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Lipid Bilayer Phase Transition: Density Measurements and Theory

(biomembranes/rotational isomeric order-disorder theory)

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ABSTRACT **The overall change of density for dipalmitoyl lecithin bilayers agrees with a general order-disorder theory and yields about seven gauche rotations per molecule for the biologically relevant high-temperature phase. The shape of the curve of density against temperature is similar to the result of an exact calculation on a specific model, which gives a 3/2-order phase transition.**

There is much interest in the hydrocarbon-chain disordering transition in lipid bilayers because many biomembranes live above (in the high-temperature disordered phase) or within the transition (1, 2). Below the transition the hydrocarbon chains are in the relatively well-ordered and well-understood all *trans* state (3). Measurements of transition changes coupled with molecular model descriptions provide information about the biologically relevant phase in comparison to the low-temperature phase (4). A particular molecular quantity of interest is the average number of gauche rotations in the fluid state. The dipalmitoyl lecithin (DPL) system is the best system for this study because the transition is sharper for such highly homogeneous systems, allowing more precise measurements of the overall changes without the uncertainty of baseline corrections, and the homogeneity simplifies theoretical considerations.

Recent theoretical work (4) has elucidated two points requiring experimental testing. First, a general model (of order-disorder, rotational isomeric type) predicts, from the calorimetric data of Hinz and Sturtevant (5), that the relative density change of the bilayer should obey the inequality $2\% < \Delta\rho/\rho < 5\%$, and further allows a computation of the number of gauche rotations n_g per molecule when $\Delta\rho/\rho$ is known. Second, an exact statistical mechanical calculation of a simplified but still reasonably realistic model yields a highly asymmetric transition as a function of temperature (4). Although this latter point could have been an artifact of the simple theoretical model, the calorimetric measurements (5) indicate a similar asymmetry.

For investigation of these points a differential densitimeter was constructed as follows. The DPL dispersion was placed in a glass container which has a mercury piston that separates the inside contents from the outside water and which allows the inside volume to change as the inside contents expand. A matched glass container with identical mercury piston was filled with water. The containers were suspended from opposite sides of a double-pan balance. The containers were also immersed in a water bath. Any changes in weight were due to a density change of the DPL with respect to water, since all other weight changes cancel. The temperature of the bath was controlled by a Haake heater with control of ±0.003°C. The temperature of the bath was measured with a Beckman mercury thermometer calibrated in 1/100 degrees. The 1/e equilibration time for the apparatus plus sample was found to be 23 min from warming and cooling runs through the transition; therefore, at least 2 hr were allowed to establish equilibrium at a new temperature; longer times did not change the measured values. Weight errors were less than 1 mg.

1 g of DPL was obtained from Calbiochem and dispersed by hand shaking in pH 7 water above the transition temperature. The transition observed in this work is the sharpest yet reported, and this is a good indication of the purity of the sample. During the course of the experiment the sample was not subject to mechanical stirring. Initially, the dispersion was 5% by weight; over the course of 3 weeks the dispersion settled to the bottom, expelling water, until it became a 25% dispersion by weight.

Fig. 1 shows the weight difference between the DPL dispersion and an equal volume of water as a function of tem-

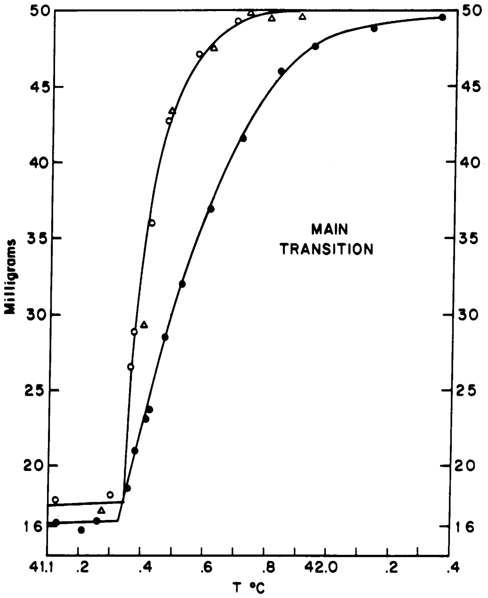

FIG. 1. The relative difference in weight between an equal volume of water and 1 g of DPL dispersion at the main transition. All measurements were made in equilibrium. The *open circles* are a sequence of increasing temperatures and the *triangles* are a sequence of decreasing temperatures, both for about a 10% dispersion. The *solid circles* are for a 25% dispersion.

Abbreviation: DPL, dipalmitoyl lecithin.

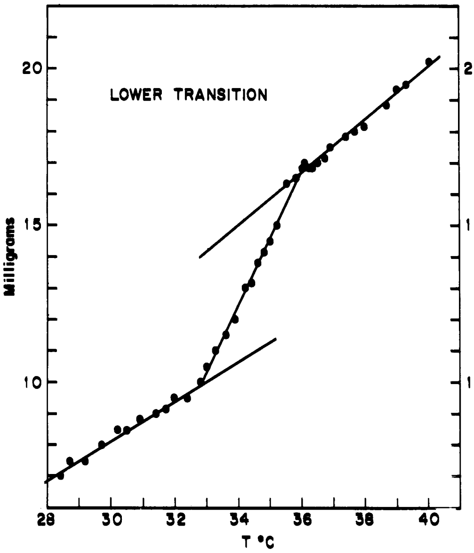

FIG. 2. The relative difference in weight as a function of temperature between an equal volume of water and 1 g of DPL dispersion for the lower transition. A heating rate of 3.25°/hr was used, and the dispersion was about 25% by weight. The numerical values of the constant slopes above and below this lower transition are not meaningful due to small mismatches in the glass containers.

perature. All points are equilibrium points. The early runs show a very sharp transition with total width about 0.4°. The last run shows a wider transition of about a degree or so, although half the change takes place over only 0.2° due to the asymmetry of the transition. No conclusions concerning the time dependence of the width of the transition have yet been drawn, but it can be concluded that all curves show the same striking degree of asymmetry.

From the data in Fig. 1, and also from nonequlibrium heating and cooling runs, I obtain $\Delta\rho/\rho = 0.035 \pm 0.003$ which is safely within the bounds provided by the general theory. From this measurement the general theory (4) also predicts that there are on average about seven gauche rotations per DPL molecule above the transition. This may be compared to a value of 12 gauche rotations if the molecules did not interact with each other. Melchior and Morowitz (6) have also measured the relative density change to be 0.026 ± 0.001, and Sheetz and Chan (7) have measured it to be 0.04. Although agreement is far from perfect, all these results agree with the theoretical bounds.

In Fig. 2 is shown the smaller, lower transition that has been observed in calorimetry, but not previously in density measurements. For this lower transition I obtain $\Delta\rho/\rho \simeq 0.004$.

The asymmetry of $\rho(T)$ shown in Fig. 1 is qualitatively consistent with a specific model calculation (4). For all first derivatives G' of the free energy G, such as G' equals volume V or entropy S, this exact calculation gives $G' = G_0' =$ constant for $T < T_c$ and $G' = G_0' + c(T - T_c)^{1/2}$ for $T > T_c$ (asymptotically close to T_c). Although this sort of transition was discovered theoretically about 10 years ago (8), it is still not well known outside the area of cooperative phenomena. It is not a first-order transition nor is it the usual kind of second-order or lambda or higher order transition. For example, the transition does not obey the scaling-homogeneity "laws" for the specific heat critical exponents since $\alpha' = 0$ and $\alpha = {}^1/_2$ for this transition (9). I suggest that this be called a 3/2-order transition because (*a*) the lowest derivative of the free energy to be discontinuous is the third derivative with respect to $(T - T_c)^{1/2}$ (this is in the spirit of the Ehrenfest definition of order) and (*b*) from the low-temperature side there are no critical fluctuations, consistent with a first-order transition, while from the high-temperature side there are large fluctuations, consistent with a second-order transition; averaging the two sides gives 3/2. However gimmicky such terminology needs be, it does serve to emphasize the fact that this is not the usual phase transition.

The 3/2-order transition is intimately involved with the excluded volume effect. Below the transition the system is locked into place due to the excluded volume interactions. At T_c a cooperative unlocking takes place. *A priori*, one might expect the unlocking to occur simultaneously resulting in a first-order transition and undoubtedly this is often the case for many real systems with excluded volume interactions. But there is also the possibility of this 3/2-order transition, which exhibits a large specific heat above T_c. The width of this high-temperature tail obviously varies under different experimental conditions, so there is no great cause for alarm that the solution to the simple model gives too wide a transition. Rather, the good agreement of the theoretical T_c with the observed T_c [with no free parameters (4)], the observed asymmetry of $\rho(T)$, and the overall change in ρ all indicate that we are on the right track to a detailed understanding of the lipid bilayer phase transition.

NOTE ADDED IN PROOF

The observed asymmetry in the transition is also consistent with about a 5% mixture of stearic fatty acid chains. However, the Calbiochem atomic analysis is more consistent with myristic fatty acid impurities which would give the opposite asymmetry.

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