Cholesterol-Induced Variations in the Volume and Enthalpy Fluctuations of Lipid Bilayers

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ABSTRACT The sound velocity and density of suspensions of large unilamellar liposomes from dimyristoylphosphatidylcholine with admixed cholesterol have been measured as a function of temperature around the chain melting temperature of the phospholipid. The cholesterol-to-phospholipid molar ratio x_c has been varied over a wide range ($0 \leq x_c \leq 0.5$). The temperature dependence of the sound velocity number, of the apparent specific partial volume of the phospholipid, and of the apparent specific adiabatic compressibility have been derived from the measured data. These data are particularly discussed with respect to the volume fluctuations within the samples. A theoretical relation between the compressibility and the excess heat capacity of the bilayer system has been derived. Comparison of the compressibilities (and sound velocity numbers) with heat capacity traces display the close correlation between these quantities for bilayer systems. This correlation appears to be very useful as it allows some of the mechanical properties of membrane systems to be calculated from the specific heat capacity data and vice versa.

INTRODUCTION

During the past decades, cholesterol-phospholipid interactions have been a topic of current wide interest (for reviews see Finean, 1990; Vist and Davis, 1990; Mouritsen and Jørgensen, 1994; McMullen and McElhaney, 1995). The interest in this topic has been renewed recently due to work by Aloia et al. (1993) who showed that the viral envelope of the human immunodeficiency virus (HIV) is characterized by a cholesterol-to-phospholipid (C/P) molar ratio that is increased by a factor of 2.5 with respect to that of the host cell surface membrane.

Various physical and biochemical techniques have demonstrated a considerable influence of cholesterol on functional and structural properties of lipid bilayers (see Yeagle, 1985, for a review). Recently the discussion has been particularly focused on the mechanisms of phase separation in cholesterol-phospholipid bilayers. A variety of detailed experimental studies led to a widely accepted phospholipid-cholesterol phase diagram (Ipsen et al., 1987; Vist and Davis, 1990; Mouritsen and Jørgensen, 1994), indicating that the amphiphilic cholesterol is able to induce both order and disorder in a phospholipid bilayer. As cholesterol interacts differently with the translational and the conformational degrees of freedom of the phospholipid molecules, a liquid-ordered phase (Ipsen et al., 1987) has been postulated to exist at cholesterol contents above 20 mol %. Though exhibiting a high degree of conformational order, this phase lacks translational order. At low cholesterol contents, cholesterol is neither able to reduce the conformational order in the gel phase nor able to enhance the order in the phospholipid alkyl chains in the fluid phase. At $x_c < 7.5$ mol % C/P, no macroscopic phase separation between phases of different cholesterol content occurs. Rather, cholesterol is enriched in the interfaces of fluid and gel lipid domains, thus reducing the cooperativity of the transition (Cruzeiro-Hansson et al., 1990; Mouritsen and Jørgensen, 1994). In contrast to the above view, McMullen and McElhaney (1995) have pointed out that cholesterol may be able to induce phase separation characteristics even at a very low C/P ratio starting from 2 mol %. These authors proceeded from an alternative phase diagram. Their analysis was based on a detailed study of dipalmitoylphosphatidylcholine (DPPC) vesicles of different cholesterol content using differential scanning calorimetry (DSC) followed by a decomposition of endoterms. A strong enhancement of volume fluctuations at moderate cholesterol concentrations ($x_c < 20$ mol %) has been shown by Michels et al. (1989) using ultrasonic absorption measurement. These authors also pointed out a cholesterol-induced enhancement of the size of lipid domains. Computer simulations performed by Mouritsen and co-authors (e.g., Mouritsen and Jørgensen, 1994) demonstrated that the formation of domains (for example, areas in the gel state dispersed in the fluid phase) is enhanced by the presence of cholesterol at low C/P ratios only. Hence, the study of the special characteristics of cholesterol-phospholipid interactions at relatively low cholesterol content seems to be most important for our understanding of the phase separation mechanism in lipid bilayer systems.

In this work, sound velocity, density, and heat capacity measurements have been performed to study the properties of large unilamellar dimyristoylphosphatidylcholine (DMPC) liposomes of different cholesterol content when passing the main phase transition. Ultrasonic velocimetry

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had been already applied to liposome suspensions of DPPC with cholesterol added (Sakanishi et al., 1979). However, those authors did not examine the region below 14 mol % C/P, which is of particular interest here. Heat capacity curves for DMPC/cholesterol suspensions have been reported in the literature (Estep et al., 1978; Mabrey et al., 1978; Sackmann, 1995). However, for a quantitative evaluation and for a comparison with sound velocity data we re-measured the heat capacity of the suspensions. We found a close inverse relation of the sound velocity number profiles (Eq. 1) and the corresponding excess heat capacity profiles. This relation has been rationalized by theoretical thermodynamic means, linking the heat capacity changes to the compressibility.

MATERIALS AND METHODS

Preparation of liposomes

Large unilamellar liposomes (LUVETs) were prepared from DMPC (Sigma Chemical Co., St. Louis, MO) and cholesterol (Merck, Darmstadt, Germany) by means of the extrusion method (MacDonald et al., 1991). For this purpose, we used the LiposoFast-Basic extruder (Milsch Equipment, Laudenbach, Germany) containing a polycarbonate filter, the pores of which were ~100 nm in diameter. According to the analysis by MacDonald et al. (1991), this method yields uniform liposomes with a relatively small size distribution. The liposomes of DMPC and cholesterol were prepared at a temperature (34°C) above the phase transition temperature ($T_m \approx 24$°C) of DMPC. The DMPC concentration was 2 mg/ml throughout. LUVETs were prepared in doubly distilled water. Lipid dispersions were checked for the phosphate content after extrusion using an assay based on Rouser et al. (1970).

Ultrasonic velocimetry

Ultrasonic velocity was measured using a fixed-path differential velocimeter consisting of two almost identical acoustic cavity resonators (Sarvazyan, 1982) operated at frequencies ν around 7.2 MHz. The resonance frequencies of the cells were measured with the aid of a computer-controlled phase-sensitive feedback circuit. The sample volume was 0.7 ml. The resonator cells were equipped with magnetic stirrers to ensure homogeneous samples during the measurements. One resonator contained the liposome solution, whereas the other one was filled with the reference liquid (doubly distilled water). When starting a series of measurements, the resonance frequencies of the resonators have been always compared with one another by filling both cells with the reference. As the intensity of the sonic signal was small throughout (the pressure amplitude in the ultrasonic wave was less than 0.01 bar), any effects of the sound wave on structural properties of the biocolloids were avoided. As usual in ultrasonic velocimetry (Sarvazyan and Chalikian, 1991), the sound velocity number, defined by the equation

$$[u] = (u - u_0)/(u_0\hat{c}) \tag{1}$$

has been derived from our experimental data. In Eq. 1, u and u_0 denote the sound velocity of the solution and of the solvent, respectively, and $\hat{c}$ is the solute concentration in mg/ml.

Density measurements

A high-precision densitometer system (DMA 60 with 602 M, Anton Paar KG, Graz, Austria) operating according to the vibrating tube principle (Kratky et al., 1973) has been used to determine the density ρ of the liposome solutions. All densities have been measured at the same time as the corresponding sound velocities, having the temperature of the densitometer and the ultrasonic resonators stabilized by circulating the same thermostat fluid to the cells. Apparent specific partial volumes φ_v have been calculated from the density data using the relation

$$\varphi_v = \frac{1}{\rho_0}\left(1 - \frac{\rho - \rho_0}{\hat{c}}\right) = \frac{1}{\rho_0} - [\rho], \tag{2}$$

where subscript 0 again refers to the solvent and $[\rho] = (\rho - \rho_0)/(\rho_0\hat{c})$ denotes the density number.

Differential scanning calorimetry

Heat capacity traces were recorded on a MicroCal (Northhampton, MA) MC-2 high-sensitivity differential scanning calorimeter at scan rates of 5°/h.

Experimental errors

The uncertainty in the concentration of the phospholipid suspensions was smaller than 0.25%. The temperature of the cells was controlled to within ±0.02 K. The relative error in the resonance frequencies of the ultrasonic resonator cells was ±100 Hz. The relative error in the sound velocity resulting thereby is $\sim 1.5 \times 10^{-5}$. Repeated measurements showed that the reproducibility of the velocity number is better than 10^{-5} ml/mg. The relative error in the density data is smaller than 10^{-5}. Hence, the compressibilities derived from the u and ρ data are accurate to within 4×10^{-5}. Depending on the phospholipid concentration, the error in the specific partial volume varies between 4×10^{-6} ml/mg and 4×10^{-5} ml/mg. The experimental error in the c_p curves is mainly determined by the baseline determination. As the pure DMPC profile has a large heat capacity, the error is small. However, in the samples with high cholesterol content, the heat capacity is small, and the error in the total enthalpy may be up to 10%.

RESULTS AND DISCUSSION

Pure DMPC suspensions

In Fig. 1, the sound velocity number $[u]$ of some suspensions of large unilamellar liposomes from DMPC in water is displayed as a function of temperature between 15°C and 40°C. There is a small difference in the $[u]$ data at different phospholipid concentrations. Nevertheless, the deviation of the sound velocity of suspensions from that of the solvent may be taken to almost linearly increase with phospholipid content $\hat{c}$. In the complete temperature range, the sound velocity u of a given suspension does not vary by more than 0.6% with the most concentrated sample (20 mg/ml) and not more than 0.06% in the case of the smallest DMPC concentration (2 mg/ml).

Two sets of data are displayed in Fig. 1, and they differ from another by the temperature of sample preparation. With one set, the extrusion procedure has been performed at a temperature above T_m, with the other one at T below T_m. Just at the lowest DMPC content (2 mg/ml), differences between the $[u]$-versus-T relations of the two samples exceed the experimental error in the sound velocity number. It is indeed likely that the size distribution of the vesicles and thus the compressibilities of the suspensions depend upon the sample temperature during the extrusion process. It is

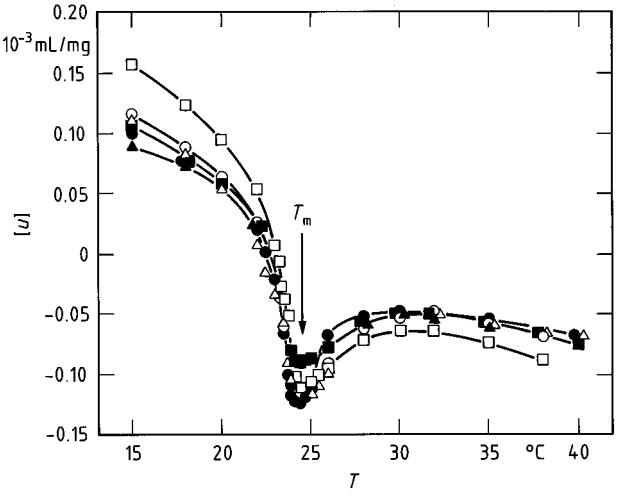

FIGURE 1 The sound velocity number $[u]$ versus temperature T for aqueous suspensions of LUVETs from DMPC. The lipid concentration is 20 mg/ml (▲, △) 10 mg/ml (●, ○), and 2 mg/ml (■, □), respectively. Closed and open symbols are used to discriminate between two sets of suspensions. One had been prepared at a temperature below T_m (15°C; ▲, ●, ■), the other one above T_m (34°C; △, ○, □). The error in the individual $[u]$ data is on the order of the size of the figure symbols.

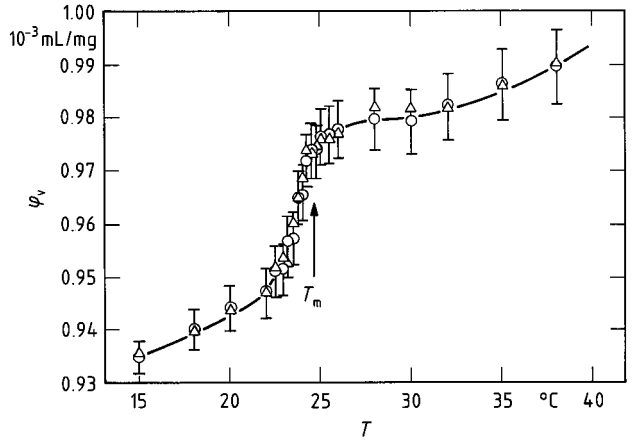

FIGURE 2 The apparent specific partial volume φ_v of two suspensions of DMPC in water displayed versus temperature T for the 10 mg/ml (○) and the 20 mg/ml (□) samples that had been prepared at 34°C.

interesting to notice that the heat capacity profiles of lipid dispersions also depend on the size distribution. On grounds of the present sound velocity data, it is, however, not possible to comment definitively on a potential effect of the sample temperature in the extrusion procedure on the acoustic properties of the DMPC vesicle suspensions.

Around the chain-melting temperature T_m of the phospholipid bilayers, the sound velocity number has a relative minimum (Fig. 1). The sound velocity of the suspensions may be expressed by the adiabatic compressibility κ_s and density ρ of the samples according to

$$u = (\rho\kappa_s)^{-1/2} \tag{3}$$

Due to (Schaaffs, 1963)

$$\kappa_s = \kappa_T c_v/c_P, \tag{4}$$

the adiabatic compressibility is related to the isothermal compressibility κ_T and the heat capacities c_v and c_P at constant volume and constant pressure, respectively, so that

$$u = \left(\frac{c_P}{c_v\rho\kappa_T}\right)^{1/2} \tag{5}$$

Hence, the minimum in the sound velocity number reflects the effects from both the increasing heat capacity c_P (van Osdol et al., 1991) and isothermal compressibility κ_T on approaching T_m.

Besides the overall tendency to increase with temperature, the apparent specific partial volume of the DMPC/water system (Fig. 2) exhibits a step-like change by ~3% around T_m, corresponding to an increase in the volume of the phospholipid molecules from 1069 Å^3 at $T < T_m$ to 1101 Å^3 at $T > T_m$. This change in the molar volume is in fairly good agreement with the volume change of pure hydrocarbons at the phase transition of the chains ($\Delta V/V = 2. . . 6\%$; Schaerer et al., 1955; Pechold et al., 1966). It is somewhat larger than the volume change of stacked lamellar structures of DPPC in water ($\Delta V/V = 1.4\%$; Träuble and Haynes, 1971; $\Delta V/V = 3.3\%$; Liu and Kay, 1977).

For the aqueous solutions of DMPC vesicles, the temperature dependence of the apparent specific adiabatic compressibility φ_κ defined by

$$\varphi_\kappa = \frac{1}{\kappa_{s0}} \frac{\kappa_s V - \kappa_{s0} V_0}{\hat{c} V} \tag{6}$$

is shown in Fig. 3. In Eq. 6, the subscript 0 denotes quantities that refer to the solvent. Using the adiabatic compressibility numer $[\kappa_s] = (\kappa_s - \kappa_{s0})/(\kappa_{s0}\hat{c})$, the apparent specific

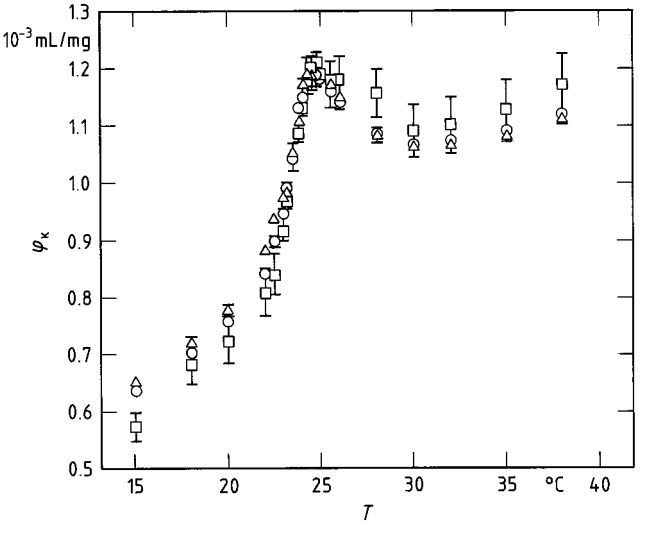

FIGURE 3 Plot of the apparent specific adiabatic compressibility φ_κ as a function of temperature T for DMPC samples of three different concentrations (see Fig. 1 for the symbols).

compressibility can be expressed as

$$\varphi_\kappa = \varphi_v + [\kappa_s] \tag{7}$$

As a result of the increasing lateral dimensions of the DMPC molecules in the LUVET bilayer at T_m, the apparent specific compressibility increases significantly with temperature ($T < T_m$). Quite remarkably, φ_κ decreases by ~10% when going from 25°C, slightly above T_m, to 30°C. In this temperature range, φ_v remains almost constant (Fig. 2) whereas κ_{s0} decreases from 0.448 to 0.441×10^{-9} m^2 N^{-1}. We therefore conclude that the decreasing φ_κ values result from a decrease in κ_s when going from 25°C to 30°C. The heat capacity c_P decreases also in this temperature range (see, e.g., Fig. 5). According to Eq. 4, the temperature dependence in the κ_s data above T_m seems thus to reflect the isothermal compressibility of the bilayer systems, which is related to the volume fluctuations of the samples (Hill, 1960),

$$\kappa_T = \frac{1}{\bar{V}}\left(\frac{\partial V}{\partial p}\right)_T = \frac{\overline{V^2} - \bar{V}^2}{\bar{V}RT}, \tag{8}$$

and which decreases with T. Here R denotes the gas constant.

Relation between the compressibility and excess heat capacity of bilayer systems

To gain deeper insights into the fluctuations in thermodynamic parameters of bilayer systems it is useful to theoretically consider the relation between the adiabatic and isothermal compressibility (Eq. 4) for phospholipid membrane systems. Analogous to Eq. 8, the heat capacity of a system is given by the fluctuations in the overall enthalpy H. These fluctuations reflect the mean square deviation of the distribution of states around the mean value. The fluctuation theorem leads to

$$c_p = \left(\frac{\partial H}{\partial T}\right)_p = \frac{\overline{H^2} - \bar{H}^2}{RT^2} \tag{9}$$

The adiabatic compressibility κ_s can be readily related to the heat capacity c_p and the isothermal compressibility (Eq. 8) if the temperature dependence of the volume $\bar{V}$ is known (Wilson, 1957; Lumry and Gregory, 1986):

$$\kappa_s = \kappa_T - \frac{T}{\bar{V}c_p}\left(\frac{d\bar{V}}{dT}\right)_p^2 \tag{10}$$

Using a vibrating tube densitometer, Anthony et al. (1981) have shown that close to the melting transition of the lipid chains the change in volume and excess enthalpy ΔH of the lipid molecules to a very high degree are proportional to each other. Hence, approximately

$$\overline{\Delta V(T)} = \gamma\overline{\Delta H(T)} \tag{11}$$

holds, which leads to two simple approximately valid relations between the isothermal compressibility, the volume expansion coefficient, and the heat capacity of lipid bilayers (Heimburg, 1998):

$$\Delta\kappa_T(T) = \frac{\overline{\Delta V^2}}{\bar{V}RT} = \frac{\gamma^2 T\overline{\Delta H^2}}{(V_0 + \gamma\Delta H)RT^2} = \frac{\gamma^2 T\Delta c_p}{(V_0 + \gamma\Delta H)} \tag{12}$$

Here V_0 is the lipid volume in the gel state and $\Delta\kappa_T$ is the excess isothermal compressibility linked to fluctuations in the lipid state.

Hence the temperature dependence of the isothermal volume compressibility is a simple function of the heat capacity change Δc_p at the transition. This is a nontrivial statement, as it requires that $\overline{\Delta V^2} = \gamma^2\overline{\Delta H^2}$. However, this can be shown to be true if Eq. 11 is valid at all temperatures, meaning that all relevant substates of the partition function fulfill the proportionality relation (Heimburg, 1998). Furthermore, one obtains

$$\frac{d\Delta V(T)}{dT} \approx \gamma\frac{d(\Delta H(T))}{dT} = \gamma\Delta c_p, \tag{13}$$

which, together with Eqs. 10 and 12, results in a simple relation for the adiabatic compressibility of the membrane:

$$\kappa_s(T) \approx \frac{\gamma^2 T\Delta c_p}{(V_0 + \gamma\Delta H)} - \frac{\gamma^2 T\Delta c_p^2}{(V_0 + \gamma\Delta H)c_p} = \kappa_T(T)\left(1 - \frac{\Delta c_p}{c_p}\right) \tag{14}$$

if the temperature dependence of V_0, which is small as compared with ΔV at the transition, is neglected.

Here c_p denotes the heat capacity of the complete sample whereas Δc_p is the excess heat capacity of the bilayer at the melting transition of the lipid chains. The excess heat produced during compression of the lipid membranes has to be distributed into the environment with capacity c_p. This is a time-dependent process. With periodic excitations, c_p thus becomes dependent upon the frequency, following relaxation characteristics (van Osdol et al., 1991). In equilibrium, when the system is excited by a sinusoidal signal of very low frequency, c_p is just the calorimetric heat capacity of the total system, including the aqueous environment, and the adiabatic compressibility is given by Eq. 14.

If the volume fraction f_w of water in the suspension is much larger than that of the lipid, f_l, then for very low frequencies $\Delta c_p/c_p \approx 0$ and the system is close to the isothermal case (Schaaffs, 1963):

$$\kappa_s(\nu \to 0, f_l \to 0) \approx \kappa_T \tag{15}$$

Hence at very low frequencies the sound velocity (Heimburg and Marsh, 1996)

$$u = (\kappa_s\rho)^{-1/2} = (\kappa_T\rho)^{-1/2} \tag{16}$$

is given by the isothermal compressibility. The sound velocity, therefore, is frequency dependent. A significant deviation from Eq. 16 is expected if the $\Delta c_p/c_p$ term in Eq. 14

is not negligibly small as compared to 1. This is the case at the heat capacity maximum around T_m (where Δc_p is large) and/or at high frequencies ν (where $c_p(\nu)$ is small). For these reasons, Eq. 16 should lead to reasonable results for small values of Δc_p, for example, in a transition of low cooperativity as found for mixtures of DMPC with cholesterol.

Suspensions of DMPC/cholesterol vesicles: simulating the sound velocity profiles from the excess heat capacity

The sound velocity numbers $[u]$ of some DMPC/cholesterol mixtures with water are shown as a function of temperature in Fig. 4. With increasing cholesterol content, the relative minimum in the $[u]$-versus-T relation becomes broader. The heat capacity profiles of extruded DMPC/cholesterol mixtures are given in Fig. 5. Similar curves were discussed in the literature, for example, by Estep et al. (1978) and Mabrey et al. (1978). The heat capacity profiles of the suspensions of extruded DMPC/cholesterol vesicles reflect rather complex melting characteristics that can be reproduced, however, by multiple measurements, including different samples. The overall melting enthalpy is strongly dependent on the cholesterol content, being 28.5 kJ/mol in pure DMPC and only 5.2 kJ/mol of DMPC at $x_c = 0.43$. Furthermore, the excess heat capacity displays a sharp peak at 24.5°C up to $x_c \approx 0.12$, which totally disappears at $x_c \geq 0.25$. The same features can be found in the experimental sound velocity number profiles (Fig. 4).

In the previous paragraph we discussed the effect of the high-frequency excitation of the ultrasonic field compared with the slow relaxation of heat in the melting regime, being in the range of seconds. As relaxation is slow, we will now assume that both lipid membranes and aqueous buffer may be considered as being adiabatically uncoupled. Thus, no heat will be transferred between membranes and water in the microsecond time regime. In this case, the adiabatic

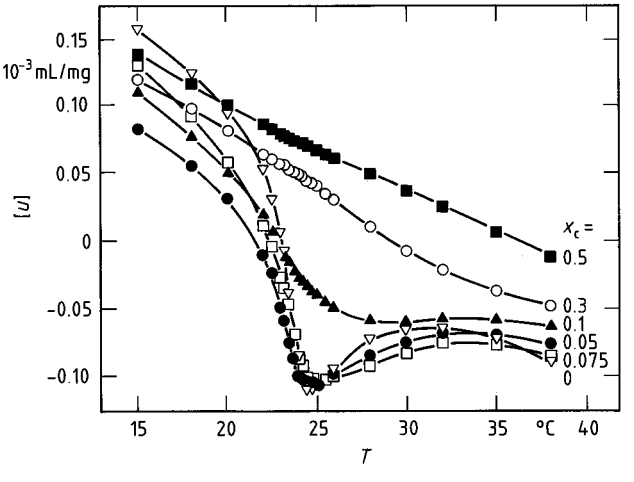

FIGURE 4 The sound velocity numer $[u]$ of aqueous suspensions of LUVETs from DMPC (2 mg/ml) with admixed cholesterol ($0 \leq x_c \leq 0.5$) displayed versus temperature T.

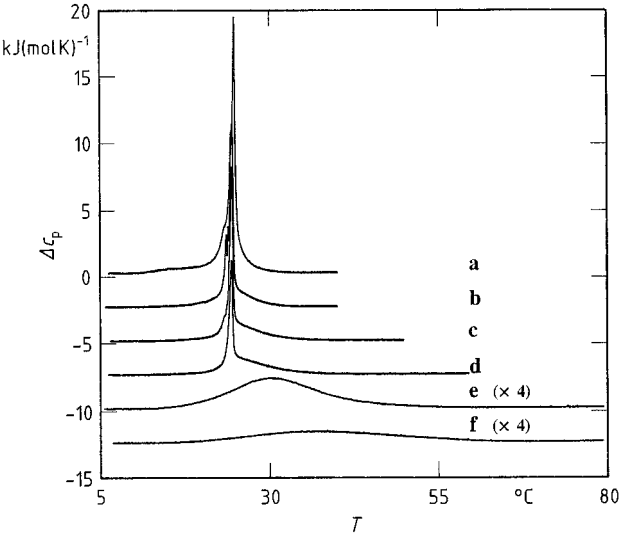

FIGURE 5 Excess heat capacity profiles of DMPC/cholesterol vesicles with (*a*) $x_c = 0$; (*b*) $x_c = 0.053$; (*c*) $x_c = 0.081$; (*d*) $x_c = 0.111$; (*e*) $x_c = 0.25$; (*f*) $x_c = 0.429$. Curves a–d were recorded using a scan rate of 5°/h; curves e–f were with 60°/h. As both latter curves have low overall enthalpies, they were multiplied by a factor of four.

compressibilities of water and lipid become additive properties:

$$\kappa_S = f_{lipid}\kappa_{S,lipid} + f_{H_2O}\kappa_{S,H_2O}, \tag{17}$$

where f_{lipid} and f_{H_2O} are the volume fractions of lipid and water, respectively. The adiabatic compressibility of the lipid can be approximated using Eq. 14:

$$\kappa_{S,lipid} = (1-f)\kappa_{T,gel} + f\kappa_{T,fluid} + \Delta\kappa_T\left(1 - \frac{\Delta c_p}{c_{p,0} + \Delta c_p}\right) \tag{18}$$

Here f is the fractional degree of lipid melting as determined from the Δc_p profiles and $c_{p,0}$ is the heat capacity of the lipid hydrocarbon chains, which is roughly independent of the melting process. It has been found by Blume (1983) to have values between 1200 and 1700 J/(mol *K*), determined for DMPC and DPPC. Δc_p is the experimental excess heat found in Fig. 5. It can adopt values much larger than the specific heat of the hydrocarbon chains. The adiabatic compressibility of water can be found in the literature. Equation 18 was derived from Eq. 14 by neglecting the thermal expansion coefficient of the lipid chains outside the melting regime. $\kappa_{T,gel}$ and $\kappa_{T,fluid}$ are the intrinsic compressibilities of the gel and fluid state, respectively. The relative volume change of DMPC upon melting is 3.6%, adding up to changes in the pretransition and in the main transition (Nagle and Wilkinson, 1978). The heat of the complete melting process is 28,500 J/mol DMPC, yielding $\gamma = 8.9 \times 10^{-4}$ cm^3/J.

With Eqs. 17 and 18, we were able to calculate the ultrasonic velocities from the heat capacity profiles, using an adiabatic compressibility of $\kappa_{T,gel} = 3 \times 10^{-11}$ cm^2/dyne in the gel state, $\kappa_{T,fluid} = 5.1 \times 10^{-11}$ cm^2/dyne in the

fluid state, and a hydrocarbon heat capacity of $c_{p,0} = 1650$ J/(mol K). These compressibility values are close to the isothermal compressibilities found for DPPC vesicles, being $\kappa_{T,gel} \leq 3.3 \times 10^{-11}$ cm^2/dyne and $\kappa_{T,fluid} = 11\text{–}17 \times 10^{-11}$ cm^2/dyne (Liu and Kay, 1977). The parameter γ was taken to be constant for DMPC (independent of cholesterol content) and to be zero for cholesterol. This implies the assumption that the overall volume change in the transition is reduced upon the addition of cholesterol, as the overall melting enthalpy is strongly reduced. The results of this calculation are given in Fig. 6, being in reasonable agreement with the experiment. Reproduced are especially the lower limit cutoff of the velocity numbers at low cholesterol content and the absence of a velocity number anomaly at high cholesterol content. The sharp velocity reduction at 24.5°C seems to be extended over a broader temperature interval in the experimental profiles as compared with the calculation, which may be due to the very simplifying assumptions on the basic parameters of the system.

As we argued in the theoretical section, the compressibility and the heat capacity are linked to the fluctuations in the system states. In recent decades, in several articles it has been proposed that heat capacity profiles in the presence of cholesterol may be deconvolved into two melting peaks, representing lipid domains of different composition (see, for example, Estep et al., 1978; Mabrey et al., 1978; Tampé et al., 1991; McMullen et al., 1993 McMullen and McElhaney, 1995; Huang et al., 1993). Similarly, mixtures of single lipids with integral peptides have been deconvolved that way (Zhang et al., 1995). This deconvolution implies that two lipid domains coexist and melt independently. In contrast, Heimburg and Biltonen (1996) have shown that heat capacity traces of lipid peptide mixtures, which apparently contain two components, may be simulated with a simple two-state lipid model. The asymmetry in the heat capacity profiles is caused by a change in the interaction of the lipids with the peptides in different phases, thus leading to a temperature-dependent mixing behavior. From this point of view, the conclusions drawn from the deconvolution of the heat capacity traces are incorrect. Rather, the anomalies of the heat capacity represent the fluctuations of the system, which reflect complex mixing behavior. Statistical thermodynamics studies on lipid-cholesterol interaction have taken a similar view (Ipsen et al., 1987, 1989; Cruzeiro-Hansson et al., 1990). Their conclusion highlighted the influence of cholesterol on the thermodynamic fluctuations and the interfacial energy of the gel and fluid lipid domains, which determine the transition cooperativity. The phase diagram derived on these grounds is clearly incompatible with the decomposition of the specific heat into different components. Sackmann (1995) adopted the DPPC phase diagram by Ipsen et al. (1989) for the DMPC-cholesterol system.

The apparent specific compressibilities displayed in Fig. 7 show that at low cholesterol content the compressibility of the membranes below as well as above T_m appears to be enhanced with respect to pure DMPC vesicles. This is in accordance with the idea that small concentrations of cholesterol tend to decrease the surface tension between suggested gel and liquid domains, respectively, on the membrane and to promote the formation of domains and thus decrease the cooperativity of the transition (Sackmann 1995; Mouritsen and Jørgensen, 1994; Michels et al., 1989). At large cholesterol-to-phospholipid molar ratio x_c, the phase transition is not cooperative. The melting is more or less a continuous process with temperature. Thus, the lipid compressibility also undergoes a continuous change from a

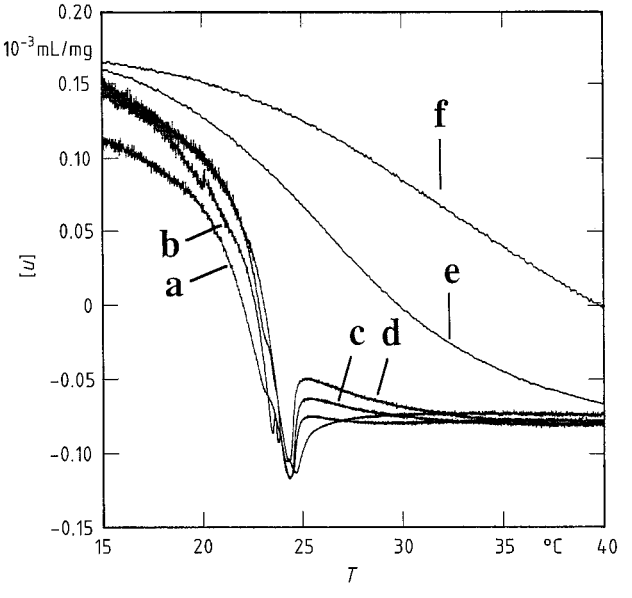

FIGURE 6 Ultrasonic velocity numbers of DMPC/cholesterol vesicle suspensions with (*a*) $x_c = 0$; (*b*) $x_c = 0.053$; (*c*) $x_c = 0.082$; (*d*) $x_c = 0.111$; (*e*) $x_c = 0.25$; (*f*) $x_c = 0.429$, calculated using the curves in Fig. 5. They show a behavior that is very similar to the experimentally obtained profiles in Fig. 4.

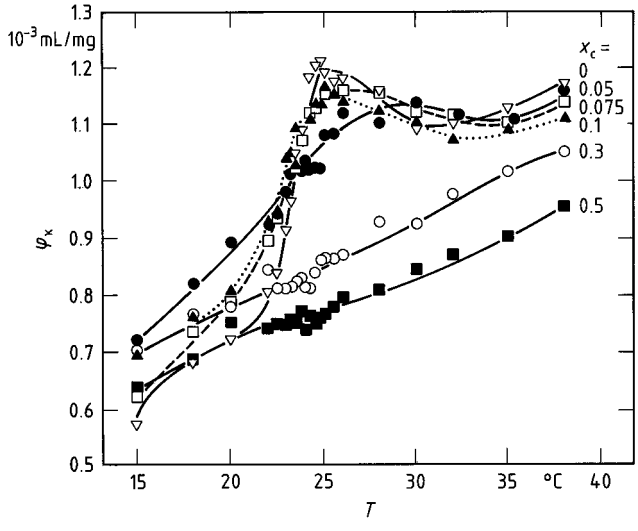

FIGURE 7 The apparent specific adiabatic compressibility φ_κ for aqueous suspensions of DMPC/cholesterol vesicles ($0 \leq x_c \leq 0.5$) shown as a function of temperature T. The concentration of DMPC is 2 mg/ml.

rigid to a more compressible state due to the different compressibilities of the gel and the fluid lipid states. No significant anomalies of the compressibility linked to fluctuations in state are expected. Below T_m, φ_κ is enhanced with respect to the pure DMPC membrane; above T_m it is reduced. Hence, the gel phase becomes more liquid and the liquid phase appears to be more rigid at high cholesterol content.

CONCLUSIONS

We have shown that the ultrasonic velocity numbers of DMPC/cholesterol mixtures display a surprisingly good correlation with the heat capacity traces given in Fig. 5. To explain this correlation we have derived a theoretical relation between the compressibility and heat capacity of membranous systems near the lipid chain-melting transition temperature. In general, no straightforward relation between the heat capacity and compressibility of a system exists because volume and inner energy are two functions with nontrivial functional connection. In an ideal gas, for example, the volume and the compressibility can change without change of the inner energy and the heat capacity. In membranous systems close to the chain-melting transition, however, volume and inner energy have experimentally been shown to be proportional functions of the temperature. The relation between the two quantities is extremely useful as it allows one to predict the mechanical and ultrasonic velocity properties of a membranous system if the specific heat is known. The implications of this finding are discussed in detail by Heimburg (unpublished manuscript).

Protein insertion has been shown to influence the calorimetric behavior of lipid melting. A corresponding change in the compressibilities can be expected. Lipid mixtures display very lipid-dependent characteristics. This should be reflected in the excess compressibility. The phase diagram of DMPC and dimyristoyl glycerol displays a stoichiometric compound at a 1:1 mixing ratio with a very cooperative melting at a well defined temperature (Heimburg et al., 1992). Thus, a pronounced minimum of the sound velocity number at this temperature is predicted.

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