower than those observed for doubly charged phosphatidic acids in the pressure jump experiments [2]. The time constants in Fig. 3 resemble more the two slower relaxation times in the pressure jump experiment. It cannot be excluded that a third faster process is also present in the stopped-flow experiment, but not observable because its time constant is shorter than the dead time of our instrument.

The transition from the fluid to the ordered gel state can be induced by mixing the DMPA vesicle suspension with 0.1 mM HCl as schematically shown in Fig. 4. The time-course of the absorbance after rapid mixing can in most cases be

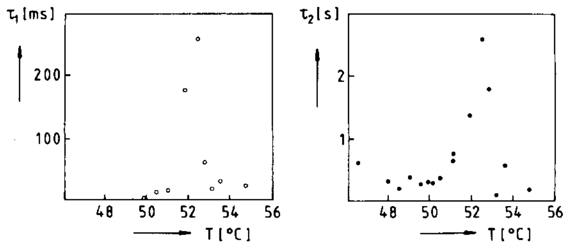

Fig. 3. Temperature dependence of the time constants τ_1 and τ_2 of the ordered-fluid transition induced by a pH jump from 6 to 10. The error margins for the time constants are approx. ±5% at the transition midpoint and increase to approx. ±10% below and above this temperature.

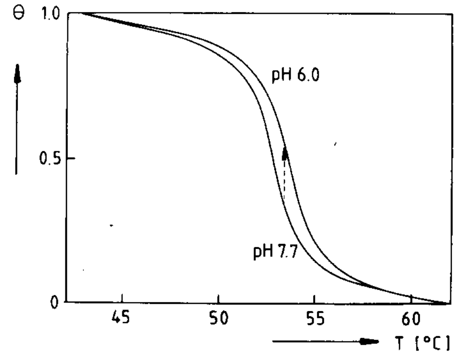

Fig. 4. Scheme of the change in the degree of transition θ for a pH jump from 7.7 to 6.

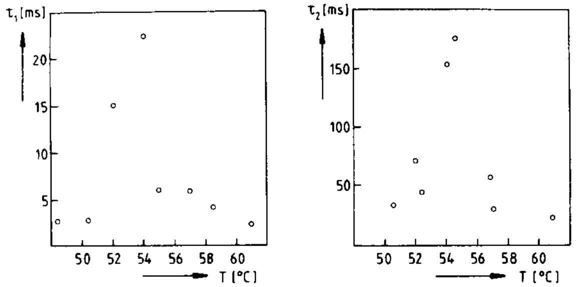

Fig. 5. Temperature dependence of the time constants τ_1 and τ_2 of the fluid-ordered transition induced by a pH jump from 7.7 to 6.

fitted with three exponentials. However, the slowest process in the second time range shows no distinct dependence on temperature and seems to be related to aggregation of the vesicles. Only the two faster processes display the characteristic maximum at T_m as shown in Fig. 5. As the shift in T_m for a pH jump from 7.7 to 6 is very small a distinction whether the maximum of the time constants is at the T_m of the initial or final state cannot be made.

The transition from the fluid to the ordered state can also be triggered by the addition of divalent ions, as they bind strongly to the charged headgroups. The binding of Ca^{2+} and Mg^{2+} leads to lowering of the surface charge density with a concomitant increase in the transition temperature [3]. We measured the dependence of T_m on the Mg^{2+} concentration of our DMPA vesicle suspension recording the absorbance at 360 nm. Our results agree with those published by Träuble and Eibl [3]. Fig. 6 shows schematically the change in the degree of transition after mixing a 0.5 mM DMPA vesicle suspension with a 0.2 mM $MgCl_2$ solution. The interpretation of the results of the kinetic experiments is somewhat ambiguous in this case due to slow diffusion of Mg^{2+} through the bilayer and aggregation of vesicles after Mg^{2+} binding. From the time dependence of the absorbance at least three different processes could be resolved. Their time constants are in the 10–100 ms, 0.5–1 s and 2–5 s range. As the two slower processes are probably partly due to aggregation, only the fastest process can definitely be assigned to the transition. As shown in Fig. 7 the time constant displays the characteristic maximum at T_m. Thus it can be excluded that Mg^{2+} permeation is the rate-limiting step for this process, as in this case the time constant should decrease at T_m as a consequence of enhanced ion permeation at the transition midpoint [15,16].

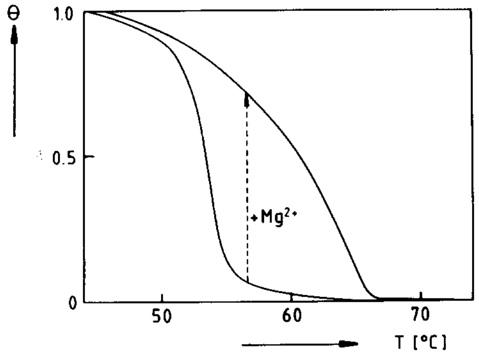

Fig. 6. Scheme of the change of the degree of transition θ for the Mg^{2+}-induced fluid-ordered transition. A 0.5 mM DMPA vesicle suspension was mixed with a 0.2 mM $MgCl_2$ solution.

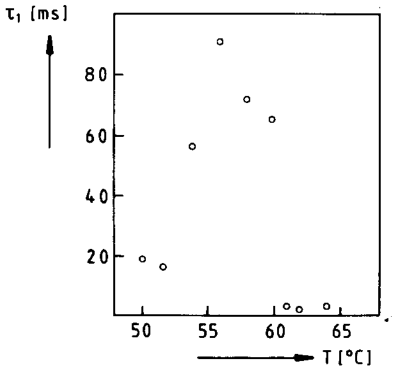

Fig. 7. Temperature dependence of the time constant τ of the fastest process of the Mg^{2+}-induced fluid-ordered transition.

Discussion

In our previous pressure jump relaxation study on the transition kinetics of DMPA vesicles we found a complex dependence of the time constants on pH and electrolyte concentration. In all cases we found two or three relaxation processes, respectively, each separated by approximately one order of magnitude [2]. The aim of this study was to test, whether results obtained from relaxation experiments, where the rapid change of a physical parameter, in our case pressure, triggers the phase transition, are comparable to results from a completely different type of experiment, where the change of a chemical variable, i.e. pH or ion concentration, induces the transition. In the only study of this type by Strehlow and Jähnig [11] the time dependence of the fluorescence anisotropy of the probe diphenylhexatriene was recorded after triggering the transition of the methyl ester of phosphatidic acid by H^+ or Ca^{2+}. Diphenylhexatriene is sensitive to changes of the orientational order of the lipid chains [11]. Strehlow and Jähnig observed only one relaxation time in the 10–200 ms range. This process has a similar time constant as the slower process we observed in the H^+-induced transition. However, a direct comparison between these two sets of data is not possible, as we used DMPA, which has completely different transition characteristics than the methyl ester of DMPA. As shown in our previous paper [2], changes in headgroup structure lead to large variations in the kinetic behaviour, in particular when strong attractive headgroup interactions are present. The differences between the two results seem therefore be related to the different chemical structure of the two lipids and not to inherent differences in the type of process observed either by recording the absorbance or the fluorescence anisotropy of diphenylhexatriene, respectively. As Gruenewald [17] has shown, DPH does detect two relaxation processes in phosphatidylcholine vesicles as observed before in the pressure jump experiment with turbidity detection [1].

Comparing the results of our previous pressure jump experiments with DMPA with those reported here for the electrostatically induced transition, the obvious differences are that in this case the structure of the initial state determines the transition kinetics. The time constants for the OH^--induced transition are much slower than those observed in a pressure jump experiments of vesicles at pH 10. As mentioned above, the system obviously relaxes directly to the liquid-crystalline state without first adopting the equilibrium gel state structure at this particular pH. The similarities are, that we definitely observe more than one relaxation process as in the pressure jump experiment. A direct comparison of the time constants derived from both types of experiments is not possible due to the inherent differences in the initial states from which the transition proceeds.

In the stopped-flow experiment the system is in a non-equilibrium state after the rapid mixing process. As shown by Strehlow and Jähnig [11] the kinetics can be described using classical theories for relaxations from non-equilibrium states. Following these theories two different mechanisms can be distinguished, depending on whether the non-equilibrium state is unstable or metastable [18,19]. For the first case the relaxation time τ depends on the degree of dissociation α according to

$$\tau \sim (\alpha' - \alpha_T)^{-1/2} \tag{1}$$

where α' is the degree of dissociation after the pH jump, which is fixed in our type of experiment, and α_T is the degree of dissociation, which would lead to a transition at this particular temperature T. α_T can be taken from a plot of the dependence of T_m on α (see Fig. 8), which is constructed from a titration curve of DMPA and a plot of the pH dependence of the transition temperature T_m [3,11].

For the second type Strehlow and Jähnig [11] found the following relation between τ and α:

$$\tau \sim \exp[\text{const.}/(\alpha' - \alpha_T)] \tag{2}$$

For the fluid-ordered transition of methyl phosphatidic acid Strehlow and Jähnig found a dependence of τ on α according to Eqn. 2 and concluded that the relaxation mechanism is of the 'heterophase' fluctuation type.

Plots of the time constants of Fig. 3 (transition

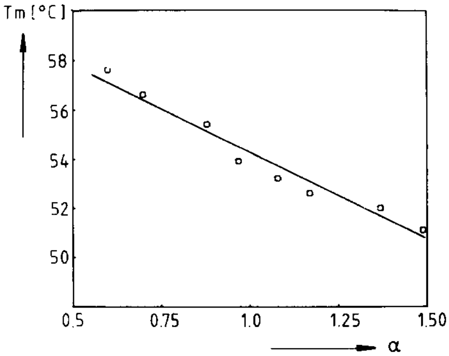

Fig. 8. Dependence of the transition temperature T_m of DMPA on the degree of dissociation α.

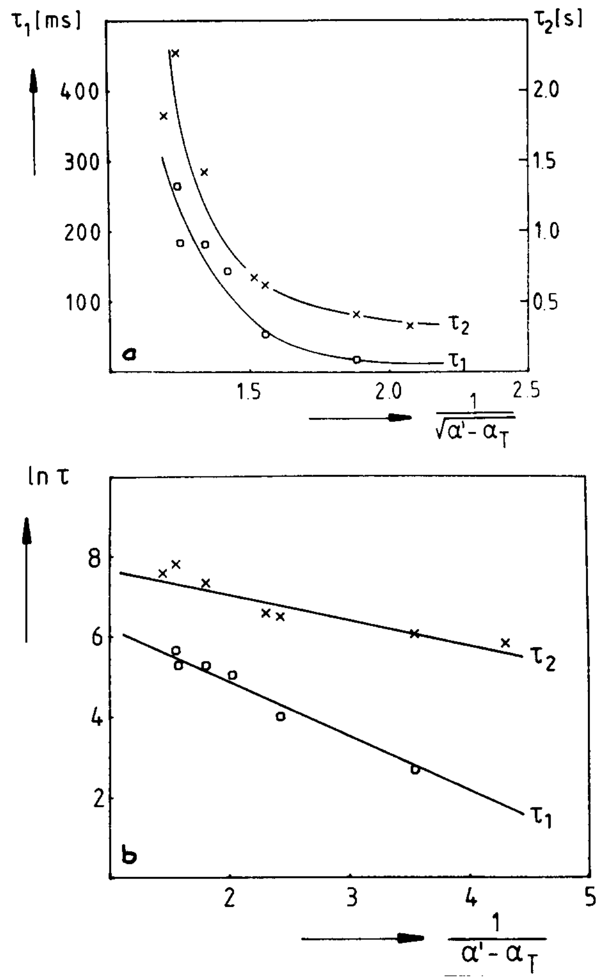

Fig. 9. (a) Plot of the time constants τ_1 and τ_2 of the OH$^-$ induced transition according to Eqn. 1. (b) Plot of the time constants according to Eqn. 2. See text for further explanations.

from the ordered to the fluid state) according to Eqns. 1 and 2 are shown in Fig. 9. It is evident that only a plot according to Eqn. 2 gives a straight line for both time constants. The same result is obtained when the time constants for the H$^+$-induced transition from the fluid to the ordered state are plotted. Thus for DMPA vesicles both transitions, i.e. the ordered-fluid as well as the fluid-ordered, start from a metastable non-equilibrium state. This is in agreement with the results of Strehlow and Jähnig and the observations in the pressure jump experiments. Thus nucleation is the rate-limiting step in the transition. The differences in the time constants between OH$^-$- and H$^+$-induced transition can be interpreted in the following way. The addition of protons facilitates better packing of the lipid molecules due to increased hydrogen bonding between headgroup, whereas the addition of hydroxyl ions produces doubly charged headgroups which cannot be hydrogen donors. The possibility to form an increased number of hydrogen bonds obviously accelerates the nucleation step, so that the transition from the fluid to the ordered state proceeds faster than the transition from the ordered to the fluid phase in the case of the pH jump into the alkaline pH region. Here clusters of DMPA in the fluid state have to be formed, but they cannot be stabilized by hydrogen bond interactions. The results obtained for the Mg^{2+}-induced transition can be interpreted in a similar way. They agree with those reported by Strehlow and Jähnig for the Ca^{2+} induced transition of methyl phosphatidic acid [11]. However, we observe additional slower processes, which we think are due to aggregation phenomena, but it cannot be excluded that they obscure other processes which are still related to the transition.

The results of our stopped-flow experiments show, that the electrostatically induced transition is also complex, as two or more relaxation processes can be resolved. The two time constants evaluated differ by approximately one order of magnitude. This is in agreement with our previous pressure jump experiments of the same system [2]. It shows, that the phase transition mechanisms for transitions induced by the change of a physical parameter or a chemical variable are very similar.

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