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FAR INFRARED ABSORPTION OF THE WATER–LECITHIN SYSTEM

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SUMMARY

Transmission spectra through a thin water–dipalmitoylphosphatidylcholine system show a decrease in the absorption of far-infrared radiation at normal incidence after thermal cycling through the transition temperature. It is suggested that the absorption may be due to the dipoles of the choline groups, and that the data may be explained by analogy with the infrared absorption in ionic crystals.

INTRODUCTION

The water–lecithin system forms a simple model system of biological membranes. When a lipid–water system is heated, it undergoes a phase transition from crystalline state at a temperature which increases with increasing hydrocarbon chain length and decreases with increasing water content, A limiting transition temperature (Kraft point) is reached for water contents larger than about 30%. For water–dipalmitoylphosphatidylcholine this transition temperature is about 41 °C[1–3]. Above the transition temperature the polar head groups have a higher degree of rotational freedom and the hydrocarbon chains are in a more liquid-like state[1,2]. Thus, if there is an infrared absorption due to vibrations of the positive charges on the choline groups with respect to the negative charges of the phosphate groups, a change of this absorption may be expected at the transition temperature.

EXPERIMENTAL

Far-infrared absorption measurements between 8 and 200 $\mathrm{cm^{-1}}$ (1250–50 μm wavelength) were made using a modified[4] Beckman/R.I.I.C. FS-720 Michelson interferometer and a ^{3}He-labelled cooled bolometric detector[5]. Samples were placed in polyethylene-windowed cells of 19 mm diameter, The optical path length was either 0.1 ± 0.01 mm or 0.2 ± 0.01 mm. Sample temperature was controlled to ± 0.5 °C by a copper enclosure heated by circulating water. Because the interferometer operates in a single-beam mode, ratios of the transmission spectra at various temperature were performed to determine the relative absorption strengths.

The samples were approx. 1:1 by weight of double-distilled water (pH 7.0, $\rho = 10^{-7}\ \Omega\cdot$cm) and β,γ-dipalmito-L-α-lecithin (purum) from Fluka, Buchs, Switzerland. They were prepared by sonicating the constituents for 2 h at full power in an ultrasonic bath (E/Mc Corp., Model BP1) operating at 20 kHz. The resulting gel was placed into the open sample holder and the windows were clamped in place.

RESULTS

The total transmitted intensity, I_0 (t), over the entire frequency interval (up to 200 cm^{-1}) was measured as a function of sample temperature. The results are shown in schematically in Fig. 1. Starting with the sample in the gel phase at 24 °C (for the previous 24 h), heating causes a continuous decrease in transmitted signal until $t=39\pm1$ °C; here I_0 (39 °C)$=0.75$ I_0 (24 °C). Slight additional heating results in a sharp increase in signal until I_0 (42 °C)$=1.2$ I_0 (24 °C); there is a 60% increase in signal over 3 °C of temperature rise. Further heating causes the signal to drop gradually until I_0 (60 °C)$=0.9$ I_0 (24 °C). Holding the sample at 60 °C for 1 h produced no further changes in transmitted intensity. Next the sample was slowly cooled (over a period of 1 h) to 24 °C. The signal increased gradually, without any sharp transitions, to I_0' (24 °C)$=1.45$ I_0 (24 °C), where I_0' denotes the value of intensity transmitted through the sample after one thermal cycle. Holding the sample at 24 °C for 1 h produced no further changes in signal. Additional slow heating to 60 °C and subsequent cooling to 24 °C caused the signal to vary smoothly between 1.45 I_0 (24 °C) at 24 °C and 0.9 I_0 (24 °C) at 60 °C. No sudden changes in signal occur, other than the one that occurs during the initial heating.

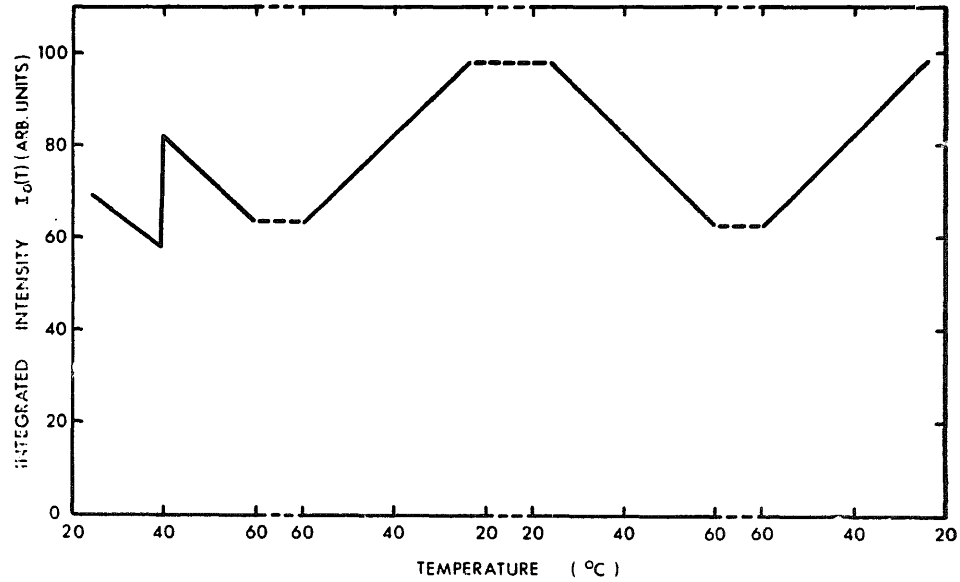

Fig. 1. Schematic diagram of a typical thermal history of a 0.2-mm thick sample of lecithin + 49.7% water. The sample starts at room temperature at the left side of the figure and is alternately heated and cooled.

We interpret these results as the destruction of the ordered solid phase at $t \gtrsim 40$ °C (which is the Kraft point). This permits increased transmission of infrared radiation in the new, more disordered phase. This phase persists even when the sample is returned to and held at room temperature for up to 1 h. The destruction of the ordered phase is not permanent; it re-establishes itself overnight at room temperature. Hysteresis effects in liquid crystals have been observed previously[6].

Measurements of the frequency spectrum of the transmitted radiation were also made, at an instrumental resolution of 2.4 cm^{-1}. Because of the very low signal levels encountered, several transmission spectra were taken at each temperature and the results averaged digitally. Typical results for a sample of 49.7% water 0.2 mm thick are shown in Fig. 2. Spectra are shown for the sample at room temperature before and after thermal cycling to 60 °C, as well as one spectrum taken with the sample at 60 °C. It is clear that energy is absorbed at high frequencies in the solid phase before thermal cycling, when compared with the sample at either room temperature after thermal cycling, or at 60 °C.

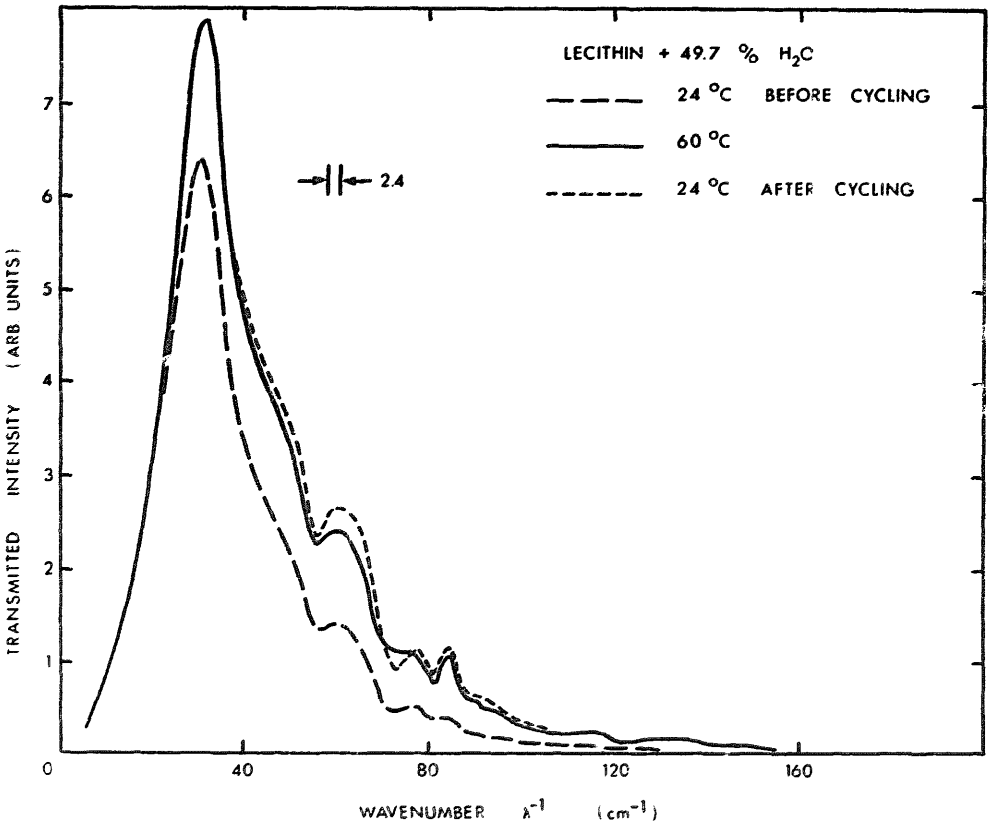

Fig. 2. Transmission spectra of a 0.2-mm thick sample taken at three points in its thermal history. Instrumental resolution of 2.4 cm^{-1} is indicated. Spectra are reliable to ±3% between 10 and 60 cm^{-1} and are reliable to better than ±8% at higher frequencies up to 100 cm^{-1}.

To quantify the amount of absorption, the transmitted intensities at 24 °C (before cycling) and at 60 °C were divided digitally by the intensity transmitted at 24 °C (after cycling). The resultant ratios are displayed in Fig. 3. The comparison of room temperature spectra in Fig. 3 shows a marked increase in absorption at higher frequencies. Comparison of the spectrum at 60 °C suggests a slight frequency-dependent absorption.

Because of the high absorption at frequencies $> 100\ \mathrm{cm}^{-1}$, the signal-to-noise ratios of the data in Fig. 3 are very poor $\gtrsim 100\ \mathrm{cm}^{-1}$. Samples one-half as thick (0.1 mm) improved the situation considerably. The results are shown in Fig. 4 for a sample at 24 °C before thermal cycling to 60 °C. Also shown is the ratio of this spectrum with one taken at 24 °C after thermal cycling. Although the signal-to-noise ratio is still poor, a peak in the absorption at 95–100 cm^{-1} is clearly evident.

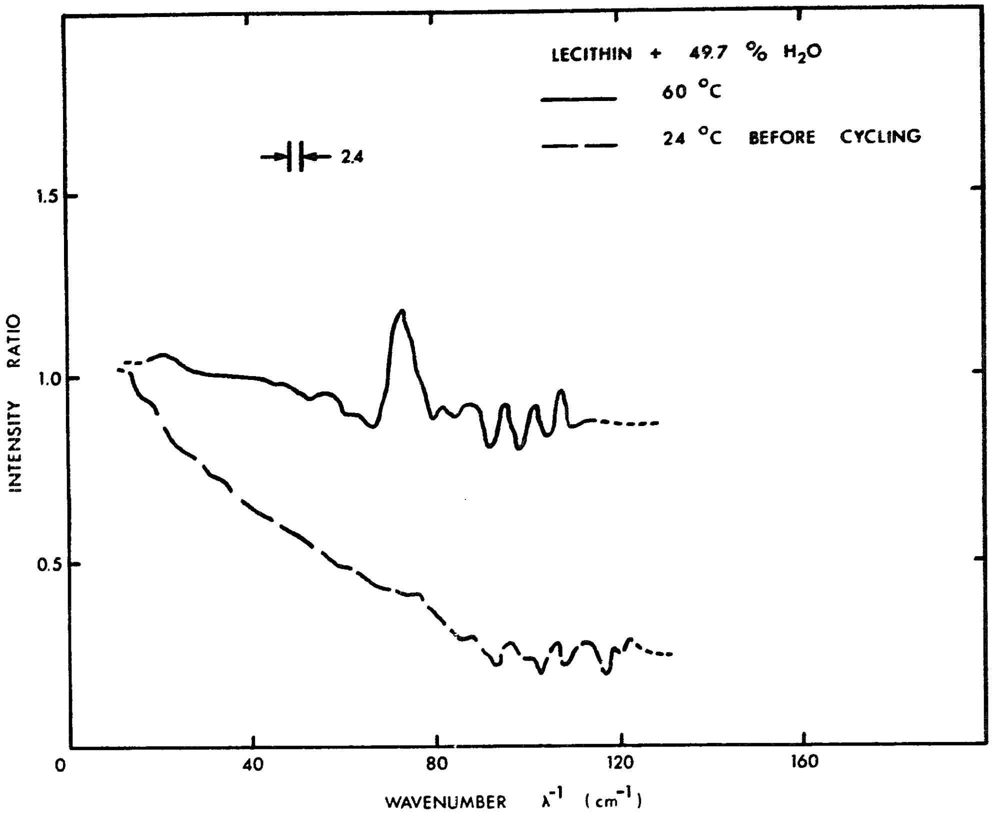

Fig. 3. Ratios of the intensity spectra display in Fig. 2. The spectrum taken at 26 °C after thermal cycling has been divided into the two other spectra. Ratios are reliable to ±5% between 10 and 60 cm^{-1} and are reliable to better than ±11% at higher frequencies up to 100 cm^{-1}. The structure near 75 cm^{-1} at 60 °C is due to a temperature-dependent absorption in water.

DISCUSSION

We concentrate our attention on the ratios which compare spectra taken at the same temperatures. In this way, all effects associated with the sample holder, such as temperature-dependent absorption in the polyethylene windows, will divide out. From Fig. 3, we see that the additional strong absorption associated with the solid phase is strongly frequency-dependent and peaks near 100 cm^{-1}.

Although no specific attempts were made to produce alignment of the lipid planes, some preferential alignment parallel to the hydrophobic windows is expected. Since the radiation is incident normally to the windows, the alignment of the layers is perpendicular to the direction of propagation of the radiation. For absorption to occur, the E-vector of the radiation must be directed along the oscillating dipoles which are responsible for the absorption. We suggest that it is the vibrations of the positive charges on the choline groups with respect to the negative charges on the phosphate groups which cause the absorption. A simple model calculation shows this to be plausible. We assume that the dipoles of the choline group act like an isolated Einstein oscillator, and that the negative group is anchored to the heavy (approx. 700 amu) hydrocarbon chain. Then the reduced mass μ of the oscillator is approximately that of the positive $N^+(CH_3)$ part of the choline group, or about 59 amu. In order to estimate the effective force constant for the oscillator, we draw a parallel between this system and the alkali halides, highly ionic crystals. A typical

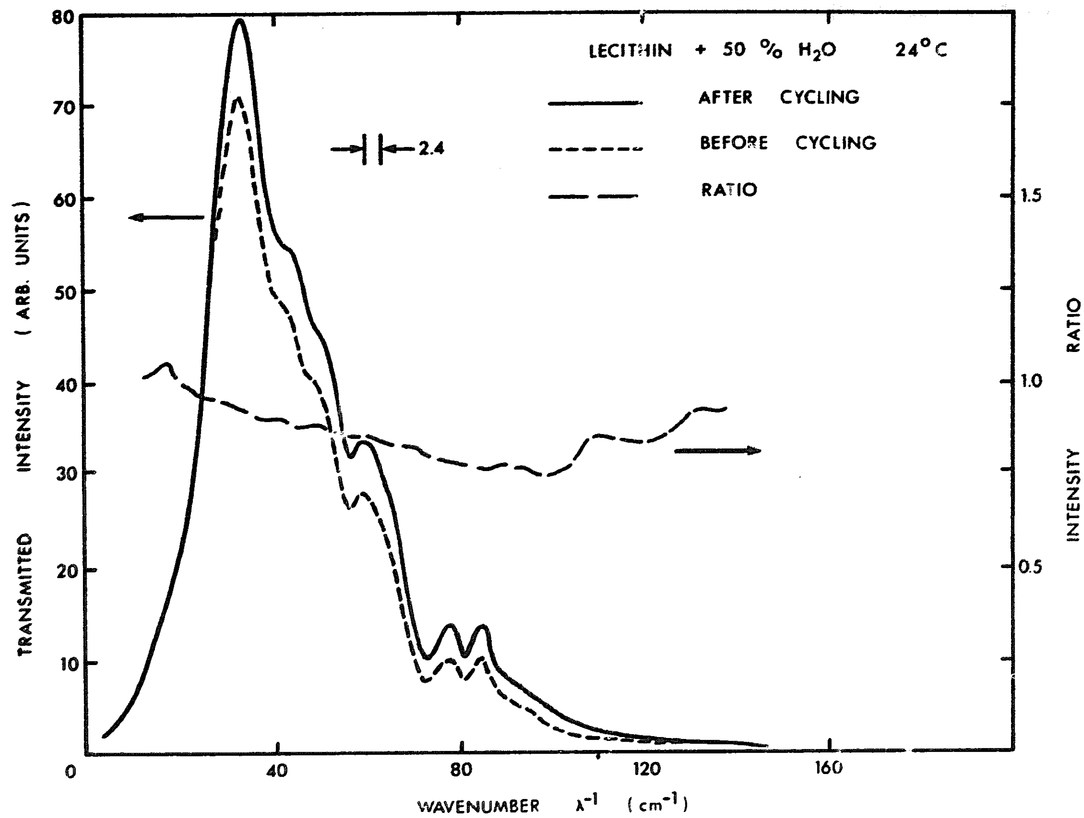

Fig. 4. Transmission spectra of a 0.1-mm thick sample taken at 24 °C before and after thermal cycling to 60 °C. Also shown is the ratio of transmitted intensities: *I* (before)/*I* (after). The ratio is reliable to better than ±2% from 10 to 100 cm^{-1} and is reliable to better than 4% at higher frequencies up to 120 cm^{-1}.

force constant[7] is that of NaCl : $1.5 \cdot 10^4$ dynes/cm. So for our system where the reduced mass is 59 amu, the characteristic frequency is given by

$$\omega_0^{\,2} = \frac{K}{\mu} = \frac{1.5 \cdot 10^4\ \text{dynes/cm}}{59\ \text{amu} \times 1.67 \cdot 10^{-24}\ \text{g}} = 1.52 \cdot 10^{26}\ (\text{rad/s})^2$$

or $\omega_0 = 1.2 \cdot 10^{13}$ rad/s and $\nu_0 = \omega_0/2\pi \simeq 2 \cdot 10^{12}$ Hz$=67$ cm^{-1}.
This is satisfactory order-of-magnitude agreement with our observed value of 100 cm^{-1} and all that can be expected of our highly simplified model.

Because the absorption due to the dipoles is stronger when the sample is in the solid phase, our model predicts that the dipoles lie parallel to the plane of the layers in this phase. When the sample is in the lamellar liquid crystalline phase, the orientations of the dipoles are more randomized. Then only a component of their dipole moments is accessible to the electric field vector of the radiation and the net absorption decreases. The orientation which we infer from our measurements is in agreement with the suggestions of others[8–10], but counter to a model proposed for monolayers[11]. In the ultraviolet and visible part of the spectrum increased transmission above the phase transition may be expected due to decreased scattering[3]. However, this effect should be quite negligible at the long wavelengths (> 50 μm) under consideration in our work.

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REFERENCES

1 Ladbrooke, B. C. and Chapman, D. (1969) *Chem. Phys. Lipids* 3, 304–367

2 Luzatti, V. (1968) in *Biological Membranes* (Chapman, D. ed.), pp. 71–123, Academic Press, London

3 Träuble, H. (1971) *Naturwissenschaften* 58, 277–284

4 Clayman, B. P. (1971) *Rev. Sci. Instr.* 42, 1409–1410

5 Clayman, B. P., Kirby, R. D. and Sievers, A. J. (1971) *Phys. Rev.* B 3, 1351–1364

6 Träuble, H. (1971) *Naturwissenschaften* 58, 277–284

7 Kittel, C. (1971) in *Introduction to Solid State Physics*, p. 181, Wiley, New York

8 Pethica, B. A. (1965) *Soc. Chem. Ind. Monogr.* 19, 85

9 Hanai, T., Haydon, D. A. and Taylor, J. (1965) *J. Gen. Physiol.* 48, 59–63

10 Hanai, T., Haydon, D. A. and Taylor, J. (1965) *J. Theoret. Biol.* 9, 278–296

11 Shah, D. O. and Schulman, J. H. (1967) *J. Lipid. Res.* 8, 277–233