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PRETRANSITIONS IN THE HYDROCARBON CHAINS OF PHOSPHATIDYLETHANOLAMINES

A WIDE ANGLE X-RAY DIFFRACTION STUDY

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Summary

The hydrocarbon chain packing of fully hydrated phosphatidylethanolamine multilayers is investigated by X-ray diffraction. An analysis of the wide angle reflections (short spacings) as a function of temperature indicates that, apart from the well-known ordered-disordered lipid phase transition, a second transition takes place at lower temperatures. This transition, which is in the present paper referred to as the pretransition, is characterized by a transformation of the hydrocarbon chain packing. A first model for the chain lattice is presented, which gives rise to the expectation that similar pretransitions might be found with other phospholipids.

Introduction

There have been many studies dealing with the ordered-disordered lipid phase transition. In the present paper this transition, in which the hydrocarbon chains change from an ordered structure to a more liquid-like state, is referred to as the main phase transition. A number of calorimetric studies have also noted the existence of a pretransition for phosphatidylcholines [1—3], and for phosphatidylglycerols [4]. Interpreting optical probe studies Gebhardt et al. [5] proposed a pretransition in phosphatidic acid. As NMR studies of phosphatidylcholine indicated that the choline group was not associated with the pretransition, it was suggested that the phosphatidylcholine pretransition should be attributed to the hydrocarbon chains [6].

The pretransition of phosphatidylcholine has been related to a change in the wide angle X-ray diffraction lines and thus to a change in the packing of the hydrocarbon chains [7]. Whereas this change is well-documented [8], varying

Abbreviations: T_m, main phase transition temperature; T_p, pretransition temperature.

results have been reported for the small angle diffraction lines at T_p [7,9]. It is therefore not yet clear, whether the chains become perpendicular or remain tilted at the pretransition. The pretransition of phosphatidylcholine has been associated with the appearance of regular band patterns in the plane of the bilayer (so-called "ripples"), both in small angle X-ray diffraction [7] and in freeze-fracture studies [10]. The rippled structure was identified by the appearance of additional small angle reflections and was found only at temperatures between the pretransition and the main transition. In the case of phosphatidylglycerol, which is also known to show a rippled phase [4], additional low temperature phases have been reported recently [11].

The present X-ray diffraction study investigates the wide angle reflections of fully hydrated phosphatidylethanolamine multilayers as a function of temperature. A chain lattice transformation, similar to that reported for the phosphatidylcholine pretransition, was found at low temperatures. Therefore, in the present paper, this transformation is referred to as a pretransition, although none of the calorimetric measurements of phosphatidylethanolamine have yet shown a heat capacity change at low temperatures. The appearance of regular band patterns, which always points to the existance of a pretransition, was found not to be an indispensable feature of this transformation.

Materials and Methods

L-β,γ-Dipalmitoyl-α-phosphatidylethanolamine (puriss.) and L-β,γ-distearoyl-α-phosphatidylethanolamine (puriss.) were obtained from Fluka, Neu-Ulm and were used without further purification. In order to check the purity of the lipid, thin-layer chromatographic plates prepared with Merck "silica gel 60 HR" were used. The solvent system employed was $CHCl_3/CH_3OH/25\%\ NH_3$ (65 : 30 : 3, v/v). The plates were developed with "Dittmer" spray [12] followed by charring. Normal loadings showed a single spot; an overloading of 0.1 mg, however, revealed traces of free fatty acids. Care was taken that in all the samples the amount of these impurities as estimated from the thin-layer chromatographic plates stayed below 1%.

For the diffraction experiments a Guinier camera (operating under vaccuum) with a bent quartz crystal monochromator was used (R. Huber, 8211 Rimsting, West Germany). The monochromator was set ot isolate the $CuK\alpha_1$ line (λ = 1.5405 Å). The X-ray tube (AEG type F 50/21) with a focal size of 0.4 × 8 mm was operated at 50 kV and 20 mA. The use of the X-ray line focus reduced the beam width to 0.04 mm. In order to limit the axial divergence, Soller slits were employed in the diffracted beam [13]. The film cylinder had a diameter of 114.6 mm. The instrumental line broadening and the line broadening due to the sample thickness (0.3 mm) was estimated by the results obtained with finely powdered $CaWO_4$. The resolution of the set-up was found to be better than 0.2° for the 20° (2θ) range. Thus, lines in the 4 Å region with no intrinsic broadening and of similar intensity could be seperated as long as the line centres were 0.04 Å apart.

The lipid samples were prepared by adding 40 μl H_2O to 20 μg dry lipid and storing the samples at $T > T_m$ for 30—60 min. The samples were then sealed with Teflon between two mica plates and transferred to the X-ray sample

holder, which could be thermostated by a circulating liquid. Before the X-ray exposures were started, the samples were kept for 15 min at the desired temperature. The usual exposure times of the photographic films (Kodak, "Kodirex, une face") were 15 and 120 min. Teflon lines were used as an internal reference. The density of the reflections was scanned with a Joyce-Loebl microdensitometer type 3CS. The ratio of the observed long spacings (1, 1/2, 1/3, 1/4 etc.) indicated that the lipid was in the lamellar phase. In the densitometer traces only the strongest reflections could be demonstrated, although further orders could be seen visually.

Results

The X-ray diffraction lines of fully hydrated distearoyl phosphatidylethanolamine as a function of temperature are shown in Fig. 1. Whereas hardly any change in the position of the small angle lines (Fig. 1a) is found below T_m, the

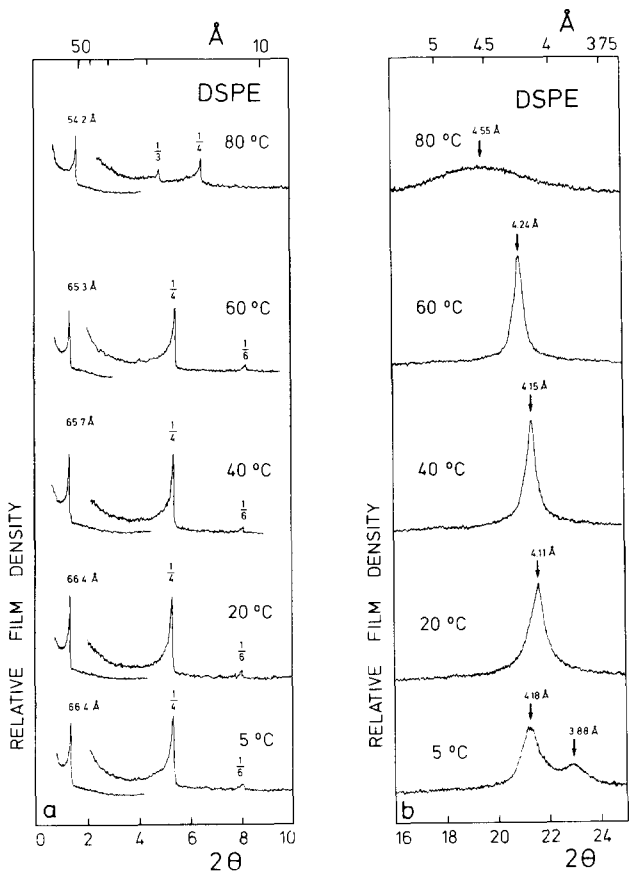

Fig. 1. X-ray diffraction lines of fully hydrated distearoyl phosphatidylethanolamine (DSPE) multilayers as a function of temperature. The reflections of the small and wide angle region are shown in a and b, respectively. The film density (relative units) is plotted against the diffraction angle 2θ and the corresponding Bragg spacings. For innermost reflection ($2\theta < 2$) a shorter exposure time was used.

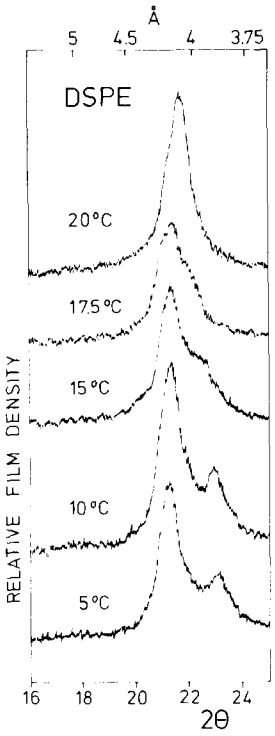

Fig. 2. Wide angle X-ray diffraction lines of the same system as in Fig. 1 at temperatures between 5 and 20°C. DSPE, distearoyl phosphatidylethanolamine.

wide angle reflections show a distinct temperature dependance (Fig. 1b). At 5°C a strong line at 4.18 Å is followed by a weaker reflection at 3.88 Å. With increasing temperature the weaker line moves towards smaller angles, until at around 20°C only a single sharp line at 4.11 Å is visible (Fig. 2). This change is, in the present paper, defined as being characteristic for the pretransition.

With further increasing temperature the single line becomes sharper and moves towards smaller angles, until is reaches a value of around 4.30 Å just below the main transition temperature (T_m (distearoyl phosphatidylethanolamine) = 71°C, [14]). At the same time a slight decrease in the lamellar spacings can be seen (Fig. 1a). At 80°C ($T > T_m$) the diffuse line at 4.55 Å and the change of the small angle reflections towards smaller lamellar spacings indicate that the lipid is in the disordered state. The temperature dependence of the long and the short spacings between T_p and T_m is similar to, although somewhat smaller than, the one reported for dipalmitoyl phosphatidylcholine [15].

The results obtained with dipalmitoyl phosphatidylethanolamine are analogous to the one for distearoyl phosphatidylethanolamine, but, as the wide angle line splitting takes place in the range of the H_2O freezing point, the two lipid reflections are overlapped by sharp ice peaks. (For the appearance of ice peaks at temperatures below zero degrees see Costello and Gulik-Krzywicki [16].)

In the following paragraphs a first model is adopted to describe the observed changes in the wide angle diffraction lines. The model is slightly different to

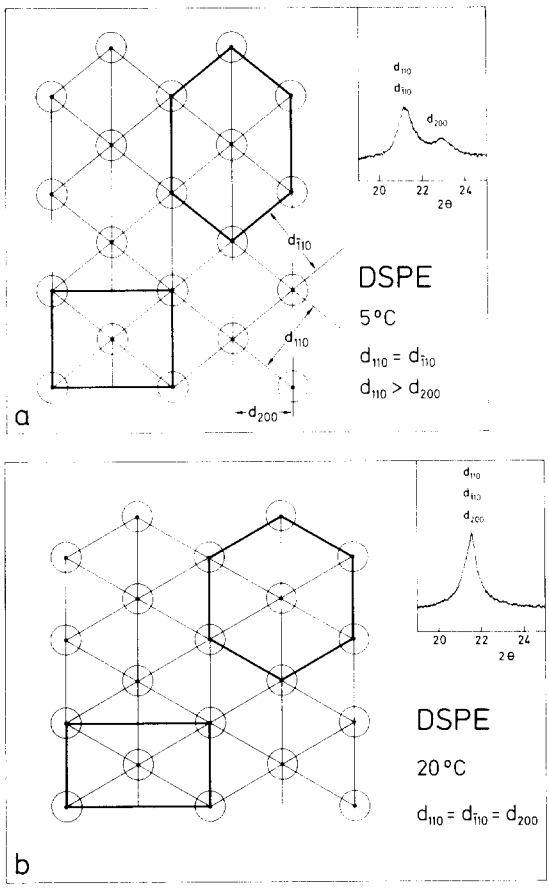

Fig. 3. Model for the hydrocarbon chain packing of phosphatidylethanolamine below (a) and above (b) the pretransition. Inserts show the corresponding wide angle diffraction lines. DSPE, distearoyl phosphatidylethanolamine.

the one suggested for phosphatidylcholine [8]. Figs. 3a and 3b show the chain lattice in a plane perpendicular to the chain axes below and above the pretransition. The chains are indicated as circles. At the bottom of the lattice the cross-section through a unit cell is marked, whereas the corresponding hexagon is indicated in the top right-hand corner or the lattice. The numbering of the d_{110}, $d_{\bar{1}10}$, and d_{200} lattice planes is shown in Fig. 3a.

In Fig. 3b (T = 20°C, $T > T_p$) it can be seen that the chains are stacked in a hexagonal way, which gives rise to a single wide angle diffraction line. The lattice spacings d_{110}, $d_{\bar{1}10}$, and d_{200} are identical. At 5°C, however, the chains are not stacked hexagonally (Fig. 3a). Here d_{110} and $d_{\bar{1}10}$ are equal and form the 4.18 Å line, whereas d_{200} is smaller and lies at 3.88 Å. The actual type of this plane lattice is rectangular I [17]. Increasing the temperature from 5°C causes an anisotropic expansion of the lattice in the direction perpendicular to the d_{200} plane, because d_{200} increases whereas d_{110} and $d_{\bar{1}10}$ stay constant. (In Fig. 3a the d_{110}/d_{200} ratio is drawn larger that the measured ratio, in order to demonstrate the different lattice types.)

From the above model the area per phospholipid molecule (in a plane perpendicular to the chain axes) can be calculated as

$$F = \frac{2d_{110}^2}{\sin 60^\circ} \text{ for the hexagonal and}$$

$$F = 4d_{200}^2 \tan \left(\arcsin \frac{d_{110}}{2d_{200}}\right) \text{ for the rectangular I}$$

lattice. As the area at 20°C (F (20°C) = 39.0 Å^2) is hardly different to the area calculated for 5°C (F (5°C) = 38.5 Å^2) and the long spacings do not change, there is therefore very little change in volume between these two temperatures, assuming the hydration to remain constant. The lack of volume change might constitute a difficulty in detecting the phosphatidylethanolamine pretransition by other techniques.

The model, as it is presented here, does not differentiate between different tilt angles. If the chains are tilted, as in the case of phosphatidylcholine, the packing in the plane perpendicular to the chain axes will be quite different to the packing parallel to the plane of the bilayer.

Discussion

A common feature of the phosphatidylethanolamine and the phosphatidylcholine pretransition is that the hydrocarbon chains assume a configuration of hexagonal symmetry. However, considerable differences exist between phosphatidylethanolamine and phosphatidylcholine, both in the temperature of the pretransition and in the temperature of the main transition. Furthermore, in phosphatidylcholine a rippled phase is found between T_p and T_m. A possible reason for these remarkable differences could lie in different angles of tilt. Whereas for phosphatidylcholine a tilt angle of 28° has been measured [18], a tilt angle has not yet been reported for phosphatidylethanolamine.

The conformation of hexagonal symmetry in the case of phosphatidylcholine might be sterically hindered by the rather large angle of tilt. Therefore T_p (phosphatidylcholine) is higher than T_p (phosphatidylethanolamine). As the hydrocarbon chain interaction is reduced with increasing tilt angle owing to end group effects, one would then expect the main transition temperature to decrease with increasing tilt angle. This might be one reason why T_m (phosphatidylcholine) $< T_m$ (phosphatidylethanolamine). (It is interesting to note that, in the case of phosphatidylcholine, the chain tilt increases with increasing hydration [8] and at the same time a decrease in T_m is found [1].)

The reason for the packing with hexagonal symmetry is not yet clear. In paraffins the hexagonal lattice is known to be due to a long axes rotation of the paraffin molecules [19,20]. However, it has been pointed out that the hexagonal symmetry found in phospholipids cannot be due to a rotation of the chains [21]. It would be interesting to investigate whether the pretransition of phospholipids is associated with a change in the motion of the lipid molecules. For example, the fact that the water structure of phosphatidylcholine becomes more isotropic at T_p [22] could be caused by an increase in the lipid mobility.

As the real cross-section of hydrocarbon chains is not circular, but is prop-

ably more elliptical [19,20], the direction of the individual chains might have a defined configuration at temperatures below T_p. At present it does not seem possible to define the subcell of the chains, and so the chains in Fig. 2a are still drawn with a circular cross-section. (For different subcells and packing possibilities of hydrocarbon chains see res. 23 and 24.) The exact carbon chain packing has been reported for dilauroyl phosphatidylethanolamine-acetic acid crystals [25].

A closely-packed lattice of chains with elliptical cross-section is probably not a hexagonal lattice. Other phospholipids should then, at low enough temperatures, assume a hydrocarbon chain packing, which is also not a hexagonal arrangement. However, as many phospholipids show a hexagonal packing at room temperature, it seem likely that other phospholipids also show a pretransition at lower temperatures. In fact pretransitions were found in the course of this study also with dipalmitoyl phosphatidic acid.

Besides the familiar ordered-disordered phase transition temperature, the actual position of the pretransition could be another feature by which the state of phospholipid multilayers can be characterized. Two phospholipids could show the same main transition temperature T_m, but a large difference in the pretransition temperature T_p would indicate that the structure of the two lipids is in fact quite different.

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