Application of Pressure Perturbation Calorimetry to Lipid Bilayers

Heiko Heerklotz and Joachim Seelig
Department of Biophysical Chemistry, Biozentrum, University of Basel, CH-4056 Basel, Switzerland

ABSTRACT Pressure perturbation calorimetry (PPC) is a new method that measures the heat consumed or released by a sample after a sudden pressure jump. The heat change can be used to derive the thermal volume expansion coefficient, α_V, as a function of temperature and, in the case of phase transitions, the volume change, ΔV, occurring at the phase transition. Here we present the first report on the application of PPC to determine these quantities for lipid bilayers. We measure the volume changes of the pretransition and main transition of 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC), and the thermal expansivity of the fluid phase of DMPC and of two unsaturated lipids, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine and 1,2-dioleoyl-*sn*-glycero-3-phosphocholine. The high sensitivity of PPC instrumentation gives accurate data for α_V and ΔV even upon the application of relatively low pressures of ~5 bar.

INTRODUCTION

We know from daily experience that heating up a body leads to an expansion of its volume. The reverse experiment, usually not encountered in daily life, is physically equivalent: volume reduction by application of an isotropic pressure increases the temperature of an isolated body. Very high pressures are usually needed to generate noticeable heat effects. However, the recent development of pressure perturbation calorimetry (PPC) has made it possible to measure even extremely small temperature changes induced by pressure jumps of a few bar. Although the method has been developed primarily for studies of protein folding and unfolding, it also offers interesting perspectives for lipids and membranes. In the present study, we demonstrate that PPC can be used to measure the thermal expansion coefficient, α_V, of lipid bilayers as a function of temperature, the effect of pressure on the phase transition temperature, T_m, and the volume changes, ΔV, associated with different types of lipid phase transitions. Pressure changes of ~5 bar are induced in the millisecond time range. The heat changes induced by a pressure increase and by a pressure decrease are recorded. We have previously measured heat changes of lipid bilayers caused by application of osmotic pressure (Nebel et al., 1997). PPC confirms earlier results but is more versatile and easier to use. Calorimetric measurements with periodic pressure perturbations (Johnson et al., 1986) and pressure jumps (Heimburg et al., 2001) have also been used to characterize the kinetics of the lipid gel-to-liquid crystal transition.

Submitted August 30, 2001 and accepted for publication November 16, 2001.

Address reprint requests to Joachim Seelig at Dept. of Biophysical Chemistry, Biozentrum, Univ. of Basel, Klingelbergstrasse 70, CH-4056 Basel, Switzerland. Tel.: +41-61-267-2190; Fax: +41-61-267-2189; E-mail: joachim.seelig@unibas.ch.

0006-3495/02/03/1445/08 $2.00

MATERIALS AND METHODS

Materials

1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC), 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC), and 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC) were purchased from Avanti Polar Lipids (Alabaster, AL) and used without further purification. Phosphorylcholine chloride calcium salt was from Sigma (St. Louis, MO). TRIS buffer and NaCl were from Fluka (Buchs, Switzerland). The specific volumes of the lipids used for the data evaluation were 1.0 mL/g for POPC, 0.998 mL/g for DOPC, and 0.978 mL/g for DMPC and were calculated on the basis of molecular volumes at 30°C published by Nagle and Tristram-Nagle (2000). The partial specific volume of the phosphorylcholine salt was estimated roughly as 0.61 mL/g. This follows from the fact that 4538 g of water and 0.762 g of the salt yield a solution of 5 mL.

The lipid was dried from a stock solution in chloroform by a gentle stream of nitrogen followed by application of vacuum over night. Multilamellar vesicles (MLV) were prepared by addition of buffer (10 mM TRIS, 100 mM NaCl, pH 7.4) followed by vortexing and three freeze–thaw cycles. Small unilamellar vesicles (SUV) with an average diameter of 30 nm were prepared by sonication of MLV as described elsewhere (Nebel et al., 1997). Large unilamellar vesicles (LUV) with a diameter of 100 nm were prepared by extrusion using polycarbonate filters with 100-nm pore size (MacDonald et al., 1991). The lipid concentration, typically 10 or 20 mM, was adjusted gravimetrically and proven by a phosphorus assay (Böttcher et al., 1961).

Differential scanning calorimetry

A VP differential scanning calorimeter by MicroCal (Northampton, MA), was used (Plotnikov et al., 1997). The volume of the sample cell was ≈0.52 mL. The samples were scanned at rates of 1 or 3 K/h (MLV), 6 K/h (LUV), and 10 K/h (SUV) in the high gain mode, measuring the heat required for the desired temperature increase. Under these conditions, the effects of the scan-rate on the shape of the transition peak are negligible. Baseline subtraction and normalization with respect to scan rate and concentration are automatically performed by the instrument software, yielding the temperature-dependent molar heat capacity of the lipid vesicles, C_p. The $C_p(T)$ curve was fully reversible at subsequent runs. The standard VP differential scanning calorimeter (DSC) instrument includes a pressurizing cap that allows application of up to ~1.8 bar to the cells to avoid air bubbles at elevated temperatures. For experiments up to 5.5 bar, the newly designed PPC accessory was used.

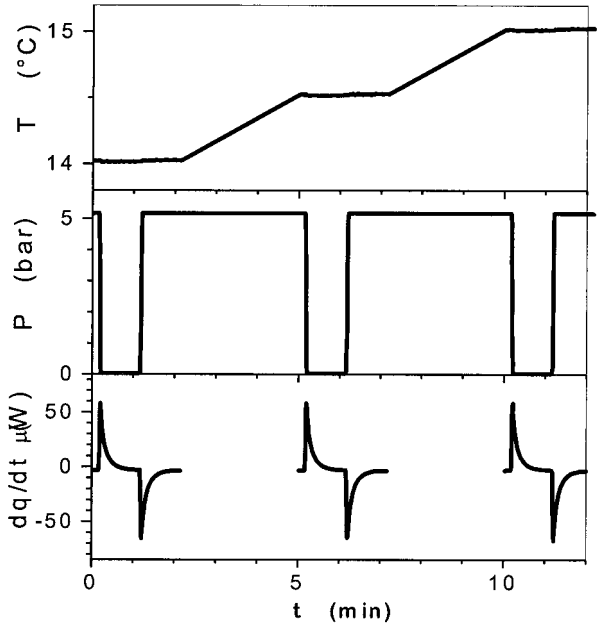

FIGURE 1 Schematic diagram of a PPC experiment. The figure illustrates the time courses of the cell temperature, T, the pressure, P, and the compensation power (dq/dt) upon 3 (out of typically 20–100) pressure jumps (downward and upward). Integration of dq/dt yields two data points for $q(T)$ at a given pressure change.

Pressure perturbation calorimetry

Following the DSC experiment, the same sample can be used without reloading for the PPC experiment. Pressures of up to 5.5 bar are applied to the DSC cell in a software-controlled manner from a nitrogen tank. The procedure is illustrated by Fig. 1, which displays 3 out of typically 20–100 pressure jumps. Each pressure jump starts with the equilibration of the calorimeter in the isothermal mode (high gain, low noise mode) at the desired temperature (tolerance set to 0.02 K) and at high pressure (typically 5.3 bar). The software then initiates a pressure release to ambient pressure (0 bar). The temperature of the cell is kept constant by active compensation of the heat change caused by the pressure jump. The compensation power returns to the baseline typically within one minute and integration of the supplied power versus time yields the heat consumed or released by the sample. After complete equilibration, automatically checked by an adjustable slope criterion, an upward pressure jump is performed as the PPC controller reconnects the PPC cell with the nitrogen tank. For both experiments temperature, pressure, and heat flow are recorded as a function of time (with a 1-s filter). The heat peaks caused by the upward and downward pressure jumps differ in sign but should agree in absolute values. After equilibration, the calorimeter is automatically heated or cooled to the next desired temperature and the next two pressure jumps are performed. The short-term peak-to-peak noise of the temperature is ~0.01 K. The pressure jumps may induce slight shifts in temperature of the order of −0.02 K (pressure release) and +0.04 K (pressure increase). This is well within the accuracy of ±0.05 K specified by the manufacturer, but can be a problem if very sharp phase transitions with $\Delta T_m \leq 0.1$ K are to be investigated.

All data points were fully reproducible and no hysteresis effects occurred. At the end of the experiment, the integrated heats were evaluated using the instrument software. Three control experiments were performed, namely water (sample cell) versus water (reference cell), buffer versus buffer, and buffer versus water. The results of these controls were fit by second-order polynomials and were taken into account for the evaluation of the thermal expansion coefficient $\alpha_V(T)$ of the lipid phase.

THEORY

Basics of pressure perturbation calorimetry

The heat of a reversible process, dQ_{rev}, is related to the entropy change, dS, at the temperature T,

$$dQ_{rev} = T dS. \quad (1)$$

Differentiation with respect to pressure, p, yields

$$\left(\frac{\partial Q_{rev}}{\partial p}\right)_T = T\left(\frac{\partial S}{\partial p}\right)_T. \quad (2)$$

From $dG = V dp - S dT$, it follows that

$$\left(\frac{\partial S}{\partial p}\right)_T = -\left(\frac{\partial V}{\partial T}\right)_p. \quad (3)$$

Eq. 2 can thus be rewritten as

$$\left(\frac{\partial Q_{rev}}{\partial p}\right)_T = -T\left(\frac{\partial V}{\partial T}\right)_p. \quad (4)$$

The thermal expansion coefficient of volume V is defined as

$$\alpha_V = \frac{1}{V}\left(\frac{\partial V}{\partial T}\right)_p, \quad (5)$$

and can thus be determined from an isothermal measurement of the heat consumed or released upon a small pressure change:

$$\alpha_V = -\frac{\Delta Q_{rev}}{TV\Delta p}. \quad (6)$$

In the present experiments, ΔQ_{rev} is the heat difference between the sample cell, containing lipid in buffer, and the reference cell, containing buffer only, upon a pressure change, Δp. For the evaluation of the α_V of the lipid phase, the contribution of the buffer must be subtracted. This is done by the instrument software on the basis of the volume fraction of the lipid in the cell (calculated from the lipid concentration and the partial specific volume) and the temperature-dependent α_V of the pure buffer. The latter is determined by an additional experiment with buffer in the sample cell and water in the reference cell.

Lipid phase transitions

Lipid–water systems are characterized by the formation of various mesophases. Transitions between these phases can be induced by temperature, lipid concentration, salt, etc. The best known example is the gel-to-liquid crystal transition, observed for a large variety of synthetic lipids and studied extensively with x-ray diffraction (Luzzati and Husson, 1962; Janiak et al., 1979; for a review, see Nagle and Tristram-Nagle, 2000) and DSC (van Deenen, 1965; Chap-

man et al., 1967; de Kruijff et al., 1972; Albon and Sturtevant, 1978; for a review, see Koynova and Caffrey, 1998).

Mesomorphic phase transitions are characterized by enthalpy changes, ΔH, and volume changes, ΔV. Monitoring the phase transition with DSC shows a dramatic increase in the heat capacity at the phase transition. The area under the DSC peak at the phase transition yields the corresponding change in enthalpy, ΔH,

$$\Delta H = \int_{T<Tm}^{T>Tm} C_p \, dT. \tag{7}$$

The relative volume change, $\Delta V/V$, can be derived analogously by measuring the thermal expansion coefficient as a function of temperature,

$$\frac{\Delta V}{V} = \int_{T<Tm}^{T>Tm} \alpha_V \, dT. \tag{8}$$

As an alternative approach to measure ΔV, it should be recalled that the application of pressure shifts the phase transition temperature as described quantitatively by the Clausius-Clapeyron equation (Anthony et al., 1981; Landwehr and Winter, 1994),

$$\frac{dT_m}{dp} = \frac{\Delta V}{\Delta S} = T_m \frac{\Delta V}{\Delta H}. \tag{9}$$

Accordingly, the temperature shift, ΔT_m, upon a pressure change, Δp, can be used to calculate ΔV, provided the transition enthalpy is also measured.

RESULTS

Figure 2 summarizes PPC experiments performed with multilamellar DMPC vesicles and three controls. The exothermic response of the various systems to a 5.31-bar pressure increase is plotted as a function of temperature. In addition, the figure contains the result of the corresponding pressure release experiments. Because pressure release produces positive (endothermic) heat peaks, the latter data points were included in Fig. 2 with a reversed sign. This allows a direct superposition with the compression experiments. An excellent agreement between the two types of experiments is obtained at the resolution of the temperature scale used in Fig. 2.

As anticipated, the control experiments water versus water and buffer versus buffer reveal almost no heat changes, indicating a well-balanced cell system. In contrast, buffer versus water leads to a small exothermic heat upon compression, indicating differences in the thermal expansivity of the two solutions. The most dramatic effect is, however, observed for the lipid versus buffer experiment. In particular, as the temperature approaches the gel-to-liquid phase transition of DMPC at 23°C, the thermal heat flow increases, leading to a maximum heat change of ~−25 mcal at the midpoint of the transition.

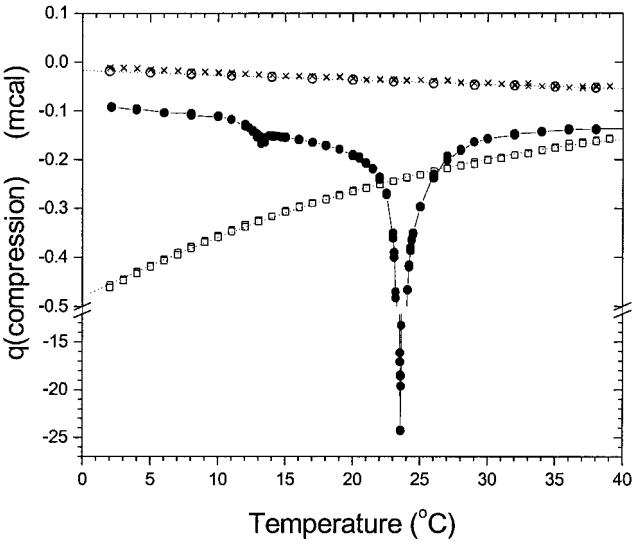

FIGURE 2 Measured heat changes in a PPC experiment. (●), 11 mM DMPC versus buffer; (□), buffer versus water; (○), buffer vs. buffer; and (×) water versus water. Pressure jumps of 5.3 bar to and from ambient pressure. Heats measured upon pressure release (endothermic) are shown with reversed sign. These data are used to compute $\alpha(T)$ as shown in Figs. 3 and 4.

We briefly illustrate the evaluation of α_V from the data of Fig. 2. In the range of 2–10°C, the heat measured for an 11-mM DMPC suspension is ~−0.1 mcal. The lipid volume is $\sim 3.8 \times 10^{-9}$ m^3 ($V_{cell} = 0.52$ mL) and the pressure change, $\Delta p = 5.3 \times 10^5$ N/m^2. Using Eq. 6, the thermal expansivity is calculated as $\alpha_V \sim 0.7 \times 10^{-3}$ K^{-1}.

A more precise evaluation is obtained by fitting the control measurements with second-order polynomials, which can then be used to calculate the effect of the replacement of buffer by lipid. The resulting α_V versus temperature curve for multilamellar DMPC vesicles is shown in the upper left panel of Fig. 3. The most conspicuous aspect of Fig. 3 is the more than 200-fold increase of α_V in the region of the phase transition reaching a maximum of $\alpha_V \sim 170 \times 10^{-3}$ K^{-1} at the transition temperature $T_m = 23.6$°C. The middle and lower left panels display the PPC results for large and small unilamellar vesicles (note the different scales). The width at half height for MLVs is $\Delta T_{1/2} = 0.1$ K. Unilamellar vesicles exhibit a broader transition (LUVs, $\Delta T_{1/2} = 0.4$ K; SUVs, $\Delta T_{1/2} = 1.0$ K) of reduced height. However, the areas underneath the transition peak are the same as observed for MLVs because the smaller peak height is compensated by the larger width. In fact, LUVs and SUVs show two transitions: a low-amplitude transition at the temperature of multilamellar dispersions and an intense and broader transition at 0.5° higher temperature. The low-amplitude transition can be traced back to a small percentage of multilamellar starting material still remaining in the

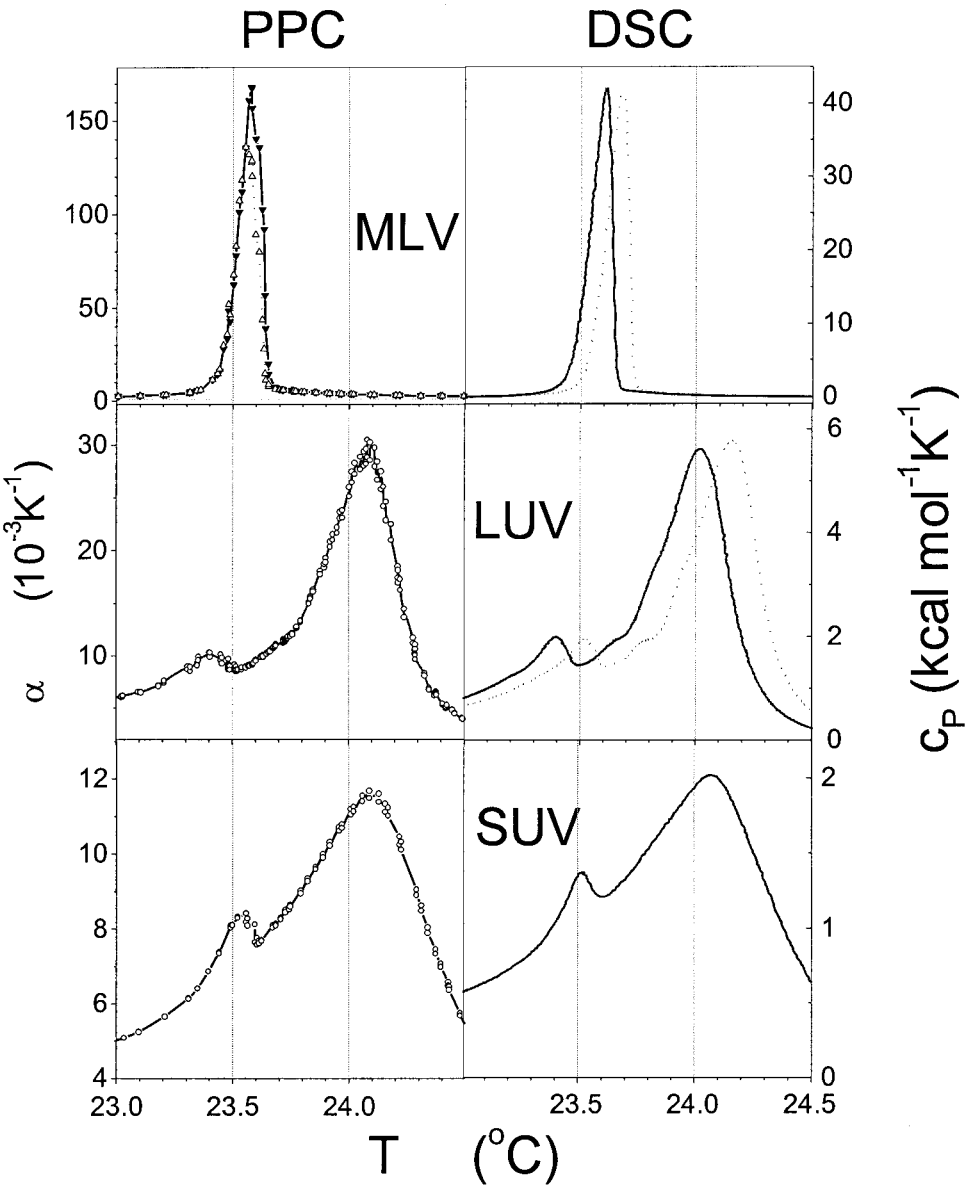

FIGURE 3 Thermal expansion coefficient, α_V, measured by PPC (*left column*) and molar heat capacity, C_p, measured by DSC (*right column*) versus temperature, T, in the main transition region of DMPC. *Upper row:* Multilamellar vesicles (*MLV*). PPC data measured upon pressure increase (*left*, Δ) and decrease (*left*, ▼) and DSC curves at 1.8 (*right*, *solid*) and 5.4 bar (*right*, *dotted*). *Middle row:* Large unilamellar vesicles (*LUV*). PPC data of both pressure increase and decrease (*left*, ○) and DSC curves at ambient pressure, $p = 0$ (*right*, *solid*) and at $p = 5.5$ bar (*right*, *dotted*). *Bottom row:* Small unilamellar vesicles (*SUV*). PPC data (*left*, ○) and DSC curve at $p = 0$ (*right*, *solid*). The area underneath the peaks represents the relative volume change, $\Delta V/V$ (*left*) and the enthalpy change, ΔH (*right*).

LUV or SUV preparation. The broadening of the phase transition of LUVs and SUVs has been explained in terms of curvature effects and the lack of interlamellar constraints (Mason et al., 1983; Heimburg, 1998).

From the area underneath the α_V-T transition curve (after baseline subtraction) the relative volume change of the lipid phase at the main transition is calculated according to Eq. 8, yielding $\Delta V/V = 3.1\%$ for LUV and 3.0% for SUV. MLVs exhibit two transitions, the pretransition from the gel to the ripple phase at ~14°C and the main transition from the ripple to the liquid crystalline phase at ~24°C. The PPC curve reveals both transitions, but the volume change associated with the pretransition is much smaller and the two peaks overlap to some extent. This is illustrated in Fig. 4, showing the data below and above the main transition in high resolution. The PPC data in Table 1 refer to the area under the peak below 18°C (approximately assigned to the pretransition) and above 18°C (main transition). MLVs exhibit slightly different transition curves for pressure increase ($\Delta V/V = 3.0\%$) and pressure release ($\Delta V/V = 2.6\%$, cf. Fig.

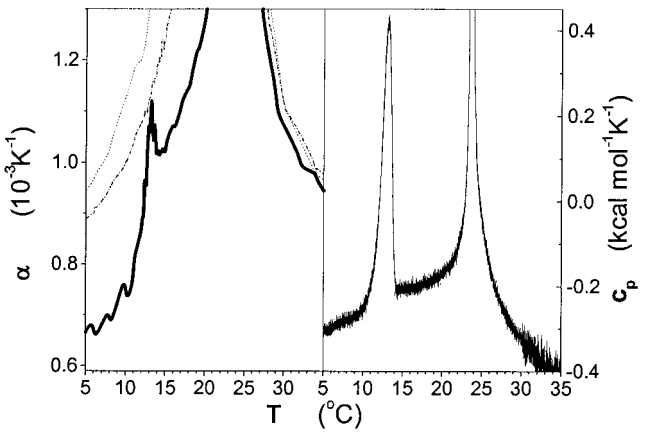

FIGURE 4 Zoomed view of the pretransition region of DMPC measured with PPC (*left*) and DSC (*right*) at $p = 0$. MLV (*solid*), LUV (*dash-dot*) and SUV (*dotted*).

3, *upper left panel*). This is caused by small temperature instabilities of the instrument that become visible for very sharp transitions.

Figure 3 (*right panels*) also contains the DSC scans of the samples recorded at different constant pressures. The area underneath the $C_p(T)$ curve of MLV at 0 bar yields a transition enthalpy of $\Delta H = 1.0$ kcal/mol for the pretransition and $\Delta H = 6.3$ kcal/mol for the main transition. Even though the pressure is small, the very high resolution of the DSC instrument allows a precise measurement of the pressure-induced shift of the transition temperature, T_m. An increase of T_m from 23.59°C at atmospheric pressure to 23.70°C at 5.31 bar is observed. A linear regression of data from five curves measured at different pressures yields a slope of $dT_m/dp = 21$ K/kbar. Using the Clausius–Clapeyron equation (Eq. 11), the volume change is calculated as $\Delta V = 19$ mL/mol and the corresponding relative volume change is $\Delta V/V = 2.8\%$ (cf. Table 1). The pretransition temperature is slightly shifted from 13.58°C at 0 bar to 13.65°C at 5.31 bar (13 K/kbar), which implies a volume change of 1.9 mL/mol (≈0.3%) according to Eq. 11. The thermal expansion coefficient of DMPC in the fluid phase is $\alpha_V \approx 0.9 \times 10^{-3}$ K^{-1} and is identical for MLVs, SUVs, and LUVs. In the gel phase, MLVs have a somewhat smaller expansivity of $\sim 0.7 \times 10^{-3}$ K^{-1} (cf. Fig. 4).

The influence of unsaturation on the thermal expansivity α_V of lipid bilayers was also investigated. Almost all naturally occurring lipids contain *cis*-double bonds in one of their fatty acyl chains. Due to their unfavorable geometry, *cis*-double bonds prevent the orderly packing of the saturated fatty acyl chains and lead to an expansion of the cross-sectional area per lipid molecule. The introduction of a *cis*-double bond also shifts the gel-to-liquid phase transition temperature dramatically toward lower temperatures.

Figure 5 shows the PPC curves for POPC (one *cis*-double bond) and DOPC (two *cis*-double bonds). The thermal expansivities of the two membrane systems are of the order of

TABLE 1 Thermodynamic parameters of the gel-to-liquid crystal transition and the pretransition of DMPC multilamellar (MLV), large (LUV), and small (SUV) unilamellar vesicles as measured by DSC (at ambient pressure) and PPC

	MLV			LUV		SUV	
	DSC	PPC	Literature	DSC	PPC	DSC	PPC
Gel-to-liquid crystal transition							
T_m (°C)	23.59	23.58	23.6*	24.02	24.08	24.08	24.10
$\Delta T_{1/2}$ (K)	0.1	0.1		0.4	0.4	1.0	1.1
ΔH (kcal/mol)	6.3		6.0*	5.5		4.8	
ΔV_L (mL/mol)	*19*	*19*		*20*	*21*		*20*
$\Delta V/V$ (%)	*2.8*	2.8	3‡	*3.0*	3.1		3.0
ΔV/molecule (Å^3)	*31*	*31*			*34*		*33*
dT_m/dP (K/kbar)	21	21	22†	26	*27*		*30*
			18.3§				
Pretransition							
T_m (°C)	13.58	13	13.7*				
$T_{1/2}$ (K)	1.1						
ΔH (kcal/mol)	1.0		1.2*				
ΔV_L (mL/mol)	*1.9*	*1.3*					
$\Delta V/V$ (%)	*0.3*	0.2					
dT_m/dP (K/kbar)	13	*9*	12§				

Parameters not directly measured but derived indirectly are shown in italic letters. The reference volume for ΔV/molecule is 1101 Å^3 reported for DMPC at 30°C by Nagle and Tristram-Nagle (2000).

*Koynova and Caffrey (1998).

†Landwehr and Winter (1994).

‡Böttner and Winter (1993).

§Prasad et al., (1987).

The pre-transition can only be detected for multilamellar but not for unilamellar vesicles.

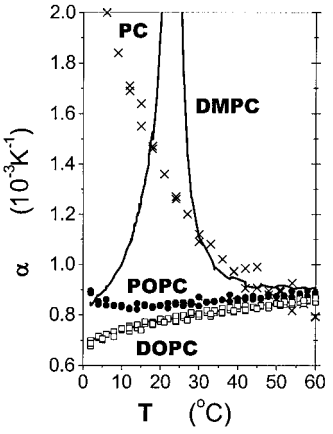

FIGURE 5 Thermal expansion coefficients, α_V, versus temperature, T, for large unilamellar vesicles of DMPC (*solid line*), POPC (●), and DOPC (□), and a solution of phosphorylcholine chloride calcium salt (×).

$\alpha_V = (0.7–0.9) \times 10^{-3}\ K^{-1}$ for unilamellar and multilamellar vesicles composed of either POPC or DOPC.

An aqueous solution of phosphorylcholine chloride calcium salt was studied as a model for the polar head group of the phospholipids. Its expansivity decreases from 0.3 mL/(mol·K) at 10°C to 0.15 mL/(mol·K) at 60°C. The corresponding curve of $\alpha_V(T)$ calculated with a partial specific volume of 0.61 mL/g estimated volumetrically is shown in Fig. 5.

DISCUSSION

Membrane volume changes

PPC measures the relative volume change, $\Delta V/V$, associated with a lipid phase transition. Two experimental approaches are possible. In the first, $\Delta V/V$ is obtained directly by the integration of the α_V versus T-curve; in the second, the phase transition temperature T_m is measured with DSC, with and without the application of external pressure. From the shift in T_m, the volume change, ΔV, is calculated with the Clausius–Clapeyron equation. Inspection of Table 1 reveals an excellent agreement of the two methods for the main transition of DMPC. The small discrepancy observed for the pretransition can be traced back to the relatively small changes and low signal-to-noise of α_V at the pretransition and the overlap of the peaks from pre- and main transition.

The volume changes and phase transition shifts of a variety of lipids have been measured in calorimeters equipped with special high-pressure cells, withstanding pressures up to almost 100 bar (Prasad et al., 1987; Mason and O'Leary, 1990; Landwehr and Winter, 1994). These earlier results are compared with the present measurements for DMPC in Table 1. A very good agreement between the various types of measurements is obtained.

Thermal expansion coefficient α_V

PPC allows a fast and accurate measurement of the thermal volume expansion coefficient $\alpha_V = (1/V)\ (\partial V/\partial T)_p$ as a function of temperature. For DMPC MLV bilayers, α_V was determined as $0.7 \times 10^{-3}\ K^{-1}$ below the pretransition and as $0.9 \times 10^{-3}\ K^{-1}$ at temperatures above the main transition. For POPC and DOPC, i.e., bilayers containing *cis*-double bonds, α_V was found to be in the same range (cf. Fig. 5). The α_V of lipid bilayers is comparable to the thermal expansivity of pure liquid hydrocarbons (e.g., tetradecane $\alpha_V = 0.9 \times 10^{-3}\ K^{-1}$, Lide, 1998) whereas that of water is an order of magnitude smaller with $\alpha_V = 0.2 \times 10^{-4}\ K^{-1}$ at 6°C.

The results obtained with PPC may be compared with other techniques that also measure α_V. The most direct, but also more laborious, approach is dilatometry. Nagle and Wilkinson (1978) report $dV/dT = 0.83 \times 10^{-3}\ mL g^{-1}\ K^{-1}$ for DMPC, 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC), and 1,2-diastearoyl-*sn*-glycero-3-phosphocholine (DSPC), which converts to $\alpha_V = 0.83 \times 10^{-3}\ K^{-1}$ based on lipid density of 1.0 g/mL. A more recent volumetric measurement of DMPC by Böttner and Winter (1993) yields $\alpha_V = 1.2 \times 10^{-3}\ K^{-1}$ at 30°C and $0.7 \times 10^{-3}\ K^{-1}$ in the gel phase, in agreement with the present results.

A calorimetric measurement of α_V has been possible by applying osmotic gradients across the bilayer membrane (Nebel et al., 1997). Osmotic compression produced an exothermic effect, osmotic expansion an endothermic response. The α_V derived from compression of POPC vesicles was $1.27 \times 10^{-3}\ K^{-1}$. Physical pressure, as applied in the present study, and osmotic pressure are not exactly comparable, because the solution environment of the membrane is different in the two experiments. Nevertheless, the two methods produce qualitative and quantitative similar results.

In summary, pressure perturbation calorimetry, dilatometry, and osmotic compression lead to consistent results concerning α_V. Among these techniques, pressure perturbation calorimetry is easiest to use, in particular, because the temperature course of α_V can be determined in a single experiment.

Molecular interpretation of α_V

A systematic measurement of α_V as a function of membrane composition and external parameters holds promise for a detailed molecular interpretation. In particular, it should be noted that the volume expansivity α_V is related to the linear expansivity α_ℓ and the area expansivity α_A according to

$$\alpha_V = \alpha_\ell + \alpha_A, \tag{10}$$

with

$$\alpha_\ell = \frac{1}{\ell}\left(\frac{d\ell}{dT}\right)_p \quad \text{and} \quad \alpha_A = \frac{1}{A}\left(\frac{dA}{dT}\right)_p. \tag{11}$$

This follows from the fact that the partial volume, V, of a lipid in the membrane is the product of its projected length,

ℓ, along the bilayer normal and its average cross-sectional area, A. Independent methods are available to measure α_ℓ and α_A.

Changes in membrane thickness can be determined with high precision either from chain segmental order parameters using ^{2}H-NMR or from x-ray or neutron scattering data. Published values range from $\alpha_\ell \approx -1.5 \times 10^{-3}$ K^{-1} for DMPC (Sankaram and Thompson, 1992) over $-(2 \pm 0.5) \times 10^{-3}$ K^{-1} for DPPC (Seelig and Seelig, 1974; Salmon et al., 1987) to -2.9×10^{-3} K^{-1} for DSPC (Sankaram and Thompson, 1992). α_ℓ is negative because the average length of the hydrocarbon chains shrinks upon thermal excitation.

Likewise, the area expansivity of lipid bilayers has been measured using the micropipette aspiration technique yielding $\alpha_A = 2.4 \times 10^{-3}$ K^{-1} for lecithin (Kwok and Evans, 1981), 3.3×10^{-3} K^{-1} for 1-stearoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (SOPC) at 15°C and 4.2×10^{-3} K^{-1} for DMPC at 35°C (Evans and Needham, 1987). Comparing the present α_V data with the earlier α_ℓ and α_A results shows that Eq. 10 is only approximately fulfilled. Because α_ℓ was obtained consistently by three independent methods, it is likely that the micropipette technique overestimates the membrane expansivity by a factor 1.5–2.

The thermal expansivity of the lipid is composed of contributions from the head groups, the glycerol backbone, and the acyl chains, weighted with their partial volumes. The expansivity of the head group as reflected in the model compound phosphorylcholine salt is larger than 10^{-3} K^{-1} at low temperature, which can be explained by the thermal release of densely packed, bound water. Its effect on the overall expansivity of the lipid is however limited because the volume of the phosphorylcholine moiety (<260 Å^3) is less than 20% of that of the total DMPC (1101 Å^3). Nevertheless, the glycerol backbone and the acyl chains must compensate the large α_V factor of the lipid head group.

The possibility to precisely measure α_V and also its temperature dependence does not only allow a critical cross-check of existing data but may be used for a systematic study of the influence of the structure of the hydrocarbon chains (length, number of double bonds, branching) or the polar head groups. The role of sphingomyelin, which is at present under investigation as a component of putative "lipid rafts" (London et al., 2000; Schutz et al., 2000), is of special interest.

Another application of PPC could be the measurement of volume changes, ΔV, of chemical reaction equilibria. The application of pressure shifts the equilibrium constant of a chemical equilibrium according to $\mathrm{d} \ln K/\mathrm{d}p = -\Delta V/RT$. For a pressure change of 5 bar and a reaction volume of 10 mL, the equilibrium constant would be changed by 0.2%. If the reaction is characterized by a sufficiently large ΔH, even such small changes could be detected by high sensitivity scanning calorimetry.

This work was supported by the Swiss National Science Foundation grant #31.58800.99.

We are indebted to Halina Szadkowska for excellent technical assistance.

REFERENCES

Albon, N., and J. M. Sturtevant. 1978. Nature of the gel to liquid crystal transition of synthetic phosphatidylcholines. *Proc. Natl. Acad. Sci. U.S.A.* 75:2258–2260.

Anthony, F. H., R. L. Biltonen, and E. Freire. 1981. Modification of a vibrating tube density meter for precise temperature scanning. *Anal. Biochem.* 116:161–167.

Böttcher, C. J. F., C. M. van Gent, and C. Fries. 1961. A rapid and sensitive sub-micro phosphorus determination. *Anal. Chim. Acta.* 24:203–204.

Böttner, M., and R. Winter. 1993. Influence of the local anesthetic tetracaine on the phase behavior and the thermodynamic properties of phospholipid bilayers. *Biophys. J.* 65:2041–2046.

Chapman, D., R. M. Williams, and B. D. Ladbrooke. 1967. Physical studies of phospholipids VI. Thermotropic and lyotropic mesomorphism of some 1,2-diacylphosphatidylcholines (lezithins). *Chem. Phys. Lipids.* 1:445–475.

de Kruijff, B., R. A. Demel, and L. L. van Deenen. 1972. The effect of cholesterol and epicholesterol incorporation on the permeability and on the phase transition of intact *Acholeplasma laidlawii* cell membranes and derived liposomes. *Biochim. Biophys. Acta.* 255:331–347.

Evans, E., and D. Needham. 1987. Physical properties of surfactant bilayer membranes: thermal transitions, elasticity, rigidity, cohesion, and colloidal interactions. *J. Phys. Chem.* 91:4219–4228.

Heimburg, T. 1998. Mechanical aspects of membrane thermodynamics. Estimation of the mechanical properties of lipid membranes close to the chain melting transition from calorimetry. *Biochim. Biophys. Acta.* 1415: 147–162.

Heimburg, T., P. Grabitz, and V. Ivanova. 2001. The coupling of relaxation times to the heat capacity of lipid vesicles studied by pressure jump calorimetry. *Biophys. J.* 80:502 (Abstract).

Janiak, M. J., D. M. Small, and G. G. Shipley. 1979. Temperature and compositional dependence of the structure of hydrated dimyristoyl lecithin. *J. Biol. Chem.* 254:6068–78.

Johnson, M. L., W. W. van Osdol, and R. L. Biltonen. 1986. The measurement of the kinetics of lipid phase transitions: a volume-perturbation kinetic calorimeter. *Methods Enzymol.* 130:534–551.

Koynova, R., and M. Caffrey. 1998. Phases and phase transitions of the phosphatidylcholines. *Biochim. Biophys. Acta.* 1376:91–145.

Kwok, R., and E. Evans. 1981. Thermoelasticity of large lecithin bilayer vesicles. *Biophys. J.* 35:637–652.

Landwehr, A., and R. Winter. 1994. High-pressure differential thermal analysis of lamellar to lamellar and lamellar to non-lamellar lipid phase transitions. *Ber. Bunsenges. Phys. Chem.* 98:214–218.

Lide, D. R. 1998. CRC Handbook of Chemistry and Physics. CRC Press, Boca Raton, FL. 6–128.

London, E., D. A. Brown, and X. Xu. 2000. Fluorescence quenching assay of sphingolipid/phospholipid phase separation in model membranes. *Methods Enzymol.* 312:272–290.

Luzzati, V., and F. Husson. 1962. The structure of the liquid–crystalline phases of lipid–water systems. *J. Cell Biol.* 12:207–219.

MacDonald, R. C., R. I. MacDonald, B. P. Menco, K. Takeshita, N. K. Subbarao, and L. R. Hu. 1991. Small-volume extrusion apparatus for preparation of large, unilamellar vesicles. *Biochim. Biophys. Acta.* 1061: 297–303.

Mason, J. T., C. Huang, and R. L. Biltonen. 1983. Effect of liposomal size on the calorimetric behavior of mixed-chain phosphatidylcholine bilayer dispersions. *Biochemistry.* 22:2013–2018.

Mason, J. T., and T. J. O'Leary. 1990. Effects of headgroup methylation and acyl chain length on the volume of melting of phosphatidylethanolamines. *Biophys. J.* 58:277–281.

Nagle, J. F., and S. Tristram-Nagle. 2000. Structure of lipid bilayers. *Biochim. Biophys. Acta.* 1469:159–195.

Nagle, J. F., and D. A. Wilkinson. 1978. Lecithin bilayers. Density measurement and molecular interactions. *Biophys. J.* 23:159–175.

Nebel, S., P. Ganz, and J. Seelig. 1997. Heat changes in lipid membranes under sudden osmotic stress. *Biochemistry.* 36:2853–2859.

Plotnikov, V. V., J. M. Brandts, L. N. Lin, and J. F. Brandts. 1997. A new ultrasensitive scanning calorimeter. *Anal. Biochem.* 250:237–244.

Prasad, S. K., R. Shashidhar, B. P. Gaber, and S. C. Chandrasekhar. 1987. Pressure studies on two hydrated phospholipids—1,2-dimyristoyl-phosphatidylcholine and 1,2-dipalmitoyl-phosphatidylcholine. *Chem. Phys. Lipids.* 43:227–235.

Salmon, A., W. Dodd, G. D. Williams, J. M. Beach, and M. F. Brown. 1987. Configurational statistics of acyl chains in polyunsaturated lipid bilayers from H-2 nmr. *J. Am. Chem. Soc.* 109:2600–2609.

Sankaram, M. B., and T. E. Thompson. 1992. Deuterium magnetic resonance study of phase equilibria and membrane thickness in binary phospholipid mixed bilayers. *Biochemistry.* 31:8258–8268.

Schutz, G. J., G. Kada, V. P. Pastushenko, and H. Schindler. 2000. Properties of lipid microdomains in a muscle cell membrane visualized by single molecule microscopy. *EMBO J.* 19:892–901.

Seelig, J., and A. Seelig. 1974. Deuterium magnetic resonance studies of phospholipid bilayers. *Biochem. Biophys. Res. Commun.* 57:406–411.

van Deenen, L. L. M. 1965. Phospholipids and biomembranes. *Prog. Chem. Fats Lipids.* 8:1–127.