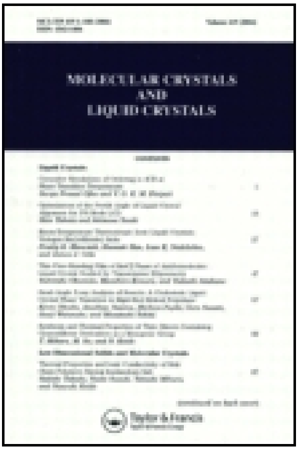

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Hysteresis at the L_α-$P_{\alpha\beta}$ Phase Transition in Hen Egg Lecithin

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Infrared spectroscopy in the OH and CH stretching region of very thin samples of hydrated egg yolk lecithin reveals an hysteresis at the transition between the two liquid crystalline phases L_α (higher temperature phase) and $P_{\alpha\beta}$. Both of these phases have a lamellar structure with alternating hydrophilic and hydrophobic layers, but they differ as to the state of the interiors of the layers. The hysteresis can be explained in terms of a coupling between the structures and the dynamics of adjacent layers.

Lecithins (1,2-diacyl phosphatidylcholines) are one of the main constituents of the cell membrane and have been used by many investigators as model systems of the cell membrane. Hydrated lecithins, at moderate water content, can form, depending on the temperatures, a variety of smectic-like lyotropic phases where bimolecular layers of lecithin are intercalated between water layers. The polar moieties of the lecithin molecules are at the surfaces of the bilayers, in contact with the water layers, and the alkyl chains of the lecithin are in the interior of the bilayers.

The processes that take place in a living cell membrane are very complex[1,2] and it is often difficult to determine the origin of the effects arising from the various membrane components unless data are available from physical studies on simpler artificial systems whose structure and composition, although different from that of the cell membrane, are known and predetermined. Furthermore a study of lyotropic mesophases gives matter for speculation that is of fundamental physical interest.[7]

In this paper we report the study of a transition between two liquid-crystalline phases of hydrated egg yolk lecithin by means of infrared spectroscopy in the frequency range of the OH and CH stretching vibrations. Between the room temperatures phase L_α and the lower temperature phase, $P_{\alpha\beta}$, an hysteresis has been observed that is accounted for by a coupling between the geometrical and the dynamical states of adjacent hydrophobic and hydrophilic layers.

EXPERIMENTAL

Egg yolk lecithin was obtained by the Singleton method. Weighed amounts of water were added to egg lecithin in order to obtain a hydrated hen egg yolk lecithin (hEL) with a 10% water content. This was allowed to equilibrate for two days at room temperature and was then stored at −18°.

Samples suitable for infrared spectroscopy were prepared by smearing, at room temperature, a convenient amount of hEL between two AgCl windows with no spacer in between. The sandwich was then clamped in a cell holder. It was not possible to measure accurately the thickness of the samples, but we estimated it by comparing the heights of some moderately intense absorption lines (in the range 1000–2000 cm^{-1}) given by the samples so obtained with those of a sample obtained with a 11 μm teflon spacer between the AgCl plates. Thicknesses as low as about 2 μm were obtained.

Absorbance spectra were obtained with a Beckmann IR 12 spectrometer. The AgCl windows were continuously bathed in a stream of dry nitrogen in order to avoid water condensing on them during the low temperature experiments.[8] The temperature stability at the cell holder was better than 0.1°. The temperature was cycled between room temperature and about −20° using temperature sweeping rates as low as about 0.3°/hour.

Neither specimen was removed from the measuring apparatus during the time necessary to perform an entire temperature cycle (i.e. several days). The design of the cell holder and of the thermostat allowed no accidental movement of the specimen during this time.

RESULTS

Figure 1A shows the room temperature absorbance spectrum of hEL (estimated sample thickness ~3 μm). According to the usual assignments, the broad (300 cm^{-1}) band centered at 3390 cm^{-1} is due to the OH stretching vibrations.[3] The narrow bands are due to the symmetric (2850 cm^{-1}) and to the antisymmetric (2930 cm^{-1}) stretching vibrations of the CH_2 groups of the alkyl chains.[4] The weak peak at 2965 cm^{-1} (barely visible in Figure 1A) can be attributed to the antisymmetric stretching of the terminal CH_3 groups.[4]

Figure 1B shows the absorbance spectrum of the same sample at −20°. All the band intensities have increased and, while no change was measured in the width and in the center frequency of the CH bands, the OH band appeared broader than that obtained at room temperature (400 cm^{-1}) and was de-

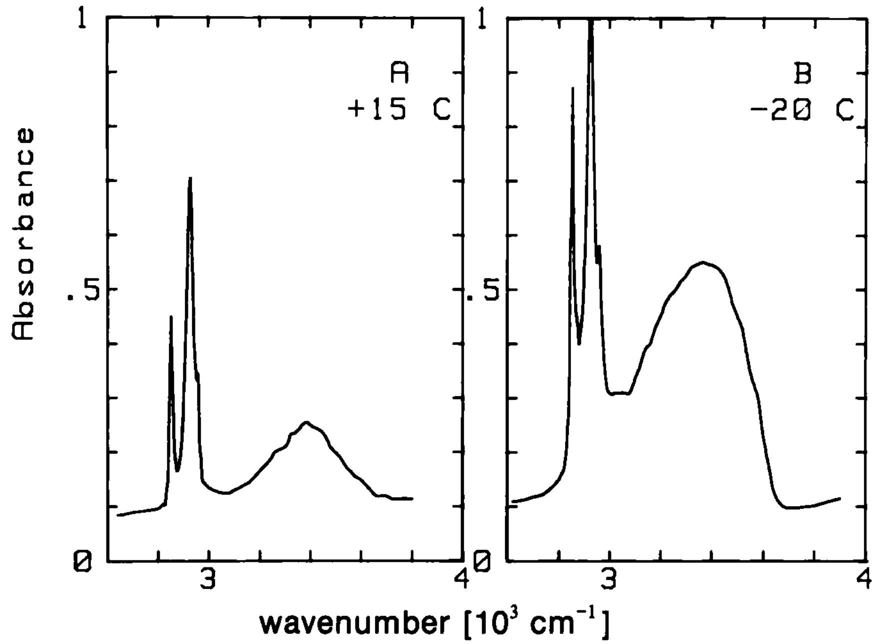

FIGURE 1 Absorbance spectrum of hydrated (10% water content) hen egg yolk lecithin. Sample thickness ~2 μm. Spectral resolution <5 cm^{-1}. A) room temperatures. B) −20°C.

formed, as well as being centerd at a frequency 55 cm^{-1} lower, i.e. at 3335 cm^{-1}.

On cycling the sample temperature between room temperature and about −20°C an hysteresis loop was observed in the band intensities, as shown in Figure 2 (where for comparison purposes all the band intensities were normalized to their room temperature value). The loops of the two CH bands are very similar to each other, with smooth transitions, but are not coincident: in fact the (CH sym. str.)/(CH antisym. str.) band intensity ratio was a decreasing function of the temperature. The OH band intensity exhibited sharper transitions than the CH bands.

The hysteresis loop of Figure 2 was obtained with very low cooling and warming rates, of the order of about 0.3°/hour. In addition, before taking each spectrum, the sample was allowed to reach equilibrium for a few hours. Fast cooling rates shifted the cooling branch of the hysteresis to a lower temperature and made it steeper. The warming branch of the hysteresis loop was little affected by the warming rate.

DISCUSSION

In hEL at room temperature, the OH stretching band is centered on a frequency about 50 cm^{-1} lower than that of pure water and its molar extinction

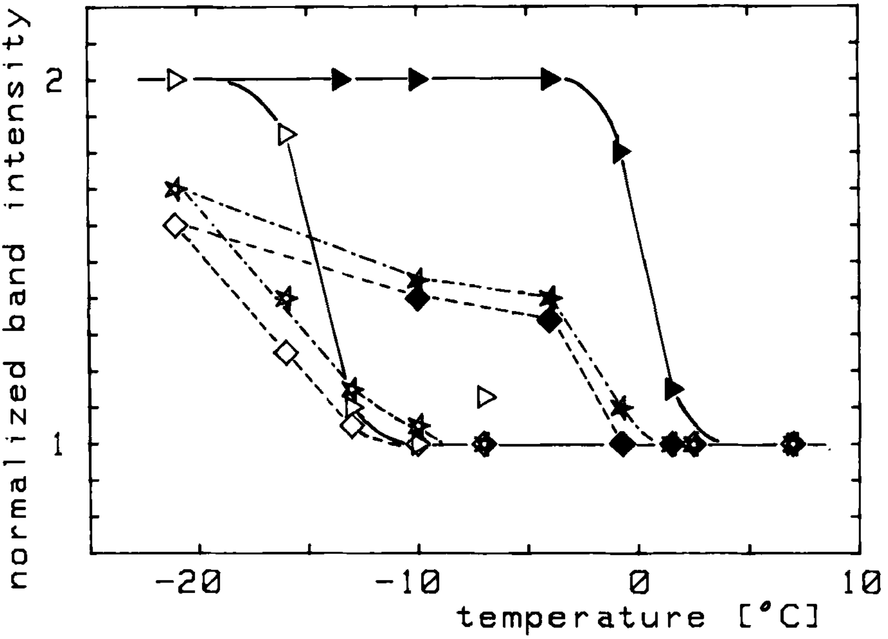

FIGURE 2 Temperature hysteresis loops of the intensity of the bands of the sample of Figure 1. The band intensities have been normalized to their room temperature value for the sake of comparison. Triangles: OH stretching. Diamonds: CH antisymmetric stretching. Stars: CH symmetric stretching. Open symbols: cooling. Bold symbols: warming. Lines are intended as a guide for the eye only.

coefficient is ~3 × 10^5 cm^2/mole (calculated assuming absorbance −0.15; sample thickness ~2 μm; average water density within the sample ~0.1 g/cm^3). It has been reported[12] that the peak absorptivity of the OH stretching band of ice at 100°K is 3.3 × 10^4 cm^{-1}. This corresponds to a molar extinction coefficient of 5.9 10^5 cm^2/mole. It has also been reported[3] that in liquid water the molar extinction coefficient of the same band is about 0.6 10^5 cm^2/mole. Although this figure was obtained by extrapolation using H_2O/D_2O mixtures and may not be strictly comparable, we consider it to be a reasonable approximation to the value for pure H_2O. We conclude that in hEL at room temperature the water molecules are in a bound state, in agreement with the results of other workers.[7] The shift to lower frequency together with the increase in the intensity and in the width of the OH stretching band show that at −20° the water molecules are even more bound (but not as much as in ice, where the OH stretching band occurs near 3200 cm^{-1}). The frequency shift of the OH stretching band is related to the strength of the hydrogen bonds.[3]

The occurrence of an hysteresis in the OH as well as in the CH bands (Figure 2) shows that a phase transition takes place and that the transition affects both the polar and the lipid regions.

The smoothness of the transitions of the CH bands and the temperature dependence of their intensity in the low temperature phase (warming branch of the hysteresis) can be more easily discussed after a description of the structure of the two phases between which we observe the hysteresis.

According to the phase diagram reported by Luzzati and Tardieu, hEL, at room temperature and at our water content, is in the L_α phase, while at −20° it is in the $P_{\alpha\beta}$ phase. The L_α phase is characterized by a cylindrical symmetry around the normal to the layers and by alkyl chains in a molten state. In the $P_{\alpha\beta}$ phase the alkyl chains are predominantly in a rigid state, but a temperature dependent fraction of them containing the shortest and the more unsaturated chains are still in the molten state. In the $P_{\alpha\beta}$ phase, the molten chains are segregated from the rigid chains to form a two-dimensional lattice within the lecithin bilayers.[5] This segregation may be due to the fact that the transition from the rigid to the molten state of a system of alkyl chains occurs at higher temperatures as the chain length increases.[6]

Let us neglect for the moment the smoothness of the two transitions that form the hysteresis loop; this will be discussed later, together with the temperature dependence in the $P_{\alpha\beta}$ phase.

The jump observed in the CH stretching absorption at a transition reflects the change of state of the alkyl chains, as they pass from a molten state to a more solid-like state (or vice versa). A configurational transition greatly affects the intermolecular motions. These, by several possible mechanisms, determine the intensity of the absorption of the intramolecular vibrations, but have little effect on the force constants, and hence on the absorption frequency.[13] It has been shown, for example, that intermolecular motions introduce an anharmonic contribution to the local field which, while only affecting the absorption frequency to a very small extent, causes the absorption of the intermolecular vibrations to decrease appreciably as the temperature increases.[14] This effect has been experimentally observed with liquids.[15,16] We would expect its enhancement at a liquid–solid transition.

The peculiar structure of the $P_{\alpha\beta}$ phase accounts for the smoothness of the transitions as well as for the temperature dependence of the CH absorption in the $P_{\alpha\beta}$ phase. The coexistence of rigid and molten alkyl chains in proportions that are temperature dependent causes the CH absorption to be temperature dependent because the CH bonds contribute to the absorption with different weightings depending on whether they are in a molten or in a solid-like domain.

Provided that the cooling rate is slow enough to allow the segregation process to take place, the L_α to $P_{\alpha\beta}$ phase transition is expected to occur smoothly over a quite wide temperature range. Quenching, on the other hand, causes the transition to occur more abruptly and presumably because of the greater local heterogeneity of the system of alkyl chains, it occurs at a lower temperature than for slow cooling.

For a more detailed picture we must consider also the water molecules. These form a sub-system which interacts with the alkyl chains *via* the polar heads of the lecithin molecules and which is kept in a fluid-like state by the diffusive motions of the lecithin molecules. On cooling, however, the water molecules undergo a transition to a more bound state. This transition occurs in a narrow temperature range because the water molecules form a homogeneous system. The reslt of the transition in the polar layers is a slowing down of the diffusion of the lecithin molecules; condensation nuclei are allowed to form in the lipid bilayers and the segragation process is started.

The hydrophilic and the hydrophobic moieties of the lecithin molecule are linked by quite flexible bonds, so that the system of alkyl chains and the system formed by water plus the polar heads of the lecithin molecules generally behave more or less independently. In the proximity of a phase transition, however, the interaction becomes significant and, bearing in mind the layered structure of hEL, it is to be expected that the thermal motions of the two subsystems will be coupled. We believe that this kind of crossed feedback between the two sub-systems causes a synergism[10] which produces the observed hysteresis. Hysteresis of this kind may be a general phenomenon exhibited by a wide range of inhomogeneous systems.[9]

SUMMARY

The model we propose for the L_α to $P_{\alpha\beta}$ phase transition is consistent with our experimental data: the smoothness of the transition of the CH bands is explained by invoking a segregation process that occurs among the lecithin molecules according to the length and the saturation of their alkyl chains, while the water molecules, which form a homogeneous system, exhibit a sharp transition. The segregation is made possible by the lateral diffusion of the lecithin molecules. This, in the condition of our experiment, is a very slow process.[11] In the L_α phase the aqueous layers are fluid and therefore interactions across the hydrophobic/hydrophilic boundary offer no hindrance to the easy lateral diffusion of the lecithin molecules within a layer. Conversely, if this diffusion is occurring at an appreciable rate it will keep the water layers disordered and fluid. This positive feedback will delay the phase change when the temperature is lowered. If we start with the more ordered $P_{\alpha\beta}$ phase however, the decreased fluidity of the aqueous layers hinders diffusion in the lipid layers and the absence of the disruptive effects of diffusion in the lipid regions reciprocally tends to stabilize the structure of the aqueous regions, delaying the phase change as the temperature is raised. Thus, the hydrophobic and hydrophilic layers tend to stabilize each other in either the fluid disordered L_α state or in the more ordered $P_{\alpha\beta}$ state and an hysteresis results.

The model is consistent with the results of other investigators.[5-7] However, it is still incomplete; further experiments, a few of which are under way, are necessary in order to gain a detailed knowledge of the interaction between the polar and the lipid layers, as well as that between the water molecules and polar heads of the lecithin molecules.

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