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THERMODYNAMIC PROPERTIES OF MIXTURES OF DEUTERATED AND UNDEUTERATED DIPALMITOYL PHOSPHATIDYLCHOLINES (DIFFERENTIAL SCANNING CALORIMETRY/LIPID BILAYERS/MEMBRANES)

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ABSTRACT

In need for nonperturbing but spectroscopically unique environments for studies of lipid/lipid and lipid/protein interactions, we have investigated the thermodynamic properties of deuterated and undeuterated dipalmitoyl phosphatidylcholines as well as the mixtures of both. The data have been collected using the highly sensitive differential scanning calorimetry. From the results of these measurements we conclude that mixtures of deuterated and undeuterated lipids behave ideally. The transition enthalpies, transition entropies and the cooperative unit are given as a function of the input ratio of the components and the phase diagram is constructed, showing that the deuterated lipids are suitable as nonperturbing probes.

INTRODUCTION

In the study of biomembrane structure there is a need for nonperturbing, but spectroscopically unique, environments for studies of lipid/lipid and lipid/protein interactions. Such systems can, in principle, be provided by deuteration of one or more components. The utility of deuterated lipids for Raman [1,2] and NMR [3] studies has been demonstrated. However, with the exception of the work of Stahling et al. [4], few if any studies have been conducted on the comparison of hydrocarbon systems with deuterocarbon systems. In this paper we report the first set of thermodynamic data for dipalmitoyl d_{62} phosphatidylcholine and mixtures of this compound with the undeuterated compound. Comparison is also made with the lecithin in which only the number two chain is deuterated.

The data have been collected using the high sensitivity differential adiabatic scanning microcalorimeter, the utility of which has been demonstrated by Hinz and Sturtevant [5] in the investigation of phase transitions of lipids and lipid mixtures. From the evidence of the calorimetric data we conclude

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that mixtures of deuterated and undeuterated lipids behave ideally. Thus the introduction of the deuterated component to a complex lipid mixture is nonperturbing, validating the use of these systems for spectroscopic investigations.

MATERIALS AND METHODS

Dipalmitoyl phosphatidylcholine (Sigma) and dipalmitoyl d_{62} phosphatidylcholine (Lipid Specialities, Inc.) were routinely purified on a column of Sephadex LH-20 (95% ethanol, 37° C). Purified in this way, the lipids demonstrated a sharp main melting transition and a pre-melting transition in the differential scanning calorimetry. The compound 1-palmitoyl 2-palmitoyl d_{31} phosphatidylcholine was prepared by the acylation procedure of Cubero-Robles [6] starting with commercially obtained (Cal-Biochem) lysopalmitoyl phosphatidylcholine and palmitic d_{31} acid (Merk, Sharpe and Dome). The product was also purified on Sephadex LH-20 and was shown by Raman spectroscopy to contain equivalent amounts of deuterated and hydrated chains. Dispersions of the pure phospholipid were prepared by dissolving a weighed amount of material in twice recrystallized benzene, removing the solvent with a stream of nitrogen and suspension (to a concentration of approximately 0.5 mg ml^{-1}) in water. The material was heated to 60° C and vortexed. The heating—vortexing sequence was repeated three times. To assure homogeneous mixture the component phospholipids in benzene were mixed, frozen and lyophylized. The sample was dispersed in water at a temperature above T_M and subjected to low-power bath sonication for approximately 30 s. This procedure was chosen for the mixtures because it was found that when the solvent was removed with nitrogen, differential solubility in benzene of the two classes of phospholipids produced physical separation of lipids prior to dispersion. A solution containing equal molar amounts of dispersions of the deuterated and undeuterated phosphatidylcholines was prepared by gentle mixing of solutions of the pure dispersion components. The concentration of phospholipid in each dispersion was determined by phosphate analysis by the method of Chen et al. [7]. The analyses were performed in duplicate on the benzene stock solutions and on solutions removed from the microcalorimeter following differential scanning calorimetry. All calorimetric scans were performed with the Privalov calorimeter [8] at 1° C min^{-1} scan rates.

Specific heat values for the calorimetric transitions were determined by comparing the transition area to the area of a calibration mark introduced into the scan after each run of the sample.

RESULTS AND DISCUSSION

Figure 1 shows the calorimetric scan of the deuterated and undeuterated phosphotidylcholine: (1) as pure components, (2) a mixture of dispersions containing the pure components, and (3) a dispersion prepared from a thoroughly mixed solution containing 0.5 mole% of the same components. The melting points of the deuterated and undeuterated lipids are seen to dif-

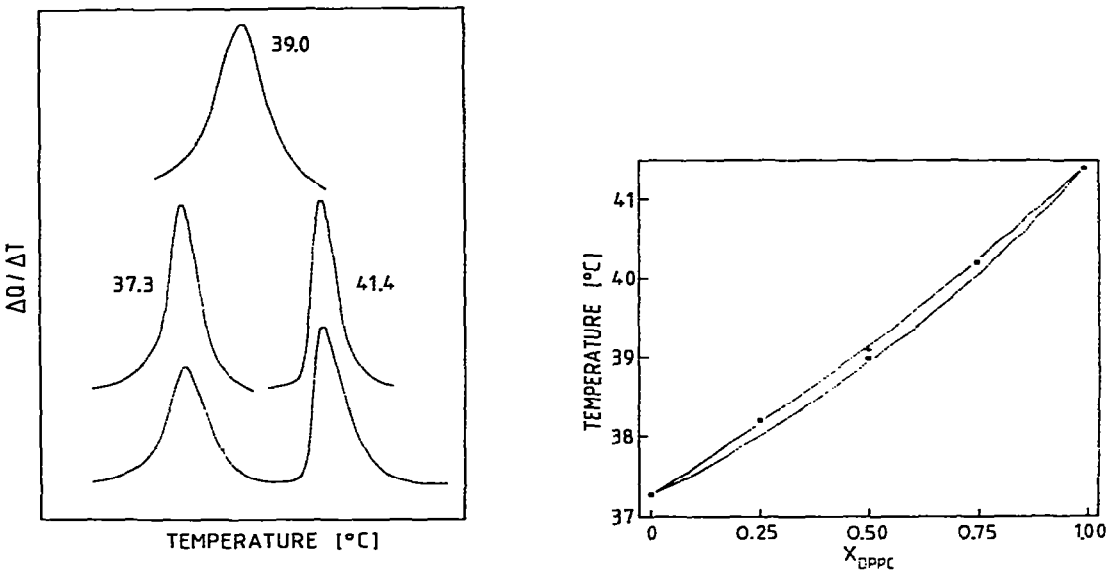


Fig. 1. Differential calorimetric scan for the main melting transitions of DPPC (lower trace) and an equimolar mixture of DPPC and DPPC-d_{62} dispersions: dispersions of pure DPPC and pure DPPC-d_{62} (run separately and traced for figure) (middle trace); dispersion prepared from a thoroughly mixed equimolar combination of DPPC and DPPC-d_{62} (upper trace).

Fig. 2. Mid-transition main melting points for mixtures of DPPC and DPPC-d_{62}. "X" at $X = 0.5$ is the value for 1-palmitoyl, 2-palmitoyl-d_{62} phosphatidylcholine. Solid curve is the theoretical determined phase diagram for the system DPPC/DPPC-d_{62}.

fer by 4°C. When dispersions of the pure components are gently mixed, no fusion of the system is evident and two distinct melting transitions are observed at 37.3°C (corresponding to the deuterated lipid) and 41.4°C (the undeuterated lipid). No mixing occurs in the heterogeneous population of pure dispersion on the time scale (h) of the calorimetric measurement.

Comparison of the two 50 : 50 mixtures prepared prior to and subsequent to dispersion allows the possibility of detailed study of the kinetics of both fusion of populations of phospholipids of identical chain length as well as studies of lateral phase separations within the plane of the bilayer.

When a 50 : 50 mixture of pure components is prepared prior to dispersion and then subjected to differential scanning calorimetry, a single sharp main transition is observed at 39°C, a temperature exactly intermediate between the main transitions of the two pure components. A similar behavior is also seen for the premelt transition which occurs at 31.6°C. The data suggested that the two components mixed ideally. To test this hypothesis we prepared mixtures of the pure components at various mole % fractions and determined their melting behavior. The resulting phase diagram is shown in Fig. 2. The full phase diagram for both the main transition and the pre-transition indicates that mixing is ideal throughout the concentration ranges studied. The phase diagram was computed according to the procedure of Selz [9] as used by Mabrey and Sturtevant [10].

TABLE 1

Thermodynamic parameters for aqueous dispersion of deuterated and undeuterated phospholipids

Sample	T (°C)	ΔH_{cal} * (kcal mole^{-1})	ΔH_{vH} (kcal mole^{-1})	ΔS (cal deg^{-1} mole^{-1})	Cooperative unit **	T (°C)	ΔH_{cal} * (kcal mole^{-1})	ΔH_{vH} (kcal mole^{-1})	ΔS (cal deg^{-1} mole^{-1})	Cooperative unit **
DPPC	41.4	8.8	839	28.0	95	35.4	1.2	338	3.8	323
DPPC-d_{62}	37.3	10.9	712	35.3	65	28.8	1.2	226	3.8	192
DPPC/ DPPC-d_{62}										
0.75	40.2	9.0	648	28.8	72	32.8	1.2	236	4.1	189
0.50	39.0	8.9	361	28.5	41	31.6	1.2	190	4.0	154
0.25	38.2	9.8	639	31.5	65	29.0	1.2	226	3.6	187
DPPC-d_{31}	39.1	9.6	827	30.7	86	30.9	1.3	281	4.2	216

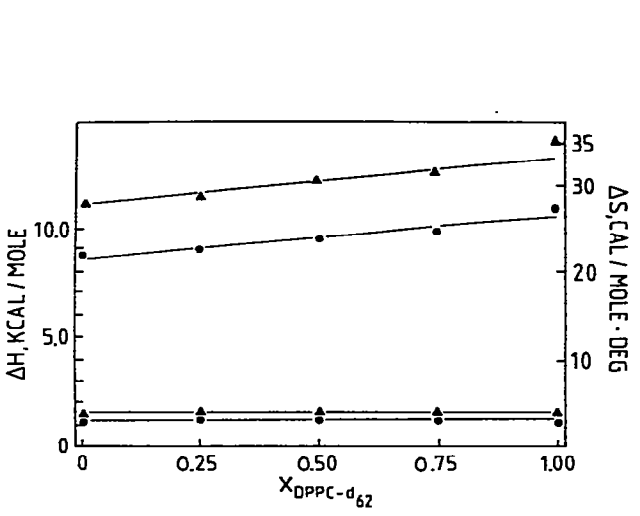
* ΔH_{cal} is the calorimetric ΔH, Δ_{vH} is the van't Hoff ΔH and is equal to $6T_m^2R(dQ/dT)$.

** The cooperative unit is the ratio of ΔH_{vH} to ΔH_{cal} and may be thought of as the minimum number of molecules undergoing a simultaneous transition.

The experimental phase diagram was constructed from the mid-point melting temperatures and is bracketed by the theoretical phase diagram constructed from the experimentally determined enthalpies of transition assuming ideal mixing of liquid and solid phases and ignoring heats of mixing. It should be noted that the temperature of the pre-melting transition is further depressed by deuteration than is the temperature of the main melting transition. Further, the temperatures of both the melting and pre-melting transitions in 1-palmitoyl, 2-palmitoyl d_{31} phosphotidylcholine lie exactly midway between the transition temperatures of the pure components. The data derived from the differential scanning calorimetry are summarized in Table 1.

The data in Table 1 show that both ΔH and ΔS are different for the deuterated and undeuterated lipid, a result confirmed by the plot of ΔH and ΔS vs. transition temperature (Fig. 3). The fact that the melting points of the deuterated lipids change only slightly despite rather large changes in the ΔH of transition, indicates there must be relatively large changes in the ΔS of transition as shown in Fig. 2. ΔH for the pre-transition peaks is essentially identical for all mixtures within the limits of experimental error of our method.

It is not obvious to us why both the entropy and enthalpy of transition are higher for the deuterated lecithin. Possible explanations might include subtle differences in packing or in the zero-point energy resulting in differences in the mean diameter of the deuterocarbon chains as opposed to the hydrocarbon chains. This may result in slight differences in packing efficiency of interchain distances between deuterated and undeuterated compounds. Note that the phospholipid deuterated only in the number 2 chain



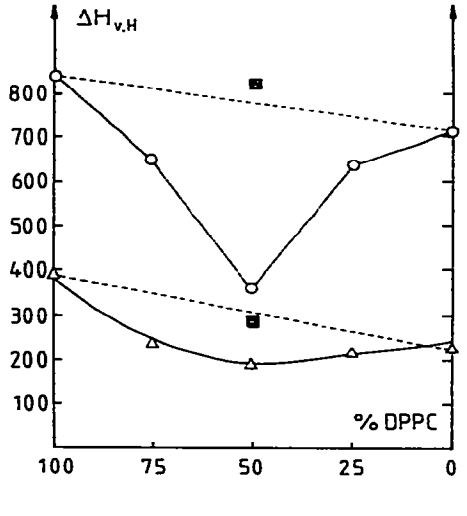


Fig. 3. Transition enthalpies (●) and entropies (▲) for the main melt (upper lines) and premelt (lower lines) for mixtures of DPPC and DPPC-d_{62}.

Fig. 4. Transition enthalpies (van't Hoff) as a function of the input ratio (upper line) and transition entropies as a function of the input ratio of the constituents. △, Premelting peak; ○, main peak; ■, DPPC deuterated only in the number 2 chain.

falls on the lines between the deuterated and undeuterated compounds. This suggests that the enthalpy and entropy contributions of the two chains of the phospholipid are exactly additive in this case.

From the data on patch sizes, calculated from the ΔH (van't Hoff) of transition for the main transitions of the pure phospholipid compounds, it may be seen that the cooperative unit (or patch size) involved in the melting transitions of HH and DD differ substantially, and that the patch size for the compound with only the number 2 chain deuterated is exactly intermediate between the patch size of the pure hydrated and pure deuterated compounds. The fact that the apparent width of the transition for the 50 : 50 HH—DD mixture is substantially greater than the width of the transition for HD may be caused by two possible sources. The simplest origin for this may be the well-known broadening of melting transitions in all ideal mixtures in which both the liquid solution is ideal and the solid solution is ideal, originating from enrichment of the crystalline phase at the melting temperature at equilibrium in the higher melting component of the ideal mixture. However, we have calculated this curve if the expected broadening of the melting transitions occurred and this effect is not sufficient to account for the width of this transition for the 50 : 50 HH : DD mixture (cf. Fig. 4).

CONCLUSIONS

Deuterated and undeuterated dipalmitayl lecithins have been shown to mix very nearly ideally and thus provide useful nonperturbing systems with which to study complex lipid/lipid and lipid/protein interactions. The compound 1-palmitoyl, 2-palmitoyl phosphatidylcholine behaves in all respects as a unique compound, with thermodynamic properties exactly intermediate between those of the pure deuterated and pure undeuterated lecithin. The difference between the thermodynamic parameters for deuterated and undeuterated lecithins is clearly real, but is as yet not fully understood. Our data also show that by use of deuterated lipids it is possible to manipulate melting points of complex phospholipid mixtures without resorting to components with different chain lengths, different head groups, or degree of unsaturation. This concept should prove of value to studies of vesicle fusion and kinetics of exchange of phospholipids between separate bilayers as catalyzed by phospholipid exchange enzymes.

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