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CALORIMETRIC AND FOURIER TRANSFORM INFRARED SPECTROSCOPIC STUDIES ON THE INTERACTION OF GLYCOPHORIN WITH PHOSPHATIDYLSERINE/DIPALMITOYLPHOSPHATIDYLCHOLINE-d_{62} MIXTURES *

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Glycophorin has been isolated in pure form from human erythrocyte membranes and reconstituted into lipid vesicles composed of binary mixtures of bovine brain phosphatidylserine (PS) and acyl-chain perdeuterated dipalmitoylphosphatidylcholine (DPPC-d_{62}). The effect of protein on lipid melting behavior and order has been monitored with differential scanning calorimetry and Fourier transform infrared spectroscopy (FT-IR). The phase diagram for PS/DPPC-d_{62} is consistent with that previously reported for PS/DPPC (Stewart et al. (1979) Biochim. Biophys. Acta 556, 1–16) and indicates that acyl chain perdeuteration does not greatly alter the lipid mixing characteristics. The use of deuterated lipid allows the examination of lipid order by FT-IR of each lipid component in the binary mixtures as well as in the ternary (lipid/lipid/protein) systems. Addition of glycophorin to a 30:70 PS/DPPC-d_{62} binary lipid mixture results in a preferential glycophorin/PS interaction leading to bulk lipid enriched in DPPC-d_{62}. This is revealed in two ways: first, through cooperative calorimetric transitions increased in temperature from the binary lipid system and second, through FT-IR melting curves of the DPPC-d_{62} component which shows transitions increased in both onset and completion temperatures in the presence of protein. In addition, non-cooperative melting events are observed at temperatures below the onset of phase separation. The FT-IR data are used to assign these non-cooperative events to the melting of the PS component. For the 50:50 lipid mixture with protein, two transitions are observed in the DSC experiments. The IR results indicate that both lipid components are involved with the lower temperature event.

Introduction

Studies of lipid-protein interaction in reconstituted systems have been widely undertaken in order to understand the structural basis for the dependence of membrane protein function on the physical state and chemical structure of the phospholipids in contact with protein (for reviews, see Refs. 1 and 2). To date, most of the reconstitution experiments have been accomplished with a single lipid class. While much important information has been extracted from these studies, the complexity of the reconstituted lipid environment does not approach that in native membranes. The simplest lipid systems which can exhibit the types of phase

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Abbreviations: DSC, differential scanning calorimetry; FT-IR, Fourier transform infrared spectroscopy; DPPC, 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine; DPPC-d_{62}, acyl-chain perdeuterated DPPC; PS, phosphatidylserine; DMPC, 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine; NMR, nuclear magnetic resonance; PE, phosphatidylethanolamine.

behavior and melting characteristics of in vivo systems and yet be amenable to detailed physical investigation are binary lipid mixtures [3]. Determination of lipid phase characteristics in such systems has been most conveniently accomplished with differential scanning calorimetry (DSC), although many spectroscopic approaches including magnetic resonance, electron spin resonance, parinaric acid or chlorophyll A fluorescence and vibrational spectroscopy have all been utilized. (For a review of the theory and practical determination of lipid mixing properties, see Refs. 3 and 4). A wide variety of mixing characteristics have been noted.

DSC techniques, while giving the best thermodynamic data about the melting process [4], suffer from three deficiencies. First, no information about lipid order may be inferred from the data. Second, as lipid melting processes become less cooperative (in the presence of protein, for example), the DSC signals become progressively more difficult to detect, and finally in complex lipid systems there is no way of assigning a particular lipid melting event to a particular component or combination of components in the system (if such an assignment is possible). Fourier transform infrared (FT-IR) spectroscopy is well-suited as a technique which complements calorimetric measurements and offers a solution to the above deficiencies. The method directly monitors acyl chain configuration without the use of probe molecules, and provides a snapshot of the entire lipid population on a short time scale. Since the C-H stretching vibrations of the acyl chains (along with C-^{2}H vibrations of deuterated chains) are sensitive to lipid order [5], deuteration of the acyl chains of one of the lipid components in a binary mixture allows the observation of the order of each component [6]. These data can then serve to complement the calorimetric studies. In addition, the FT-IR experiment measures the signal from all the lipid in the sample regardless of the cooperativity of the process leading to the lipid melting. The utility of FT-IR for studies of lipid-protein interaction has been demonstrated in earlier work from our laboratories [6,7].

The current study involves an FT-IR and calorimetric investigation of a membrane protein reconstituted into well-defined vesicles composed of binary mixtures of lipids, with the aim of determining whether membrane proteins select particular regions of physical order or chemical structure in a complex lipid environment.

The protein chosen is glycophorin, an integral transmembrane sialoglycoprotein from the human erythrocyte, which has been chemically well characterized [8,9] and so is a logical choice for biophysical studies. The molecule (M_r 31 000) is composed of 131 amino acids whose sequence has been determined and 16 oligosaccharide chains which form 60% of its weight. The protein is readily prufied and reconstituted into lipid vesicles [10]. Studies by Van Zoelen et al. [11] and by Grant and McConnell [12] have demonstrated that the lipid is not excluded from lipid gel phases, however, it is more efficiently reconstituted with liquid-crystalline lipids [13]. A variety of physical studies have examined the effect of glycophorin on single lipid components, primarily dipalmitoyl- or dimyristoylphosphatidylcholine [14,15]. The effect on other lipid classes or lipid mixtures has been less explored. (For two exceptions see Ong and Prestegard [16] and Taraschi et al. [17]).

The binary lipid system chosen for the current investigation is bovine brain phosphatidylserine (PS)/acyl chain perdeuterated dipalmitoylphosphatidylcholine (DPPC-d_{62}). Phase characteristics for the system (with proteated acyl chains) have been reported by Stewart et al. [18].

Experimental

Materials. Bovine brain PS and DPPC-d_{62} were purchased from Avanti Polar Lipids (Birmingham, AL) and evaluated for purity by TLC. Lipids were assayed for chain length distribution by gas chromatography of their methylated acyl chains. The PS was found to contain major amounts of stearate and oleate with minor quantities of other components.

Neuraminidase from *Clostridium perfringens* (Type VI) and trypsin were purchased from Sigma Chemical Co. (St. Louis, MO). Sepharose 4B was the product of Pharmacia Fine Chemical (Piscataway, NJ).

Sample preparation. Glycophorin was isolated from human erythrocyte membranes using methods previously described in detail [7]. Polyacrylamide gel electrophoresis of the purified protein

gave patterns consistent with those previously reported [19].

Glycophorin was incorporated into vesicles of varying PS/DPPC-d_{62} ratios according to the procedure of MacDonald and MacDonald [10]. Lipid and protein were simultaneously solubilized in $CHCl_3/CH_3OH/H_2O$ (6.5 : 2.5 : 0.4, v/v). Solvent was removed by N_2 drying and evacuation under reduced pressure.

The lipid-protein film was rehydrated in a buffer consisting of 100 mM NaCl/10 mM Tris/0.1 mM EDTA/1 mM histidine (pH 7.4) and vesicles collected by centrifugation. The concentration of the lipid-protein complexes in aqueous buffer was approx. 5–10% (w/w) for all the samples described here. Reconstitution of samples in ^2H_2O for some IR experiments was carried out as above except that the rehydration buffer was made up in ^2H_2O.

Sample characterization Lipid phosphorus was determined by the method of Chen et al. [20], while protein was determined as sialic acid following the method of Warren [21].

The relative amounts of DPPC-d_{62} and PS in the complexes were determined either by initial lipid weights or by gas chromatography as described above. Demonstration of the association of lipid and protein in the complexes and removal of residual uncomplexed lipid and protein was accomplished on a column of Sepharose 4B. Aliquots were collected and assayed as described [6]. Asymmetry of orientation of glycophorin in the vesicles was also accomplished as described [6].

FT-IR spectroscopy. Typical samples for FT-IR were examined in a Harrick cell (50 μm path length) equipped with CaF_2 windows. Spectra were recorded on a Digilab FTS-11 Fourier transform infrared spectrometer equipped with a HgCdTe detector. Two hundred fifty interferograms were collected, co-added, apodized with a triangular function, and Fourier transformed to give a resolution of 4 cm^{-1}. Temperature control was as described [22].

Frequencies were determined using a center of gravity routine to an accuracy of 0.01 cm^{-1} [23]. Underlying H_2O or ^2H_2O bands were subtracted prior to the frequency measurements. [6]. Alternatively, derivatives were used to accomplish the same purpose of minimizing the effects of non-linear baselines on the measured frequencies [24].

Differential scanning calorimetry. Calorimetry measurements were made on a Micro-cal MC1 unit. Sample volumes of 0.70 ml containing 1.4–3.0 mg for pure lipid samples and slightly less for lipid/protein complexes, were injected into the sample cell with the same volume of buffer used in the reference cell. Samples were scanned with heating rates of between 24 and 35 K/h after 1–2 h equilibration time in the instrument. Duplicate runs of the same sample gave onset and completion temperatures reproducible to 0.2 K. Independent samples gave data reproducible to 0.5 K. Heat capacities were calibrated with a standard electronic pulse and checked with pure lipid suspensions. Enthalpies were determined either by cutting and weighing the area under the heat capacity vs. temperature curve, or by computer collection of the data (using an A-D converter from Interactive Structures, Inc., Bala Cynwyd, PA, and an Apple II + Computer) followed by numerical integration. Typical values for the transition enthalpies of multilamellar vesicles are: DMPC, 4.9 ± 0.3 kcal/mol; DPPC 7.1 ± 0.5 kcal/mol. Phase boundaries for the main gel-liquid crystal transformation of PS/DPPC-d_{62} mixtures were approximated from the set of onset and completion temperatures.

Results

PS/DPPC-d_{62} mixtures

DSC scans for pure DPPC-d_{62}, pure PS, and several mixtures of the two are given in Fig. 1. The curves are heating scans obtained with a heating rate of about 35 K/h. DPPC-d_{62} exhibits its main endothermic (gel-liquid crystal) transition with an onset temperature of 35.4 °C and a completion temperature of 37.8 °C, in good agreement with data reported by Klump and co-workers [25]. The measured enthalpy of the transition, 7.4 kcal/mol, is lower than that reported in previous work. Also visible is a weak broad pretransition with a peak at about 29 °C. PS shows a single broad peak over the range 5–15 °C, interpreted as the main chain melting event. For binary mixtures containing 11.5 mol% PS or more, a separate peak is no longer visible for the pretransition of the phosphatidylcholine. As the mole fraction of PS is increased, the melting of the binary mixtures shows a broad-

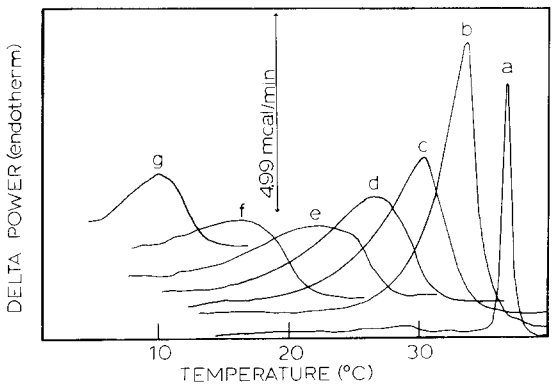

Fig. 1. Differential scanning calorimetry data for PS/DPPC-d_{62} binary mixtures. Samples were made up in a buffer consisting of 100 mM NaCl/10 mM Tris/0.1 mM EDTA/1 mM histidine. Mole fractions of DPPC-d_{62} in the samples were: a, 1.00; b, 0.885; c, 0.73; d, 0.58; e, 0.415, f, 0.20; g, 0.00. Scan rates varied slightly but were generally about 35 K/h. The enthalpies cannot be directly compared from the data from sample to sample as the amount of lipid in the cell varied from run to run. The excess heat capacity cannot be plotted directly because of the slight variations in scan rate.

ening of the transition range (compared with the pure components) and reduction in onset and completion temperatures for the main transition. A partial phase diagram is given in Fig. 2. Effects due to the pre-transition have been omitted. At high levels of PS, the onset temperature is difficult to define accurately, leading to some uncertainties in the construction of the solidus curve for the system. The FT-IR data, as discussed below, lend insight into this problem, which may be traced to non-cooperative introduction of disorder into the acyl chains prior to the main melting event. In general, the DSC results presented here indicate that the perdeuteration of the acyl chains produces rather small alterations in the shape of the phase diagram for the binary lipid mixture (compare with Ref. 18). The slight differences are traced to the fact that the transition temperature of DPPC is itself lowered by about 5 K upon perdeuteration of the acyl chains. The mixing properties of the PS with DPPC are not altered. Similar conclusions have been reached for the DPPC/DPPE system [26].

The 2000–2300 cm^{-1} region of the infrared spectrum for a 1 : 1 binary lipid mixture at various temperatures is shown in Fig. 3. The spectral features in this figure arise from the C-^{2}H stretching modes of the DPPC-d_{62} in the binary mixture. The C^2H_3 groups give rise to bands at 2212 (asymmetric stretch) and at 2169 cm^{-1} (symmetric stretch), while the stronger bands at 2194 and 2089 cm^{-1} arise from antisymmetric and symmetric C^2H_2 stretching modes, respectively [27]. The spectral features in the C-H stretching region (2800–3000 cm^{-1}) arise from the proteated acyl chains of the PS component. The asymmetric and symmetric CH_3 stretching modes appear near 2956 and 2872 cm^{-1}, while the antisymmetric and sym-

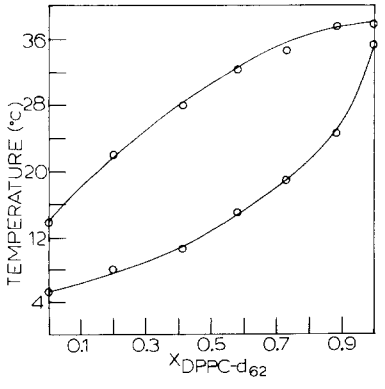

Fig. 2. Partial phase diagram for PS/DPPC-d_{62}, constructed from the set of onset and completion temperatures for the gel-liquid crystal phase transition. No attempt is made to include the existence regime of the pre-transition for high levels of DPPC-d_{62}.

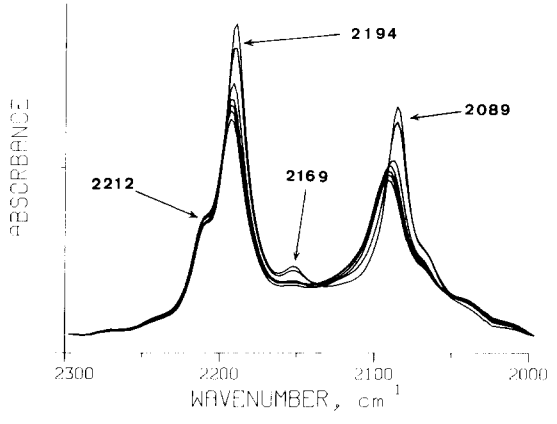

Fig. 3. Temperature dependence of the infrared spectra of the C-^{2}H stretching region for a 1 : 1 binary lipid mixture PS/DPPC-d_{62}. Spectra decrease in peak height with increasing temperature and are plotted in 5 K intervals over the range 2–55 °C.

metric CH_2 stretching bands are observed at 2920 and 2850 cm^{-1} [5]. In addition, there is a broad Fermi resonance band centered at about 2900 cm^{-1} [28].

Variations in temperature produce changes in the bandwidth and frequency for both C-H and C-^{2}H stretching modes which have been used to characterize the melting behavior of the individual lipid components [6]. Sharp discontinuities over very small temperature intervals in plots of frequency or intensity versus temperature have been empirically correlated with highly cooperative phase changes in the hydrocarbon chains of both *n*-alkanes and phospholipids [5,29]. An example of such a highly cooperative transition is shown in Fig. 5 for DPPC-d_{62}; the calorimetric data for DPPC-d_{62} (Fig. 1) show a similar melting behavior. Hydrocarbon chains also exhibit less cooperative phase changes which show a sigmoid-shaped discontinuity in plots of frequency or intensity versus temperature [29]. These less cooperative changes are observed in the infrared melting curves of phospholipids (i) in which the acyl chains are heterogeneous [30], (ii) where the addition of protein causes changes in hydrocarbon packing [7], or (iii) in binary mixtures of lipids which broaden the phase transition [6]. In each case, the decrease in cooperativity can be confirmed by calorimetric studies. In the present case, pure bovine PS undergoes a broadened cooperative melting from 8 to 16 °C associated with its gel to liquid-crystal phase transition as seen by DSC and FT-IR (Figs. 1, 4). The broadening in this case arises from the heterogeneity and polyunsaturation of the PS acyl chains.

A comparison of the DSC and FT-IR data for binary PS/DPPC-d_{62} mixtures reveals differences in the way the two methods sense the phase transitions and/or phase separation of the binary mixture. For the 50 : 50 PS/DPPC-d_{62} mixture, the DSC data exhibit onset and completion temperatures of 13 and 30 °C (as interpolated from Fig. 2). The FT-IR data for the individual components of the 50 : 50 binary mixture allow a detailed examination of the melting behavior of both constituents and a correlation with the DSC data. The PS component of the 50 : 50 binary mixture shows a broad, sigmoid-shaped melting profile between 10–25 °C (Fig. 4) that can be associated with a non-cooperative transition of the acyl chains (see above). The data for the DPPC-d_{62} component (Fig. 5) show a transition at 20–30 °C broadened from that of pure DPPC-d_{62} but still exhibiting a well-defined onset temperature, unlike that of the PS compenent. The DSC data (Fig. 1d) for a similar PS/DPPC-d_{62} binary mixture show a region of slowly increasing excess heat capacity on

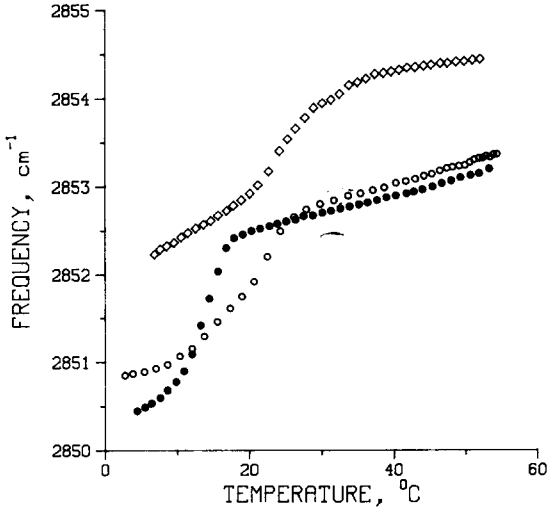

Fig. 4. Plots of the temperature-induced variation in the CH_2 symmetric stretching frequency for pure PS multibilayers (●), a 50 : 50 PS/DPPC-d_{62} mixture (○), and for a 30 : 70 PS/DPPC-d_{62} mixture (◇).

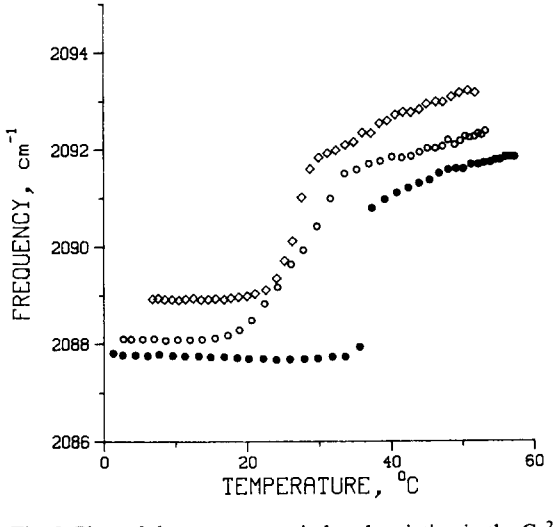

Fig. 5. Plots of the temperature-induced variation in the C-$^2H_2$ symmetric stretching frequency for pure DPPC-d_{62} multibilayers (●), a 50 : 50 PS/DPPC-d_{62} mixture (○), and for a 30 : 70 PS/DPPC-d_{62} mixture (◇).

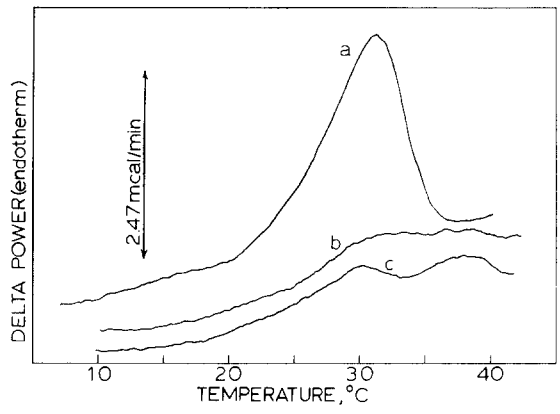

Fig. 6. Differential scanning calorimetry traces for: (a) PS/DPPC-d_{62} 30 : 70, lipid/protein ratio = 160 : 1. (b) PS/DPPC-d_{62} 40 : 60, lipid/protein ratio = 110 : 1. (c) PS/DPPC-d_{62} 50:50, lipid/protein ratio = 70:1. Scan rates about 35 K/h. The areas under the curves are not directly comparable as the sample quantities differed

the low temperature side of the transition, rendering the entire contour asymmetric. The infrared results suggest an assignment of this feature to the PS component, which (as shown by the FT-IR data) undergoes the non-cooperative introduction of disorder into its acyl chains between 10 and 25 °C. Conversely, the large endothermic event between 25 and 30 °C seen in the DSC (Fig. 1d) must be assigned to the transition of the DPPC-d_{62} component of the mixture, as deduced from its FT-IR melting curve (Fig. 5). In such a manner, the combination of FT-IR and DSC is able to distinguish the cooperativity of the melting behavior in the individual components of the mixture.

Addition of glycophorin produces large changes in both the FT-IR and DSC data which are dependent on the lipid composition of the vesicles into which the protein has been inserted. The calorimetry data for some ternary systems are shown in Fig. 6. FT-IR plots of the temperature-induced variations in the C-H and C-^{2}H stretching modes are shown in Figs. 7A and B for the 50 : 50 : 1 (PS/DPPC-d_{62}/glycophorin) ternary system and the 50 : 50 binary lipid mixture, and in Figs, 8A and B for the 30 : 70 : 0.6 (PS/DPPC-d_{62}/glycophorin) ternary system and the 30 : 70 binary lipid mixture. The DSC data for a 50 :50 lipid mixture at a lipid/protein ratio of 70 : 1 (Fig. 6c) shows a broad transition with 2 distinct peaks in the excess heat capacity. The onset temperature is 18 °C (however, a slight upward slope is noted from 13–18 °C), while the two peaks appear at 29 and 38 °C. The corresponding FT-IR melting behavior is shown in Fig. 7 (lipid/protein ratio 100 : 1). The DPPC-d_{62} component in the ternary system (Fig. 7B) has its onset temperature (20–21 °C) shifted

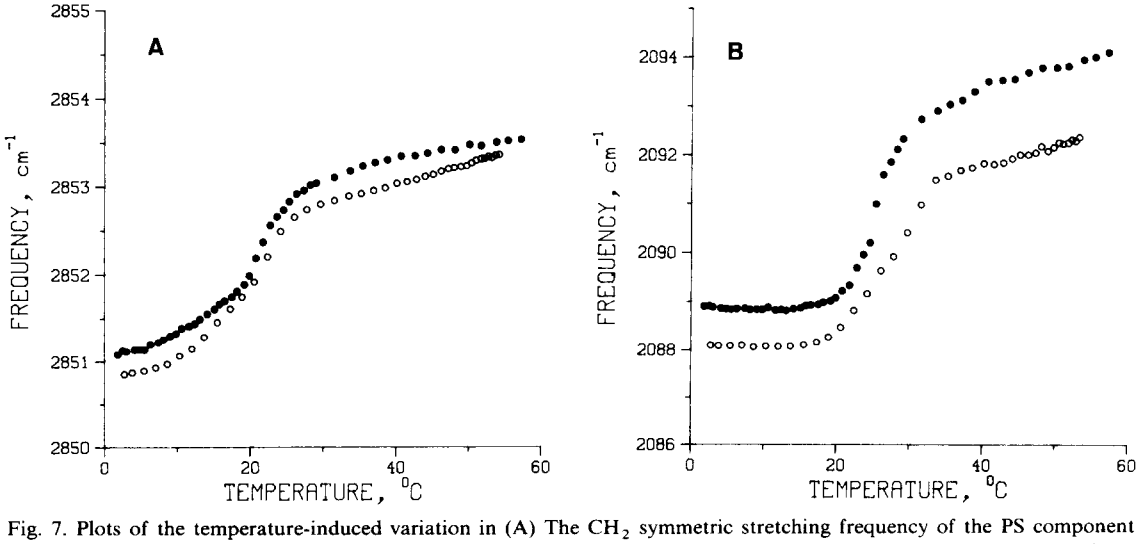

Fig. 7. Plots of the temperature-induced variation in (A) The CH_2 symmetric stretching frequency of the PS component in a 1 : 1 binary combination with DPPC-d_{62} (○), and in the ternary system PS/DPPC-d_{62}/glycophorin (●), PS/DPPC-d_{62} mole ratio is 1 : 1, lipid/protein mole ratio is 100 : 1. (B) The C-$^2H_2$ symmetric stretching frequency of the DPPC-d_{62} in a 1 : 1 binary combination with PS (○), and in the ternary system described in the caption to Fig. 7A (●).

up from the binary lipid mixture (where the onset is 16–17 °C). The ternary system data then show steep changes until 32 °C. There is no evidence of non-cooperative processes below the onset temperature although a continuous increase in the frequency is observed above 32 °C. In contrast, the PS component in the ternary system (Fig. 7A) shows significant non-cooperative melting from 6–20 °C, followed by more cooperative events from 20–28 °C. The FT-IR data thus suggest an assignment for some of the calorimetric events. The initial upward slope in the DSC endotherm between 13 and 18 °C probably arises from the melting of the PS, while the first cooperative calorimetric peak contains contributions from both lipid components. The origin of the peak at 37–38 °C in the DSC is unclear, but may result from residual melting of DPPC-d_{62} domains. There is some evidence for melting of the DPPC-d_{62} between 32 and 40 °C in the FT-IR data (Fig. 7B) but the slope of the data over that temperature range is close to that observed above 40 °C, so that the variation between 32 and 40 °C may simply reflect non-specific thermal effects.

The observed increase in the onset of melting for the DPPC-d_{62} in the ternary system, suggests enrichment of the bulk lipid phase in this component, and hence a specific protein-PS interaction. Clearer evidence for selection of PS by glycophorin comes from the data for a ternary system PS/DPPC-d_{62}/glycophorin (PS/DPPC-d_{62} = 30 : 70, lipid/protein = 160 : 1). For this sample, the DSC (Fig. 6a) onset temperature (20–21 °C) is increased from its values in the binary lipid mixture of the same composition (about 17 °C, as taken from the phase diagram in Fig. 2). The completion temperature is also raised in the complex from 34 to 36 °C. These increased temperatures in the complex suggest that those lipids capable of undergoing cooperative melting processes (and hence seen with greater sensitivity in the DSC experiment) have been enriched in the higher melting component.

The FT-IR data for this ternary system (Fig. 8A and B) are consistent with the above interpretation and show different effects of protein on each lipid [30]. The PS exhibits broad, non-cooperative melting behavior in the binary lipid mixture (Fig. 8A). The presence of protein accentuates the non-cooperativity of the PS melting by increasing the transition range.

In contrast to the PS component the infrared melting curve for the DPPC-d_{62} component in the presence of protein demonstrates a 6 K increase in

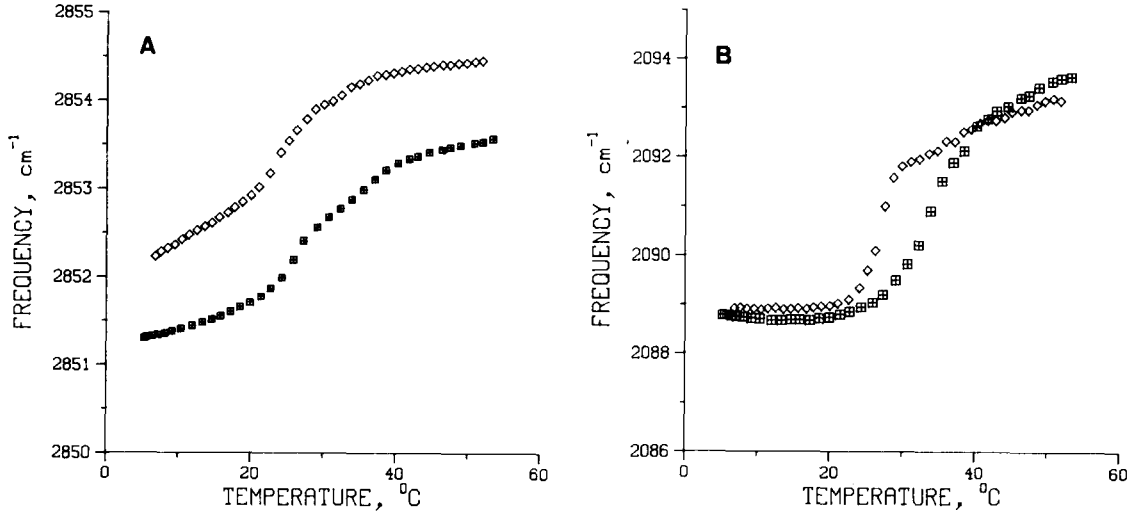

Fig. 8. Plots of the temperature-induced variation in (A) The CH_2 symmetric stretching frequency of the PS component in a 30 : 70 binary combination with DPPC-d_{62} (◇), and in the ternary system PS/DPPC-d_{62}/glycophorin (⊞), PS/DPPC-d_{62} mole ratio is 30 : 70, lipid/protein mole ratio is 160 : 1. (B) The C^2H_2 symmetric stretching frequency of the DPPC-d_{62} component in a 30 : 70 binary combination with PS (◇), and in the ternary system described in the caption to Fig. 8A (⊞).

melting temperature when compared with the binary mixture (Fig. 8B). In addition, the onset temperature is significantly raised. Both the DSC data for the system and the FT-IR data for the components indicate that addition of glycophorin to the binary lipid mixture produces a preferential interaction with the PS component. Consequently, the residual bulk lipid becomes enriched in the DPPC-d_{62} and undergoes a phase transition as observed by FT-IR (for that component) as well as by DSC (for the melting of the system) at a higher temperature than in the 30 : 70 binary lipid mixture.

Further comparison of the FT-IR data with the DSC reveals that the broad non-cooperative calorimetric event between 10 and 20 °C (Fig. 6a) is due to the (protein-associated) PS in the mixture. The FT-IR data (Fig. 8) show that only the PS component in the system undergoes melting events over this range of temperature.

Discussion

The most important result of the current work is the demonstration that glycophorin preferentially interacts with the PS component in binary mixtures of PS/DPPC-d_{62}. The data at PS/DPPC-d_{62} ratios of 30 : 70 reveal this interaction in the DSC experiment by an upward shift in the transition temperature for the cooperative melting process upon the addition of protein. The FT-IR data elaborate upon the calorimetric result and explore the molecular order and melting behavior of each lipid component. The DPPC-d_{62} transition is increased in both midpoint and onset temperature in the ternary system consistent with its being excluded from the vicinity of protein, while the PS melt is rendered less cooperative by glycophorin. Data for the 50 : 50 lipid mixture in the presence of protein are more difficult to interpret, but tend to support the idea of preferential selection of the PS.

There are not many studies in the literature concerning the effect of membrane proteins on binary lipid mixtures. A notable exception is the work of Boggs et al. [31], who used DSC to detect phase separation induced in PS/DPPC mixtures by lipophilin from the myelin of human white matter. The results are strikingly similar to those reported here (especially Fig. 6a). A strong preferential selection of PS by the lipophilin was observed. Chapman et al. [32] have investigated the partitioning of Gramicidin A into mixtures of 1,2-dilauroyl-*sn*-glycero-3-phosphocholine/ DPPC mixtures. At low protein concentrations, the polypeptide preferentially associated with the lower melting regions of the bilayer whereas at higher levels a mixing of the two lipid was noted.

There have been several investigations of glycophorin-lipid interaction pertinent to the current work. Armitage et al. [33] and Van Zoelen et al. [34], demonstrated that after lithium diiodosalicylate extraction of glycophorin from the erythrocyte membrane, some anionic lipid remains tightly bound to protein. A preferential interaction with anionic lipid (PS) has been demonstrated in direct fashion in the current study.

Two recent investigations in reconstituted systems concerning the interaction of glycophorin with lipid classes other than the phosphatidylcholines have appeared. Ong and Prestegard [16] used NMR methods to demonstrate a slight preference of protein for the phosphatidylethanolamine (PE) in PC/PE mixtures. In addition, Taraschi et al. [17] demonstrated that glycophorin exerts a bilayer stabilizing effect on the bilayer-non-bilayer transition of cardiolipin.

The few physical studies in this area, have tended to demonstrate that membrane proteins will interact with a particular class of lipids. In the current case, it is not clear whether the selection is based on a physical property of the lipid component (PS being the lower melting component), or (more likely), a particular structural feature of a lipid molecule such as charge, headgroup structure or acyl chain unsaturation. Further studies with PS of a particular chain length will be required to address this question.

The current investigations clearly show the utility of FT-IR for complementing the DSC studies. Differences in the melting behavior as observed by the two methods arise as the FT-IR senses any lipid melting process, regardless of the cooperativity, while the DSC responds with more sensitivity to cooperative events.

In addition, the use of deuterated lipids permits the monitoring of individual components in a lipid mixture. This in turn facilitates assignment of the

DSC data, which, of itself cannot be directly assigned to particular molecular components of the system. Two aspects of the current work demonstrate this approach: first, the observation of increased T_m for the DPPC-d_{62} component in the presence of protein and second, the assignment of the non-cooperative melting in the DSC experiment to the PS component alone.

A drawback of the FT-IR is that a quantitative interpretation of the data is lacking. Since the change in the FT-IR parameters normally used to monitor lipid phase alterations are not linear functions of the fraction of lipid melted, some model must be introduced for treatment of data. We have introduced a simple two state approximation and reported preliminary studies along these lines [6], and hope to present a more refined version elsewhere shortly.

Finally, the relevance of the current work to membrane structure-function relationships must be addressed. It is well documented (see Refs. 1 and 2 for reviews) that the function of membrane-bound enzymes often depends strongly on the physical structure and/or chemical nature of the lipid environment into which they are reconstituted. The current procedures enable any perferential interaction (in reconstituted systems) to be elucidated. In such cases a particular lipid component may be deuterated and its melting characteristics in a complex environment determined by FT-IR and compared with the phase behavior of the entire system as determined calorimetrically. If necessary, the procedure may be repeated for a different lipid class, until a reasonable picture of the entire system is accumulated.

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