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SPECIFIC HEATS OF LIPID DISPERSIONS IN SINGLE PHASE REGIONS

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Differential scanning calorimetry has been used for the first time to measure the specific heat, C_p, as a function of temperature in the single phase regions above and below the main phase transition temperature, T_m, for dispersions of saturated phosphatidylcholines and phosphatidylethanolamines. Within error limits C_p, when expressed per gram, does not vary in any systematic way with chain length or headgroup. Its temperature dependence in both single phase regions qualitatively resembles that of *n*-alkanes. Contributions to C_p from intrachain vibrations and interchain van der Waals' interactions have been calculated and account for nearly all the measured C_p at temperatures above T_m. However, these contributions do not yield the observed temperature dependence below T_m. It is conjectured that such a temperature dependence arises from the unhindering of chain vibrations as the lipids undergo thermal expansion, and the result of a preliminary calculation which supports this conjecture is presented.

Introduction

Calorimetric measurements on lipid systems have focused on enthalpy changes ΔH and specific heats C_p at or very close to phase transitions, such as the main gel to liquid-crystalline transition, the lower pretransition and most recently the subtransition [1]. In contrast our dilatometric studies (volume versus temperature), in addition to measuring transition volumes, also measure the coefficients of thermal expansion

$$\alpha = \left(\frac{\partial V}{\partial T}\right)_p / V$$

in the single phase regions between transitions [2,3]. In order to understand better the thermodynamic state of these phases it is desirable that specific heat measurements also be made to complement the thermal expansion measurements.

In most previous measurements (Ref. 4 and references therein) because of the low lipid concentrations used in order to focus upon the phase transitions, the specific heat a few degrees away from these transitions is observed as a flat baseline and is set at some arbitrary value (usually zero). However, the use of larger amounts of lipid in a modern DSC (differential scanning calorimeter) having the requisite reproducibility makes possible the determination of the absolute value of C_p and its temperature dependence. In the case of the Privalov DSC [5] or the Microcal DSC sample loading is done by fixed volume; in order to calculate the specific heat of the lipid part of the dispersion, the specific volumes of both water and lipid must be known. Now that specific volume data for phospholipids are readily available [2,3] there is no barrier to doing specific heat measure-

Abbreviations: PC, phosphatidylcholine; PE, phosphatidylethanolamine; DLPC, dilaurylglycerophosphocholine; DMPC, dimyristoylglycerophosphocholine; DPPC, dipalmitoylglycerophosphocholine; DSPC, distearoylglycerophosphocholine; DLPE, dilaurylglycerophosphoethanolamine; DMPE, dimyristoylglycerophosphoethanolamine.

ments in single phase regions with the present generation of differential scanning calorimeters.

Materials and Methods

Phospholipids were obtained from Calbiochem-Behring Co. and used without further purification. The DSC scans of dilute suspensions (about 1 mg/ml) exhibited comparably narrow transitions as reported in previous work and this indicates high purity of the samples, though not as high as the best sample [6]. Multilamellar vesicles were formed by suspending the lipid in water above its T_m (main transition temperature) and vortexing for a few minutes. All lipids were initially dried in a vacuum oven for several hours at 70°C before being weighed and suspended in water. Lipid concentrations were 16 to 35 mg/ml and the sample volume was 1.022 ml.

DSC scans were performed with a Microcal MC-1 calorimeter (Microcal Inc., Amherst, MA 01002). A heating rate of 12.3 K/h was used for all experiments and was carefully measured over a period of one to two hours for each experiment. The specific heat is obtained following a procedure of Privalov and Khechinashvili [7]. Let $-\Delta_t$ be the measured difference between the heat capacity of the sample and that of the water baseline (see Fig. 1). Then,

$$-\Delta_t = C_l m_l - C_w \Delta m_w \tag{1}$$

where C_l and C_w are the specific heats of lipid and water, respectively, m_l is the mass of lipid, and Δm_w the mass of water displaced by the lipid. Since the same volume is always loaded into each calorimeter cell, then

$$\Delta m_w = m_l (V_l / V_w) \tag{2}$$

where V_l and V_w are the corresponding specific volumes at the loading temperature, usually 21°C, and so

$$C_l = C_w (V_l / V_w) - (\Delta_t / m_l) \tag{3}$$

This relationship has been used previously to measure the specific heats of protein solutions by DSC [7]. This method relies on excellent baseline reproducibility since the greatest contribution to the heat capacity of the calorimeter cell arises from the solvent (water). Repeated scans of the same material left in the calorimeter were superimposable indicating calorimetric errors, which include variations in the scanning rate, of less than $3 \cdot 10^{-5}$ cal/degree. Baseline reproducibility upon repeated filling of the cells with water was better than 0.001 cal/degree. For the concentrations of lipid measured this should give rise to an error of 0.01 cal/g per degree in the value of C_l. The observed random error was larger, 0.05 cal/g per degree. The reason is that the calorimeter is loaded with a syringe which is used to withdraw a predetermined volume from a vial containing the dispersed lipid sample which is not evenly mixed like a true solution; thus the largest error is due to slightly different amounts of lipid in the calorimeter cell. (Sonicated samples, which are evenly mixed, unfortunately do not have the stability required for these experiments.) Since typical values of C_l are about 0.5 cal/g per degree the error is of order 10%. The accuracy of this method for obtaining specific heats was tested on an NaCl solution (5% w/v). Our measured values of C_p at 25 and 40°C were within 0.3% of literature values (International Critical Tables). This illustrates that the major source of error for lipids is indeed in loading an inhomogeneous dispersion.

Results

The specific heat in calories per gram per degree as a function of the temperature difference $\Delta T = T - T_m$ from the chain melting transition (T_m) is shown in Fig. 2 for saturated phosphatidylcholine and phosphatidylethanolamines with several different chain lengths. Error bars have been drawn on those curves where at least four separate samples had been examined. In other cases two or three samples were scanned.

The region between the lower and main transitions in the PCs has not been included on this graph. The specific heat in that region changes rapidly with temperature and has higher values than in the two regions shown [27]. Because of the difficulty in obtaining reliable data near 0°C, no low-temperature curve for DMPC is shown. Also, in no case were any of the PCs allowed to remain

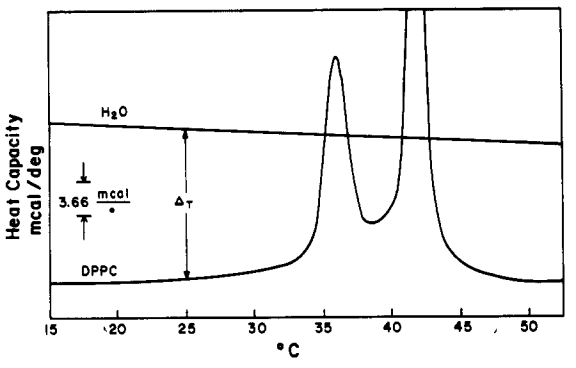

Fig. 1. Representative heat capacity curve versus temperature showing the water vs. water baseline and a DPPC vs. water scan. The quantity Δ_t is the value of the water curve minus the value of the DPPC curve.

at low temperatures for very long so that the subphase transition [1] is not present. DLPC suspensions have a T_m close to 0°C and as a consequence the low-temperature specific heat could not be measured. In addition, due to current uncertainties regarding the low-temperature phase behavior of DLPE (see Ref. 3, and Epand, R.M., personal communication), it was decided to include data only for the melted phase of this compound.

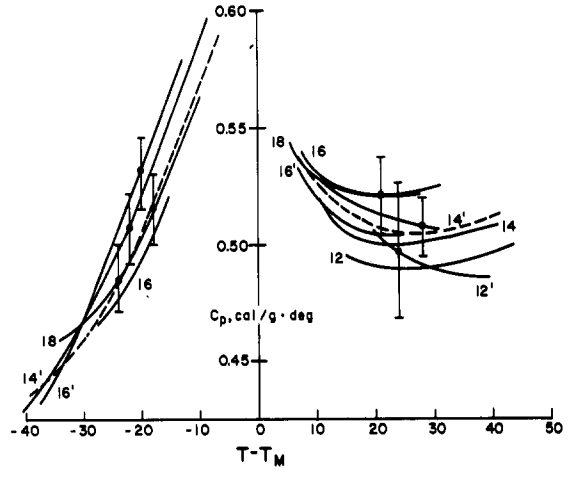

Fig. 2. Measured specific heat of phospholipids as a function of temperature difference from the chain melting point, T_m. The number beside each curve refers to the length of the hydrocarbon chain. The primed numbers refer to the phosphatidylethanolamines and the unprimed numbers to the phosphatidylcholines. The dashed line is a composite curve for all the lipid data.

Because our present DSC apparatus requires that the total heat capacity of the sample and reference cells be fairly closely matched, a wide range of lipid concentrations could not be examined. However, our data on DSPC indicated no systematic variation in specific heat with concentration in the range 16 to 30 mg/ml. This is in keeping with the lack of concentration dependence of the specific volume for these systems. (see Ref. 8, and unpublished data from this laboratory).

Within the accuracy of our measurements the specific heat per gram per degree shown in Fig. 2 is not sensitive for a given ΔT to variations in chain length or to differences in the headgroups of PCs versus PEs. Therefore, in the remainder of this paper the phrase 'specific heat of lipids' will refer to an average curve such as the one shown by dashed lines in Fig. 2. Above the transition the specific heat of lipids shows a fairly rapid decrease with increasing ΔT until it reaches a plateau for $20\,K < \Delta T < 30\,K$, followed by a slowly increasing C_p for larger ΔT. In contrast below the transition (below both the lower and the main transition for PCs) C_p increases rapidly with ΔT even at $\Delta T = -40\,K$ and the rate of increase with ΔT increases as ΔT increases.

Discussion

Let us first compare our present specific heat results to our previous volumetric results [2,3]. The latter are summarized in Fig. 3 for the coefficient of thermal expansion

$$\alpha = \left(\frac{\partial V}{\partial T}\right)_p / V$$

It is clear when comparing Fig. 3 with Fig. 2 that the specific heat and the coefficient of thermal expansion have considerably different temperature dependences. In particular, below T_m, C_p increases dramatically with T whereas α remains nearly constant. Such differences show that there is different information contained in these two sets of measurements and that C_p and α are not obviously related by simple solid state theories. For example, in Gruneisen's theory of, admittedly, far simpler solids a strongly increasing C_p requires a strongly increasing α [9,10]. (More discussion of the

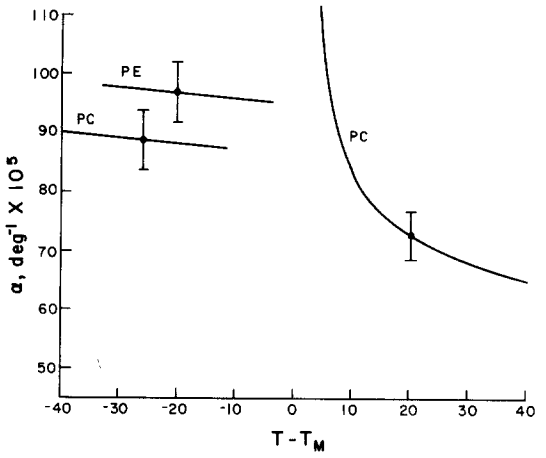

Fig. 3. Composite curves of the coefficient of thermal expansion α for phosphatidylcholines [2] and phosphatidylethanolamines [3].

Gruneisen theory will be given later.) In this discussion we try to understand why there are these differences in temperature dependence as well as to provide a quantitative basis for understanding the specific heat.

An incomplete, but still important, level of understanding comes by comparing our thermodynamic data to data from simpler systems. The particular system that we have found very useful

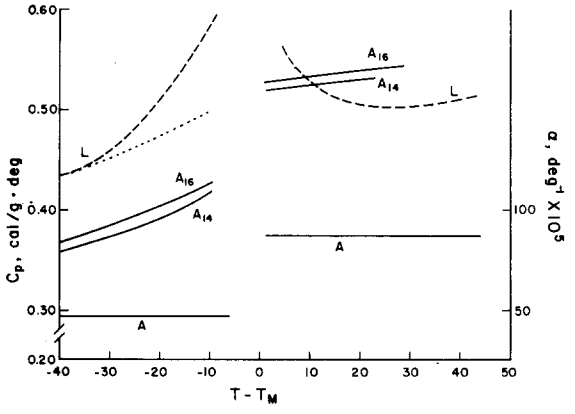

Fig. 4. Comparison of thermal data for lipids (L) and n-alkanes (A). The solid lines labelled A_{14} and A_{16} are C_p curves for tetra- and hexadecane, respectively [11]. The solid line labelled A is the average coefficient of thermal expansion of n-alkanes [12]. The dashed lines labelled L are the composite C_p date for the lipids. The dotted line is a hypothetical 'background' contribution discussed in the text.

in the past [2] when studying the main phase transition in lipids is the system of n-alkanes. Fig. 4 shows C_p for n-alkanes for $n = 14$ (tetradecane) and 16 (hexadecane) as a function of the temperature difference from the solid-liquid melting point, T_m [11]. Unfortunately, specific heat data just below T_m appear to be lacking, but the data go quite close to T_m on the high T liquid side. Also shown in Fig. 4 is a composite sketch of the values of the coefficient of expansion α above and below T_m [12]. The values are remarkably constant as depicted in the graph except for a few degrees below T_m. Comparing the data for the n-alkanes in Fig. 4 with the data for the lipids in Figs. 2 and 3 we see that an increase of C_p with increasing T in the low temperature phase while α remains nearly constant is a common feature. Nevertheless, the increase in C_p is larger for the lipids than for the n-alkanes as is seen in Fig. 4 where the composite C_p for the lipids is shown by the dashed line. Comparing the specific heat data in the high temperature melted phase it is seen that $0 < \Delta T < 20$ K there is an aftereffect of the transition in the lipids which is not present in the n-alkanes. This aftereffect is consistent with the system being close to a 3/2-order critical point [16].

A complete statistical mechanical theory of the thermodynamic properties which could quantitatively explain the C_p and α behavior is impractical for such complicated systems as lipids. What we propose to do first is to identify different sources of the specific heat and to make estimates of the sizes of their contributions as if these contributions were independent, while realizing that they are ultimately coupled. The first contribution we consider comes from molecular vibrations. Very accurate empirical formulae for these contributions to C_p are available [13] for the gas phase of alkane hydrocarbons. There are four major types of molecular vibrations of interest to us which we briefly summarize by giving their values at 20°C and also their relative rate of change (RRC) at 20°C.

$$\mathrm{RRC} = (\mathrm{d}C_p/\mathrm{d}T)/C_p \qquad (4)$$

(1) C–C–C bend: $C_p = 1.42$ cal/degree per mol and RRC = 0.002/degree. (2) C–C–C–C torsion, which includes rotational isomerism: $C_p = 2.23$

cal/degree per mol and RRC = 0.0003/degree. (3) CH_3 group: $C_p = 3.35$ cal/degree per mol and RRC = 0.0047 degree. (4) CH_2 group: $C_p = 1.29$ cal/degree per mol and RRC = 0.009/degree. The relative rates of change for these contributions do not vary rapidly in the temperature range of interest to us although the overall vibrational relative rate of change does decrease about 10% from 0°C to 40°C.

A second very important contribution to C_p comes from the work required to expand the volume of the system. In particular, as the volume increases with temperature the average van der Waals interaction U_{vdW} between molecules increases because the molecules are further apart. This contribution to C_p can be expressed as

$$\left(\frac{\partial U_{vdW}}{\partial T}\right)_p = \left(\frac{\partial U_{vdW}}{\partial r}\right)_p \left(\frac{\partial r}{\partial V}\right)_p \left(\frac{\partial V}{\partial T}\right)_p \tag{5}$$

The factor $(\partial V/\partial T)_p$ is determined experimentally from our volume measurements. The variable r is a mean separation between long parallel hydrocarbon chains and, for hexagonal arrays, is related to the volume of each CH_2 group, v_{CH_2}, by

$$v_{CH_2} = (3^{1/2}/2)\, r^2 (1.27\,\text{Å}) \tag{6}$$

A reasonable empirical formula for $U_{vdW}(r)$ which reproduces heat of sublimation data [14] is given by Salem [15]

$$U_{vdW}(r) = (1.84\ \text{kcal}/4)\left[(r_0/r)^{25} - 5(r_0/r)^5\right] \tag{7}$$

More details of the use of these formulae and their justification in disordered fluid phases are given in earlier works [2,16].

A third contribution to C_p comes from the translational and rotational degrees of freedom of the molecules. Below T_m it is natural to treat this contribution as a simple Debye term. If we assume that the lattice Debye temperature is low for a molecular crystal compared to T_m, this contribution can be approximated by R for each of 6 degrees of freedom. However, as will be discussed later, the rotational degrees of freedom are far from harmonic and so a total of $3R$ is likely to be a better approximation for this contribution.

As an example of how large the aforementioned contributions to C_p are we calculate them for *n*-tetradecane at 20°C in the liquid state (T_m = 279 K). From 12 C–C–C bends we obtain 17.04 cal/degree per mol, from 11 C–C–C–C torsions 24.5 cal/degree per mol, from 2 methyl vibrations 6.7 cal/degree per mol and from 12 methylene vibrations 15.5 cal/degree per mol, for a total vibrational specific heat of 63.7 cal/degree per mol. The C_p contribution for intermolecular van der Waals interactions obtained using $V(T)$ data gives 40.0 cal/degree per mol. Adding a 'Debye' term of $3R$ then gives 109.7 cal/degree per mol which is close to the experimental value [11] of 105.0 cal/degree per mol. This calculation illustrates the importance of including the van der Waals intermolecular interaction energy as well as the vibrational terms in the specific heat. Furthermore, when *n*-alkanes freeze α decreases by about a factor of 2. This reduces the van der Waals contribution to C_p by about 18 cal/degree per mol = 0.09 cal/g per degree for tetradecane. This is close to the observed drop in C_p seen in Fig. 4 for the *n*-alkanes at their freezing point. An analogous calculation for DMPC and DMPE at $T - T_m$ = 14 K yields a C_p which is also within 5% of the measured values. (This calculation assumes that the vibrational contribution from the head groups is equal to the contribution from the same molecular weight of hydrocarbon chains.)

In the preceding paragraph agreement between theory and experiment is encouraging. However, let us now consider the temperature dependence in the low temperature phase of the *n*-alkanes (Fig. 4). The measured RRC = 0.045/degree at ΔT = −40 K and the relative rate of change increases with increasing T whereas the calculated relative rate of change is only 0.0026/degree and decreases with increasing T. When one turns to the lipids this discrepancy becomes even greater due to the larger measured relative rate of change. Several possible explanations, not mutually exclusive, for this discrepancy will be discussed. The first possibility is that these systems undergo increasing *trans-gauche* rotational isomerism with increasing T below the transition. One cannot calculate this contribution to C_p a priori, but from the data one might hope to estimate the increase Δn_g in *gauche*

rotamers in the following way. A smooth C_p curve is drawn to represent all C_p contributions to C_p except the *trans-gauche* contribution. This 'background' curve would join the measured curve at low T and have the calculated relative rate of change up to the melting temperature. The rotameric enthalpy. ΔH_r, which is equal to Δn_g times 0.5 kcal/mol, is the area between the measured C_p and the 'background' C_p curve. Unfortunately, a plot (see (Fig. 5) of C_p data for *n*-alkanes from 0 K to T_m reveals the arbitrariness of any smooth background C_p curve. Nevertheless, with the choice of a linear background curve tangent to the data curve at 150 K, one obtains for *n*-hexadecane $\Delta H_r = 850$ cal/mol or $\Delta n_g = 1.7$/molecule. The same procedure is even more problematic for the lipids since no very low temperature data exist. Nevertheless, if one uses for a background the measured *n*-alkane C_p curve, shifted upwards to account for the extra $C_{P,vdW}$ due to the larger α, we calculate an additional $\Delta H_r = 650$ cal/mol for DPPC in the temperature range $-40\,K < \Delta T < -10\,K$ for a total $n_g = 4.7$/molecule at $\Delta T = -10$ K. For the phosphatidylethanolamines which have no lower transition one can make estimates of n_g just below the main transition ($\Delta T = -2$ K). The large extra increase in C_p in the last 8 degrees increases this estimate to $n_g = 6.8$ for DMPE just below the transition. Added to our estimates of $\Delta n_g = 4.2$ due to the transition [3], one would obtain $n_g = 11.0$ just above the transition for DMPE, which translates to a fraction of *gauche* bonds, $p_g = 0.55$. This fraction is even larger than the 0.47 that one would expect for a completely disordered hydrocarbon system, even ignoring the pentane effect which would exclude g^+g^- pairs and which would reduce p_g to 0.37 as emphasized recently by Gruen [17]. Therefore, this first explanation, followed exclusively, leads to the contradiction that the condensed matter phase of lipid bilayers just above T_m is more rotamerically disordered than the vapor.

Raman and infrared spectroscopy have been used to obtain independent estimates of n_g. The pioneering work of Gaber and Peticolas [18] suggested that many *gauche* conformers were introduced below T_m, in agreement with the model in the preceding paragraph. However, the linear calibration of the 1132 cm^{-1} line has been criticized [19,20,21,22] and it appears that the former estimates were too large. Cameron et al. [20] suggest that for DPPC, even in the intermediate phase above the lower transition, n_g is still only about 2/molecule. This is in agreement with estimates of $n_g = 1$/chain for n-$C_{21}H_{44}$ [22] in the hexagonal phase between the pretransition and the main transition. (The more precise estimates of Pink et al. [20] for DPPC of $n_g = 1.78$ at 25°C, while an improvement upon the estimates of Gaber and Peticolas [18], are based too heavily upon a crude statistical model to be so accurate; their result $n_g = 10.35$ in the L_α phase also predicts a larger n_g than would be attained in the vapor.) Therefore, it seems likely that the gradual increase in n_g in the low temperature phases is not large enough to account solely for the large increase in C_p.

We now turn to a second possible theory for the temperature dependence of C_p in the low temperature phase. As is clear in Fig. 5 the rapid increase in C_p below T_m is not some easily decoupled event presaging the onset of the phase transition. As a function of T it is a gradual change which is intimately connected to the vibrational properties of the solid. A similar kind of C_p versus T curve to the one in Fig. 5 is observed in solid benzene and the benzene C_p curve has been adequately fit by Lord et al. [10]. Their theory has three main contributions to C_p: a Debye lattice contribution, an

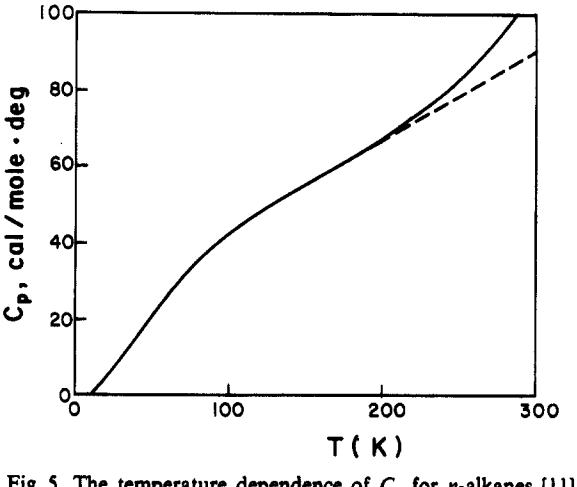

Fig. 5. The temperature dependence of C_p for *n*-alkanes [11]. The dashed line is an attempted 'background' curve which is drawn tangent to the data curve at 150 K.

intramolecular vibration contribution and a lattice expansion contribution. The first two terms in their theory are essentially the same as in our theory except that we do not have to treat the Debye contribution so carefully because it is relatively much smaller in our problem and we are mostly interested in higher temperatures. The significant difference is that in their calculation it is the lattice expansion contribution to C_p (calculated using a Gruneisen theory slightly more sophisticated than ordinary) which is most rapidly increasing at higher temperatures and which supplies the upward curvature to C_p. This theory gives

$$\alpha = \gamma C_v(\kappa/V) \tag{8}$$

where γ is the Gruneisen constant, κ is the isothermal compressibility, and κ/V is assumed to be nearly temperature independent [9,10]. Therefore, α and C_v have essentially the same temperature dependence. Of course, it is C_p not C_v that is measured. To obtain C_p one uses the thermodynamic formula $C_p = C_v + \alpha^2 T(V/\kappa)$ which with Eqn. 8 gives $C_p = (\alpha/\gamma)(V/\kappa)(1 + \alpha\gamma T)$. With the standard assumption that (κ/V) is independent of T (which is consistent with but by no means compelled by the data of Liu and Kay [23] on DPPC) then a rapidly increasing C_p requires a rapidly increasing α, as noted in the first paragraph of the discussion. Since our measured α is not rapidly increasing, this theory does not help to explain our results.

The third explanation, which is our tentative one, is based on the idea that many of the molecular vibrations in a condensed phase are hindered due to excluded volume interactions of neighboring molecules. This is already true for C–C–C bending vibrations when the central carbon is in the central third of the hydrocarbon chain. It is especially true for C–C–C torsions, even when such vibrations are restricted to the trans potential well; only the last rotameric vibration on the chain is not hindered. Unfortunately, a complete calculation would require a detailed normal mode analysis including coupling between intramolecular vibrations of neighboring molecules and even the entire lattice. However, so that one may obtain some appreciation of how the effect may alter C_p, consider motion in a harmonic potential which is absolutely hindered beyond a certain amplitude b, which in turn depends upon T,

$$U_{pot} = a\theta^2, \quad |\theta| < b(T)$$
$$= \text{infinity}, \quad |\theta| > b(T) \tag{9}$$

The quantum statistical specific heat obtained from Eqn. 9 is shown in Fig. 6. The torsion constant a and the moment of inertia were chosen to correspond to a simple C–C–C bend [13] and it was assumed that $b(T)$ increases linearly with T, $b(T) = (T - T_0)b'$. From our volume measurements and the assumption that the methylene volume $v_{CH_2} = 22.1$ Å^3 in the close-packing limit (where $b = 0$), b' was calculated as $3.7 \cdot 10^{-4}$ rad/degree and $T_0 = 200$ K for lipids. It should be emphasized that some of these parameters change for different C–C–C normal modes and for the C–C–C–C torsional modes, so Fig. 6 is only a representative graph of C_p for one degree of freedom. Nevertheless, Fig. 6 illustrates some important features and suggests at least qualitative resolution of the problem. First, the hindering of the vibrations at low T can retard the growth of C_p with increasing T even more than could be obtained from the Debye or Einstein theory. Second, as T is increased and the vibrations become unhindered, the specific heat increases more rapidly than given by the Debye or Einstein theories or the Scott empirical formulae [13]. Third, in temperature intervals in which the unhindering is proceeding especially rapidly C_p may actually exceed the high temperature classical value of R/degree of freedom. The latter two

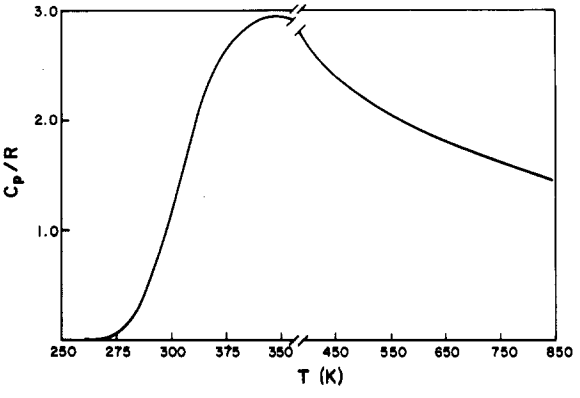

Fig. 6. Quantum statistical C_p obtained from Eqn. 9.

features are enhanced for larger rates of unhindering b'. If a reasonable fraction of intramolecular vibrations undergo unhindering in the same temperature range, then C_p can increase quite rapidly. The fact that C_p increases more rapidly for the lipids than for the alkanes below T_m is consistent with the larger measured α which is proportional to the rate of unhindering, b'. The fact that the simple theory, assuming uncoupled, unhindered vibrations and a van der Waals expansion contribution, gives a good first estimate of the magnitude of C_p, while getting the T dependence wrong, is consistent with the average hindered vibration being near $T = 290$ K in Fig. 6. Thus, hindered vibrations explain, at least in part, the anomalously large dC_p/dT in the low temperature phases of *n*-alkanes and lipids. In the disordered high Tphases, as T is increased, unhindering of the vibrations is relatively retarded for the following reason. As V increases with increasing T more *gauche* disorder is introduced causing new close contacts between molecules which hinder the vibrations, and so the effective $b(T)$ increases more slowly with T than does the free volume. The differences above T_m in Fig. 4 between C_p for lipids and C_p for *n*-alkanes is most likely due to the differences in the van der Waals interactions. The rapid decrease in C_p for lipids just above T_m is due to the sharply decreasing α. Also, in this interpretation the lower C_p for lipids compared to *n*-alkanes at $\Delta T = 30$ K is due to the smaller α.

So far, we have interpreted our results nearly exclusively in terms of the hydrocarbon tails. When the headgroups have been considered, as in the vibrational contributions to C_p, it has been assumed that they are more or less indistinguishable from the tails on a per gram basis. Here, we consider briefly special contributions to C_p from the head groups and from a water interfacial term. The primary variable that determines headgroup and water interactions is the area A per molecule. For the PCs below T_m and below the lower transition the work of Janiak et al. [24] implies that A decreases with increasing T because the tilt angle θ decreases and the relative thickness of the bilayer increases more rapidly (0.002 to 0.006/degree) than does the relative volume increase $\alpha =$ 0.0009/degree. In contrast for the phosphatidylethanolamines there is no tilt [25] and so A must increase with increasing T and V. The fact that there is little difference in C_p for these two lipid types while A behaves radically differently suggests that the contribution to C_p from headgroup interactions and water interfacial terms is only secondary. (Such a secondary effect might account for the small differences seen in Fig. 2; the sign of the differences is consistent.) This conclusion is also supported by calculations of changes in U_{head} with A and T similar to those which have been discussed in the past in connection with the main transition. Even if the area change ΔA in the range $-40\text{ K} < \Delta T < 0$ is as large as at the main transition, which is most unlikely, the associated ΔH due to electrostatic interactions or hydrogen bonding is less than 500 cal/mol for DPPC [26]. Likewise, the ΔH due to the water interfacial term is bounded above by 200 cal/mol [16]. Van der Waals interactions between head groups are not significantly different on this scale because as the head groups get further apart water fills the interstices.

Of course, in an ultimate sense the headgroups and the presence of water must be responsible for the very real differences in thermodynamic properties between lipids and *n*-alkanes. However, the mechanism responsible for these differences appears not to be one which is easily separable from the hydrocarbon tails. Rather, the primary effects of the headgroups and the interaction with water is to pin the lipid molecules to an interface. This boundary condition requires that the tails in lipid bilayers are in an anisotropic liquid crystalline state even above T_m whereas in the *n*-alkanes the tails are in an isotropic liquid state. This boundary condition affects the state of the tails which then, according to the analysis presented in this paper, determines the most important features of the specific heat.

In conclusion, it should be clear from the discussion that the theoretical explanation of the T dependence of our C_p results is tentative. We do make a suggestion, which as far as we know is new and not just for lipid systems, that the unhindering of hindered vibrations plays a significant role in the rapidly increasing C_p in the gel phase and we present the results of partial calculations to support this idea. More elaborate calculations could be done but they would involve much parameter

fitting. A complete theory must also account, simultaneously, for the nearly constant α in the gel phase, and we are not presently certain how to develop such a complete theory. In any case such extensions seem unwarranted until after exposure of the basic ideas, and the data, to other workers.

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