Biochimica et Biophysica Acta, 552 (1979) 450—456

BBA 78342

AN ULTRASONIC STUDY OF THE THERMOTROPIC TRANSITION OF DIPALMITOYL PHOSPHATIDYLCHOLINE

JEFFREY E. HARKNESS and R.D. WHITE

Department of Chemistry, University of Idaho, Moscow, ID 83843 (U.S.A.)

(Received October 3rd, 1978)

Key words: Ultrasound absorption; Thermotropic transition; Phase transition; Dipalmitoyl phosphatidylcholine; (Liposome)

Summary

The acoustic absorption of sonicated and unsonicated suspensions of dipalmitoyl phosphatidylcholine has been studied as a function of temperature at frequencies between 0.6 and 4.0 MHz. The absorption at a fixed frequency passes through a maximum at a temperature which is dependent on the sample preparation and history and somewhat dependent on the frequency of observation, with sonicated vesicles exhibiting a temperature of maximum absorption 2—3°C below that found for unsonicated dispersions. The temperature dependence of the absorption at fixed frequency suggests a cooperative process while the frequency dependence of the absorption at fixed temperature indicates that more than a single relaxation time is required to describe the acoustic absorption.

Introduction

Thermotropic phase transitions occurring in phospholipid bilayer systems have been intensively studied during the past few years [1]. While the majority of these studies have been concerned with the characterization of the thermodynamics of the transition, the molecular structure of the bilayer, or with the alteration of the fluidity of the bilayer interior, the detailed dynamics of the bilayer molecules have received less attention. It is known from NMR experiments that the correlation times for hydrocarbon chain isomerization and reorientation are in the range of 10^{-7}—10^{-10} s [2] and that head group rotations have correlation times of about 10^{-7} s [3]. Attempts to deduce the nature and rates of the kinetic steps involved in the transition have included studies by means of temperature jump experiments [4] which indicated a relaxation time of about $30 \cdot 10^{-3}$ s for unsonicated liposomes and were interpreted in

terms of a cooperative lattice model, and by means of ultrasonic absorption experiments [5,6].

The periodic oscillations in temperature and pressure of an acoustic wave perturb the equilibria between any states of a system which differ in either their volume or enthalpy. This perturbation results in an absorption of energy from the sound wave. The absorption per wavelength due to a single process $(\alpha\lambda)_e$ with a relaxation time τ is given by the expression [7]

$$(\alpha\lambda)_e = C(2\pi f\tau)/(1 + (2\pi f\tau)^2) \tag{1}$$

The constant C is independent of frequency and depends on the volume (ΔV) and enthalpy (ΔH) changes of the process, the constant pressure heat capacity (C_p) and coefficient of thermal expansion (α_p), the density (ρ), temperature (T) and adiabatic compressibility (κ) of the solution, the ideal gas constant R, and the chemical factor Γ according to the relation

$$C = \pi(\Delta V - \alpha_p \Delta H/\rho C_p)^2 \Gamma/\kappa RT \tag{2}$$

The quantity Γ is given by the dependence of the reaction affinity A on the degree of advancement ϵ:

$$\Gamma^{-1} = -(\partial A/\partial\epsilon)_{T,p}/RT \tag{3}$$

In dilute aqueous solution ΔV usually dominates due to the small value of α_p and the ΔH portion of Eqn. 2 is often ignored.

For a unimolecular process

$$A \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} B$$

the relaxation time is given by

$$\tau^{-1} = k_1 + k_{-1}$$

and the amplitude factor is

$$C = \pi(\Delta V)^2 K([A] + [B])/(1 + K)^2 \kappa RT$$

where the square brackets denote concentrations and K is the equilibrium constant. The quantity C is a maximum when $K = 1$. For a cooperative process a superposition of terms of the form given by Eqn. 1 is appropriate, and C is increased by the degree of cooperativity [8].

The temperature dependence of the amplitude factor C is controlled primarily by the temperature dependence of the equilibrium constant K. For a cooperative process the temperature dependence of K is given by $m\Delta H$ where m is the number of cooperative units with the consequence that C is a stronger function of temperature.

The results of the acoustic absorption experiments with dimyristoyl phosphatidylcholine (DMPC) vesicles near the transition temperature [5] indicate a maximum in the absorption at a temperature 4°C below the transition tempera-

ture for unsonicated DMPC liposomes. This temperature dependence was interpreted in terms of cooperative headgroup motions which occur prior to the thermodynamic transition. In contrast to this study, acoustic absorption measurements [6] in sonicated dipalmitoyl, dimyristoyl, and distearoyl phosphatidylcholines (DPPC, DMPC and DSPC, respectively) indicated in each case a maximum absorption at the thermodynamic transition temperature and were interpreted in terms of the hydrocarbon chain reorientations within the bilayer.

In this paper the results of acoustic absorption measurements of both sonicated and unsonicated DPPC dispersions are presented in order to demonstrate the dependence of the absorption on sample preparation. The character of the spectra obtained are discussed in terms of their thermodynamic and kinetic implications. Although the processes responsible for the acoustic absorption cannot be unequivocally identified, some constraints on the nature of these processes can be deduced.

Materials and Methods

DPPC was obtained from Sigma Chemical Co. (St. Louis, MO). The purity was checked by thin-layer chromatography using a $CHCl_3/CH_3OH/H_2O$ (65 : 25 : 4, v/v) solvent system. Only one spot appeared after development of the plate with I_2. However, at higher concentrations of DPPC another faint spot appeared just above the DPPC spot. This spot was determined to contain choline by use of Dragoff's choline-specific reagent.

The liposomes were prepared by dispersion of 1 mg DPPC per ml H_2O containing 0.02% NaN_3 and 10^{-5} M EDTA with a Vortex mixer at a temperature of 50° C. The dispersion was then incubated for 1 h at 50° C before use. The vesicles were prepared by sonication at 50° C of 2 mg DPPC per ml H_2O containing 0.1 M NaCl, 0.02% NaN_3 and 10^{-5} M EDTA with a Bransonic ultrasonic cleaner.

Electron micrographs of suspensions prepared in a similar way were obtained on a Zeiss Model A10 transmission electron microscope. The bilayer dispersion was mounted on 200-mesh copper grids coated with carbon on formvar by the drop method [9] and stained with 1% uranyl acetate.

Acoustic measurements were obtained with a fixed path length variable frequency ultrasonic interferrometer of a design similar to that of Eggers et al. [10]. The interferrometer was immersed in a water bath controlled to ±0.002° C longterm by a Tronac (Orem, UT) Precision Temperature Controller. The temperature was measured to ±0.05° C using a calibrated digital thermometer.

The ultrasonic absorption at several frequencies between 0.6 and 4 MHz was measured at various temperatures in the transition region of the DPPC bilayers. The absorption of the vesicles was measured for both heating and cooling through the transition. The absorption of the liposomes, due to the instability of the suspension, was measured at only four temperatures.

Results and Discussion

Measurements of the acoustic absorption have been made in two distinct preparations of DPPC as a function of frequency (600 kHz to 4 MHz) and

temperature near the phase transition temperature. The absorption per wavelength ($\alpha\lambda$) as a function of temperature at a frequency of 980 kHz for sonicated vesicles and for unsonicated liposomes is shown in Fig. 1. The data for vesicles is in good agreement with the observations of Eggers and Funck [5] for DMPC with respect to both the magnitude and the temperature dependence. They observed, as in this case, that the temperature (T_m) of maximum absorption (at frequencies below 10 MHz) occurs at temperatures distinctly below the thermodynamic 'melting' point which is 41—42° C in DPPC liposomes [11].

The frequency dependence of $\alpha\lambda$ (shown in Fig. 2) is indicative of more than one relaxation process with a mean relaxation frequency which appears to be greater than the highest frequency of our measurements. As the temperature increases above T_m, the slope $\partial \ln \alpha\lambda/\partial\omega$ increases, suggesting an increase in the mean relaxation frequency. Below T_m, the slope $\partial \ln \alpha\lambda/\partial\omega$ decreases slightly with increasing temperature. Though it is not possible to deduce a relaxation frequency from these measurements, the results are not inconsistent with the acoustic measurements of Gamble and Schimmel [6], who observe a maximum in $\alpha\lambda$ at a frequency near 15 MHz in DPPC vesicles at T_m.

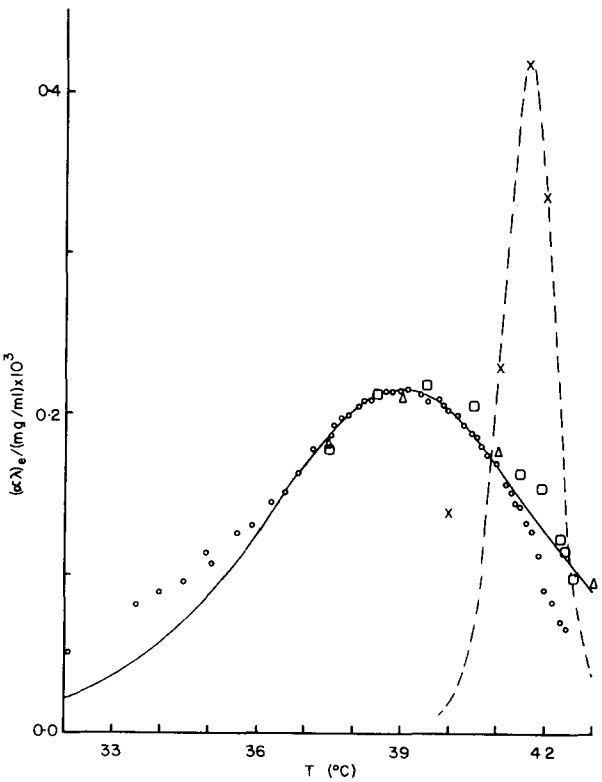

Fig. 1. The excess absorption per wavelength ($\alpha\lambda$) divided by the concentration in mg/ml at 976 kHz is displayed vs. temperature for sonicated DPPC dispersions in 0.1 M NaCl, $1 \cdot 10^{-5}$ M EDTA, 0.02% NaN_3. ○, first cooling curve; △, reheating curve; □, second cooling curve. The solid line is calculated for a two state model with ΔH^0 = 100 kcal/mol and a temperature-independent relaxation time. The data for unsonicated DPPC liposomes in $1.0 \cdot 10^{-5}$ M EDTA, 0.05% NaN_3 are represented by crosses (X) and the broken curve is calculated for a two state model with ΔH^0 = 550 kcal/mol.

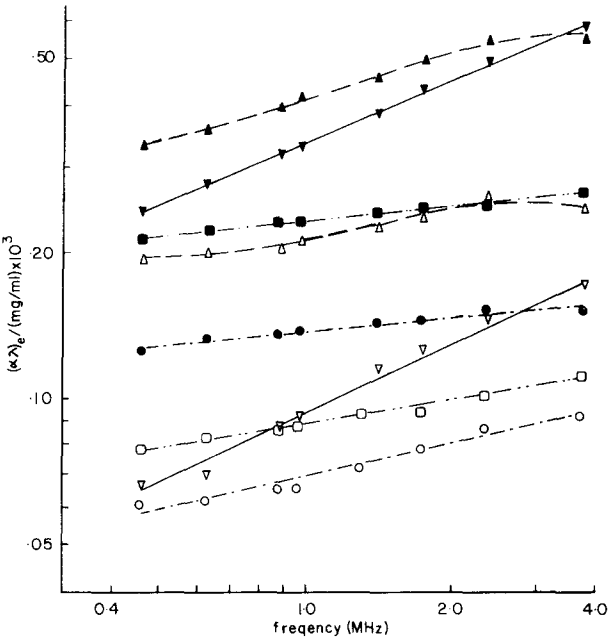

Fig. 2. The specific excess absorption per wavelength is given vs. frequency for sonicated DPPC vesicles in 0.1 M NaCl, $1.0 \cdot 10^{-5}$ M EDTA, 0.02% NaN_3 at 32.0°C (○------○), 34.0°C (□------□), 39.0°C (△------△), and 43.0°C (▽———▽). The data for unsonicated DPPC liposomes in $1.0 \cdot 10^{-5}$ M EDTA and 0.02% NaN_3 is given at 40.0°C (●------●); 41.0°C (■------■), 41.6°C (▲------▲), and 42°C (▼———▼). The curved lines have no theoretical basis.

Absorption measurements of unsonicated dispersions of DPPC show distinctly different behavior in comparison to the sonicated dispersions. As seen in Fig. 1, the dependence of $\alpha\lambda$ on temperature is much sharper and T_m is significantly higher for the unsonicated dispersions, though the frequency dependence (Fig. 2) of $\alpha\lambda$ is qualitatively similar for both preparations.

Electron micrographs of suspensions prepared similarly to those used in the ultrasound experiments indicate that the mass average diameter of the vesicles increases from about 1000 to 2000 Å on cycling through the transition. This is similar to observations in the literature [4,12] and may explain the hysteresis in the heating and cooling curves in the vesicles. This result, combined with the large difference in behavior between the liposomes and vesicles indicates a strong dependence of the acoustic properties on sample preparation and history.

The shape of the temperature dependence of the absorption can, in principle, provide some information concerning the thermodynamic nature of the processes responsible for the acoustic relaxation. Table I gives the full width at half height ΔT for the constant frequency thermograms at several frequencies. In the sonicated preparations the ΔT seems to be essentially independent of frequency between 630 kHz and 3.2 MHz with a value of about 7°C, in good agreement with the values deduced from Fig. 4 of ref. 5 and from Fig. 2 of ref. 6. For a single unimolecular process, the shape of the constant frequency thermogram is determined by the enthalpy change and activation energy in a straight forward way. If the variation in relaxation time with frequency is

ignored, the solid curve in Fig. 1 represents the temperature variation for an enthalpy change of 100 kcal/mol while the broken curve corresponds to an enthalpy change of 550 kcal/mol. A similar calculation using the calorimetric ΔH^0 of about 10 kcal/mol [11] results in a thermogram with a ΔT of about 70°C. If the widths of the curves in Fig. 1 are partially the result of the superposition of narrower curves due to the polydispersity of the sample or are broadened due to the presence of a temperature-independent background absorption, the values of ΔH^0 required would be even larger. The most obvious interpretation of the discrepancy between the ΔH^0 required to explain the temperature dependence of the acoustic absorption and the calorimetric ΔH^0 is that the process observed in the acoustic measurements is cooperative in nature, involving at least 10 molecules in the sonicated vesicles.

For processes with temperature-dependent relaxation times, the temperature of maximum absorption is a function of frequency. The variation of T_m with frequency shown in Table I may be due to such an effect and could account for at least part of the difference between the temperatures of maximum absorption observed in this work and those found at a frequency of 15 MHz [6]. It is also possible that this variation is due to a combination of the polydispersity of the sample and a size-dependent relaxation time [2,4].

Results of NMR measurements [2,3] indicated that motions of the hydrocarbon chains and head groups occur on a time scale similar to the relaxation times observed in these and other acoustic measurements, while temperature jump experiments reflect relaxations on a much slower time scale [4]. As mentioned above, the temperature profiles of the acoustic measurements are not consistent with the thermodynamics of the overall melting. This suggests that the acoustic relaxation corresponds either to a somewhat cooperative process (about 10 molecules for the vesicles) or to microscopic steps in the overall transition which have surprisingly large enthalpy changes for such a rapid process. Additionally, the dependence of the absorption on sample state, though ultimately providing a clue to the mechanism, currently prevents the attribution of the absorption to any particular process.

TABLE I

THE FREQUENCY DEPENDENCE OF THE TEMPERATURE-DEPENDENT ABSORPTION FOR DPPC VESICLES

Data are for the first cooling curve for sonicated DPPC vesicles in 0.1 M NaCl, $1.0 \cdot 10^{-5}$ M EDTA, 0.02% NaN_3.

Frequency (MHz)	T_m (°C) [a]	$(\alpha\lambda)_m \times 10^6$ (ml/mg) [b]	T_u (°C) [c]	T_l (°C) [c]	ΔT (°C) [d]
0.631	38.7	200	41.7	35.0	6.7
0.976	39.0	215	41.9	35.1	6.8
1.76	39.4	250	42.0	35.6	6.4
2.37	39.6	260	42.2	35.4	6.7
3.25	39.8	260	42.4	35.1	7.3

[a] Temperature of maximum absorption.
[b] Maximum specific excess absorption per wavelength.
[c] Temperature at which $(\alpha\lambda)_e = 1/2(\alpha\lambda)_m$.
[d] $T_u - T_l$.

Acknowledgement

This project was supported in part by B.R.S.G. grant RR07170-01 awarded by the Biomedical Research Support Grant Program, Division of Research Resources, National Institutes of Health.

References

1 Lee, A.G. (1977) Biochim. Biophys. Acta 472, 237—281
2 Petersen, N.O. and Chan, S.I. (1977) Biochemistry 16, 2657—2667
3 Seelig, J. (1978) Biochim. Biophys. Acta 515, 105—140
4 Tsong, T.Y. and Kaneshisa, M.I. (1977) Biochemistry 16, 2674—2680
5 Eggers, F. and Funck, Th. (1976) Naturwissenschaften 63, 280—285
6 Gamble, R.C. and Schimmel, P.R. (1978) Proc. Natl. Acad. Sci. U.S.A. 75, 3011—3014
7 Stuehr, J. (1974) in Techniques of Chemistry (Hammes, G.G., ed.), Vol. 6, pp. 237, John Wiley and Sons, New York
8 Schwarz, G. (1965) J. Mol. Biol. 11, 64—67
9 Haschesmeyer, R.H. and Myers, R.J. (1972) in Principles and Techniques of Electron Microscopy (Hayat, M.A., ed.), Vol. 2, pp. 101, Van Nostrand Reinhold Co., New York
10 Eggers, F., Funck, Th. and Richmann, K.H. (1976) Rev. Sci. Instrum. 47, 361—367
11 Mabrey, S. and Sturtevant, J.M. (1976) Proc. Natl. Acad. Sci. U.S. 73, 3862—3866
12 van Dijck, P.W.M., de Krujff, B., Aarts, P.A.M.M., Verkleij, A.J. and de Gier, J. (1978) Biochim. Biophys. Acta 506, 183—191