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Dilatometry and Calorimetry of Saturated Phosphatidylethanolamine Dispersions†

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ABSTRACT: The specific volumes of a series of saturated phosphatidylethanolamine dispersions with 12, 14, and 16 carbon atoms per chain have been measured in the region of the chain melting transition, T_m. The change in specific volume at T_m for the 12 and 14 carbon compounds are 0.0160 and 0.0204 mL/g, respectively. Comparisons are drawn between this class of lipids and phosphatidylcholines. In both cases, T_m extrapolates with increasing chain length to the melting point of polyethylene. Both types of lipids appear to be packed in a similar way below T_m. One major difference is that dilaurylphosphatidylethanolamine undergoes a second transition above T_m.

In an earlier paper, we showed that the combination of calorimetry and density measurements with elementary theoretical considerations can lead to a more detailed molecular picture of the main phase transition of phosphatidylcholine bilayers (Nagle & Wilkinson, 1978). We now extend these measurements and this type of analysis to another major phospholipid group—the phosphatidylethanolamines (PE).[1] While highly sensitive calorimetry of saturated PE's has been done already (Mabrey & Sturtevant, 1978), no previous report of specific volume data has appeared.

The second aspect of this work involves additional polymorphism of the saturated phosphatidylethanolamines. It has been known for some time that well-defined reversible bilayer to hexagonal (H_{II}) phase transitions occur in certain unsaturated phosphatidylethanolamines (Cullis & de Kruijff, 1976). Such additional transitions, however, have not been found in saturated PE's (Cullis & de Kruijff, 1978). Since such transitions have a possible role of importance to play in membrane functions such as fusion (Cullis & Hope, 1978), it is worthwhile to see if there are conditions under which additional phases form. The stability of such phases in the presence of lipids which remain in the bilayer phase is also a question of importance to answer.

Materials and Methods

The phospholipids used in this study were obtained from Calbiochem and were not further purified. The best measure of purity is the narrowness of the phase transition. The widths

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[1] Abbreviations used: PE, phosphatidylethanolamines; DLPE, dilaurylphosphatidylethanolamine; DMPE, dimyristoylphosphatidylethanolamine; DPPE, dipalmitoylphosphatidylethanolamine; DMPC, dimyristoylphosphatidylcholine; DPPC, dipalmitoylphosphatidylcholine.

0006-2960/81/0420-0187$01.00/0

Table I: Properties of the Main Phase Transition

	T_m' (°C)	$\Delta T_{1/2}'$ (°C)	ΔH (kcal/mol)	T_m (°C)	$\Delta T_{1/2}$ (°C)	ΔV mL/g × 10^5	α_{below} [mL/(g °C) × 10^5]	α_m [mL/(g °C) × 10^5]
DLPE	30.5	0.3	3.5	30.5	0.3	1600	85	2300
DMPE	49.1	0.5	5.7	48.9	0.5	2040	90	1550
DPPE	63.1	0.2	8.8					
DMPC	24.0	0.13	5.4	24.0	0.13	2700	83	9000
DPPC	41.4	0.14	8.5	41.4	0.15	3700	83	12000

reported here are somewhat smaller than the best literature values (Mabrey & Sturtevant, 1978). The lipid mixture examined was formed by the cosonication technique described previously (Wilkinson & Nagle, 1979).

Specific volume data were obtained by using a combination of the technique of neutral buoyancy in D_2O–H_2O mixtures with our differential dilatometer. Details of these methods can be found elsewhere (Nagle & Wilkinson, 1978). Scanning rates of 3–5 °C/h were used. Approximately 60–120 mg of lipid in 10 mL of H_2O was used for each dilatometric experiment.

Calorimetric data were obtained with a Microcal MC-1 differential scanning calorimeter. Rates of 6–10 °C/h were used unless otherwise noted in the text. Lipid samples of 1–4 mg in 1 mL of H_2O or buffer were used. The buffer concentration in all cases was 100 mM. The sharpness of a typical phase transition of commercial lipid samples as measured by the Microcal calorimeter is comparable to that measured by our dilatometer and to that measured by other instruments of high quality such as the Privalov calorimeter. To date, no samples of higher purity, such as those examined by Albon and Sturtevant (1978), have been available to test this instrument. It is notable that the transition enthalpy changes for DMPC (5.4 kcal/mol) and DPPC (8.5 kcal/mol) agree well with those of Mabrey and Sturtevant (1978) and disagree with the higher values found with less accurate instruments. It should be noted that the DSC scans do not indicate different excess specific heats in the single phase regions above and below the transition, but this is expected because of the low concentrations of lipid used. A systematic investigation of such differences is planned for a variety of lipids. Noise levels in these scans is approximately the width of the lines.

Results

The excess specific heat as a function of temperature for aqueous suspensions of DLPE, DMPE, and DPPE is shown in Figure 1. The chain melting transition is well defined by a characteristic temperature, T_m', the maximum value of the specific heat. The heating scans shown in Figure 1 were done slowly enough to be comparable to our dilatometric scans.

In Figure 2 is shown the specific volume as a function of temperature for these same three PE's. As is the case for the specific heat curves, there is an abrupt change in the specific volume at a characteristic temperature, T_m, for DLPE and DMPE. The range of our dilatometer does not permit scanning at temperatures high enough to observe the corresponding change for DPPE dispersions.

Both the specific volume and specific heat curves display an asymmetry in the transition that is usual in PE's (Mabrey & Sturtevant, 1978) and also in *n*-alkanes (Templin, 1956). That is, there is a broad low-temperature part of the transition followed by a considerably sharper high-temperature end. This same asymmetry also exists in our dilatometric cooling scans.

Our thermal data for these compounds are summarized in Table I. For the sake of comparison, comparable data on DMPC and DPPC have also been included. While agreement

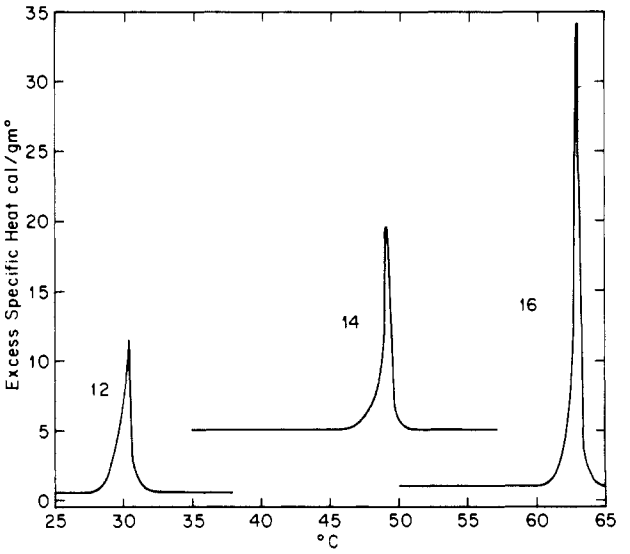

FIGURE 1: Excess specific heat vs. temperature for three phosphatidylethanolamines having chain lengths of 12, 14, and 16 carbon atoms. The choice of base line levels is arbitrary.

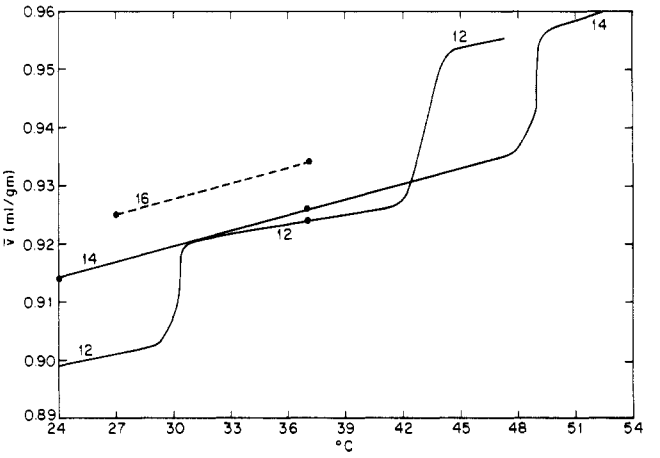

FIGURE 2: Specific volume vs. temperature for three phosphatidylethanolamines having chain lengths of 12, 14, and 16 carbon atoms. The heavy dots are flotation specific volumes and the solid lines, dilatometric scans.

between our values for T_m' and ΔH for the PE's and those published elsewhere (Mabrey & Sturtevant, 1978) is good, the lower values for T_m' that we obtain for DMPE and DPPE should be commented on. It is possible that variations in sample purity are part of the reason for the differences, but another factor is the scanning rate. Unlike the phosphatidylcholines, these transitions display a small amount of hysteresis (approximately 0.6 °C) even at heating and cooling rates as low as 2 °C/h. In particular, changing the heating rate from 6 to 12 °C/h causes an increase of 0.2 °C in the midpoint of the DPPE endotherm. Since scanning rates of 20 or more °C/h have typically been used by others, the discrepancy between our values and those published previously

Table II: Calculation of van der Waals Interactions

	r_b (Å)	r_a (Å)	$\Delta V/CH_2$ (mL/mol)	$\Delta U_{vdW}/CH_2$ (kcal/mol)	ΔU_{vdW}/mol (kcal/mol)
polyethylene[a]	4.54	5.13	3.08	0.659	
DMPC[a]	4.83_0	4.93_3	0.65	0.148	4.1
DLPE	4.86_2	4.93_{27}	0.39	0.098	2.4
DMPE	4.91_{08}	4.98_4	0.46	0.108	3.0

[a] For details, see Nagle & Wilkinson (1978).

may possibly be due to the sluggishness of the melting process in the PE's.

The melting temperatures of each lipid as measured by calorimetry (T_m') and dilatometry (T_m) are virtually the same, as are the transtion half-widths (ΔT and $\Delta T'$). Another measure of the sharpness of the transition, besides the half-width, is α_m, the maximum slope of the specific volume vs. temperature plot. As expected in comparing different PE's, smaller values of ΔT_m are associated with larger α_m values. A similar pattern for these lipids is evident in values for the cooperative unit (Mabrey & Sturtevant, 1978) which is proportional to the transition sharpness, although the cooperative unit theory only applies to symmetrical heat capacity curves.

Comparison of these thermodynamic parameters with corresponding ones for a series of phosphatidylcholines is also instructive. First, one can note that the transition enthalpy change appears little affected by changing the head group (see Table I). That is, ΔH values for the two members of each homologous pair (e.g., DMPC and DMPE) are nearly equal. There is, however, a large difference in $\Delta \bar{V}$ values between DMPC and DMPE (28%). The coefficient of expansion below T_m, $\alpha = d\bar{V}/dT$, is roughly the same for all the PE's and PC's examined so far. A more detailed comparison of the molecular structure of these two lipid classes will appear later in this paper.

The main transition of the phosphatidylcholines has certain similarities to the hydrocarbon melting transition of long-chained alkanes and polyethylene (Nagle & Wilkinson, 1978). One way to demonstrate this is to plot T_m or T_m' in Kelvin vs. $1/(N-\delta)$ where N is the number of carbon atoms per chain and δ is an adjustable parameter. For $\delta = 3$, which provides the best linear fit of both the PE and the PC data, the reciprocal N plot has an intercept ($N = \infty$) of 136 °C, which is only 2 °C lower than the transition in polyethylene.

Transition Energetics. In order to use the density data for detailed calculations of the energetics of the phase transition, it is useful to divide the volume of the lipid molecule into two parts, viz.

$$v_{lipid} = v_H + v_{HC} \quad (1)$$

where v_{HC} denotes the volume of the hydrocarbon chains, $2([CH_2]_{N-2}CH_3)$, and v_H the volume of the rest of the molecule, i.e., head group including the glycerol moiety (Tardieu et al., 1973). The hydrocarbon chain contribution to the volume is obtained from X-ray diffraction data. First, the cross section area per chain A, of DPPE can be calculated from the X-ray spacing at 20 °C, 4.15 Å (McIntosh, 1980). Since the chains are packed in a hexagonal array, $A = (2/3^{1/2})(4.15)^2$. The volume per CH_2 is obtained by multiplying by 1.27 Å, the carbon–carbon distance projected along the chain for an all-trans configuration. The volume per methylene, 25.3 Å^3, is multipled by 32 to yield the total hydrocarbon chain volume per molecule at 20 °C, 810 Å^3. Substraction from our measured specific volume at that temperature, 1056 Å^3, gives the volume of the headgroup, 246 Å^3. By assuming that v_H is independent of temperature and chain length, one can then

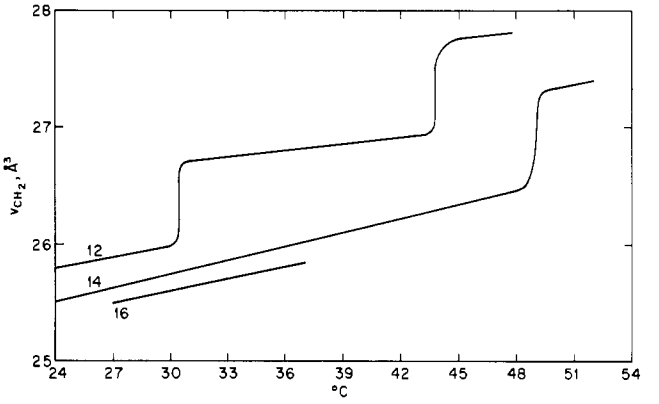

FIGURE 3: Volume per methylene group as a function of temperature for PE's having chain lengths of 12, 14, and 16 carbon atoms.

calculate v_{HC} as a function of temperature for the other members of the PE series. The results is shown in Figure 3. As expected, v_{CH_2} decreases with increasing chain length at the same temperature since the total van der Waals forces compressing the bilayer laterally are stronger for longer chains and the disordering effect of the terminal methyl group is correspondingly less.

These volume data are now in a state useful for calculating the change in the van der Waals interaction energy that occurs when the lipid bilayer expands laterally at T_m. We use a formula previously developed (Nagle & Wilkinson, 1978):

$$\Delta U_{vdW} = (2.3 \text{ kcal})((r_o/r_b)^5 - (r_o/r_a)^5) \quad (2)$$

where r_o, r_a, and r_b are the nearest neighbor chain separations at 0 K, just above thr transition, and just below the transition, respectively. The effective chain separation, r, at any temperature can be calculated from $v_{CH_2} = 1.27r^2(3^{1/2}/2)$. The results of calculations of ΔU_{vdW} are given in Table II where, for comparison, corresponding values for polyethylene and DMPC are included.

Next, the energy equation

$$\Delta H = \sum \Delta U_i + P\Delta V \quad (3)$$

is balanced. $P\Delta V$ at atmospheric pressure is approximately 10^{-4} times the measured ΔH's, and so can be neglected. The major contributions to the internal energy changes include both van der Waals interactions and rotameric disordering, so that we have

$$\sum \Delta U_i = \Delta U_{vdW} + \Delta U_{rot} + \Delta U_{other} \quad (4)$$

In the case of polyethylene and long-chain alkanes, ΔU_{vdW} and ΔU_{rot} accounted for most of the internal energy change, leaving only 5% as ΔU_{other} (Nagle & Wilkinson, 1978). However, for the PE's, one should also consider hydrogen-bonding interactions between the head groups. Nagle (1976) has analyzed the effects of electrostatic interactions and hydrogen bonding in these lipids and concluded that the contribution to ΔU from these sources would be less than or of the order of 10% ΔH. Accordingly, we set $\Delta U_{other} = 10\%\ \Delta H$ and balance the energy equation. The results are shown in Table III. Also in that

Table III: Calculation of Rotameric Properties

	ΔH (kcal/mol)	$\Delta H - \Delta U_{vdW}$ (kcal/mol)	ΔU_{other} (kcal/mol)	ΔU_{rot} (kcal/mol)	Δn_g	Δp_g
DLPE	3.5	1.1	0.4	0.7	1.4	0.09
DMPE	5.7	2.7	0.6	2.1	4.2	0.21
DMPC[a]	5.4	1.3	0.3	1.0	2.0	0.10
DPPC[a]	8.7	3.2	0.4	2.8	5.6	0.23

[a] Nagle & Wilkinson (1978).

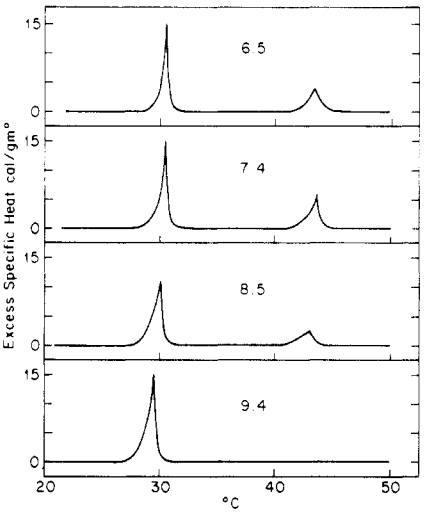

FIGURE 4: Excess specific heat vs. temperature for DLPE at four different pH values (6.5, 7.4, 8.5, and 9.4).

table are shown the change in the number of gauche rotamers, $\Delta n_g = \Delta U_{rot}/(0.5$ kcal/mol), and Δp_g, the average change in the probability of a carbon–carbon bond being gauche $[\Delta n_g/(2N - 8)]$.

High-Temperature Phase Behavior. When DLPE suspensions are scanned to temperatures approximately 12–15 °C above T_m, a second transition, T_h, can be observed with both the calorimeter and the dilatometer depending on the nature of the experimental conditions. For example, at a scanning rate of 30 °C/h, no second transition is observed for DLPE in water, at least up to 80 °C, whereas at 10 °C/h, an endotherm is evident at 43 °C. Because of this apparent dependence on scanning rate, it is not too surprising that earlier work on DLPE failed to detect T_h since it is the usual practice to scan rapidly in temperature.

Since bilayer–hexagonal phase transitions are known to occur in certain phosphatidylethanolamines at temperatures above T_m and since these transitions are very much influenced by pH (Cullis & de Kruijff, 1978), we performed DSC scans of DLPE at various pH values in two different buffer systems—Tris and sodium phosphate. Representative heating curves are shown in Figure 4. It should be noted in passing that the specific heat behavior between T_m and T_h in samples at pH 8.5 and lower was often erratic and depended on such factors as the thermal history of the sample. It is clear from Figure 4 that at pH 8.5 and below, PE bilayers undergo a second transition at approximately 43 °C. Dilatometric scans of DLPE corroborated these results. That is, two transitions were observed for DLPE in H_2O (pH between 5 and 6) in heating scans, whereas at pH 9.5 only the lower transition, T_m, appeared. When DLPE was cooled at low pH, the higher transition did not appear at 43 °C, but instead there was a very much increased specific volume change at T_m compared to the value obtained in the heating scan. This increased $\Delta\bar{V}$ was, however, approximately 25% less than the sum of the $\Delta\bar{V}$ for chain melting and higher transition. These thermal data are summarized in Table IV.

Table IV: Effect of pH on Phase Transitions

buffer	pH	T_m or T_m' (°C)	ΔH (kcal/mol)	$\Delta\bar{V}$ [(mL/g) × 10^5]	T_h (°C)	ΔH (kcal/mol)	$\Delta\bar{V}$ [(mL/g × 10^5]
Tris	9.4	29.0	3.5	1630	none		
	8.5	30.0	3.5	a	43.0	1–3.5	a
	7.4	30.5	3.3	a	43.6	3–6	a
	6.5	30.5	3.4	a	43.7	3–6	a
phosphate	9.5	28.8	a	1620	none		
	6.8	30.1	3.3	a	43.0	6	a
water	5–6	30.5	3.5	1600	43.7	1	500–2500

[a] No data available.

Calorimetric heating scans at low rates (7 °C/h) on DMPE at pH 6.8 and 9.5 showed only one endotherm between 25 and 85 °C. The transition midpoint varied slightly with pH. At high pH, T_m was approximately 1 °C lower than at low pH (buffer or water). This effect was also observed in the case of DLPE. ΔH did not appear to vary significantly with pH, in agreement with what was found for the chain melting transition enthalpy of DLPE.

If the higher transition does indeed represent a change from a bilayer to a hexagonal state (this question will be discussed later), then it is of importance to determine if such a transition occurs in natural membranes. While we have no direct evidence on this point, we did obtain thermal data for mixtures of DLPE and DMPC at low pH. Under those conditions (pH 6.8 and 7.4), DMPC should remain in a bilayer phase. The presence of the second lipid which by itself should undergo the second transition might either induce the PC to also change state or the DMPC might prevent the PE from undergoing its higher temperature transition. In fact, a 53 mol % DLPE mixture showed only one endotherm, four degrees broad and intermediate in temperature between the respective T_m's of the components. This is very similar to the DMPE–DSPC mixtures studied by Mabrey & Sturtevant (1976). No evidence was found for a second transition, at least up to 55 °C. A similar result occurred for a mixture containing 10 mol % DMPC. That is, there was no second transition.

Discussion

One object of this work is to compare the molecular structure of phosphatidylethanolamines with their choline analogues, especially at the chain melting transition. First, though, it is instructive to look at the differences in the head groups. It has been recently proposed (McIntosh, 1980) that the difference in size between the head groups of these two lipid classes is responsible for the difference in chain tilt that has been observed to occur below T_m. Our data show clearly that v_h for the PE's is indeed smaller than v_h for the PC's (cf. 246 and 344 Å^3). This measured difference can be related to the difference in structure of the head groups in the following way. The van der Waals radius of a choline group is $r_{choline} = r_{NC} + r_{CH_3} = 1.47$ Å + 2.0 Å = 3.47 Å, where we have used the covalent bond length for the nitrogen–carbon bond and the van der Waals radius for a methyl group (Pauling, 1960). We will assume that this van der Waals radius is roughly constant over a solid angle ϕ. Thus, the excluded volume of the choline group is $(\phi/3)(r_{choline})^3$. In the case of the ethanolamine group, the van der Waals radius is given by $r_{NH} + r_H = 2.21$ Å. However, the ethanolamine group will hydrogen bond to water which, for NH···O hydrogen bonds, will shorten the excluded volume radius by about 0.4,

giving an effective radius of 1.81 Å. Thus, the difference in excluded volume of the PC and PE head groups is roughtly $(\phi/3)(3.47^3 - 1.81^3)$ Å^3 = 11.95ϕ. Setting this equal to the observed difference of 98 Å^3 gives ϕ = 8.2 = 2.6π, or 30% greater than hemispherical. This value of ϕ seems well within the bounds of possibility.

While the extrapolation of T_m with reciprocal chain length to the melting temperature of polyethylene tends to identify the chain melting process in the PE's and PC's with the same type of transition in pure hydrocarbons, it is clear from our earlier work on PC's and the present study on PE's that the presence of the head group at the lipid–water interface exerts a restraining influence on the transition. That is, the change in volume per CH_2 group is much less when there is a polar group attached (compare to polyethylene in Table II). The apparent constraint imposed by the NH_3 group as measured by a reduction in the volume change is even greater than that of the $N(CH_3)_3$ group, and this could be a reflection of the hydrogen bonding that likely occurs between the protons of the amino group and those of surrounding water molecules or adjacent amino groups.

It is also of interest to compare the chain packing in each lipid class. One can compare hydrocarbon volumes in two ways: (1) at the same temperature or (2) should a law of corresponding states apply, at the same reduced temperature, $t = (T - T_m)/T_m$. For example, at 20 °C, DPPC is below its lower transition and has a molecular volume of 1140 Å^3. The molecular volume of DPPE at 20 °C, extrapolated from the data of Figure 1, is 1056 Å^3. The volume difference is 84 Å^3. But at 20 °C, t = –0.068 for DPPC. For DPPE this value of t occurs at 40.6 °C where the volume per molecule is 1078 Å^3, so that in this case the volume difference is 62 Å^3. In both cases though, the hydrocarbon volume difference is obtained by substracting the headgroup size difference of 98 Å^3. Thus, for both lipids at 20 °C, the difference in hydrocarbon volume turns out to be approximately 14 Å^3 (PE > PC), a value that is within the error limits of the data (e.g., ±0.04 Å in the X-ray spacing). However, using corresponding states gives a difference of 36 Å^3 which would appear to be larger than the error bounds. A similar result holds for the pair DMPE–DMPC in the low temperature phase. Furthermore, just above the phase transition, v_{CH_2} for DMPC is 26.7 Å^3 whereas for DMPE it is 27.3 Å^3. In contrast, the volumes per methylene agree much better when compared at the same temperature in the high-temperature phase. Thus, the hydrocarbon chains of these two types of lipids seem to pack in a very similar manner at the same temperature. There are, of course, some differences due to the restrictions of chain tilt in the PC's. Nevertheless, the similarity in structure of the hydrocarbon chain implies that the observed difference in T_m between a PE molecule and its PC analogue is attributable to head-group differences and such resulting factors as hydrogen-bond formation.

The results discussed above indicate that with respect to chain packing a PC and its PE analogue are most similar at the same temperature. This result has a bearing on free volume theories of transport and lateral mobility. For example, on the basis of such theories, one would expect that the rates of transport would be equal at the same temperature for both DMPE and DMPC where the free volumes are equal and that transport would be faster for DMPE than for DMPC at the same reduced temperature. Recent work by Derzko & Jacobson (Z. Derzko and K. Jacobson, unpublished results) supports these theories. They have shown that the lateral mobility of DPPC is greater than that of DMPC at the same reduced temperature while our previous volume measurements (Nagle & Wilkinson, 1978) showed that v_{CH_2} for DPPC is greater than v_{CH_2} for DMPC at the same t.

Below T_m the volume per methylene for both classes of phospholipids is larger than v_{CH_2} for the intermediate hexagonal phase of the alkanes (24.7 Å^3). This may be due to a greater fraction of gauche rotamers below T_m in the phospholipids compared to the all-trans configuration of the pure hydrocarbon chains. Interpretation of Raman spectroscopic data suggests some rotameric disorder below T_m for lecithins (Yellin & Levin, 1977; Gaber et al., 1978). While the extent of gauche rotamer formation is only one factor influencing the hydrocarbon volume, it seems likely that the similarity in chain packing between these two lipid classes is also reflected in similarities in trans-gauche populations.

Let us next consider the higher transition observed in DLPE suspensions. Calorimetric and dilatometric data are themselves not sufficient to permit unambiguous assignment of this transition. However, comparison with the bilayer–hexagonal transition of other PE's (Cullis & de Kruijff, 1978) does suggest that the higher transition in DLPE may be of that type. The pH dependence of the hexagonal phase observed in DLPE is similar to what occurs in unsaturated PE's. That is, as the pH is raised, the bilayer state is stabilized. Also, the hexagonal phase forms only when the acyl chains are melted, that is, above T_m. However, unlike the NMR study on DLPE, T_h occurs in the absence of cholesterol. Further spectroscopic studies are needed to resolve this question. We did not find any evidence for such a transition in either DMPE or DPPE. Apparently, the increased intermolecular attractive forces of these longer chained compounds are sufficient to stabilize the bilayer structure.

Another means of increasing the stability of the bilayer state appears to be the presence of a lipid which does not go into a hexagonal phase. A mixture containing as little as 10 mol% DMPC displayed no endotherm at 43 °C. If DLPE can form a hexagonal phase, it must do so less easily than certain unsaturated PE's where, for example, addition of up to 15 mol % PC does not prevent formation of that phase (Cullis & de Kruijff, 1978). While there may be structural differences between saturated and unsaturated PE's that are important in the formation of nonbilayer phases, it is also possible that the question of lipid miscibility plays an important role. Thus, in this study, the PE and PC are completely miscible in both low- and high-temperature phases (i.e., below and above the two-phase region), and no hexagonal phase is formed. In systems where miscibility is incomplete or absent, there would be a greater chance for an hexagonal phase to form in those regions containing almost none of the second lipid. This may have been a factor in the ability of unsaturated PE's, although this must remain a matter of speculation until a phase diagram is reported. The question of miscibility could also be of considerable importance in determining the significance of nonbilayer states in biological membranes.

Acknowledgments

We thank Dr. David Tirrell for the use of the calorimeter.

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Effects of Limited Denaturation by Heat on the Dynamic Conformation of Equine Immunoglobulin M Antibody and on Interaction with Antigen and Complement†

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ABSTRACT: In this paper, we report quantitative studies on the effects of heating equine immunoglobulin M (IgM) anti-5-(dimethylamino)naphthalene-1-sulfonyl (anti-dansyl) antibodies at 60 °C for 30 min on antigen binding and interaction with complement; parallel studies on IgM conformation and segmental flexibility were performed to localize these effects. Binding of the heated IgM to the hapten ϵ-dansyl-Lys and to the multivalent antigen dansyl$_{92}$-ficoll was measured by fluorescence enhancement and solid-phase radioimmunoassay, respectively, and found to be about half that for untreated IgM in both cases (K_0 for untreated IgM and ϵ-dansyl-Lys was 2.8×10^6 M^{-1}). Although binding of [^{125}I]C1q was only decreased 4-fold, antigen-independent complement fixation was decreased 40-fold. Antigen-enhanced complement fixation was abolished, even at IgM and antigen concentrations sufficient to compensate for reduced binding. The effects of heating on IgM conformation were determined with three independent and complementary approaches: (1) measurement of Fabμ segmental flexibility by nanosecond fluorescence depolarization; (2) circular dichroism (CD) of proteolytic fragments; and (3) binding of specific anti-Cμ_2 antibodies. Both heat-treated IgM and the (Fab′)$_2\mu$ fragment exhibited increased segmental flexibility; the latter showed the presence of a larger rotational subunit which probably includes part or all of the Cμ_2 domain. Comparison of the UV circular dichroism spectra of untreated and heated Fabμ fragments showed that only a localized change, probably in V_L, occurred on heating. In contrast, more general changes occurred in heated (Fab′)$_2\mu$ fragments. Since the only difference between Fabμ and (Fab′)$_2\mu$ is the presence of the Cμ_2 domains, the observed CD changes must have occurred in the latter. Similarly, binding measurements of specific anti-Cμ_2 antibodies to IgM showed that 2–3 times as much heated as untreated IgM was required for equivalent binding. Taken together, all of these results suggest that the conformation of the Cμ_2 domains was preferentially altered, and interactions between adjacent Cμ_2 domains within each IgM were weakened by heating the IgM. It seems reasonable, then, to implicate a structural requirement for intact, paired Cμ_2 domains in complement fixation. Since we have recently shown that optimal complement fixation occurs when several Fab's from the same IgM bind to the same antigen molecule and that a site for complement binding in addition to that for C1q can be inferred [Siegel, R. C., & Cathou, R. E. (1980b) *J. Immunol. 125*, 1910], we conclude that the role of antigen may be to stabilize an appropriate conformation of IgM for second-site binding.

Immunoglobulin M (IgM) is both the first immunoglobulin to appear in a primary immune response and the largest naturally occurring polymeric antibody with ten potential antibody binding sites (Ashman & Metzger, 1969; Metzger, 1970; Kim & Karush, 1973). Although the intrinsic affinity of each site for ligand is on the order of 10^5 M^{-1}, multivalency provides functional affinities on the order of 10^{10}–10^{11} M^{-1} (Karush, 1978). Electron micrographs of IgM bound to bacterial flagella have shown that IgM can adopt a number of different conformations of which the most striking is the staple structure, in which several Fabμ moieties are bound to the same antigenic surface (Feinstein et al., 1971). In another study, Feinstein & Munn (1969) reported that not all of the Fabμ regions of uncomplexed IgM were always seen, an observation that led them to suggest that the plane of the (Fab′)$_2\mu$ may be perpendicular to the major plane of the (Fc)$_5\mu$ ring. More recently, electron micrographs of IgM, in which significantly more detail could be seen, revealed IgM molecules with compact, asymmetric structures in which usually five Fab's radiated outward, and the other Fabμ was apparently

† From the Department of Biochemistry and Pharmacology, Tufts University School of Medicine, Boston, Massachusetts 02111. *Received June 18, 1980.* Supported by U.S. Public Health Service Grant AI-10115 and American Heart Association Grant 78-752. Taken in part from a dissertation submitted by R.C.S. in partial fulfillment of the requirements for the degree of Doctor of Philosophy, Tufts University, Medford, MA (1980). Preliminary communications of these studies appeared in Siegel & Cathou (1978a,b, 1979, 1980a).
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