Biochimica et Biophysica Acta 859 (1986) 10–14
Elsevier

BBA 73132

High pressure volumetric measurements in dipalmitoylphosphatidylcholine bilayers

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(Received October 3rd, 1985)
(Revised manuscript received March 20th, 1986)

Key words Dipalmitoylphosphatidylcholine, Phospholipid bilayer High pressure Specific volume

The one previously reported high pressure volumetric experiment on a phospholipid bilayer investigated a region of pressure between 0 and 25 MPa and obtained isothermal compressibility values for the liquid crystal and intermediate phases which differed by more than a factor of ten. We report new volumetric measurements around the main transition in dipalmitoylphosphatidylcholine (DPPC) from 0 to 100 MPa. The isothermal compressibility data for the two phases are of the same order of magnitude, and the experimentally determined coexistence curve, specific volume dependence, and volume discontinuity values are compared with the predictions of the phenomenological theory according to Sugar and Tarjan ((1982) Sov Phys. Crystallogr. 27, 4–5). Significant discrepancies between this theory and experiment are found. Finally, the data indicate that steric interactions play a more dominant role in the main transition of phospholipid bilayers than in transitions in most thermotropic liquid crystals

Introduction

Phospholipid bilayers in an aqueous dispersion have been studied intensively as a model system for biological membranes These bilayers undergo phase transitions as the temperature, pressure, and water content are varied, for example, L-α-dipalmitoylphosphatidylcholine (DPPC) dispersed in excess water possesses at least four phases at atmospheric pressure The 'subgel' phase occurs below 13°C, it is characterized by the rigid packing of the hydrocarbon chains and is the most crystalline phase of the four The 'gel' or $L_{\beta'}$ phase exists between 13°C and 34°C and differs from the 'subgel' phase by a slightly different packing of the rigid hydrocarbon chains Between the pretransition at 34°C and the main transition at 42°C, the 'intermediate', 'ripple', or P_{β} phase exists The rigid hydrocarbon chain packing of the $L_{\beta'}$ phase is still present, but the bilayers are now rippled The main transition separates this phase from the 'liquid crystal' or L_{α} phase, in which the hydrocarbon chains are no longer rigid

By far, most investigations of these transitions have been performed at atmospheric pressure with temperature change inducing the transition Much less work has been done in order to understand the effect of hydrostatic pressure on these transitions Early experiments determined the phase diagram for DPPC using a variety of methods [1–6], while more recent work has utilized adiabatic compression [7], Raman spectroscopy [8], light transmission [9], and fluorescence depolarization [10] under pressure Examples of measurements of direct biological relevance obtained under pressure may be found in Refs 11 and 12 Theoretical attempts to understand pressure-induced transitions include a special lattice model [2,13], a phenomenological theory [14], and an Ising model formalism [15]

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The one previous volumetric measurement under pressure investigated a small region of pressure and contained some questionable results, i e , the P_{β} and L_{α} phases possessed extremely different compressibilities In addition, more recent high pressure volumetric measurements in thermotropic liquid crystals have been extremely useful in characterizing the different phase transitions present [16,17] Finally, of the theoretical models, Sugar and Tarjan's phenomenological theory [14] is the only one which contains realistic predictions for high pressure volumetric experiments With these considerations in mind, we performed such volumetric measurements over a wide range of pressure and compared the data to the predictions of this model The results indicate that major disagreement between this theory and experiment exist Our measurements furnish new isothermal compressibility data and also indicate that the transition between the L_{α} and P_{β} phases is strongly influenced by steric interactions

Materials and Methods

The DPPC used in this experiment was obtained from Calbiochem and was not further purified A 10 0 ± 0 1% mixture by weight of phospholipid in distilled water was made, which was rapidly stirred while cycling the temperature above and below the main transition Such a procedure should produce multilamellar vesicles of various sizes The mixture was stored at room temperature for a few days before being used in the experiment, and showed no evidence of containing any unhydrated DPPC

This dispersion was confined in a stainless-steel bellows holding about 4 5 cm^3, which was part of a system capable of simultaneously measuring the pressure, volume, and temperature The experimental apparatus and instrumentation have been described previously [16] Although the specific volume of the sample could be measured to an accuracy of 0 00015 cm^3/g, the specific volume of the water had to be subtracted using precise equation of state data for water [18] The resulting DPPC specific volume measurements were therefore good to 0 0015 cm^3/g This same equation of state data for water was utilized with a sample of pure water in the initial calibration of this apparatus, so this subtraction procedure should not introduce a systematic error The main transition was investigated by starting at a temperature and pressure in the L_{α} phase, and then increasing the pressure in steps of about 0 7 MPa, recording the volume each time This process was performed slowly enough to keep the sample at the equilibrium temperature, and was continued until the sample was well into the P_{β} phase The entire procedure was repeated at several points along the main transition

To provide predictions which could easily be compared to our experimental data, the Landau phenomenological theory of Sugar and Tarjan [14] was completely worked out, generating isothermal specific volume data for DPPC over a wide range of pressure As a check of these calculations, we compared them to Sugar and Tarjan's results at atmospheric pressure and along the main transition curve, obtaining excellent agreement

Results

Fig 1 presents the specific volume data on DPPC for a typical isothermal experimental run (320 4 K) Similar data were obtained at 325 2 K, 330 4 K, 335 4 K and 340 3 K During each run, the temperature of the sample was constant to

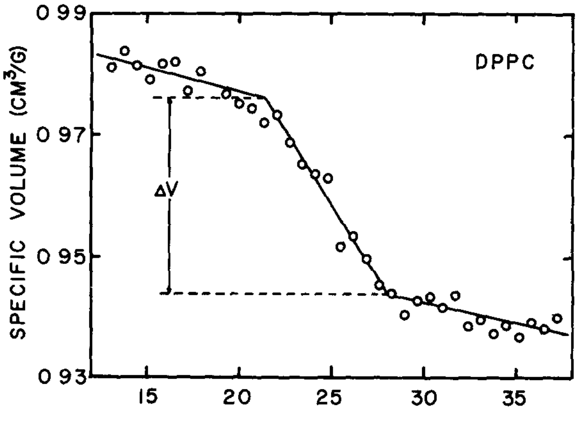

Fig 1 Typical isotherm in DPPC (320 4 K) The straight lines represent linear least-square fits to the two pure phase isotherms and the one mixed phase isotherm The midpoint of the mixed phase isotherm serves to determine the pressure, specific volume, and temperature at the main transition ΔV specific volume discontinuity at the main transition

within 0 1 K as specific volume and pressure measurements were taken The obvious scatter in the data indicates the difficulty of obtaining such data when only 10% of the sample is DPPC Although the transition appears to be quite wide in Fig 1, one must realize that the pressure scale is expanded The entire transition is on the order of 5 MPa wide, which is consistent with the results of both specific volume [4] and microviscosity [10] experiments One may convert this 5 MPa width to an estimate of the width of the transition in temperature at constant pressure by multiplying by the slope of the coexistence curve, 0 23 K/MPa [12] This procedure results in a width estimate of a little over 1 K, which is typical of some experiments on DPPC performed at atmospheric pressure, but significantly greater than other results [19] The slight asymmetry of the transition which is evident at atmospheric pressure is also quite evident here To generate values for comparison to theory, the two pure phase isotherms and the mixed phase isotherm were obtained by least squares linear fit, the midpoint of the mixed phase isotherm was used to determine the specific volume and pressure at the transition, the two intersections of isotherms gave the volume discontinuity at the transition, and the slopes of the two pure phase isotherms generated compressibility data

The results of this procedure and the prediction of theory are shown in Fig 2 Sugar and Tarjan's theory predicts that the coexistence curve should show positive curvature, and this is in fact what occurs Other theoretical models also predict this effect correctly [2,13] At any temperature and pressure along this coexistence curve, (1) the main transition takes place at a particular value of the specific volume, and (2) the specific volume abruptly increases in going from the P_{β} phase to the L_{α} phase The measured values for the specific volume at the transition and the specific volume change of the transition (volume discontinuity) are also shown in Fig 2 The specific volume and volume discontinuity are predicted to increase as the pressure and temperature are increased along the coexistence curve, while the data show that the specific volume definitely decreases while the volume discontinuity remains fairly constant, perhaps decreasing slightly It should be pointed out that absolute specific volume measurements with this apparatus are more difficult to do precisely than measurements of relative volume changes The fact that the specific volume data in Fig 2 fall below the theory curve is therefore not as important as the fact that the change with increasing temperature or pressure is in the wrong direction The volume discontinuity data does not have this problem, as the data extrapolate at low pressure to a value only slightly less than both the theory and measurements done at atmospheric pressure [19]

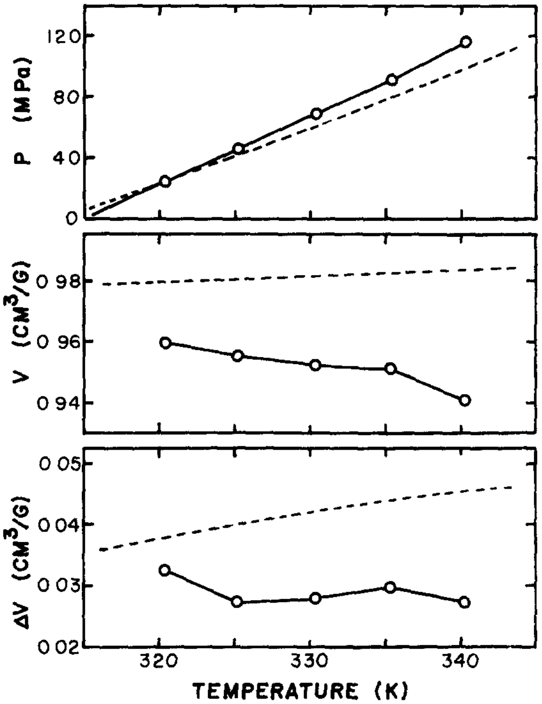

Fig 2 Pressure specific volume, and specific volume discontinuity along the main transition The circles are experimental data points, the dashed lines are the results of Sugar and Tarjan's phenomenological theory To convert V or ΔV to ml/mol, multiply by 735 1 g/mol

Discussion

The theory predicts that the L_{α} phase should have an isothermal compressibility of about 3 3 10^{-4} MPa^{-1}, while the data support a value of (7 8 ± 1 1) 10^{-4} MPa^{-1}, (average of five isothermal measurements) The isothermal compressibility for the P_{β} phase is (5 2 ± 0 8) 10^{-4} MPa^{-1} demonstrating that it is definitely less compressible than the L_{α} phase, but clearly of the same order of magnitude These values are representative of measurements in thermotropic liquid crystals comprised of molecules with fairly long hydrocarbon chains [17], but differ significantly from the results for DPPC for Ref 4 This inconsistency is difficult to reconcile, since the isothermal compressibility for the L_α phase from Ref 4 is larger than our result, whereas the isothermal compressibility for the P_β phase from Ref 4 is smaller than our result

There may be a simple reason why the isothermal compressibility of these two phases are not more different given that one phase is composed of partially molten hydrocarbon chains while in the other phase the hydrocarbon chains are rigidly packed Results of X-ray diffraction measurements under pressure have demonstrated that the bilayer thickness in the liquid crystalline phase actually increases as the pressure is increased rather than decreasing as in the intermediate and gel phases [6] This would seem to indicate that the compressibility of the liquid crystal phase is not as great as one might expect, since the straightening of the melted hydrocarbon chains brought on by a pressure increase also extends the chains, increasing the bilayer thickness The resulting volume change is therefore less than would be expected if only lateral compressibility effects were considered

A parameter which is useful in characterizing transitions in condensed matter phases is the ratio of the temperature change to the volume change along the coexistence curve This parameter

$$\gamma = -\frac{\partial(\ln T/T_0)}{\partial(\ln V/V_0)}$$

where T_0 and V_0 represent a point on the coexistence curve, is extremely important theoretically, since it is sensitive to the relative strength of steric repulsions (which are athermal) as compared to attractive interactions (which are temperature dependent) [20] The higher the value of γ, the more steric interactions play an important role in the transition Fig 3 presents the data for DPPC, showing that a γ value of about 3 4 is appropriate This value is typical of the isotropic-nematic transition in systems of rather short and inflexible molecules Steric repulsion is generally

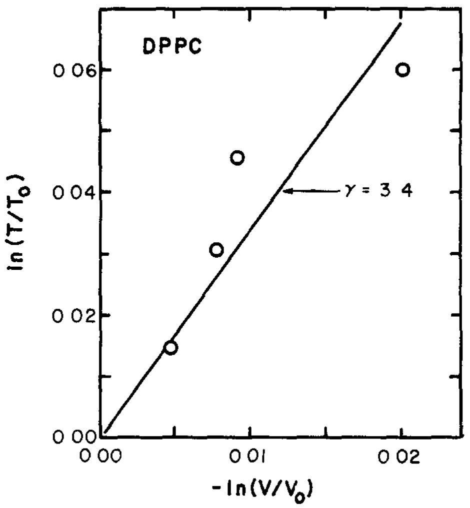

Fig 3 Temperature and specific volume changes along the main transition The straight line represents a least-square linear fit constrained to pass through the origin the γ value denotes the slope of this line

thought to be important in this transition and our results seem to imply a similar conclusion for the main transition in phospholipid bilayers Since this transition represents a change from a partially molten system of hydrocarbon chains to a phase of closely packed rigid chains, this finding is not surprising

The importance of this parameter is evident from the fact that Sugar and Tarjan's theory predicts a large negative value whereas the experiment produces a small positive value Although only positive values have been obtained experimentally in the case of thermotropic liquid crystal systems, both positive and negative γ-values have been produced by theories of the liquid crystalline phase [16] It would therefore seem that the competition between the effects of temperature and pressure on a transition is a difficult one to model theoretically

Numerous studies have demonstrated that the water between phospholipid bilayers is perturbed by the bilayers and therefore not identical to bulk water [21–23] Since this may imply a difference in specific volume, the often-used method of subtracting the volume of water using bulk water data may introduce error Although it has been argued that such an error is insignificant [21], it is useful to estimate its order of magnitude From experiments on phospholipid bilayers with varying amounts of water between the bilayers, the chemical potential difference of the water at the equilibrium bilayer separation was found to be different for the P_β and L_α phases, but both were less than one calorie per mole Since our sample contained two to three times more water, the chemical potential difference is reduced in both phases to something on the order of 0 1 calorie per mole This free energy difference can be used to estimate the amount of variation in specific volume one could expect in our experiments The energy of compression can be represented by $P\Delta V$ where P is the hydrostatic pressure and ΔV is the molar volume change Using a value for P typical of our experiments and equating the compressional energy per mole to the difference in chemical potential per mole, results in an estimated change of specific volume for the water of 0 03% compared to its bulk specific volume This translates into an error of about 0 3% in our specific volume measurements for DPPC Thus the result is clear, the effect of the difference in the water between bilayers when compared to bulk water whether in the intermediate phase or the liquid crystal phase, is of little consequence in this investigation

Conclusion

The results presented here are important for both the questions they answer and the questions they raise Certainly the new isothermal compressibility data for DPPC are important since they are so different from the one earlier reported result It is also obvious that no major changes in the volume discontinuity take place in the region studied, in agreement with the results of the first volumetric study [4], but later questioned by the adiabatic compression data [7] The lack of agreement between Sugar's and Tarjan's theory and this experiment raises questions as to why the pressure and temperature dependences are incorrect, when the theory seems to work well in describing the dependence on chain length Finally, these data point out the importance of steric interactions in the main transition This quantitative result serves as a critical test for theoretical work and should be complemented by measurements in other phospholipid systems and along other phospholipid phase transitions

Acknowledgement

The authors would like to acknowledge the valuable contribution made by C S Johnson during the early stage of this work

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