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BIOLOGICAL THERMODYNAMIC DATA FOR THE CALIBRATION OF DIFFERENTIAL SCANNING CALORIMETERS: DYNAMIC TEMPERATURE DATA ON THE GEL TO LIQUID CRYSTAL PHASE TRANSITION OF DIALKYLPHOSPHATIDYLCHOLINE IN WATER SUSPENSIONS

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ABSTRACT

Heat capacity measurements of the gel to liquid crystalline transition of phosphocholine lipid suspensions in buffer solutions were performed using a differential scanning calorimeter (DSC) over a range of experimental conditions. Since the temperatures at the transition peak maxima, i.e. transition temperatures, were observed to increase linearly with scan rate due to the response time of the DSC, they were corrected to zero scan rate and found to agree closely with the literature values. The transition temperatures and enthalpies of the seven dialkylphosphatidylcholine lipids investigated ranged from 286.6 ± 0.1 K and 15.9 ± 0.6 kJ mol^{-1} for 1,2-ditridecanoyl-*sn*-glycero-3-phosphocholine to 353.7 ± 0.1 K and 69.1 ± 3.3 kJ mol^{-1} for 1,2-ditetracosanoyl-*sn*-glycero-3-phosphocholine. At the slowest scan rates of 3–5 K h^{-1}, the transition widths at half the peak maximum were close to 0.2 K. The transition temperatures were found to be independent of the following: the ionic strength of the sodium phosphate buffer from 0.01–0.02 M, the pH of the buffer from 6 to 8, the concentration of the suspension from 0.1–1.0 mass%, the commercial source of the lipid, and storage of the lipid in a freezer for 4 years. Suspensions of the dialkylphosphatidylcholine lipids in sodium phosphate buffer solutions can be used as sources of dynamic temperature data for the calibration of DSCs.

INTRODUCTION

Differential scanning calorimeters (DSCs) designed for microcalorimetric measurements on samples of aqueous solution having a volume of 1 ml or less are used to determine the transition enthalpies, transition temperatures, and heat capacity changes accompanying conformational changes of biomolecules in solution [1,2]. Heat capacity data on the unfolding transitions of ribonuclease *a* from 312 K (pH 2) to 335 K (pH 4) [3], lysozyme from 326 K (pH 2.3) to 349 K (pH 3.9) [4], and β-lactoglobulin from 353 K (pH 2.3) to 362 K (pH 3.5) [5] in HCl–glycine buffered solutions have been reported

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for use in the calibration and testing of such DSCs. To correct for any dynamic effects of the response time of the instrument on the transition temperatures and the heat capacity data, the Tian equation was applied to the data prior to analysis using the two-state model [3–5]. To determine the accuracy of this correction for the transition temperatures, standard reference materials are available for dynamic temperature measurements in a DSC [6]. However, only two temperature points are available between 298 and 350 K and they are the melting points of solids; this makes them unsuitable for evaluation of DSCs used in microcalorimetric measurements, which usually have fixed sample cells filled through capillary tubes. The ideal transitions for such calibrations and tests would be the sharp, nearly isothermal gel to liquid crystalline phase transitions of dialkylphosphatidylcholines in water suspensions [7].

The dialkylphosphatidylcholines are found in cell membranes and can be synthetically prepared at a high level of purity by reacting L-α-glycerolphosphorylcholine with the appropriate alkyl fatty acid. Over the last 60 years, the gel to liquid crystalline phase transitions of dialkylphosphatidylcholines have been studied by a variety of methods, including differential scanning calorimetry [7–15]. The phase transitions exhibit a nearly isothermal transition enthalpy of 20–40 kJ mol^{-1} over the temperature range 273–348 K. An investigation of the use of dialkylphosphatidylcholines as a standard reference material for the calibration of DSCs [7] showed that the transition enthalpies were precise to within only 10% and, thus, unsuitable for purposes of enthalpy calibration. The transition temperatures, however, exhibited a precision of 0.05 K, and were thus suggested as sources of dynamic temperature calibration data. In this investigation, the transition temperature measurements are investigated in more detail as a function of the commercial source of the lipid, the scan rate of the DSC, and the ionic strength, pH and concentration of the suspension. In addition, the temperature range of measurements is extended from 286 to 354 K to cover the operating temperature range of most DSCs used in biological studies.

EXPERIMENTAL

The phosphatidylcholines 1,2-ditridecanoyl-*sn*-glycero-3-phosphocholine (DTPC), 1,2-ditetradecanoyl-*sn*-glycero-3-phosphocholine (commonly named 1,2-dimyristoyl-L-phosphatidylcholine, DMPC), 1,2-dihexadecanoyl-*sn*-glycero-3-phosphocholine (commonly named 1,2-dipalmitoyl-L-phosphatidylcholine, DPPC), 1,2-dioctadecanoyl-*sn*-glycero-3-phosphocholine (commonly named 1,2-distearoyl-L-phosphatidylcholine, DSPC), 1,2-dieicosanoyl-*sn*-glycero-3-phosphocholine (commonly named 1,2-diarachidoyl-L-phosphatidylcholine, DAPC), 1,2-didocosanoyl-*sn*-glycero-3-phosphocholine (1,2-dibehenoyl-L-phosphatidylcholine, DBPC), and 1,2-ditetracosanoyl-*sn*-

glycero-3-phosphocholine (1,2-dilignoceroyl-L-phosphatidylcholine, DLPC) were obtained from Avanti Polar Lipids, Inc. * (Company a) with a stated purity of > 99.0 mol% and stored in a freezer at 253 K. Samples of DMPC, DPPC, DSPC and DAPC had also been obtained from the same company at the same level of purity in 1984 and stored in a freezer. These samples had been analyzed by HPLC with a crosslinked methyl silicone gum capillary column and found to have a purity of > 99.8 mass% [7]. The phosphatidylcholines DMPC, DPPC and DSPC were also obtained from Sigma Chemical Co. (Company b) at two levels of purity, ≈ 99.0% and > 99.0%, while DAPC and DBPC were obtained at > 99% from the same company. The monobasic and dibasic sodium phosphate salts were reagent quality and the water was deionized distilled water.

Suspensions of the lipids in the buffer were prepared by mixing weighed amounts of the lipid with weighed aliquots of the Na_2HPO_4–NaH_2PO_4 buffer at 0.01 M (pH 7) or 0.02 M (pH 6, 7 and 8). The mixture was then heated in a hot water bath to a temperature 5 K above the main transition temperature of the lipid and shaken by a vortex mixer at this temperature until the lipid appeared completely suspended in the buffer. The pH of the buffer was measured using an Orion pH meter calibrated with commercial buffer solutions of known pH. Suspensions of the lipids were stored in a refrigerator at ≈ 278 K for up to a week prior to the DSC measurements.

DSC measurements were performed with a Hart 7707 differential heat conduction scanning microcalorimeter fitted with three removable sample cells and a removable reference cell (all 1 ml volume) [4,5]. Calibration of the instrument was performed by internal heaters and checked by comparison of the heats of fusion of *o*-terphenyl and diphenyl ether with literature values [7] as described previously. The heats of fusion were within 1% of the literature values at 300.15 K for diphenyl ether and 329.2 K for *o*-terphenyl [7]. The Hart DSC measurements differed by less than 0.1 K and 3% respectively from the transition temperatures and enthalpies of human serum octanoate solutions measured by Ross using a DSC designed and built by Ross and Goldberg [16]. The calibration constants determined by the internal heaters at every 10 K from 273–373 K were fitted to a second-order polynomial in temperature. The polynomial was then used to calculate calibration constants for temperatures between 273 and 373 K. Multiplication of the instrumental voltage readings by the calibration constants converted the readings into power readings. Temperature calibrations

* Certain commercial equipment, instruments and materials are identified in this paper in order to specify adequately the experimental procedure. In no case does such identification imply recommendation or endorsement by the National Institute of Standards and Technology, nor does it imply that the material, instruments or equipment identified is necessarily the best available for the purpose.

were performed at 10 K intervals with a Type E thermocouple which had been calibrated by the NIST Temperature and Pressure Division with an accuracy of 0.1 K. The sample cells contained 0.4 g of the lipid suspension and were scanned against the reference cell containing 0.4 g of the buffer solution.

The power versus temperature scans were converted into heat capacity versus temperature scans by dividing the power by the scan rate as described previously [3]. The areas under the transition peaks were obtained by integrating the heat capacity units over the temperature range of the transition peak or by integrating the power units over the time it took for the transition to occur. Since the baseline exhibited little change before and after the transition peak, a straight baseline, extrapolated between the pre- and post-transitional baselines, was used to determine the areas under the transition curves and, when the areas were divided by the number of moles of lipid in the sample, yielded transition enthalpies in kJ mol^{-1}. Some of the DPPC heat capacity versus temperature scans were corrected by the Tian equation as described in Ref. [3]. The response time of the DSC was determined by monitoring the power output from a DSC cell filled with water after turning off a 1 mJ heating pulse from the calibration heater [7]. Since the power output followed an exponential decay with time, the response was taken as the time required for the output to decrease to 36.8% of its initial value, approximately 138 s.

RESULTS AND DISCUSSION

Typical thermal scans of the dialkylphosphatidylcholines in 0.02 M sodium phosphate buffer at pH 7 are shown in Figs. 1–5. The gel to liquid crystalline transitions are narrow, with peak widths at half maximum ranging from 0.2–0.5 k. All the transitions are, however, slightly asymmetric, as evident from the rapid heat capacity increase to the maximum followed by a slower heat capacity decrease to the baseline above the transition temperature. This symmetry appears to result from the instrument response time, since it becomes more apparent with DTPC and DMPC (Fig. 1), which were scanned at faster scan rates, respectively 5.00 and 4.18 h^{-1}, than with DBPC and DLPC (Fig. 5) at, respectively, 2.88 and 2.58 K h^{-1}. This is further shown in Fig. 2 for DPPC scanned at 4.18 and 9.00 K h^{-1}.

Pre-transition peaks were also observed for the phosphocholines but are not shown in Figs. 1–5. The pre-transition peaks are broader and represent less than 15% of the area of the main transition peaks. They occur at the following temperatures: 273 K for DTPC, 288 K for DMPC, 308 K for DPPC, 324 K for DSPC, and 335.7 K for DAPC. Pre-transition peaks were not observed for DBPC and DLPC. The pre-transition consists of a structural transformation from a one-dimensional lamellar structure to a two-di-

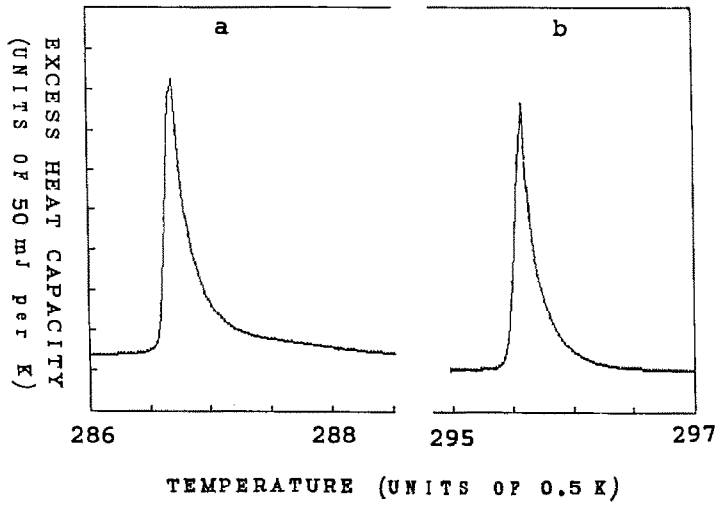

Fig. 1. (a) DSC scan of 0.4 g of 1.0 mass% suspension of DTPC in 0.02 M sodium phosphate buffer (pH 7.0) at a scan rate of 5.00 K h^{-1}. (b) DSC scan of 0.4 g of 1.0 mass% suspension of DMPC in 0.02 M sodium phosphate buffer (pH 7.0) at a scan rate of 4.64 K h^{-1}.

mensional lattice consisting of lipids distorted by a periodic ripple and increased disorder [7]. Because of their low amplitude and broadness, the pre-transition peaks were not evaluated for use as sources of dynamic temperature reference data.

Thermodynamic data on the gel to liquid crystalline transitions are presented in Tables 1–7. The DMPC, DPPC, DSPC, DAPC and DBPC

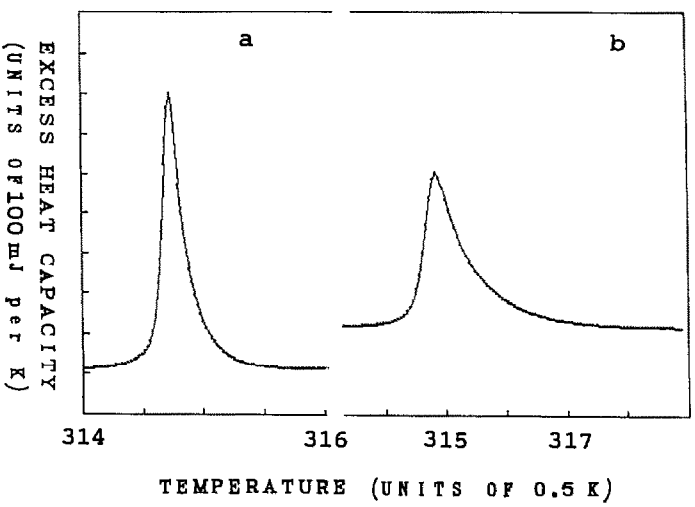

Fig. 2. (a) DSC scan of 0.4 g of 1.0 mass% suspension of DPPC in 0.02 M sodium phosphate buffer (pH 7.0) at a scan rate of 4.18 K h^{-1}. (b) DSC scan of 0.4 g of 1.0 mass% suspension of DPPC in 0.02 M sodium phosphate buffer (pH 7.0) at a scan rate of 9.00 K h^{-1}.

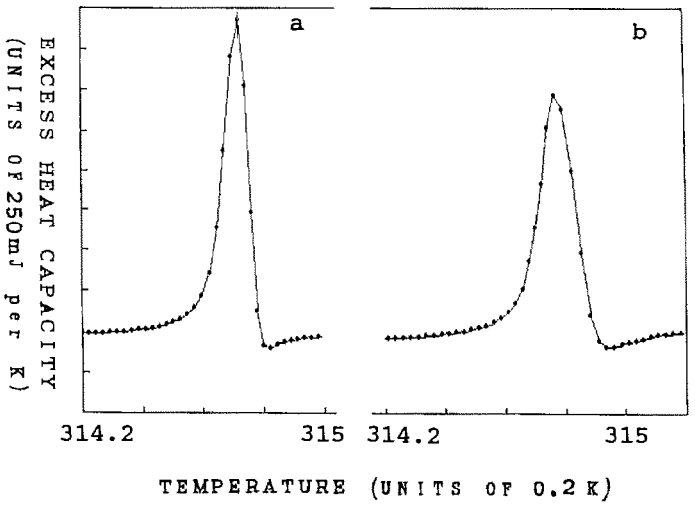

Fig. 3. (a) The Tian corrected DSC scan of the DPPC scan in Fig. 2 (a) performed at a scan rate of 4.18 K h^{-1}. (b) The Tian corrected DSC scan of the DPPC scan in Fig. 2(b) performed at a scan rate of 9.00 K h^{-1}.

samples were obtained from two different commercial sources which are designated by the numbers 1 and 2 for Company a and 3 and 4 for Company b. Samples numbered 1 were > 99 mol% pure and obtained from Company a in 1984, while samples No. 2 were > 99 mol% pure and obtained from the same company in 1989. Samples No. 3 were > 90% pure and obtained from Company b, while No. 4 were approximately 99% pure

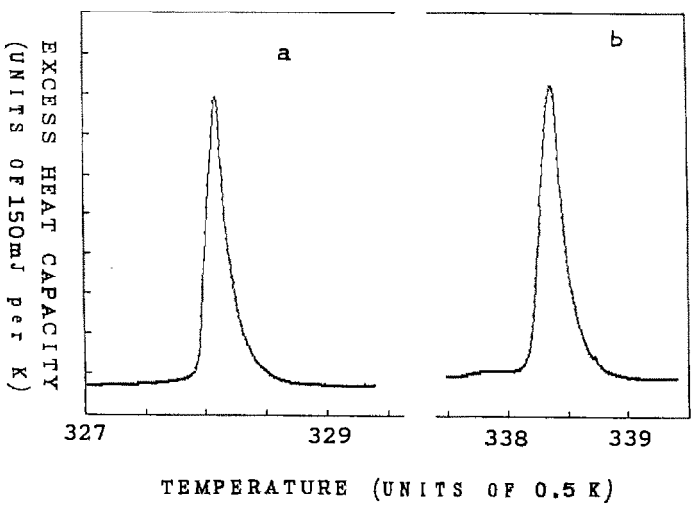

Fig. 4. (a) DSC scan of 0.4 g of 1.0 mass% suspension of DSPC in 0.02 M sodium phosphate buffer (pH 7.0) at a scan rate of 3.70 K h^{-1}. (b) DSC scan of 0.4 g of 1.0 mass% suspension of DAPC in 0.02 M sodium phosphate buffer (pH 7.0) at a scan rate of 3.39 K h^{-1}.

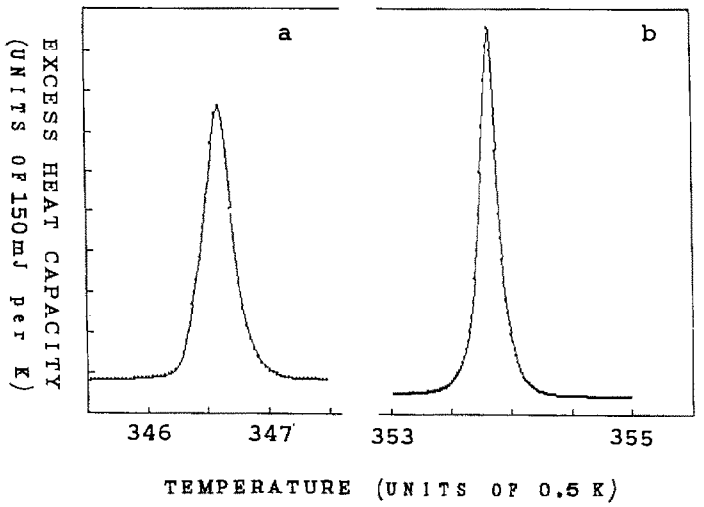

Fig. 5. (a) DSC scan of 0.4 g of 1.0 mass% suspension of DBPC in 0.02 M sodium phosphate buffer (pH 7.0) at a scan rate of 2.88 K h^{-1}. (b) DSC scan of 0.4 g of 1.0 mass% suspension of DLPC in 0.02 M sodium phosphate buffer (pH 7.0) at a scan rate of 2.58 K h^{-1}.

from the same Company. Usually, samples of the same suspension were scanned in three cells with different calibration factors, and the average value of the measurements is reported in Tables 1–7 along with the standard deviation. The transition temperature is the temperature of the peak maxi-

TABLE 1

Thermodynamic data on the gel to liquid crystal phase transition of 1,2-ditridecanoyl-*sn*-glycerol-3-phosphocholine (DTPC) in sodium phosphate buffer

Sample				Transition temperature, T (K)	Transition peak width, ΔT (K)	Transition enthalpy, ΔH (kJ mol^{-1})	Ratio ($C_p^m/\Delta H$) (K^{-1})	No. of measurements
Conc. (mass%)	I	pH	Source					
1	0.02	7	2	286.6	0.15±0.01	15.3±0.5	3.79±0.04	3
1	0.01	7	2	286.7	0.16±0.01	15.4±1.0	3.92±0.23	3
0.1	0.02	7	2	286.6	0.16±0.01	13.0±0.7	4.60±0.18	3
1	0.02	6	2	286.6	0.15±0.01	15.3±1.6	3.76±0.01	3
1	0.02	8	2	286.6	0.16±0.01	15.8±0.5	3.80±0.10	3
Scan rate 9.29 K h^{-1}								
1	0.02	7	2	286.8	0.27±0.01	16.6±0.2	2.13±0.05	3
Scan rate 13.9 K h^{-1}								
1	0.02	7	2	287.0	0.48±0.01	16.7±0.2	1.41±0.03	3

The sample size was 0.4 g and, unless otherwise specified, all scans were performed at 5.0 K h^{-1}.

TABLE 2

Thermodynamic data on the gel to liquid crystal phase transition of 1,2-ditetradecanoyl-*sn*-glycero-3-phosphocholine (DMPC) in sodium phosphate buffer

Sample				T	ΔT	ΔH	Ratio	No. of
Conc. (mass%)	I	pH	Source	(K)	(K)	(kJ mol^{-1})	($C_p^m/\Delta H$) (K^{-1})	Measure-ments
1	0.02	7	1	297.1	0.14±0.01	23.5±0.3	4.67±0.23	3
1	0.02	7	2	297.1	0.13±0.01	21.6±0.2	4.70±0.02	3
1	0.02	7	3	297.1	0.17±0.01	20.3±1.2	4.40±0.10	3
1	0.02	7	4	297.0	0.21±0.01	22.1±0.5	3.42±0.01	3
1	0.01	7	1	297.1	0.15±0.01	17.7±0.2	4.90±0.05	3
1	0.01	7	2	297.0	0.15	21.1	4.53	1
1	0.01	7	3	297.1	0.17	17.7	4.26	1
0.1	0.02	7	2	297.1	0.13	21.6	5.43	1
0.1	0.02	7	3	297.1	0.16	21.6	4.46±0.02	2
1	0.02	6	1	297.1	0.15±0.01	20.4±0.2	4.56±0.5	3
1	0.02	6	2	297.1	0.16	21.6	4.25	1
1	0.02	6	3	297.1	0.17	22.3	4.17	1
1	0.02	8	1	297.1	0.16±0.03	19.7±0.2	4.66±0.01	3
1	0.02	8	2	297.1	0.16	22.6	4.65	1
1	0.02	8	3	297.1	0.15	21.3	4.39	1
Scan rate 9.25 K h^{-1}								
1	0.02	7	1	297.2	0.24±0.02	23.8±0.2	2.66±0.05	3
Scan rate 18.5 K h^{-1}								
1	0.02	7	1	297.5	0.53±0.01	24.0±0.2	1.37±0.01	3

The sample size was 0.4 g and, unless otherwise specified, all scans were performed at 4.64 K h^{-1}.

mum, and was determined by the average temperature values of the three maximum heat capacity points of the transition. At the slowest scan rate settings employed for each lipid, this value was within 0.1 K of the temperature at the maximum heat capacity value. Determinations of the peak width at half maximum height, ΔT, were performed by taking the difference between the temperatures at half the peak maximum. Areas under the transition peaks were determined by a straight baseline extrapolated between the pre- and post-transitional baselines and, when divided by the number of moles of lipid in the sample, yield transition enthalpies in kJ mol^{-1}. Ratios of the maximum heat capacity above the baseline, C_p^m, to the peak area are also presented in the tables. Unless otherwise specified, all scans were performed at the lowest scan rate setting of the DSC to minimize the effect of scan rate on the shape of the transition peak. These scan rate settings were 5.0 K h^{-1} for DTPC, 4.64 K h^{-1} for DMPC, 4.18 K h^{-1} for DPPC, 3.70 K h^{-1} for DSPC, 3.39 K h^{-1} for DAPC, 2.88 K h^{-1} for DBPC and 2.58 K h^{-1} for DLPC.

TABLE 3

Thermodynamic data on the gel to liquid crystal phase transition of 1,2-dihexadecanoyl-*sn*-glycero-3-phosphocholine (DPPC) in sodium phosphate buffer

Sample				T (K)	ΔT (K)	ΔH (kJ mol^{-1})	Ratio ($C_p^m/\Delta H$) (K^{-1})	No. of Measurements
Conc. (mass%)	I	pH	Source					
1	0.02	7	1	314.7	0.19±0.01	32.3±0.5	3.6 ±0.5	3
1	0.02	7	2	314.7	0.14±0.01	30.6±1.5	4.89±0.02	3
1	0.02	7	3	314.7	0.26	33.5±0.7	2.98±0.01	3
1	0.02	7	4	314.7	0.16±0.01	28.2±0.9	4.27±0.02	3
1	0.01	7	1	314.7	0.14±0.01	29.7±2.4	4.79±0.07	3
1	0.01	7	2	314.7	0.16	31.3	4.54	1
1	0.01	7	3	314.7	0.26	30.0	3.05	1
0.1	0.02	7	1	314.7	0.19±0.02	32.3	3.41±0.02	3
0.1	0.02	7	2	314.7	0.15	29.5	4.80	1
0.1	0.02	7	3	314.7	0.25	30.2±1.8	3.13±0.01	2
1	0.02	6	1	314.7	0.14±0.01	29.3	4.78±0.06	3
1	0.02	6	2	314.7	0.15	28.4	4.78	1
1	0.02	6	3	314.8	0.26	29.7	3.17	1
1	0.02	8	1	314.7	0.16±0.01	32.1±2.2	4.50±0.05	3
1	0.02	8	2	314.7	0.15	21.2	4.96	1
1	0.02	8	3	314.8	0.25	29.3	3.14	1
Scan rate 9.00 K h^{-1}								
1	0.02	7	1	314.9	0.34±0.03	32.8±0.7	2.09±0.18	3
Scan rate 19.2 K h^{-1}								
1	0.02	7	1	315.2	0.60±0.02	32.3±0.7	1.16±0.03	3

The sample size was 0.4 g and, unless otherwise specified, all scans were performed at 4.18 K h^{-1}.

Dependence of the transition temperature and peak shape on scan rate

In addition to the broadening of the peak with increase in scan rate shown in Fig. 2, there is also an increase in the transition temperature, as well as a decrease in the peak height to peak area ratio. However, the transition enthalpies as determined by the areas of the transition peaks remain the same at all the scan rates.

The broadening of the peak with scan rate can be approximately corrected by the Tian equation [17], which relates an instantaneous signal such as power $[P(t)]$ to the observed signal $[E(t)]$ of a microcalorimeter as follows

$$P(t) = F[E(t) + \tau \, \mathrm{d}\, E(t)/\mathrm{d}t] \tag{1}$$

where F is a calibration factor to convert the voltage signal, E, into watts

TABLE 4

Thermodynamic data on the gel to liquid crystal phase transition of 1,2-dioctadecanoyl-*sn*-glycero-3-phosphocholine (DSPC) in sodium phosphate buffer

Sample				T	ΔT	ΔH	Ratio	No. of
Conc. (mass%)	I	pH	Source	(K)	(K)	(kJ mol^{-1})	($C_p^m/\Delta H$) (K^{-1})	Measure-ments
1	0.02	7	1	328.1	0.24±0.01	40.1±0.2	3.40±0.10	3
1	0.02	7	2	328.1	0.18±0.01	32.9±2.3	4.40±0.04	3
1	0.02	7	3	328.0	0.33±0.01	44.1±2.6	2.73±0.03	3
1	0.02	7	4	328.1	0.26±0.01	42.4±2.6	3.30±0.01	3
1	0.01	7	1	328.0	0.14±0.01	29.8±1.6	5.07±0.02	3
1	0.01	7	2	328.0	0.18	38.1	4.41	1
1	0.01	7	3	328.1	0.26	36.8	3.32	1
0.1	0.02	7	1	328.0	0.15±0.04	37.5±2.0	4.11±0.22	3
0.1	0.02	7	2	328.0	0.20±0.01	48.3	4.32	1
0.1	0.02	7	3	328.3	0.50	86.9	2.87	1
1	0.02	6	1	328.0	0.16±0.01	34.5±2.0	4.66±0.03	3
1	0.02	6	2	328.1	0.18	36.1	4.31	1
1	0.02	6	3	328.1	0.28	39.6	3.23	1
1	0.02	8	1	328.0	0.16±0.01	28.8±3.6	4.79±0.01	3
1	0.02	8	2	328.1	0.19	37.1	4.23	1
1	0.02	8	3	328.0	0.27	38.7	3.00	1
Scan rate 8.82 K h^{-1}								
1	0.02	7	1	328.2	0.66±0.01	41.6±0.8	2.24±0.01	3
Scan rate 19.4 K h^{-1}								
1	0.02	7	1	328.7	0.70±0.01	40.7±2.0	1.13±0.01	

The sample size was 0.4 g and, unless otherwise specified, all scans were performed at 3.70 K h^{-1}.

and τ is the time constant of the DSC. A solution of eqn. (1) is obtained in the following form [17]

$$E(t) = (F\tau)^{-1} \int_0^t P(u) \exp[-(t-u)/\tau] \, du$$

$$= (\alpha\tau)^{-1} \exp[-t/\tau] \int_0^t P(u) \exp(u/\tau) \, du \qquad (2)$$

where the function $P(u) \exp(u/\tau)$ represents the heating inside the cell arising from the gel to liquid crystal transition as a function of the instantaneous time, u. Employing time resolved X-ray diffraction measurements in conjunction with microwave-induced temperature jump measurements, Caffrey et al. [18] have shown that the gel to liquid crystalline phase transition of dihexadecylphosphatidylethanolamine (DHPE) occurs within 2 s. It is thus assumed that the maximum instantaneous time, u, for the phosphocholines, which are similar in structure to DHPE, is < 2 s, i.e. the maximum

TABLE 5

Thermodynamic data on the gel to liquid crystal phase transition of 1,2-dieicosanoyl-*sn*-glycero-3-phosphocholine (DAPC) in sodium phosphate buffer

Sample				T (K)	ΔT (K)	ΔH (kJ mol^{-1})	Ratio ($C_p^m/\Delta H$) (K^{-1})	No. of Measurements
Conc. (mass%)	I	pH	Source					
1	0.02	7	1	338.3	0.23±0.01	45.3±1.6	3.19±0.16	3
1	0.02	7	2	338.4	0.20±0.01	39.5±0.8	3.88±0.02	3
1	0.02	7	3	338.5	0.37±0.02	50.9±2.8	2.45±0.02	3
1	0.01	7	1	338.3	0.25±0.01	35.7±0.6	3.10±0.03	3
1	0.01	7	2	338.3	0.20	50.5	4.41	1
1	0.01	7	3	338.4	0.44	47.3	2.41	1
0.1	0.02	7	1	338.3	0.20±0.01	38.1	3.12±0.16	3
0.1	0.02	7	2	338.3	0.19	50.9	3.94	1
0.1	0.02	7	3	338.4	0.33	48.8±2.1	2.48±0.01	1
1	0.02	6	1	338.3	0.26±0.01	28.9±2.8	2.94±0.08	3
1	0.02	6	2	338.3	0.21	43.4	3.76	1
1	0.02	6	3	338.4	0.34	51.4	2.51	1
1	0.02	8	1	338.3	0.24±0.01	35.5±4.0	3.23±0.03	3
1	0.02	8	2	338.3	0.20	39.2	3.00	1
1	0.02	8	3	338.4	0.64	53.0	2.23	1
Scan rate 8.64 K h^{-1}								
1	0.02	7	1	338.5	0.39±0.05	44.2±1.7	1.81±0.17	3
Scan rate 19.4 K h^{-1}								
1	0.02	7	1	338.9	0.75	53.3±9.9	0.86±0.03	3

The sample size is 0.4 g and, unless otherwise specified, all scans were performed at 3.39 K h^{-1}.

heat absorbed by the phase transition occurs within 2 s. For large time constants on the order of 100 s, the heat absorbed by the phase transition is the same as turning on and off an "instantaneous" calibration pulse. The observed time, Δt, to reduce the maximum observed signal, $E(t)$, to half its value is thus approximately constant. If Δt is replaced by the ratio of the observed temperature difference between the maximum value of $E(t)$ and half its maximum value, ΔT, to the scan rate, dT/dt, then ΔT, which is a measure of the peak broadening, is inversely proportional to the scan rate. Since τ is the time it takes for a heating pulse to decay to 36.8% of its maximum value and ΔT is the temperature difference between the maximum and 50% of the maximum value of $E(t)$, then the ratio $\Delta t/\tau$ is close to unity. From the rate of increase of the temperature width at half the peak maximum, ΔT, with scan rate in Tables 1–7, the ratio of ΔT to the scan rate is 130 ± 16 s, which is close to the time constant of 138 s for the DSC. Thus, the broadening of the transition peak at faster scan rates arises from the slow DSC response time. In a DSC with a shorter response time, Albon and

TABLE 6

Thermodynamic data on the gel to liquid crystal phase transition of 1,2-didocosanoyl-*sn*-glycero-3-phosphocholine (DBPC) in sodium phosphate buffer

Sample				T (K)	ΔT (K)	ΔH (kJ mol^{-1})	Ratio ($C_p^m/\Delta H$) (K^{-1})	No. of Measure-ments
Conc. (mass%)	I	pH	Source					
1	0.02	7	2	346.6	0.26±0.01	71.4± 1.1	3.17±0.03	3
1	0.02	7	3	346.6	0.29	66.6± 3.1	2.81±0.01	3
1	0.01	7	2	346.6	0.26±0.01	55.0±12.6	3.23±0.06	3
1	0.01	7	3	346.6	0.30	64.2	2.73	1
0.1	0.02	7	2	346.6	0.25	82.5± 2.2	3.32±0.01	3
0.1	0.02	7	3	346.6	0.27	101.6	2.88	1
1	0.02	6	2	346.6	0.29	68.3± 1.1	3.12±0.03	3
1	0.02	6	3	346.6	0.33	61.6	2.80	1
1	0.02	8	2	346.7	0.20	70.7± 2.3	4.02±0.02	3
1	0.02	8	3	346.6	0.25	70.0	3.04	1
Scan rate 8.28 K h^{-1}								
1	0.02	7	2	346.8	0.50±0.02	70.0± 1.1	1.68±0.01	3
Scan rate 13.9 K h^{-1}								
1	0.02	7	1	347.1	0.65±0.01	70.0±2.7	1.24±0.02	3

The sample size was 0.4 g and, unless otherwise specified, all scans were performed at 2.88 K h^{-1}.

TABLE 7

Thermodynamic data on the gel to liquid crystal phase transition of 1,2-ditetracosanoyl-*sn*-glycero-3-phosphocholine (DLPC) in sodium phosphate buffer

Sample				T (K)	ΔT (K)	ΔH (kJ mol^{-1})	Ratio ($C_p^m/\Delta H$) (K^{-1})	No. of Measure-ments
Conc. (mass%)	I	pH	Source					
1	0.02	7	2	353.8	0.17±0.01	69.8±1.9	4.62±0.10	3
1	0.01	7	2	353.8	0.17	73.4±1.4	4.34±0.06	3
0.1	0.02	7	2	353.7	0.17±0.01	88.8±1.4	4.33±0.21	3
1	0.02	6	2	353.8	0.16±0.01	66.7±1.7	4.60±0.13	3
1	0.02	8	2	353.8	0.20±0.01	71.8±0.7	3.99±0.01	3
Scan rate 8.24 K h^{-1}								
1	0.02	7	2	354.1	0.41±0.01	64.3±3.4	1.96±0.05	3
Scan rate 13.8 K h^{-1}								
1	0.02	7	2	354.4	0.57±0.02	68.4±2.4	1.35±0.02	3

The sample size was 0.4 g and, unless otherwise specified, all scans were performed at 2.58 K h^{-1}.

Sturtevant [19] found that, for highly purified samples of DPPC, the observed ΔT was 0.067 K and could be only partially attributed to the time constant of the DSC. They attributed this slight broadening to the lack of perfect cooperativity between the numerous small domains in the transition of the entire sample. This may well be the case here, since the error in ΔT is on the order of ± 0.01 K. However, the narrow half widths of the transition at a scan rate close to zero show, as Albon and Sturtevant [19] concluded, that the gel to liquid phase transition of the phosphocholine suspensions is very close to an isothermal process.

DPPC, DSPC and DAPC samples from source 3, which were lower in purity, exhibited larger ΔT values at the same scan rates. Comparison of the peak shapes of these samples with purer samples from sources 1, 2, and 4 show that the onset of the transition for the source 3 samples is more gradual with increase in temperature than for the purer samples. Such asymmetry is expected for slightly impure samples [19].

The total amount of heat absorbed or transition enthalpy, $\int_0^t q(t)\,\mathrm{d}t$, is the same at different scan rates and this is shown in Tables 1–7. An increase in ΔT at faster scan rates would thus reduce the peak height to area ratio. This is also shown in Tables 1–7, where this ratio is reduced by a factor of ≈ 2 with doubling of the scan rate.

In the previous heat capacity data studies [3–5], eqn. (1) was employed to correct the observed power signals at scan rates from 5 to 20 K h^{-1}, which yielded transition temperatures and enthalpies which were independent of the scan rate. Dividing both sides of eqn. (1) by the scan rate converts the power signals to heat capacity signals. This form of eqn. (1) was used to convert the observed heat capacity signals for DPPC in Fig. 2 to the "instantaneous" heat capacity plots shown in Fig. 3. The instantaneous plots shown in Fig. 3 exhibit more similar time profiles than the observed heat capacity plots in Fig. 2. In Fig. 3, the corrected transition peak observed at the faster scan rate is slightly broader than the corrected peak observed at the slower scan rate, and may arise from the approximate nature of the Tian equation correction.

Similarly to the heat capacity correction, Flynn [20] has shown that the instantaneous transition temperature, T_m, is related to the observed transition temperature, T, via the following form of the Tian equation

$$T_m = T + \tau\,\mathrm{d}T/\mathrm{d}t \tag{2}$$

T_m scan be determined from a plot of the observed temperature vs. the scan rate. From the dependence of the transition temperature on the scanrate given at the bottom of Tables 1–7, a value of 140 ± 11 s is determined for τ, which is in agreement with the DSC time constant of 138 s determined from the decay time of a calibration heat pulse. The correction to the temperature observed at the slowest scan rate for each lipid is -0.2 K for DTPC, DMPC

TABLE 8

Summary of thermodynamic functions on the gel to liquid crystal phase transition of dialkylphosphatidylcholine in buffer suspensions

Dialkylphosphatidylcholine (alkyl chain length)	T (K)	Lit. T (Ref.)	ΔH (kJ mol^{-1})	Lit. ΔH (Ref.)	ΔS (J mol^{-1} K^{-1})	Lit. ΔS (Ref. 14)
DTPC (13)	286.4	–	15.9 ± 0.6	–	56 ± 2	–
DMPC (14)	296.9 ± 0.1	296.2 (Ladbrooke [9]) 296.9 (Hinz [10]) 297.1 (Mabrey [11]) 297.6 (Guard-Friar [14]) 297.0 (Schwarz [7]) 297.0 (Huang [15])	21.8 ± 1.3	28 (Ladbrooke [9]) 26.2 (Hinz [10]) 22.6 (Mabrey [11]) 22.8 (Guard-Friar [14]) 23.9 (Schwarz [7])	73 ± 4	75
DPPC (16)	314.6 ± 0.1	314.2 (Ladbrooke [9]) 314.9 (Hinz [10]) 314.6 (Mabrey [11]) 315.2 (Guard-Friar [14])	30.7 ± 1.6	36.4 (Ladbrooke [9]) 40.6 (Hinz [10]) 36.4 (Mabrey [11]) 35.2 (Guard-Friar [14])	98 ± 5	113

		314.6 (Schwarz [7]) 314.5 (Huang [15]) 314.3 (Jacobson [21])		38.3 (Schwarz [7])		
DSPC (18)	328.0 ± 0.1	331.2 (Ladbrooke [9]) 328.1 (Mabrey [11]) 328.2 (Guard-Friar [14]) 327.8 (Schwarz [7]) 326.7 (Huang [15])	39.2 ± 3.9	44.8 (Ladbrooke [9]) 44.4 (Mabrey [11]) 39.0 (Guard-Friar [14]) 44.0 (Schwarz [7])	120 ± 12	117
DAPC (20)	338.2 ± 0.1	338.0 (Schwarz [7]) 337.9 (Huang [15])	45.4 ± 6.2	62.4 (Schwarz [7])	134 ± 18	–
DBPC (22)	346.5 ± 0.1	348.2 (Ladbrooke [9])	68.1 ± 3.1	62.4 (Ladbrooke [9])	197 ± 9	–
DLPC (24)	353.7 ± 0.1	–	69.1 ± 3.3	–	195 ± 10	–

The average enthalpy value for DTPC excluded the lowest value of 13.0 kJ mol^{-1}; for DMPC, the two lowest values of 17.7 kJ mol^{-1}: for DPPC, the lowest value of 21.2 kJ mol^{-1}; for DSPC, the high value of 86.9 and the low values of 28.8 and 29.8 kJ mol^{-1}; for DAPC, the low value of 28.9 kJ mol^{-1} for DBBC, the low value of 55.0 kJ mol^{-1} and high values of 82.5 and 101.6 kJ mol^{-1}, and for DLPC, the high value of 88.8 kJ mol^{-1}. The temperature values from Ref. [7] were too high by 0.7 K, as determined by the thermocouple calibration, and the corrected values are presented in Table 8.

and DPPC. The correction is −0.1 K for the remaining lipids. The corrected values are given in Table 8.

Dependence of the transition temperature on concentration, pH, ionic strength and source

A decrease in the lipid concentration by an order of magnitude does not affect the transition temperature as shown in Tables 1–7. This is further substantiated by comparison of the transition temperatures with the literature values in Table 8. Similar lack of any transition temperature dependence on concentration for DMPC, DPPC and DSPC was observed by Hinz and Sturtevant [10] over the lower concentration range of 0.04–0.66 mass% and by Mabrey and Sturtevant [11] over the range 0.02–0.04 mass%. There is also close agreement of the transition temperatures for DMPC and DPPC with Ladbrooke and Chapman's values at 60 mass% [9].

There does not appear to be any effect of ionic strength on the transition temperature over the change in buffer concentration from 0.02 M to 0.01 M. This is in agreement with the lack of any ionic strength effect on the transition temperature of DPPC in solutions of 0 to 0.5 M NaCl and KCl observed by Jacobson and Papahadjopoulos [21]. At high changes in ionic strength, such as an increase in KCl concentration from 0 to 1.50 M, Sturtevant [22] observed an increase of 0.48 K in the transition temperature of DMPC. Ladbrooke and Chapman also observed that at high salt concentrations the transition moves to higher temperatures [9]. Since this behavior was similar to decreasing the water content of the suspension at high lipid concentrations, Ladbrooke and Chapman [9] attributed the increase in temperature to replacement of the free water by the salt in the suspension.

The effect of pH on the phase transition would presumably result from protonation effects on the polar phosphatidylcholine head group. Changes in pH as well as ionic strength have diverse effects on the transition temperature of pure acidic lipids such as dipalmitoylphosphatidic acid, dipalmitoylphosphatidylglycerol and phosphatidylserine [21]. However, no effect of pH on the transition temperature was observed with bilayers of electrically neutral DPPC [21], in agreement with the results presented in Table 3. Apparently, over the pH range 6–8 there is also no effect of pH on the transition temperatures of the phosphocholine lipids studied here (See Tables 1–7).

The lipid suspensions were prepared in the same manner without any further purification to determine any direct effect of source on the transition properties. Samples from the two different commercial sources yielded the same transition temperatures for DMPC, DPPC, DSPC, DAPC and DBPC, even though some of the DMPC, DPPC, DSPC and DAPC samples had been stored in a freezer over a period of 4 years. The close agreement of the

transition temperatures with the literature values in Table 8 also substantiate the lack of any transition temperature dependence on the source of the lipid samples.

Dependence of the transition enthalpy on concentration, ionic strength, pH and source

Similar to the transition temperatures in Tables 1–7, all the transition enthalpies are, within experimental error, independent of concentration, ionic strength, pH, and the source of the lipid. Average values for the transition enthalpies are also presented in Table 8. In contrast to the constant average deviation of the transition temperature of 0.1 K, the standard deviations of the transition enthalpies vary from 4% (DTPC) to 14% (DAPC). As discussed previously [7], part of the discrepancies in the experimental enthalpies can be attributed to experimental problems of transferring a suspension of the lipid in buffer to the DSC cells and to incomplete suspension of the lipid in the buffer.

Dependence of the temperature and thermodynamic functions of the transition on lipid chain length

As pointed out by Ladbrooke and Chapman [9], the primary process in the main phase transition is the change of the hydrocarbon chains from an all-*trans* orderly crystalline gel configuration to a more disordered combination of *trans* and gauche configurations in solution. Since the number of configurations would increase with alkyl chain length, there is a monotonic increase in the transition temperature and enthalpy with increase of the alkyl chain lengths of the phosphatidylcholines as shown in Table 8. Ladbrooke and Chapman [9] first observed the correlation between chain length and transition temperature and showed that it was similar to the increase in the melting points of *n*-paraffins. Phillips et al. [23] showed that the transition enthalpy increase by 2 kJ mol^{-1} per methylene group from DMPC to DBPC, which is also observed in Table 8 over the same range. However, extension of this correlation to DTPC and DLPC shows departures from this linearity to a larger change of 3 kJ mol^{-1} per methylene addition from DTPC to DMPC and almost no change in the transition enthalpy from DBPC to DLPC. Preliminary data from this laboratory on the gel to liquid crystalline transition of didodecanoyl-*sn*-glycero-3-phosphocholine where each alkyl chain contains 12 methylene groups, indicate a transition enthalpy of 6.1 kJ mol^{-1}, yielding an even larger increase of 5 kJ mol^{-1} per methylene addition from didodecanoyl-*sn*-glycero-3-phosphocholine to DTPC. Apparently, across a larger range of alkyl chain lengths, there is a very gradual decrease in the contribution of each additional methylene

group to the transition enthalpy, and the transition enthalpy approaches a constant value.

In Table 8, values of the transition entropy, ΔS, are included with the transition temperature and enthalpy. Values of ΔS were determined by assuming that the transition temperature is the temperature of the peak maximum and, since $\Delta G = 0$ at the transition temperature, $T\Delta S = \Delta H$ at the transition temperature. Phillips et al. determined an entropy change of 5.2 J K^{-1} mol^{-1} per methylene group for the gel to liquid crystal phase transition of phospholipids DMPC to DBPC, and showed that the entropy change resulted from a larger mobility of the alkyl chains in solution [23]. A similar entropy change of 6 ± 1 J K^{-1} mol^{-1} per methylene addition is also observed in Table 8 from DMPC to DSPC. Over the larger range from DTPC to DLPC, there is a gradual decrease in the entropy change per methylene addition from 8.5 K^{-1} mol^{-1} per methylene group (DTPC to DMPC) to almost 0 from DBPC to DLPC. As with the enthalpy change, at long alkyl chain lengths the entropy change from the gel state to the liquid crystal state approaches a constant value.

CONCLUSIONS

Despite the large standard deviations in the transition enthalpies and entropies for each phospholipid, the transition temperature of each lipid exhibits a standard deviation of only 0.1 K, which arises from the imprecision of 0.1 K attributable to the readability of the temperature in the thermocouple calibration. Closer examination of Tables 1–7 reveals that the standard deviation in the precision of the temperature measurements is less than this. The transition widths at half-max are 0.2–0.3 K and, thus, the phosphocholine main transitions represent nearly ideal isothermal transitions. The lack of any dependence of the transition temperature on change in ionic strength from 0.01 to 0.02 M sodium phosphate buffer concentration, change in pH from 6 to 8, change in concentration from 1 to 0.1 mass%, and source of the lipid facilitates the preparation of the suspensions under ordinary laboratory conditions. In addition, storage of the samples in the freezer for 4 years did not alter their transition temperatures. The transition temperature is simply taken as the temperature at the peak maximum, and does not involve analysis of the onset of the transition peak as with the melting points of organic solids. However, it is important to measure the transition temperature as a function of scan rate and extrapolate to zero scan rate to determine the true transition temperature.

The dependence of the transition temperature on scan rate is also another method of determining the response time of the DSC, which is needed for the Tian equation correction of the shape of a transition peak. Comparison of the instantaneous transition temperatures with literature values indicates

good agreement with the temperatures determined in other types of DSCs such as the Perkin–Elmer DSC1-B used by Ladbrooke and Chapman [9] and the Privalov DSC used by Sturtevant and co-workers [11,12,19]. Finally, the range of temperatures from 286.4 to 353.7 K covers the operational range of most microcalorimeters used in biological studies. These properties of the gel to liquid crystalline transitions of the phosphocholines make them suitable for use as sources of dynamic temperature data.

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