

# The Pressure Dependence of the Lipid Bilayer Phase Transition<sup>†</sup>

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**ABSTRACT:** The transition temperature for dipalmitoyllecithin bilayers has been determined at pressures up to 100 bars by following the volume change accompanying the transition. The increase in the transition temperature was found to be nonlinear with increasing pressure whereas the density change due to the transition was invariant with pressure up to the highest

pressure studied. The near equality of the limiting slope ( $dP/dT$ )<sub>P=1</sub> with  $\Delta H/T_m \Delta V$  at 1 atm is consistent with a first-order transition. The entropy change for the transition, as calculated from the Clapeyron equation, decreases by about 40% over the pressure range studied. The implications of these results for order-disorder theories are discussed.

Lipid bilayers undergo a phase transition associated with hydrocarbon chain disordering. The evidence for the structural changes that occur during the transition have been reviewed in a recent paper (Nagle, 1973a). Briefly, the hydrocarbon chain has been shown to undergo a partial transformation from trans to gauche forms during the transition.

Generally dipalmitoyllecithin (DPL)<sup>1</sup> has been selected for the study of such transitions because of the sharpness of its transition, its overall homogeneity, and its availability in a purified form. Calorimetric (Chapman *et al.*, 1967; Hinz and Sturtevant, 1972) and density measurements (Nagle, 1973b) on DPL indicate that two transitions are involved: one at 35° and a second (the main transition) at 41.5° at 1 atm. This work deals only with the main transition. Both the calorimetric and density measurements indicate a definite asymmetry with respect to temperature. A recent statistical mechanical calculation using a microscopic order-disorder model for this system (Nagle, 1973a) yields a highly asymmetric transition as a function of temperature which is called a  $\frac{3}{2}$  order transition. The same kind of observed asymmetry led to the suggestion that the transition was not first order but rather  $\frac{3}{2}$  order (Nagle, 1973b).

The first purpose of this work is to test that conclusion by measuring the pressure dependence of the bilayer phase transition. If the Clapeyron equation is not obeyed, then the transition cannot be first order, although, unfortunately, the converse is not necessarily true. The Clapeyron equation can be tested readily by comparing the limiting value of  $dP/dT$  with that predicted from the known values of  $\Delta V$  and  $\Delta H$  at 1 atm.

## Experimental Section

The details of the volumeter design (Srinivasan, 1972; Srinivasan and Kay, 1974), the pressure vessel, and the high-pressure system (Pribadi, 1971) are given elsewhere, and only a brief description of the apparatus and the method of its operation is given here.

The volumeter consists of two concentric stainless steel cylinders of 1 in. and 0.375 in. diameter. The outer cylinder is closed and contains about 32 ml of the sample to be com-

pressed while the inner cylinder (welded to the outer cylinder) serves as a sleeve through which a tight-fitting piston moves smoothly under pressure. An O-ring on the piston isolates the sample from the compressing fluid (Marcol 90 oil). An insulated electrical contact mounted on the piston slides continuously on a resistance wire attached to the upper part of the outer cylinder. With a constant current passing through this wire, the partial voltage (representing the position of the piston inside the sleeve) and the total voltage across the wire are measured by a digital voltmeter. The ratio of these two voltages is directly proportional to the volume of the sample in the volumeter. The proportionality factor was determined by a calibration with pure water using known density data (Kell and Whalley, 1965). Our calibration had a reproducibility of 0.02% in the total volume and a detectability of better than  $2 \times 10^{-3}$  ml.

The DPL was used as obtained from Calbiochem. The volumeter was filled with a 2% dispersion of DPL (0.64 g) in conductivity grade water (specific conductance  $1 \times 10^{-7}$  ohm<sup>-1</sup> cm<sup>-1</sup>; pH 7). The dispersion was prepared by shaking at a temperature above the transition. Temperature control was obtained by placing the stainless steel pressure vessel, containing the volumeter and the pressurizing oil, in an oil bath regulated to 0.01°. The absolute temperature was measured by a calibrated Beckman thermometer. The pressure to the nearest bar was determined by means of a Heise gauge, calibrated against a dead-weight gauge. All volume measurements were carried out under isothermal and isobaric conditions. At each pressure the starting temperature was about 40°. The temperature was raised a few tenths of a degree, the pressure was reset if necessary, and the voltages were recorded after the system again reached thermal equilibrium. Owing to the thick walls of the pressure vessel, approximately 2 hr were required to reach thermal equilibrium as indicated by a constant voltage ratio. The volume, calculated from the voltage ratios and the calibration plot, was suitably corrected for the thermal expansibility and the isothermal compressibility of steel since our calibration was made at 1 atm and some fixed temperature. In the present study, results of measurements at 1, 25, 45, 70, and 100 bars (1 bar = 0.987 atm) are reported.

## Results

The corrected volume changes recorded for runs at 1 and 100 bars are shown in Figure 1. These two runs, at the extremes of the pressure ranges measured, are shown as typical results since the data at the other pressures show identical behavior.

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<sup>1</sup> Abbreviation used is: DPL, 1,2-dipalmitoyllecithin.

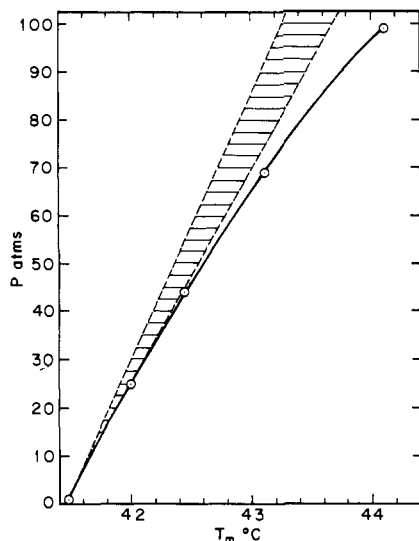


FIGURE 1: Typical volume changes during the transition at 1 and 100 bars. The broken line is the expansibility line for pure water. Concentration of DPL, 2% w/w;  $T_m$ , transition temperature.

Two features stand out in Figure 1. A base line can be established before and after the transition by drawing the best straight line through the experimental points. In each case, it was found that this line was parallel to the expansibility curve for pure water. For this purpose an average expansibility of  $4.0 \times 10^{-4} \text{ deg}^{-1}$  was used for the temperature interval studied here (Kell and Whalley, 1965).

The second feature to be noted in Figure 1 is that the transition is not sharp as required by one which is first order, but instead covers a range of about  $0.8^\circ$  in each case although the change in volume during the transition process follows a linear path with temperature. The point of intersection of the base line before the transition with the best straight line through the points during the transition was taken as a measure of the transition temperature. This procedure is based on the following rationale. More precise density measurements on DPL at 1 atm (Nagle, 1973b) suggest that it is possible that the width of the transition is affected by the inhomogeneous settling of the DPL dispersion during the course of the measurements. For a 10 and 25% by weight dispersion of DPL the transition was found to occur over a temperature range of about  $0.5\text{--}1^\circ$ , respectively. However, in both cases the onset of the transition occurred at exactly the same temperature, whereas the temperature corresponding to the midpoint of the transition differed by  $0.2^\circ$ . This could be an indication that the asymmetry found in the high-temperature side of the transition in the density measurements would be reduced considerably if the DPL could be kept completely dispersed during the measurements. Experiments that will test this conclusion are now underway.

The measured values of the transition temperature  $T_m$  and the corresponding change in volume are given in Table I. From duplicate runs the error in  $T_m$  is estimated to be  $0.06^\circ$  and that in  $\Delta V$  to be  $0.003 \text{ ml g}^{-1}$ . Our one atmosphere values can be compared to those from a number of other investigators. Our average values,  $\Delta V = 0.032$  and  $T_m = 41.48$ , agree reasonably well with those of Nagle,  $\Delta V = 0.35 \pm 0.003$  and  $T_m = 41.35$  (Nagle, 1973b). On the other hand, our values are outside the error limits when compared to  $\Delta V = 0.026 \pm 0.001$  (Melchior and Morowitz, 1972) and  $\Delta V = 0.040$  (Sheetz and Chan, 1972). A direct comparison of our  $T_m$  with the dilatometry and the calorimetric results (Hinz and Sturtevant, 1972) is not possible owing to the fact that  $T_m$  was selected not as the tempera-

TABLE I: Transition Temperature  $T_m$  and the Corresponding Change in Volume.

$P$ (atm)	$T_m(^{\circ}\text{C})\Delta V$ ( $\text{mol g}^{-1}$ )	$dP/dT$ (atm $\text{deg}^{-1}$ )	$\Delta S$ (eu)
1	41.45	0.0320	
	41.43	0.0325	46
	41.56	0.0320	26 <sup>a</sup>
25	42.00	0.0320	45
44	42.38	0.0336	41
	42.50	0.0302	23
69	43.11	0.0352	34
99	44.10	0.0325	26
			15

<sup>a</sup>  $\Delta S = 29 \text{ eu}$ , calculated from  $T_m = 41.5^\circ$  and the calorimetric  $\Delta H = 9.2 \text{ kcal mol}^{-1}$ .

ture of initial rise but as some temperature during the transition, generally the half-way point. However, an inspection of the various values quoted shows good general agreement around  $41.5$ . One point of divergence from many of the previous investigations is the lack of asymmetry during the transition as shown in Figure 1.

It is clear from the volume changes recorded in Table I that within our experimental error of about 10%, the volume change is independent of pressure. The experimental transition temperatures are plotted as a function of pressure in Figure 2. The hatched lines indicate the best estimate that can be made at the present time of the limiting slope at  $P = 1 \text{ atm}$  as calculated from the Clapeyron equation

$$dP/dT = 0.0413\Delta H/T_m \Delta V \text{ (atm deg}^{-1}\text{)} \quad (1)$$

Using the mean value of  $\Delta H = 9.2 \text{ kcal mol}^{-1}$ , taken from two recent calorimetric studies (Hinz and Sturtevant, 1972; Phillips *et al.*, 1969) and  $\Delta V$  from Table I, we calculated  $(dP/dT)_{P=1} = 51 \pm 6 \text{ atm deg}^{-1}$  with the estimated error resulting mainly from our value of  $\Delta V$ . In Figure 2, it can be seen that the experimental values of  $T_m$  approach this slope at  $P = 1 \text{ atm}$  within the experimental error. The limiting slope was calculated by fitting the transition temperature to the third degree polynomial in  $P$

$$T = 41.46 + 0.0216P - 1.50 \times 10^{-5}P^2 + 6.83 \times 10^{-7}P^3 \quad (2)$$

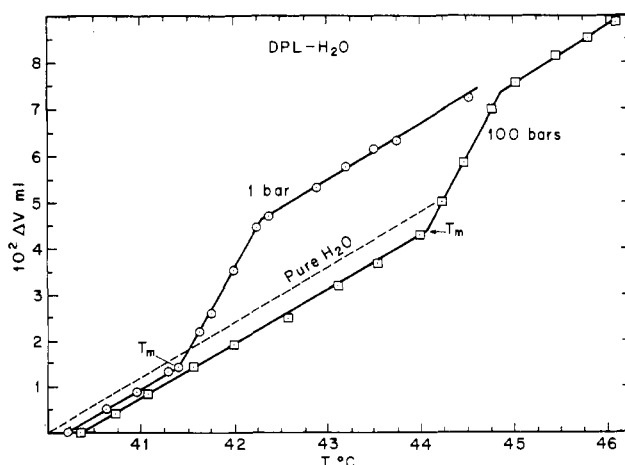


FIGURE 2: Pressure-temperature diagram for DPL-H<sub>2</sub>O system at the transition point.

Differentiation gave  $(dP/dT)_{P=1} = 46 \pm 9 \text{ atm deg}^{-1}$  which is in reasonable agreement with the value of 51 calculated from the Clapeyron equation, a result that is consistent with a first-order transition, although higher order transitions are not ruled out.

Our calculated values of  $dP/dT$  and those for  $\Delta S$  based on the assumption that the Clapeyron equation holds at each pressure are included in Table I. We estimate our error in these two quantities to be about 20%. The important point to note is that at the highest pressure the  $\Delta S$  has decreased by about 42% or about twice the experimental error. Consequently, it is clear that the entropy change during the transition decreases with pressure, which from thermodynamic considerations would appear reasonable. A simple calculation (Nagle, 1973a), based on a combinatorial expression for the total number of configurations available to two strands of 14 bonds each with two possible gauche rotations at each bond, indicates the entropy change reported here corresponds to 5 and 2.5 gauche rotations during the transition at 1 and 100 bars, respectively, if all configurations are available. However, due to steric interactions, all configurations are not available to the system and it has been estimated that the average number of gauche rotations at 1 bar should be 8 and in proportion 4 or 5 at 100 bars.

Although the entropy change with pressure is reasonable, the invariance with pressure of the volume change through the transition is puzzling. In the general theory (Nagle, 1973b) the enthalpy change is approximately (since  $P\Delta V$  is small)  $\Delta H \simeq \Delta U = En_g + a_{vdw}[1 - (V/V_0)^{-5/2}]$ , where the  $En_g$  term accounts for the change in the rotational isomeric energy for  $n_g$  gauche rotations of energy  $E = 0.5 \text{ kcal/mol}$ . The second term accounts for the energy increase due to expansion against the van der Waals interactions between chains. For  $\Delta V/V = 0.032$  the van der Waals term amounts to about 5.6 kcal/mol. For one atmosphere the measured  $\Delta H$  is taken to be about 9.2 kcal/mol which leaves 3.6 kcal/mol for the  $En_g$  term. This yields  $n_g \simeq 7$  for the high temperature disordered phase, which is a reasonable result. However, the difficulty is that the computed value of  $\Delta H = T_m\Delta S$  at  $P = 99 \text{ atm}$  from Table I gives only 5.2 kcal/mol which leaves no energy to activate any gauche rotations. One possible reconciliation of the general theory and the experiment is that  $a_{vdw}$  decreases with increasing pressure, although we do not see at this time how to justify such a large change in  $a_{vdw}$  for such a small change in pressure. Needless to say, direct measurements of  $\Delta H$  as a function of pressure would be very welcome, since the Clausius-Clapeyron equation need not hold at all pressures if the transition is not first order.

The experimental results for the transition under pressure can also be related to the exact theoretical results for a special lattice model (Nagle, 1973a). The theory gives the transition temperature  $T_m$  by the formula (eq 4.3 with a minus sign and factors of 2 corrected)  $-2kT_m \ln [1 - 2e^{-E/kT_m}] = 4p + 3a_{vdw}$ , where  $4p = 0.001 \text{ kcal/mol}$  for 1 atm and  $a_{vdw} = 1.8 \text{ kcal/mol}$ . For 1 atm this theory gives a slope  $dP/dT = 143 \text{ atm/deg}$  which is reasonably close to the measured value of 40

atm/deg, considering the differences in  $\Delta H$  and  $\Delta V$  of the model compared to the experimental  $\Delta H$  and  $\Delta V$ . But there is also a qualitative discrepancy which is more informative. According to the theory  $dP/dT$  should increase by about 6% from 0 to 100 atm, whereas experimentally  $dP/dT$  decreases by about 40%. Again, it is possible to reconcile theory and experiment by allowing  $a_{vdw}$  to be a function of  $P$ . To second order we may write  $a_{vdw} = a_0 + bP + cP^2$ . To obtain a 40% decrease in  $dP/dT$  from 0 to 100 atm, it is necessary that the parameter  $c$  be about  $2.5 \times 10^{-6} \text{ kcal/atm}^2$ , which only involves an overall change in  $a_{vdw}$  of less than 0.5%. The parameter  $b$  does not change the relative curvature in  $dP/dT$ . However,  $b$  must be greater than  $-0.001 \text{ kcal/atm}$  or else  $dP/dT$  becomes negative at low pressures. Thus, in this specific theory  $a_{vdw}$  cannot decrease by more than 2% and is more likely to increase. This is at variance with the demands of the general theory discussed in the last paragraph. Clearly, study of the transition under pressure is important for the molecular theory of lipid bilayers and biomembranes.

#### Added in Proof

Unpublished data of Dr. J. R. McColl of Yale University is in agreement with our  $dP/dT$  result at one bar and our result that  $\Delta V$  is independent of pressure. However, McColl finds no change in  $dP/dT$  for pressures up to 600 bars. This latter result is far easier to understand theoretically than our decreasing  $dP/dT$ .

#### References

- Chapman, D., Williams, R. M., and Ladbroke, B. D. (1967), *Chem. Phys. Lipids* 1, 445.
- Hinz, H., and Sturtevant, J. M. (1972), *J. Biol. Chem.* 247, 6071.
- Kell, G. S., and Whalley, E. (1965), *Phil. Trans. Roy. Soc. London* 258, 565.
- Melchior, D. L., and Morowitz, H. J. (1972), *Biochemistry* 11, 4558.
- Nagle, J. F. (1973a), *J. Chem. Phys.* 58, 252.
- Nagle, J. F. (1973b), *Proc. Nat. Acad. Sci. U. S.* 70, 3443.
- Phillips, M. C., Williams, R. M., and Chapman, D. (1969), *Chem. Phys. Lipids* 3, 234.
- Pribadi, K. S. (1971), Ph.D. Thesis, Carnegie-Mellon University.
- Sheetz, M. P., and Chan, S. I. (1972), *Biochemistry* 11, 4573.
- Srinivasan, K. R. (1972), Ph.D. Thesis, Carnegie-Mellon University.
- Srinivasan, K. R., and Kay, R. L. (1974), to be published.

<sup>2</sup> In a private communication J. M. Sturtevant has indicated that his published value for the transition enthalpy of DPL should be lowered by 3.8% to 9.3 kcal mol<sup>-1</sup> on the basis of the use of an incorrect molecular weight. He has also indicated that enthalpies as low as 9.0 kcal mol<sup>-1</sup> have been obtained with different lots of DPL. The value used here is perhaps a little high but not sufficiently so as to affect the final results significantly.