brief communication

Effects of headgroup methylation and acyl chain length on the volume of melting of phosphatidylethanolamines

Jeffrey T. Mason and Timothy J. O'Leary
Department of Cellular Pathology, Armed Forces Institute of Pathology, Washington, DC 20306-6000 USA

ABSTRACT The change in volume associated with the gel to liquid-crystalline phase transition for phosphatidylethanolamines of various chain lengths and headgroup methylation was determined by measuring the pressure dependence of the phase transition temperature and computing the volume change by using the Clausius-Clapyron equation. The volumes thus obtained were comparable to those previously obtained by using scanning dilatometry. The melting volume was larger for lipids with longer acyl chains, as found previously. The melting volume for a series of *N*-methylated dipalmitoylphosphatidylethanolamines (DPPEs) did not increase monotonically with increasing headgroup methylation. Instead, the melting volume increased in the order *N,N*-dimethyl-DPPE < *N*-methyl-DPPE < DPPE < dipalmitoylphosphatidylcholine. This unanticipated result is hypothesized to result from the competing effects of headgroup methylation on molecular volume and hydrogen bonding on the volume of melting.

INTRODUCTION

Phosphatidylcholines (PCs) and phosphatidylethanolamines (PEs) are the two major constituents of mammalian plasma membranes. In some tissues PEs may be sequentially methylated by methyltransferases, yielding *N*-methyl-PEs, *N,N*-dimethyl-PEs, and, finally, PCs (1). The partially methylated PEs demonstrate gel to liquid-crystalline phase transition temperatures between those for the corresponding PCs and PEs (2–5); however, the transition enthalpies remain relatively constant (3). Trimethylation of PE to form PC increases the volume change accompanying the gel to liquid-crystalline phase transition (6). Finally, *N*-methylation causes the bilayer lipid to spontaneously hydrate when exposed to water, rather than remaining in the crystalline form (2, 4).

Two effects of *N*-alkylation have been postulated to result in these changes. First, the addition of methyl groups to the PE nitrogen is thought to significantly decrease its hydrogen bonding and dipolar interactions (2, 4–6). This effect has been attributed to addition of the first methyl group even though amine hydrogen atoms remain available for hydrogen bonding until the headgroup is fully methylated. Second, *N*-alkylation increases the bulk of the headgroup (5–7). The relative importance of the effects of headgroup bulk and hydrogen bonding capability on bilayer properties have not been completely resolved. Silvius and co-workers have demonstrated that many chemical modifications that increase the bulk of the headgroup decrease the gel to liquid-crystalline transition temperatures regardless of the particular modification (2). However, whereas methylation of the ethanolamine nitrogen results in greater stability of the hydrated form of the lipid, modification at the headgroup carbons promotes lipid dehydration. The exact details of headgroup alkylation are important; *N*-ethyl-dimyristoylphosphatidylethanolamine (*N*-ethyl-DMPE) can form a high melting temperature crystalline phase similar to that of PE in addition to a subgel phase, in contrast to the *N*-methyl derivatives, which can form hydrated subgel phases but not dehydrated crystals. As a result of these studies on lipids with modified headgroups, Silvius and co-workers concluded that changes in hydrogen bonding have only a minimal effect on lipid interactions (2).

It is not obvious why increasing headgroup bulk should cause the volume of melting to increase, because the increasing chain disorder associated with an increase in headgroup size is presumably promoting a decrease in the phase transition temperature and because the effect of headgroup packing should be less significant in the liquid-crystalline than in the gel phase. To clarify the relative importance of changes in hydrogen/ionic bonding interactions and in headgroup bulk associated with headgroup *N*-methylation, we have measured the volume change associated with the gel to liquid-crystalline phase transition in dipalmitoylphosphatidylethanolamine (DPPE), *N*-methyl-DPPE, *N,N*-dimethyl-DPPE, and dipalmitoylphosphatidylcholine (DPPC) by using high sensitivity differential scanning calorimetry (DSC) at vari-

The opinions expressed in this paper are those of the authors and are not to be construed as official or as expressing the views of the Department of the Army or the Department of Defense.

ous hydrostatic pressures. We have also measured the volume of melting for the series of even-chain saturated PEs, dilauroylphosphatidylethanolamine (DLPE), DMPE, DPPE, and distearoylphosphatidylethanolamine (DSPE). We compare the effects of *N*-methylation and increasing chain length on the volume change associated with melting and discuss the implications of our results for understanding headgroup structural effects and for understanding the effects of small nonpolar molecules on membrane phase transitions.

EXPERIMENTAL

Lipids were obtained from Avanti Polar Lipids (Pelham, AL) and from Sigma Chemical Company (St. Louis, MO). All phospholipids were analyzed by thin-layer chromatography as described previously (8). In each case, only a single spot, corresponding to authentic phospholipid, was detected at a loading of 1 μmol of lipid. The phospholipids were dried in a vacuum desiccator over anhydrous calcium sulfate for 48 h. Between 5 and 6 mg of phospholipid was added to previously weighed calorimeter cells. The exact phospholipid dry weight was then determined by reweighing the cells. A volume of 180 μliters of 50 mM KCl was added to the cells, which were then sealed, and the total weight was recorded. This weight was compared with the weight of the cells after the calorimetric scans. In all cases no significant evaporation of water from the cells was detected. After the cells were placed in the calorimeter, the samples were fully hydrated by cycling several times through a temperature interval encompassing the gel to liquid-crystalline and crystalline to liquid-crystalline transitions of the phospholipids.

Calorimetric measurements were performed using a Hart Scientific 7707 series (Provo, UT) differential scanning colorimeter. A special cell was fabricated, which could be attached to a helium tank by stainless steel high-pressure liquid chromatography tubing. The system pressure was measured with an Omega Engineering (Stamford, CT) PX-105 transducer with a model DP-354 indicator. Thermograms were obtained on fully hydrated lipids at a heating rate of 20 K/h; enthalpies of transition were calculated by using software provided by the manufacturer. During these experiments, one of the calorimeter cells was maintained at 14.7 psi throughout the experiments. The lipid transition temperatures and enthalpies used in the melting volume calculations were averages of the values obtained from the lipid in this cell.

The performance of this apparatus was compared with the performance of a similar instrument described by Mountcastle et al. (9). The effects of pressure on the DPPC phase transition temperature as determined by our instrument were virtually identical to those reported by these workers (9). The baselines from our instrument were flatter, however, and the enthalpies determined in our instrument were almost identical to those obtained without the pressure assembly.

Thermograms were obtained at pressures ranging from 14 to 1,000 psi, and occasionally up to 1,400 psi. After determination of the peak maximum for each transition, dT_m/dP was determined by linear regression. The enthalpy of transition, ΔH_{cal}, from the unpressurized cell was used to calculate the entropy of transition ΔS via the relationship $\Delta S = \Delta H_{cal}/T_m$; ΔV was then calculated from the Clausius-Clapyron relationship

$$\Delta V = \Delta S\,(dT_m/dP). \quad (1)$$

RESULTS AND DISCUSSION

Representative thermograms for *N*-methyl-DPPE at various pressures are shown in Fig. 1. Thermograms for other lipids were qualitatively similar. Increasing hydrostatic pressure caused an increase in the temperature of the gel to liquid-crystalline phase transition, but there was no change in either the shape of the melting curve or in its enthalpy. The change in phase transition temperature with pressure for DMPE is illustrated in Fig. 2. The change in transition temperature is linear with pressure; there is only a very small scatter of experimental data around the optimal regression line. Similar linearity was seen for all lipids at pressures below 1,000 psi. Hence, the Clausius-Clapyron equation may be used to calculate the volume change associated with lipid melting. The phase transition temperature, enthalpy of melting (ΔH_{cal}) at ambient pressure, and ΔV are presented in Table 1 for all lipids studied. The volume changes associated with melting of DPPC, DMPE, and DLPE determined in this experiment are very similar to values previously reported

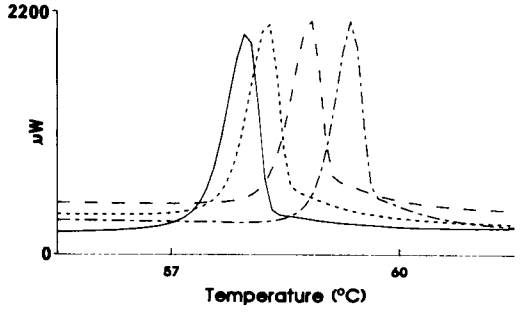

FIGURE 1 Thermograms showing the gel to liquid-crystalline phase transition of *N*-methyl-DPPE at 20 psi (*solid line*), 200 psi (*short dashes*), 600 psi (*long dashes*), and 1,000 psi (*alternating long and short dashes*).

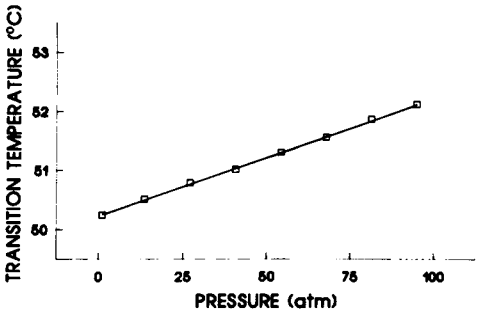

FIGURE 2 Phase transition temperature (T_m) of DMPE as a function of pressure. Squares show the experimental data, and the solid line shows the least-squares regression for this data.

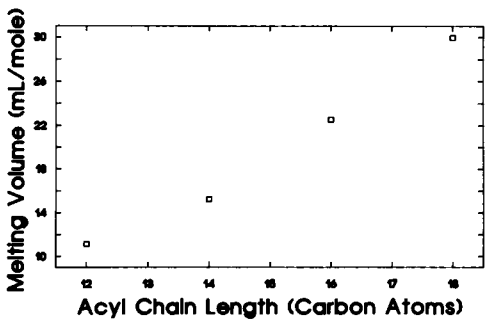

FIGURE 3 Melting volume for symmetric PEs as a function of acyl chain length.

by using dilatometry or calorimetry (6, 9, 10). Our values are virtually identical to those previously reported for DPPC, but they are ~20% higher than Wilkinson and Nagle's original values for the melting of DLPE and DMPE (6). This discrepancy apparently results from dehydration of the PEs to form a crystalline phase (2, 11–13), which resulted in an artifactually low estimate of the melting volume in the early densitometric experiments (13). The melting volume that we have determined for DMPE is actually somewhat lower than the later measurement of Wilkinson and Nagle (13), although within the limits of experimental error.

The melting volume which we obtained for DPPE is considerably smaller than that which would be inferred on the basis of adiabatic compression experiments reported by Russel and Collings (14). These authors have concluded that the value that they have measured for dP/dT is artifactually low, as a result of lipid settling in their sample chamber. Their values for the melting volume of DPPC are similar to those which we report here; significantly, their DPPC samples did not settle as rapidly as did their DPPE samples.

The effects of increasing acyl chain length on the volume of melting are illustrated in Fig. 3. There is an increase in the volume associated with melting as the chain length increases. This latter relationship is not surprising, as the entropy of transition results in large part from the change in the number of conformational states available to the hydrocarbon chain in the gel and liquid-crystalline phases. It seems reasonable that the change in the number of accessible chain states should increase as the volume increases; such an assumption is implicit in a number of theoretical models for the gel to liquid-crystalline phase transition.

The effects of increasing head group methylation on the volume of melting are illustrated in Fig. 4. There is a small decrease in the volume change associated with melting as the number of nitrogen methyl groups increases from 0 to 2. Upon addition of a third methyl group, there is a dramatic increase in the volume of melting. The most plausible explanation for these changes involves consideration of both headgroup size effects and hydrogen bonding. Increasing headgroup size by addition of nitrogen methyl groups may be expected to increase the hydrocarbon chain disorder in the gel phase, while having little or no effect on the chain disorder in the expanded liquid-crystalline phase. Upon melting, therefore, a greater change in the hydrocarbon chain disorder would be expected for the less highly methylated lipid series.

TABLE 1 Thermodynamic parameters for the gel to liquid-crystalline transition

Lipid	dT_m/dP	T_m	ΔH	ΔV
	K/atm.	*°C*	*kcal/mol*	*mL/mol**
DLPE	0.01947	30.4	3.9	11.13 (9.26)
DMPE	0.01990	47.7	5.8	15.22 (16.74)
DPPE	0.02200	63.1	8.1	22.54
DSPE	0.02680	73.4	10.2	29.96
N-methyl-DPPE	0.02106	57.5	7.8	21.07
N,N-dimethyl-DPPE	0.01544	48.2	8.7	17.72
DPPC	0.02482	41.6	8.2	27.39 (27.78)

*Values in parentheses were taken from reference 6 (DLPE), reference 10 (DMPE), and reference 13 (DPPC). The melting volume was calculated from dT_m/dP, ΔH, and T_m using the Clausius-Clapyron equation.

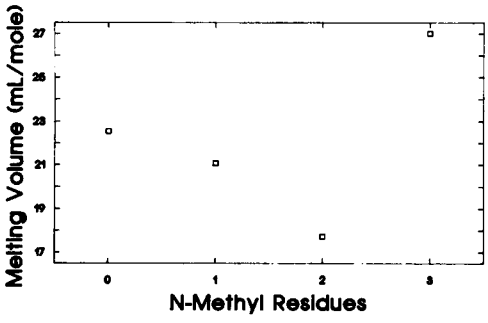

FIGURE 4 Melting volume for DPPE derivatives as a function of the degree of *N*-methylation.

Infrared spectroscopic measurements do in fact provide evidence for greater conformational disorder in the gel phase of more highly methylated PE derivatives (5). For this reason, one would expect that the effect of increasing headgroup methylation would be a decrease in ΔV as seen with the addition of the first two methyl groups to PE.

If the headgroup bulk alone was responsible for determining this volume change, PCs would be expected to have a smaller ΔV than the corresponding PEs or partially *N*-methylated derivatives. However, addition of the third methyl group to the lipid headgroup also destroys the ability of this headgroup to form hydrogen bonds with neighboring headgroups and reduces the strength of potential ionic interactions. The result is expected to be a pronounced decrease in the change of hydrogen bond strength associated with lipid melting and a consequent increase in ΔV. Such an increase in ΔV might be expected to result in an increase in ΔS due to the change in the number of accessible chain conformations. This effect may be compensated by a decrease in the entropy of melting, which results from liberation of oriented hydrogen-bonded headgroups during lipid melting in the PEs. The differences in the entropy of transition for DPPE, *N*-methyl-DMPE, *N,N*-dimethyl-DMPE, and DPPC are very small. Because this entropy contains contributions from the aqueous environment and the headgroup region, as well as from the hydrocarbon chain region, this interpretation of the effect of headgroup size and hydrogen bonding is not necessarily inconsistent with the increase in entropy of melting as one goes from PE to PC.

Whether or not this analysis is absolutely correct, it is clear from this and other experimental data that the effects of headgroup methylation are quite complex and somewhat different than previously accepted (4, 6). Addition of the first methyl group to the PE headgroup results in the inability of the lipid to maintain an unhydrated crystalline phase in the presence of water (2). Progressive methylation of the PE headgroup results in a progressive lowering of the gel to liquid-crystalline transition temperature (1–5). However, the effect of this headgroup methylation on the volume of melting is complex; progressive methylation of the DPPE headgroup results in a slight decrease in melting volume until addition of the final methyl group to form DPPC, which results in an increase in melting volume. This biphasic effect on melting volume apparently reflects the fact that not until addition of the third methyl group is the ability to hydrogen bond lost and that this loss of hydrogen bonding ability is associated with an increase in the melting volume of the lipid membrane.

This work was supported in part by grant GM40155 from the National Institutes of Health and by a grant from the American Registry of Pathology.

Received for publication 27 December 1989 and in final form 26 March 1990.

REFERENCES

1. Hirata, F., and J. Axelrod. 1980. Phospholipid methylation and biological signal transmission. *Science (Wash. DC).* 209:1082–1090.
2. Silvius, J. R., P. M. Brown, and T. J. O'Leary. 1986. Role of headgroup structure in the phase behavior of amino phospholipids. 1. Hydrated and dehydrated lamellar phases of saturated phosphatidylethanolamine analogues. *Biochemistry.* 25:4249–4258.
3. Mulukutla, S., and G. G. Shipley. 1984. Structure and thermotropic properties of phosphatidylethanolamine and its *N*-methyl derivatives. *Biochemistry.* 23:2514–2519.
4. Gagne, J., L. Stamatatos, T. Diacovo, S. W. Hui, P. L. Yeagle, and J. R. Silvius. 1985. Physical properties and surface interactions of bilayer membranes containing *N*-methylated phosphatidylethanolamines. *Biochemistry.* 24:4400–4408.
5. Casal, H. L., and H. H. Mantsch. 1983. The thermotropic phase behavior of *N*-methylated dipalmitoylphosphatidylethanolamines. *Biochim. Biophys. Acta.* 735:387–396.
6. Wilkinson, D. A., and J. F. Nagle. 1981. Dilatometry and calorimetry of saturated phosphatidylethanolamine dispersions. *Biochemistry.* 20:187–192.
7. Pascher, I., and S. Sundell. 1986. Membrane lipids: preferred conformational states and their interplay. The crystal structure of dilauroyl-*N,N*-dimethylphosphatidylethanolamine. *Biochim. Biophys. Acta.* 855:68-78.
8. Mason, J. T., A. V. Broccoli, and C. Huang. 1981. A method for the synthesis of isomerically pure mixed chain phosphatidylcholines. *Anal. Biochem.* 113:96–101.
9. Mountcastle, D. B., R. L. Biltonen, and M. J. Halsey. 1978. Effect of anesthetics and pressure on the thermotropic behavior of multilamellar dipalmitoylphosphatidylcholine liposomes. *Proc. Natl. Acad. Sci. USA.* 75:4906–4910.

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

11. Chang, H., and R. M. Epand. 1983. The existence of a highly ordered phase in fully hydrated dilauroylphosphatidylethanolamine. *Biochim. Biophys. Acta.* 728:319–324.

12. Suezaki Y., S. Kaneshina, and I. Ueda 1983. Statistical mechanics of pressure-anesthetic antagonism on the phase transition of phospholipid membranes: interfacial water hypothesis. *J. Colloid Interface Sci.* 93:225–234.

13. Wilkinson, D. A., and J. F. Nagle. 1984. Metastability in the phase behavior of dimyristoylphosphatidylethanolamine bilayers. *Biochemistry.* 23:1538–1541.

14. Russell, N. D., and P. J. Collings. 1982. High pressure measurements in phospholipid bilayers using adiabatic compression. *J. Chem. Phys.* 77:5766–5770.