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CALORIMETRIC INVESTIGATIONS OF LIPID PHASE TRANSITIONS. I. THE WIDTH OF TRANSITION

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ABSTRACT

Calorimetric calibration measurements with electrical heat pulses and with the crystallization process of hexadecane have been performed. It is shown that only under favorable conditions and for the lowest scanning rates is it possible to determine the true width of the main phase transition of aqueous dispersions of multilamellar dimyristoyllecithin-liposomes. Hence a cooperative unit involving more than 1700 molecules is deduced which is indicative of a first-order phase transition with only very little impurity broadening.

INTRODUCTION

A central point of the experimental and theoretical work on lipid phases and the changes between them is the question of the order and, related to that, the cooperativity of the phase transitions [1–7]. In particular, calorimetric measurements have been analysed, within certain assumptions, to yield so-called cooperative units, i.e., the number of lipid molecules that undergo a simultaneous transition [3,6–9]. However, one should be careful with such experimental estimations of cooperative units; e.g., Mabrey and Sturtevant published cooperative units of the main phase transition for three lecithins of different chain length differing by a factor of four: from 980 for dilauroylphosphatidylcholine (C 12-chains) to 260 for dipalmitoylphosphatidylcholine (C 16-chains, DPPC) [6,10]. A simple calculation, however, shows that for all three lipids the sharpness of the transition, expressed as the temperature width at half-height, $\Delta\tau'$ [9] is almost identical ($\Delta\tau' \approx 0.35$ K). Bearing in mind that the true width of the main phase transition of DPPC can be as narrow as 0.067 K [3], the question is left as to whether only the limited instrumental resolution has been analysed.

Before results are discussed concerning the transition width of dimyristoylphosphatidylcholine (DMPC, C 14-chains), therefore, calibration measurements, performed in order to test the temporal response of the heat conduction calorimeter, are presented. In particular, a check had to be made

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of how narrow the phase transition temperature range can be without the measured curve being affected by the instrumental resolution.

It turned out that in studies with lipid dispersions only the lowest possible scanning rates, under favorable conditions, allowed the determination of the width of the main phase transition without any distortion of the measuring curve, ΔT vs. t, by the instrument.

MATERIALS AND METHODS

All measurements were performed with a differential temperature scanning calorimeter [11] (Triflux, Thermanalyse, France). The main features of this twin instrument are schematically depicted in Fig. 1.

The thin-walled aluminum containers for sample (S) and reference (R) are thermally connected to the surroundings (T_0) by the resistors R_{OS} and R_{RO}, respectively, and to each other by the resistor R_{SR}. The instrument is operated by scanning the environmental temperature, T_0, at a constant rate, α

$$T_0(t) = T_B + \alpha t \tag{1}$$

where T_B is the temperature at the beginning of the scan, i.e., $t = 0$. The sample container temperature, T_S, and the temperature difference between sample and reference container, ΔT, are recorded.

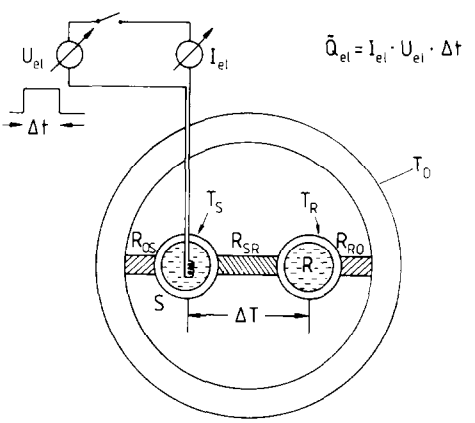

Fig. 1. Schematic drawing of the twin calorimeter with sample (S) and reference cuvette (R). R_{OS}, R_{SR}, and R_{RO} are thermal resistors between surroundings and sample, sample and reference, and reference and surroundings, respectively. T_0 is the (scanned) environmental temperature, T_S and T_R the sample and reference cuvette temperature, respectively. The temperature difference between sample and reference, $\Delta T = T_S - T_R$, is measured. Also shown are the schematics of the calibration set-up that allows the application of electrical heat pulses $\tilde{Q}_{el} = I_{el}\ U_{el}\ \Delta t$ via a heating coil of a miniature light bulb immersed into the water-filled sample cuvette.

The main advantage of this calorimeter is the wide range of possible scanning rates: the lower limit for both heating and cooling is $\alpha = 0.004$ K min^{-1}, the upper limit for heating is 2 K min^{-1}, and for cooling is 0.5 K min^{-1}. For better environmental temperature stability the instrument was placed within two temperature-stabilized boxes, the outer one being kept at $-18°C$.

Hexadecane (Merck) and DMPC (Fluka) were used without purification. For the dispersions, different amounts of lipid were weighed directly into the aluminum containers. After the addition of the appropriate volume of H_2O (pH 5.8, millipore quality), the containers were sealed with a rubber stopper through which a tiny hole had been drilled in order to allow for pressure equilibration at higher temperatures. The containers were heated to a temperature above the main phase transition of the lipid and shaken for several minutes. This procedure gave stable milky dispersions composed of multilamellar liposomes as checked by electron microscopy. The reference cuvette was filled with pure water until it reached the same weight as the sample cuvette. Thus, it was assumed to best equalize the heat capacities of sample and reference.

RESULTS AND DISCUSSION

Calibration measurements

As the "true" width of the main phase transition of dimyristoyllecithin is of interest, tests were made to evaluate which experimental parameters influence the temporal response of the instrument. It also had to be evaluated whether it is possible (under favorable conditions) to measure the phase transition without any convolution with the instrumental response function.

For this purpose two sets of calibration measurements were performed. In a first series short (δ-function-like) heat pulses were produced electrically by the heating coil of a miniature light bulb which was immersed in the water-filled sample cuvette of the calorimeter (see Fig. 1) and connected to an external voltage source, U_{el}.

The use of electrically controlled heat pulses for calibration measurements has several advantages: (1) the duration of the thermal event, Δt, can be varied purposefully. In particular, very short pulses are possible; (2) the amount of heat dissipated in the sample cuvette can be varied over many orders of magnitude without changing the sample; (3) any scanning rate, α, can be used, including $\alpha \equiv 0$, i.e., equilibrium conditions. While scanning, one can freely choose (4) the moment, and (5) the temperature of the calibration pulse; (6) in principle, it is also possible to mimic endothermic processes by using miniature peltier-elements [11]; (7) with a heating coil immersed in the solvent-filled sample container the calibrations can be

carried out under the same conditions as the measurements, i.e., with the heat sources inside the sample. This is not the case for the instrument-integrated resistors for calibration pulses.

A typical calorimetric curve, ΔT vs. t, after a short heat pulse ($\Delta t = 140$ ms) is shown in Fig. 2 (——); and with five times expanded time-scale (– · – · –). The sample was in thermal equilibrium with the surroundings, i.e., $\alpha \equiv 0$, at a temperature $T_0 = -10.4°C$. At that temperature the water in the container was, of course, frozen. At $t = 0$, a heat pulse of $\tilde{Q}_{el} = 5.5 \times 10^{-3}$ cal was applied. After a short delay time, $\tau_0 = 2.2$ s, the signal increases exponentially with a time constant $\tau_1 = 8.7$ s and decreases again exponentially to the base-line with a much longer time constant, $\tau_2 = 59$ s. An easily obtainable parameter for the instrumental response time is the full width at half maximum, $\Delta\tau$, which in this case is $\Delta\tau = 64$ s. The temporal behavior of a measuring-signal is qualitatively the same for all samples and all experimental conditions provided the thermal event, Δt, is short in comparison to the instrumental response time, $\Delta\tau$.

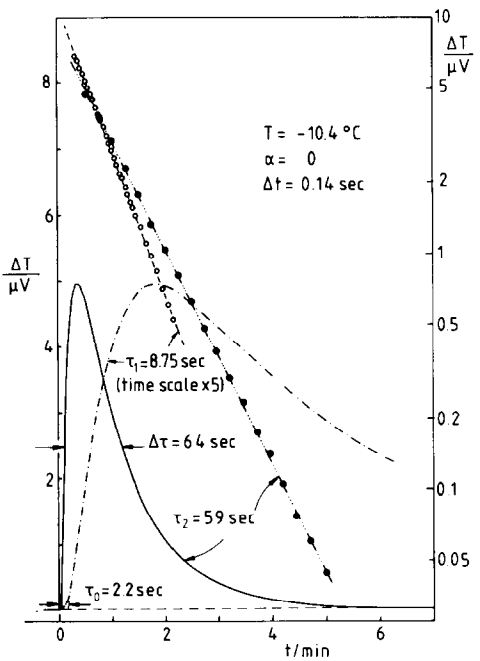

Fig. 2. Calorimetric curve obtained after the application of a heat pulse (duration $\Delta t = 140$ ms) at $t = 0$ (———), left ordinate; – · – · –, same curve on a 5 times expanded time scale). The straight lines are obtained by a semi-logarithmic plot of the exponential decrease of the ΔT-signal to the base line (– · · · ● · · · –, right ordinate). (○) Semi-logarithmic plot of the exponential increase of the calorimeter signal (-- ○ --, right ordinate, 5 times expanded time scale). The calorimeter was in equilibrium with the environment at $T_0 = -10.4°C$.

The different time constants correspond to different equilibration processes between the heating coil, the sample, the reference and the environment [11]. As such they depend on heat capacities and thermal resistances and vary, therefore, for different experimental conditions such as sample material, sample volume, temperature, etc. Some of the results obtained for such calibration measurements are outlined in the following.

First, it was checked how the instrumental response time depends on the temperature. These data are shown in Fig. 3 for two different sample volumes: the heating coil was immersed in about 350 μl pure H_2O (●) and in 500 μl of an aqueous dilute DMPC dispersion (○). Several aspects are noteworthy: (1) above 0°C where the solvents are liquid the response time decreases with increasing temperature. It can be shown [11] that the equilibration processes are, to a first approximation, proportional to the heat capacity, C_p, and inversely proportional to the heat conductivity, λ. Inserting the corresponding values for H_2O at 0°C and 75°C [12,13] results in a reduction of $\Delta\tau$ by about 20% at the higher temperature, in good agreement with the present results; (2) at the liquid–solid phase transition of water the response time decreases by some 30%. Comparing, at 0°C, the heat capacities and the heat conductivities of water and ice, respectively, a reduction of $\Delta\tau$ would be expected by a factor of eight. This discrepancy is attributed to the reduced heat transfer at the interface between ice and the container wall compared to the interface between water and the container wall. This effect again dramatically slows down the equilibration process. It also conceals the decrease of $\Delta\tau$ with decreasing temperature expected for ice because C_p decreases and λ increases; (3) for all temperatures, the smaller the sample volume, the lower the response time. This immediately demonstrates the

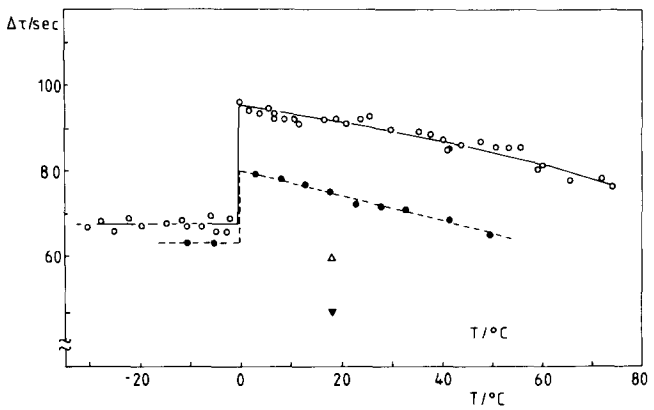

Fig. 3. Response time of the calorimeter, $\Delta\tau$, (defined in Fig. 2) as a function of temperature for different calibration pulses and different sample volumes. (○), electrical heat pulse ($\Delta t = 140$ ms) in 500 μl of a dilute lipid dispersion; (●), electrical heat pulse in ca. 350 μl H_2O; (△), crystallization of 1.4 mg hexadecane in 35 μl H_2O; (▼), crystallization of 1.4 mg hexadecane.

limited applicability of such externally triggered heat pulses. For a full immersion of the heating coil a minimum H_2O volume of about 300 μl is needed. For studies with lipid dispersions, however, considerably lower sample volumes are possible thereby increasing the temporal resolution of the instrument.

In a second series of calibration measurements, therefore, hexadecane ($C_{16}H_{34}$) slightly dispersed in H_2O was used. The use of hexadecane is well suited for this purpose for several reasons: (1) the physical nature of its solid–liquid phase transition is similar to the chain melting main phase transition of lipids [4]; (2) its transition temperature ($T_m = 18.1°C$) is sufficiently close to that of DMPC ($T_m = 23.15°C$); (3) the added water mimics the heat capacity and heat conductivity of an aqueous lipid dispersion. A disadvantage of commercially available long chain alkanes is their insufficient purity. This broadens their melting transition beyond limits that would be tolerable for the present calibration purposes. The crystallization process is, however, a sufficiently fast thermal event to allow calibration of the calorimeter even for the lowest cooling rates.

For hexadecane, the influence of low sample volumes on the instrumental response time, $\Delta\tau$, is also shown in Fig. 3. For 1.4 mg $C_{16}H_{34}$ in 35 μl H_2O, $\Delta\tau$ is as low as 60 s (Δ). A further reduction of the response time could be achieved by further reducing the sample volume (i.e., the volume of the added water) as is shown for $C_{16}H_{34}$ without water (▼). Volumes smaller than 35 μl, however, are not sufficient to cover the bottom of the sample containers completely which is a prerequisite for reproducible heat conduction properties.

An additional artefact that broadens calorimetric transition curves can be demonstrated with $C_{16}H_{34}$ provided it is heterogeneously distributed in the water (which happens mostly for larger H_2O volumes). A consequence of the principles of operation of a heat conduction calorimeter is the set-up of temperature gradients which then exist also between the sample and the container. This leads to a temperature distribution within the sample so that at different points within the sample the transition temperature may be reached at different times. For a heterogeneously distributed sample, therefore, a whole sequence of single thermal events can be observed. This is shown in Fig. 4 for 1.4 mg hexadecane in 435 μl H_2O. For a homogeneously dispersed material like lipid vesicles in water this causes an additional broadening because the single events can no longer be resolved. Instead, the transition front is observed as it moves through the sample.

A most important parameter for studies of the widths of lipid main phase transitions is the scanning rate and its influence on the response time, $\Delta\tau$. Figure 5 shows the result. With electrical heat pulses (○, sample volume 500 μl, $T = 25°C$) $\Delta\tau = 90$ s is obtained, independent of the scanning rate. No difference was observed between heating and cooling scans. Here, one of the advantages of such a calibration set-up is quite obvious: independent of the

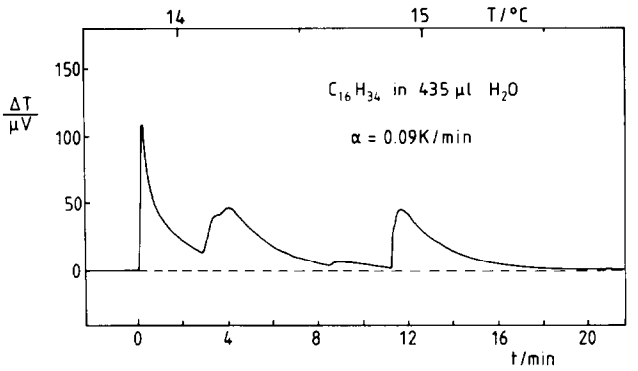

Fig. 4. Calorimetric curve obtained during the crystallization of hexadecane heterogeneously distributed in 435 μl H_2O. Time-dependent temperature gradients in the sample cuvette are the reason for this behavior: hexadecane droplets at different sites in the cuvette crystallize at different times.

scanning rate, δ-function-like heat pulses can be produced so that the temporal response of the calorimeter can be obtained even for the lowest rates. For any thermal event connected to a phase transition this is not fulfilled a priori: supposing the instrumental response time, $\Delta\tau$, is in the order of 60 s, for a scanning rate of 0.004 K min^{-1} this means that the natural width of the phase transition has to be much smaller than 4/1000 K in order to be short in comparison to the response time. Fortunately, this was indeed the case for the crystallization process of the hexadecane sample. Therefore, it was possible to obtain the response function of the calorimeter also for sample conditions that were adequate for the DMPC studies. Figure

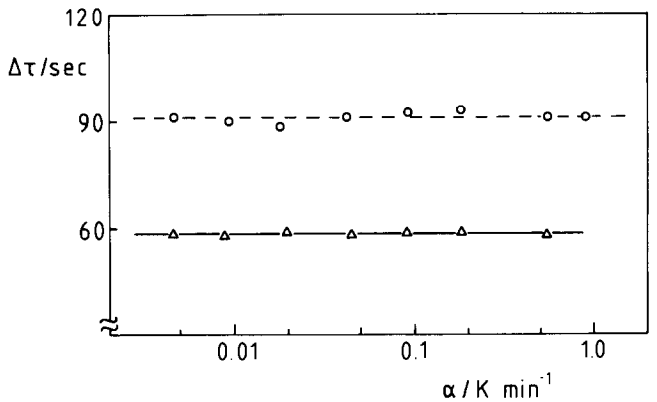

Fig. 5. Calorimeter response time, $\Delta\tau$, as a function of the scanning rate α for different calibration pulses. (○), electrical heat pulse in 500 μl dispersion, applied at 25°C; (△), crystallization of 1.4 mg hexadecane in 35 μl H_2O.

5 shows that again down to the lowest cooling rates the response time, $\Delta\tau$, does not depend on the scanning rate (Δ, 1.4 mg $C_{16}H_{34}$ in 35 μl H_2O). At all scanning rates the calorimetric curves were essentially identical and of the same form as that obtained with an electrical heating pulse (Fig. 2).

DMPC-dispersions

A typical measuring curve obtained with a DMPC-dispersion at a high scanning rate is shown in Fig. 6 (3.0 μmol DMPC in 35 μl H_2O, $\alpha = 0.19$ K min^{-1}). Beside the pre-transition peak at 13°C the main phase transition around 23°C can be identified. Its calorimetric curve shows the same qualitative features as the calibration curves, i.e., for these high scanning rates the transition is fast compared to the instrumental resolution. This changes gradually as the scanning rate is reduced. The transition curve gets more and more symmetrical until, for the lowest rates, a fully symmetrical (Gaussian) transition is observed (see Fig. 9A). Parallel to this change in the curve form, a gradual increase in the width of the calorimeter curve is found. This is demonstrated in Fig. 7. Here the temperature width, $\Delta\tau'$ (K), is plotted as a function of the scanning rate, α, for different sample volumes. Each sample contained 3.0 μmol DMPC, the amount of added water was 35 μl (∇), 435 μl ($\triangle$), and 785 μl ($\blacktriangle$), respectively. According to

$$\Delta\tau' = \alpha\Delta\tau \tag{2}$$

a constant response time, $\Delta\tau$ (s) is represented in this plot by a straight line of slope 1. The dotted line in Fig. 7 corresponds to the instrumental response for a sample volume of 35 μl (see Fig. 5). By comparing this line with the data points of a lipid sample with identical volume it is apparent that for scanning rates > 1 K min^{-1} the observed widths of the transition curves are

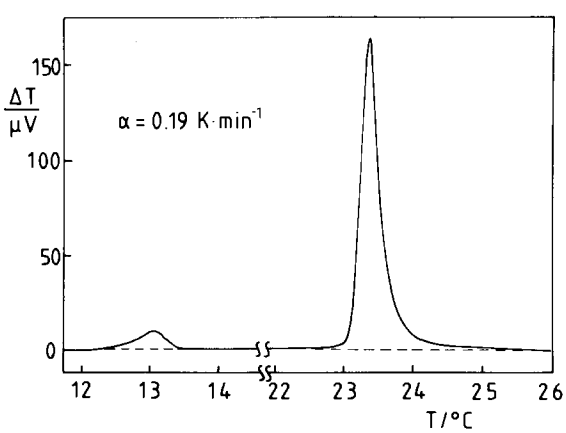

Fig. 6. Calorimetric curve obtained with 3.0 μmol DMPC in 35 μl H_2O at a heating rate of $\alpha = 0.19$ K min^{-1}.

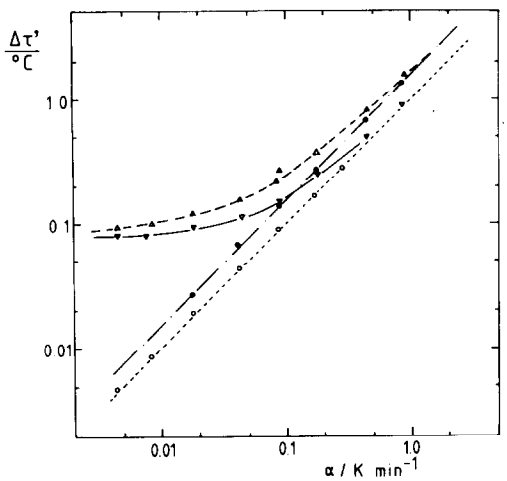

Fig. 7. Widths of transition curves, $\Delta\tau'$, for different samples and different calibration pulses as a function of the scanning rate α. (--- ○ ---), crystallization of 1.4 mg hexadecane in 35 μl H_2O, the data points are obtained from the same measurements as presented in Fig. 5 (△), according to $\Delta\tau' = \alpha\Delta\tau$; (– ·●· –), electrical heat pulses in 500 μl H_2O, see also Fig. 5 (○); triangles represent measurements of 3 μmol DMPC, dispersed in different amounts of water: (▽), 35 μl; (△), 435 μl; (▲), 785 μl.

fully determined by the instrument. For slower heating and cooling rates the finite temperature width of the lipid main transition becomes more and more dominant until, for the two lowest scanning rates ($\alpha = 0.004$ and 0.01 K min^{-1}), the measured temperature width of the transition is independent of the scanning rate. The instrument is, for the lowest rate, about a factor of 20 "faster" than the phase transition. Therefore, it is possible only for these low scanning rates to determine the true width of the phase transition without any convolution with the instrumental response function. The reduced resolution for higher sample volumes (e.g., 500 μl H_2O, (●) and (– · – · –), see also Fig. 5) prevents the measurement of the true transition width of high-volume samples even for the lowest possible scanning rates (see the data points of the sample with 3.0 μmol DMPC in 435 μl H_2O (△)). A further reduction of α by at least a factor of 2 would be necessary in order to measure the intrinsic $\Delta\tau'$.

In principle, it would be possible also for intermediate scanning rates to determine the desired width of the lipid phase transition by a deconvolution procedure. For that purpose, however, it would be necessary to know exactly the correct instrumental response, which is not a universal function but depends instead on the sample conditions themselves.

If, under favorable conditions and for the lowest scanning rates, the measured transition width is a pure lipid property, then it should not depend on the amount of lipid involved in the transition. This was tested by taking

calorimetric curves of different quantities of DMPC which were each dispersed in 35 μl H_2O in order to ensure identical instrumental conditions. Figure 8 shows the results for 0.56 μmol DMPC (●), 3.0 μmol (△), and 9.35 μmol (○), respectively. For comparison, the instrumental response is also shown (——). Indeed, for all scanning rates, the widths do not depend on the amount of lipid.

If a broader transition is observed, like the pretransition of DMPC, then the apparent width becomes independent of the scanning rate at higher rates (▽). (Due to the small heat associated with the pretransition its calorimetric curves could not be analysed for scanning rates $\leqslant 0.02$ K min^{-1}.) For the higher rates, however, it joins the resolution curve as does the width of the main phase transition.

Now that it is apparent that the calorimeter allows the determination of the intrinsic width of the main transition of DMPC dispersions it makes sense to analyse the data in terms of van t'Hoff enthalpies, ΔH_{vH}, and cooperative units, CU.

Within certain assumptions (two-state system, enthalpy change up to any temperature proportional to the fraction converted, unit activity coefficient) ΔH_{vH} can be expressed as a function of the fraction, θ, of lipid that underwent its phase change at a given temperature

$$\Delta H_{vH} = \frac{RT^2}{\theta(1-\theta)} \frac{d\theta}{dT} \tag{3}$$

At the midpoint of the transition where $\theta = 1/2$ and, by definition, $T = T_m$ the following is obtained

$$\Delta H_{vH} = 4RT_m^2 \left(\frac{d\theta}{dT}\right)_{T=T_m} \tag{4}$$

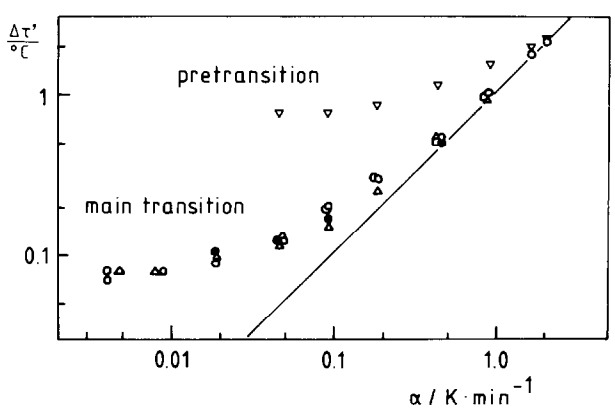

Fig. 8. Transition width, $\Delta\tau'$, of the pretransition (▽) and of the main transition of DMPC dispersions. Different symbols represent the data points for samples of different amounts of lipid, each, however, dispersed in 35 μl H_2O: (●), 0.56 μmol DMPC; (△), 3.0 μmol; (○), 9.35 μmol. (——), Calibration measurements with $C_{16}H_{34}$ in 35 μl H_2O.

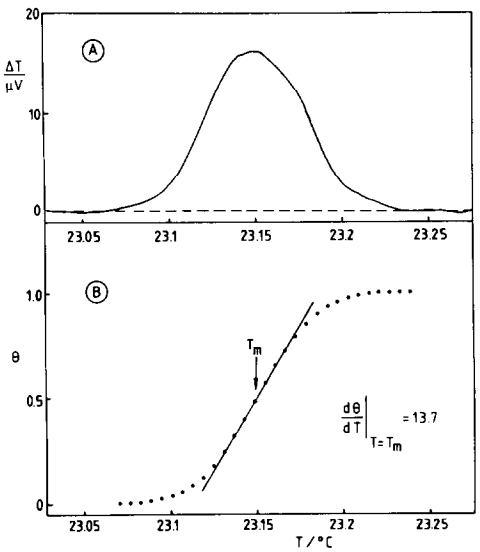

Fig. 9. (A) Calorimetric curve, ΔT vs. T, for 3.0 μmol DMPC in 35 μl H_2O obtained at a heating rate $\alpha = 0.004$ K min^{-1} (for comparison, see also Fig. 6). (B) Fraction of lipid, θ, that underwent its phase change at a given temperature, T, obtained by integrating the curve in A and normalizing it to unity. Maximum slope is reached at the phase transition temperature, T_m.

$d\theta/dT$ can be derived by integrating the calorimetric transition curves taken at sufficiently slow scanning rates. An example is shown in Fig. 9B. From the slope $d\theta/dT$ at the phase transition temperature $T = T_m$ one obtains $\Delta H_{vH} = 9.56 \times 10^3$ kcal mol^{-1}. The cooperative unit is then given by

$$CU = \frac{\Delta H_{vH}}{\Delta H_{cal}} \tag{5}$$

where ΔH_{cal} is the calorimetrically determined enthalpy of the process. As

TABLE 1

Summary of the thermodynamic data of DMPC

	T_m (°C)	T_m (K)	ΔQ (kcal mol^{-1})	$\Delta\tau'$ (K)	$(d\theta/dT)_{T=T_m}$ (K^{-1})	ΔH_{vH} (kcal mol^{-1})	Cooperative unit	
							This work	Literature
DMPC								
Pretransition	12.8	286.0	1.0	0.75	1.3	8.45×10^2	845	280 [a]
Main transition	23.15	296.3	5.6	0.07	13.7	9.56×10^3	1710	333 [a]
DPPC								
Main transition								1400 [b]

[a] Ref. 10.
[b] Ref. 3.

will be discussed in a forthcoming paper [14], only the lower heating and cooling rates allowed the identification of the measured exchanged heat as the enthalpy change at the main phase transition. $\Delta H_{cal} = 5.6$ kcal mol^{-1} was obtained. According to eqn. (5) it is calculated that a cooperative unit CU = 1710. These values are summarized and compared with literature data in Table 1.

CONCLUSIONS

It has been shown that only under certain limited conditions and only for the slowest scanning rates does the heat conduction calorimeter allow the determination of the true width of the main phase transition of DMPC multilamellar liposomes in excess water. This width and, calculated from that, the cooperativity of the transition are similar to values reported for DPPC [3]. The question remains as to what brings about the limited cooperativity. Impurity caused asymmetries as in the transition curves of DPPC [3] were not observed with the DMPC sample. However, within the quasi-chemical approximation of the Ising model it can be shown [15] that the cooperative unit may never exceed a value determined by the mole fraction of defects, x, according to

$$CU \leqslant \frac{1}{x(1-x)} \tag{6}$$

Hence, the present value of the cooperative unit (CU = 1710) would be the consequence of only 0.06 mol% defects. If the finite width of the transition is interpreted as being the result of a defect-broadened, but true isothermal, first-order transition this would mean that the DMPC sample under investigation was extremely pure (99.94%). Moreover, this is a lower limit for the chemical purity because lattice defects (physical impurities) would equally reduce the CU as predicted by eqn. (6) by a reduction of the next-neighbor interaction [15].

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