Interaction of Glycophorin with Phosphatidylserine: A Fourier Transform Infrared Investigation†

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ABSTRACT: Glycophorin, from the human erythrocyte membrane, has been isolated in pure form and reconstituted into unilamellar vesicles with bovine brain phosphatidylserine (PS). Fourier transform infrared spectroscopy has been used to monitor the protein conformation as well as the effect of protein on lipid order and melting. Glycophorin, at levels of 1 mol %, nearly abolishes the gel to liquid-crystal phase transition seen in pure PS vesicles between 8 and 16 °C by inducing significant disorder into the lipid gel phase. A transition of reduced magnitude remains between 14 and 22 °C in the lipid/protein complexes. Evidence is presented for specific interaction of glycophorin with the interfacial region of PS. In general, the effects on lipid melting produced by protein at the 1 mol % level are more pronounced than those noted in a previous study of glycophorin/phosphatidylcholine interactions [Mendelsohn, R., Dluhy, R. A., Taraschi, T., Cameron, D., & Mantsch, H. H. (1981) *Biochemistry 20*, 6699–6706]. Two bands are observed for the protein amide I (C=O stretching) mode. A main feature at 1653 cm^{-1} indicates that the bulk of the secondary structure is random coil or α-helical. A weaker shoulder at 1675 cm^{-1} suggests the occurrence of a small proportion of the β-sheet form. The results confirm circular dichroism studies of Schulte & Marchesi (1979) [Schulte, T. H., & Marchesi, V. T. (1979) *Biochemistry 18*, 275–280]. Fourier transform infrared (FT-IR) studies of a ternary complex of PS/dipalmitoylphosphatidylcholine-d_{62} (DPPC-d_{62})/glycophorin indicate that the glycophorin preferentially interacts with the PS component. The melting of the DPPC-d_{62} and PS components may be separately monitored. DPPC-d_{62} has a reduced transition width and increased melting temperature in the ternary system (two lipids + protein) compared with the binary lipid mixture. The utility of deuterated phospholipids in FT-IR studies for monitoring the preferential partitioning of proteins in complex lipid environments is demonstrated.

In view of the marked dependence of membrane protein function on the physical state and chemical structure of phospholipid in contact with protein (Gennis & Jonas, 1977), a variety of physical techniques has been used to investigate the structural and dynamic alterations that occur during lipid/protein interaction in reconstituted systems [for an overview, see Parsegian (1982)]. Vibrational spectroscopy, in particular Fourier transform infrared (FT-IR)[1] spectroscopy, is useful in this context (Lord & Mendelsohn, 1981; Mendelsohn et al., 1981). The technique directly monitors phospholipid conformation without the use of probe molecules and provides a snapshot of the entire lipid population on a rapid time scale. Previous studies [see Cameron et al. (1981) and references cited therein] have demonstrated that large changes take place in the set of vibrational frequencies during the lipid gel–liquid-crystal phase transition. In addition, studies of the peptide bond frequencies permit monitoring of protein secondary structure.

Glycophorin, an integral transmembrane sialoglycoprotein from the human erythrocyte membrane, has been chemically well characterized and so is a logical choice for biophysical studies. The molecule (molecular weight 31 000) is composed of 131 amino acids whose sequence has been determined (Tomita & Marchesi, 1975) and 16 oligosaccharide chains which form 60% of its weight. The protein is readily isolated in pure form, and a straightforward reconstitution procedure into lipid vesicles has been published (MacDonald & MacDonald, 1975). Studies by van Zoelen et al. (1978a,b) and by Grant & McConnell (1974) have demonstrated that the protein is not excluded from lipid gel phases; however, it is more easily reconstituted with liquid-crystalline phases of lipids (MacDonald, 1980). Recently, Ruppel et al. (1983) have suggested from studies of glycophorin with dimyristoylphosphatidylcholine (DMPC) that immiscibility of the protein takes place about 15 °C below the T_m for the pure lipid. Brulet & McConnell (1976), using ^{13}C NMR spectroscopy, showed that in complexes of glycophorin with dipalmitoylphosphatidylcholine (DPPC) the lipid had increased motional freedom compared with the pure DPPC. FT-IR studies of DMPC/glycophorin (Mendelsohn et al., 1981) revealed that the lipid phase transition is progressively broadened and shifted to lower temperatures as the proportion of protein in the vesicles increased. In a recent investigation (Dluhy et al., 1983a), it was demonstrated that the FT-IR spectroscopic data could be used to construct partial phase diagrams for binary lipid mixtures and ternary systems containing two lipids plus protein.

Although most of the reconstitution experiments with glycophorin have been accomplished with phosphatidylcholines (PC's) [for two exceptions, see Ong & Prestegard (1982) and Taraschi et al. (1983)], it is of importance to investigate the interactions of glycophorin and other membrane proteins with a variety of lipid classes in order to decide whether the protein selectively partitions into regions of particular chemical structure of physical order in a complex lipid environment.

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[1] Abbreviations: FT-IR, Fourier transform infrared; DPPC, 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine; DPPC-d_{62}, acyl chain perdeuterated DPPC; DMPC, 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine; PS, bovine brain phosphatidylserine; NMR, nuclear magnetic resonance; DMPC-d_{54}, acyl chain perdeuterated DMPC; PE, phosphatidylethanolamine; T_m, temperature of the gel to liquid-crystal phase transition; TLC, thin-layer chromatography; Tris, tris(hydroxymethyl)-aminomethane; EDTA, ethylenediaminetetraacetic acid.

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Toward that end, the current FT-IR study involves glycophorin interaction with phosphatidylserine (PS) from bovine brain as well as initial measurements of a ternary system involving glycophorin, PS, and acyl chain perdeuterated dipalmitoylphosphatidylcholine (DPPC-d_{62}).

Materials and Methods

Materials. Bovine brain PS and DPPC-d_{62} were purchased from Avanti Polar Lipids (Birmingham, AL) and checked for purity by TLC. Lipids were assayed for chain length distribution by gas chromatography of their methylated acyl chains as previously described (Dluhy et al., 1983a). The PS contained major amounts of oleate and stearate 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 and Sephadex LH-20 were products of Pharmacia Fine Chemicals (Piscataway, NJ).

Sample Preparation. Glycophorin was prepared from human erythrocytes by using methods previously described in detail (Mendelsohn et al., 1981). Polyacrylamide gel electrophoresis of the purified protein gave patterns consistent with those previously published (Furthmayr et al., 1975).

Glycophorin was incorporated into vesicles with PS according to the procedure of MacDonald & MacDonald (1975). The protein and PS were simultaneously solubilized in $CHCl_3$/MeOH/H_2O (6.5:2.5:0.4 v/v). Solvent was removed by N_2 drying followed by evacuation under low pressure. From this stage on, care was taken to prevent the lipid/protein film from coming into contact with oxygen. 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 were collected by centrifugation at 15 000 rpm for 1 h. Lipid phosphorus was determined by the method of Chen et al. (1956), while glycophorin was determined as sialic acid following the procedure of Warren (1959). Reconstitution of complexes in D_2O was carried out as described above except that the rehydration buffer was made up in D_2O. Occasionally, samples for FT-IR were further exchanged in D_2O just prior to use.

Sample Characterization. In ternary complexes, the relative amounts of DPPC-d_{62} and PS in the complexes were determined by gas chromatography of their methylated acyl chains. Removal of multilamellar (protein-free) lipid and uncomplexed protein from the samples was accomplished on a column of Sepharose 4B. Aliquots were collected and analyzed as previously described (Dluhy et al., 1983a). Asymmetry of protein incorporation in the vesicles was also accomplished as described in the above reference.

Negative-stain electron microscopy was carried out in order to assay the size homogeneity of the vesicles. The experiments were performed on a Phillips EM 200 microscope operating at 600 kV with initial magnifications ranging from 30000× to 65000×. Vesicles in buffer were placed on a Formvar-filmed and heavily carbon-coated grid for 5 min, and the excess was blotted off. Phosphotungstic acid was added (1% for 5 min) and the preparation air-dried before use.

FT-IR Spectroscopy. The FT-IR experiments were performed as described previously (Dluhy et al., 1983a). Binary samples of PS/glycophorin were examined in D_2O solution so that no subtraction of background was required in the C–H stretching region. In order to enhance visualization of the highly overlapped spectral features in the 1600–1750-cm^{-1} spectral region, spectra were Fourier self-deconvoluted to remove the inherent line width from the bands while preserving their frequency positions and relative intensities (Kauppinen et al., 1981a,b). Samples were about 5–10% by weight and were contained in a 50-μm cell with CaF_2 windows as described (Dluhy et al., 1983a).

Results

Biochemical Characterization of Complexes. Glycophorin/PS complexes were formed according to the procedures of MacDonald & MacDonald (1975) and fractionated on Sepharose 4B. The fractionated vesicles consisted of a population of unilamellar spheres with a size distribution (as observed in the electron microscope) ranging from 400 to 900 Å. At least 80% of the vesicles had diameters in the range 400–550 Å. Attempts at further size fractionation resulted in oxidation of the PS which was difficult to control.

Evidence for complex formation was the same as previously reported for the glycophorin/PC interaction (Dluhy et al., 1983a); when the lipid/protein complex is chromatographed on Sepharose 4B, the glycophorin coeluting with lipid has a retention time much reduced from free protein. Cleavage of those portions of the protein external to the vesicles with trypsin or neuraminidase followed by sialic acid determination suggested that about 90% of the sialic acid residues are oriented toward the exterior of the vesicle. This level of asymmetry is comparable to that observed in glycophorin/PC systems (Ong & Prestegard, 1982).

Infrared Spectroscopy. (A) PS/Glycophorin. Infrared spectra of the C–H stretching regions at various temperatures for pure PS and for a 105:1 PS:glycophorin complex are shown in panels A and B, respectively, of Figure 1. The main spectral features in Figure 1A arise from the acyl chains of PS. The asymmetric and symmetric methyl C–H stretching bands appear near 2956 and 2872 cm^{-1} (Cameron et al., 1980) while the antisymmetric and symmetric CH_2 stretching bands are observed near 2920 and 2850 cm^{-1}. Additional features observed include the C–H stretching band from unsaturated PS chains near 3010 cm^{-1} and a board Fermi resonance band (Snyder et al., 1977) centered at about 2900 cm^{-1}. Similar spectral features are seen in the PS/glycophorin complex (Figure 1B). However, the peak intensity at 2920 cm^{-1} compared with that of the 2850-cm^{-1} band is greatly increased in the complex due to the presence of underlying broad features arising from protein C–H stretching vibrations.

Variations in temperature produce alterations in the line width and frequency of the various bands which have been used to monitor changes in the physical state of the lipid acyl chains (Cameron et al., 1980). In the current study, the CH_2 symmetric stretching band near 2850 cm^{-1}, free from interference due to underlying protein bands, is used to monitor the lipid acyl chains. Temperature-induced variations for the frequency of this band for PS, and for 60:1 and 105:1 PS:glycophorin complexes are shown in Figure 2 while the temperature-induced variation in the half-widths of this band for PS and for the 105:1 complex is plotted in Figure 3. Included for comparison in Figures 2 and 3 are data for a Ca^{2+}/PS complex.

Clear evidence for a phase transition in pure PS vesicles occurring between 8 and 16 °C is apparent from the cooperative change in the frequency or half-width of the band at 2850 cm^{-1} with temperature as shown in Figures 2 or 3. The observation is consistent with earlier differential scanning calorimetry studies of Stewart et al. (1979) and Jacobson & Papahadjopoulos (1975). The origin of the frequency increase noted in Figure 2 which occurs upon the introduction of disorder into the lipid hydrocarbon chains has been discussed by Snyder et al. (1982) and can be traced to changes in the interaction constants between C–H stretching coordinates on adjacent methylene groups when the lipid physical state is

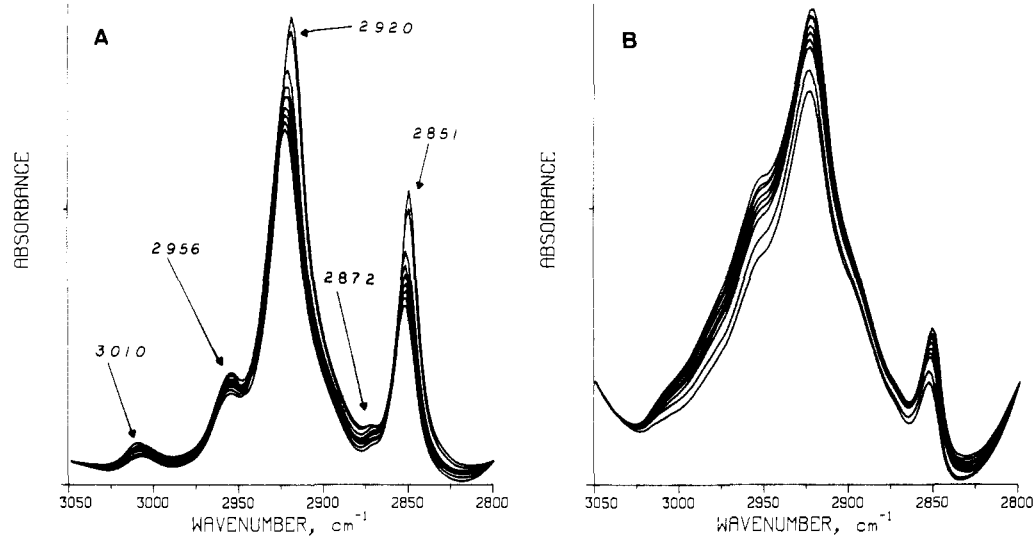

FIGURE 1: Overlaid plots illustrating the temperature dependence of the infrared spectra of (A) the C–H stretching region for pure PS and (B) the C–H stretching region for a 105:1 PS:glycophorin complex. Spectra decrease in peak height with increasing temperature and are plotted in intervals of about 6 °C over the range 5–55 °C. The assignment of the indicated spectral features in (A) is given in the text. The intensities in (A) and (B) are on a relative scale and cannot be compared directly.

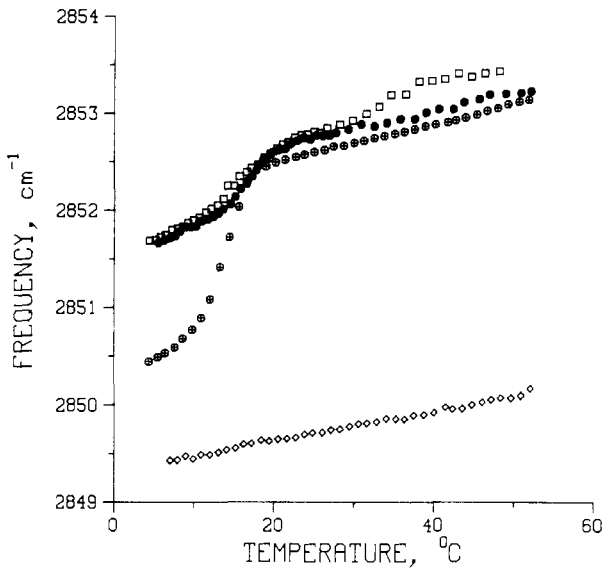

FIGURE 2: Plots of frequency vs. temperature for the symmetric CH_2 stretching mode near 2850 cm^{-1} for pure PS vesicles (⊕), for a 105:1 PS:glycophorin complex (□), for a 60:1 PS:glycophorin complex (●), and for PS/calcium (◇).

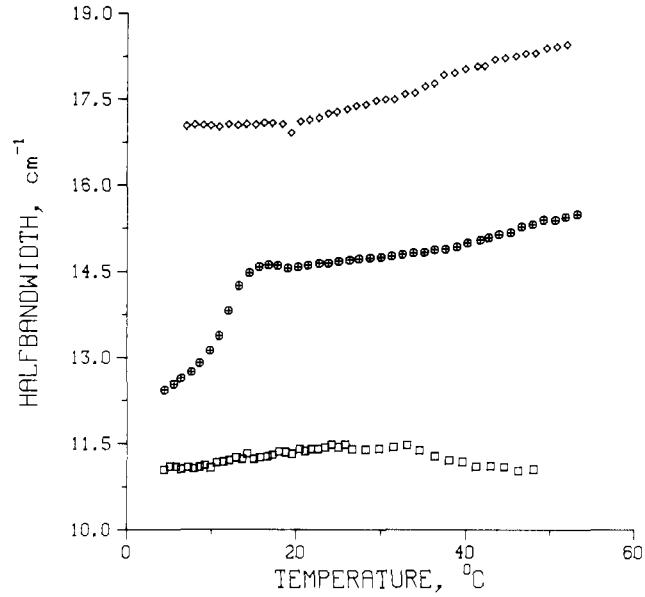

FIGURE 3: Plots of bandwidth (full width at half-height) for pure PS vesicles (⊕), for a 105:1 PS:glycophorin complex (□), and for PS/calcium (◇). The peak used for the measurement is the 2850-cm^{-1} band in each case.

altered. Addition of glycophorin induces marked changes in the lipid melting profile (Figure 2). At temperatures below 8 °C, where pure PS is in the gel state, the PS in the complex shows a C–H frequency indicative of significant disorder having been induced by protein. The gel–liquid-crystal phase transition is greatly reduced in amplitude although evidence remains for remnants of a cooperative lipid melting event between 14 and 22 °C. These residual melting processes are small in amplitude; however, they are quite reproducible and are seen in both 60:1 and 105:1 complexes. It is noted that no transitions can be observed calorimetrically at these levels of protein. At temperatures above the T_m for pure PS, the lipid in the complex shows a frequency only slightly changed by protein, suggesting that lipid order in the liquid-crystalline state is not significantly altered by protein and that glycophorin acts primarily to perturb the gel state. The effect of glycophorin on lipid phase behavior is markedly different from that of Ca^{2+} (Dluhy et al., 1983b). The latter, while also acting to abolish the lipid phase transition, significantly orders the acyl chains, as shown in Figure 2 by a reduction in the frequency of the 2850-cm^{-1} band in the presence of calcium at all temperatures.

Further evidence for the near-abolition of the cooperative melting process in the lipid as glycophorin is introduced is seen from the temperature dependence of the half-width of the 2850-cm^{-1} band (Figure 3). For pure PS, the half-width undergoes a sharp temperature-induced discontinuity over the range 8–16 °C which is abolished both by calcium and by protein. It is noted that calcium and glycophorin induce opposite changes in the magnitude of the line width of this CH_2 stretching band. The former induces an increase at all temperatures and the latter a decrease. It is difficult to correlate the absolute magnitude of changes in the half-width with any specific alterations in the structure of the lipid acyl chains, as the widths may be sensitive to many aspects of lipid structure, homogeneity, rates of motion, and environment (Snyder et al., 1982).

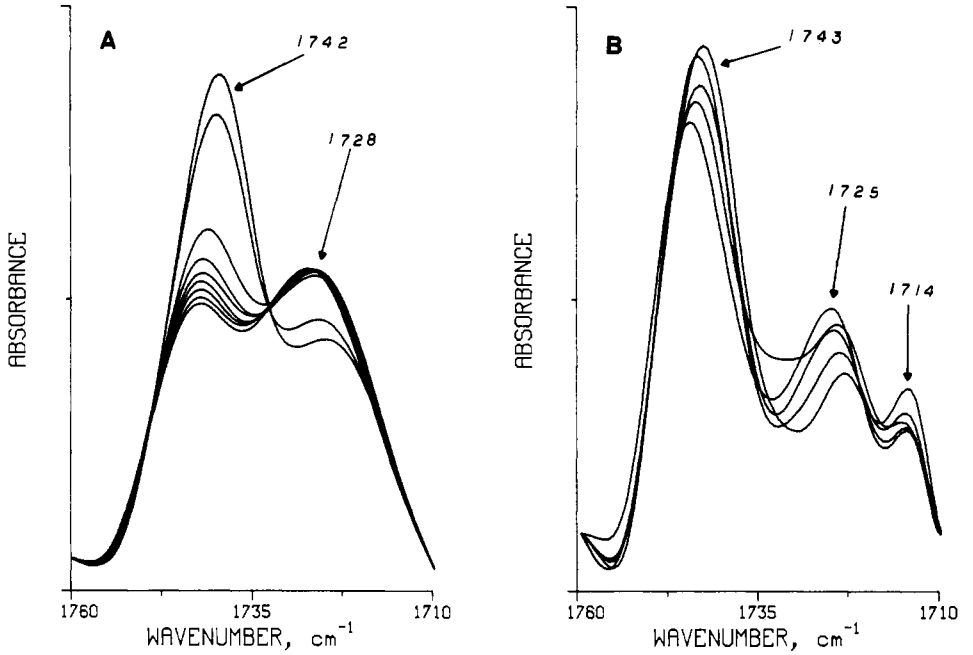

FIGURE 4: Overlaid plots illustrating the temperature dependence of the infrared spectra of (A) the C=O stretching mode of the lipid ester carbonyl for pure PS vesicles. The peak near 1742 cm^{-1} is reduced significantly in peak height as the temperature is increased. (B) The C=O stretching mode of a 105:1 PS:glycophorin complex. Note the presence of a new spectral feature near 1714 cm^{-1} upon the addition of protein. The temperature intervals over which the data are plotted are as in Figure 1. The data have been Fourier self-deconvoluted by removal of a 20-cm^{-1}-wide Lorentzian band shape and the bandwidths reduced by a factor of 1.8 (Kauppinen et al., 1981a,b).

In addition to data concerning lipid acyl chain structure, the current study yields information about the lipid interfacial region and protein secondary structure. The spectral region 1700–1750 cm^{-1} includes the C=O stretching mode of the lipid ester carbonyl (Figure 4A,B). For pure PS (Figure 4A), two features are noted near 1742 and 1728 cm^{-1}. The former is reduced significantly in peak height as the temperature is increased. Levin and co-workers (Levin et al., 1982; Mushayakarara et al., 1982; Mushayakarara & Levin, 1982) have assigned these features in phosphatidylcholines to particular acyl chains. The frequency at 1742 cm^{-1} is assigned to the C=O mode of the *sn*-1 chain with a trans conformation in the carbon–carbon bond adjacent to the ester grouping, while the 1728-cm^{-1} C=O frequency of the *sn*-2 chain suggests the presence of a gauche bend in that position. The observed frequency difference reflects the structural inequivalence of the chains, the *sn*-2 chain initially extending in a direction perpendicular to the *sn*-1 and then developing a gauche bend in order to render the two chains parallel (Pearson & Pascher, 1979).

Addition of glycophorin induces large changes in both the frequency and intensity patterns in the C=O spectral region (Figure 4B). The relative intensity of the higher frequency component is much increased from pure PS, while a new spectral feature is noted at 1714 cm^{-1}. Two types of related structural perturbation have been shown by Levin and co-workers to produce downward shifts in lipid carbonyl frequencies from the value of about 1728 cm^{-1} seen for C=O bonds adjacent to gauche bends. Insertion of a bond into a more polar environment (increased dielectric constant) results in a C=O frequency of about 1716 cm^{-1} (Mushayakarara et al., 1982), while participation of C=O in a hydrogen bond results in a frequency of about 1708 cm^{-1} (Mushayakarara & Levin, 1982). The current observed frequency of 1714 cm^{-1} is consistent with an *sn*-2 carbonyl in an environment more polar than in free PS, possibly involved in a weak H bond. It is noted that a band still remains at 1728 cm^{-1}, suggesting that not all the PS is interfacially perturbed by the protein.

The spectral region 1600–1700 cm^{-1} is quite complex and contains features from both the lipid carboxylate and protein amide I bands (Figure 5A,B). The spectrum of pure PS (Figure 5A) shows a carboxylate band near 1620 cm^{-1} whose frequency increases as the PS undergoes its gel–liquid-crystalline transition. Several new features are observed in the spectrum upon complexation of the PS with glycophorin (Figure 5B). The intense band near 1650 cm^{-1} is assigned to the amide I (primarily peptide bond C=O stretching) modes of glycophorin. In order to improve visualization of the spectral features which are highly overlapped in Figure 5B, a 30-cm^{-1} Lorentzian band has been Fourier self-deconvolved from the data, and the bands have been reduced in width by a factor of 1.8 (Kauppinen et al., 1981a,b). The results are replotted in Figure 6. The observed frequency (1653 cm^{-1}) is appropriate for the amide I vibration of peptide residues with α-helical or random-coil secondary structures (Lord & Mendelsohn, 1981). Other spectral features more clearly revealed upon deconvolution are bands near 1625 cm^{-1} and a shoulder on the amide I band near 1675 cm^{-1}. The intensity of the former relative to the lipid carbonyl indicates that it cannot be soley assigned to the PS carboxylate but must have a contribution from protein. Confirmation of this comes from the IR spectrum of sialic acid (not shown), which has a strong spectral feature in the 1625-cm^{-1} region. The sialic acid residues on glycophorin therefore contribute to the intensity of the 1625-cm^{-1} band in the complex. The shoulder at 1675 cm^{-1} arises from protein amide I vibrations of peptide bonds in β-sheet conformations (Lord & Mendelsohn, 1981). Consistent with this view, Schulte & Marchesi (1979) have examined the conformation of glycophorin and its constituent tryptic peptides by using circular dichroism. In addition to 27% helix and 63% random coil, 10% β form was observed, primarily arising from the hydrophilic peptides T1A and T3A located extracellularly in the native erythrocyte. Evidence for the correctness of the current FT-IR assignment comes from the relative intensities of the 1653- and 1675-cm^{-1} bands. The observed ratio of 8 or 9 to 1 is consistent with 90% helical plus

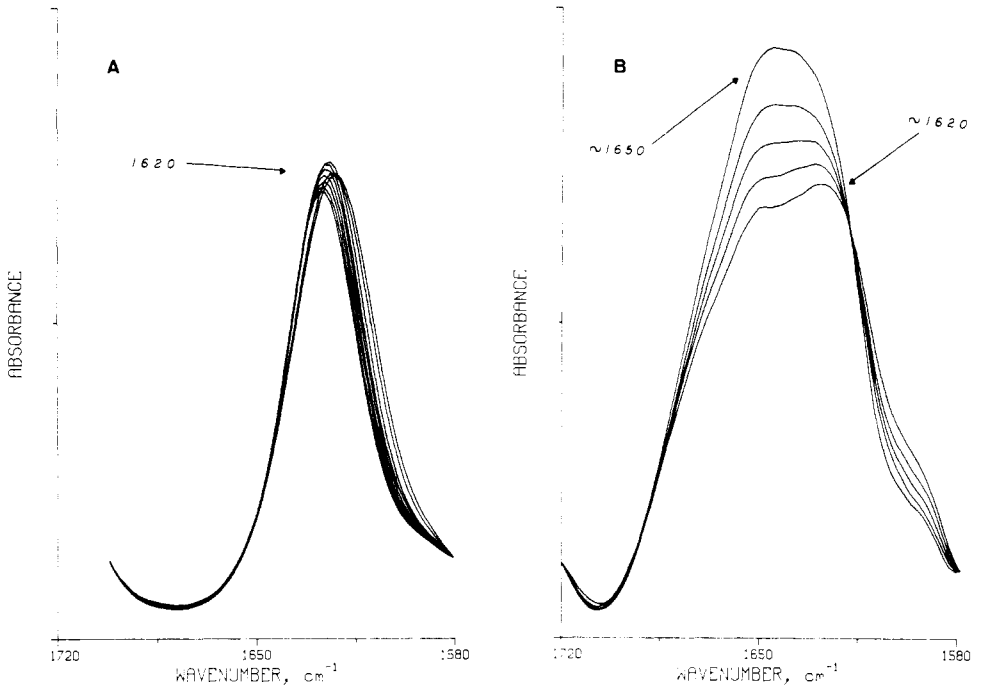

FIGURE 5: Overlaid plots illustrating the temperature dependence of the 1600–1700-cm^{-1} region for (A) pure PS vesicles. The 1620-cm^{-1} band arises from the antisymmetric COO^- stretching vibration. (B) A 105:1 PS:glycophorin complex. The intense band near 1650 cm^{-1} arises from protein amide I stretching vibrations. Spectra have not been subject to Fourier self-deconvolution.

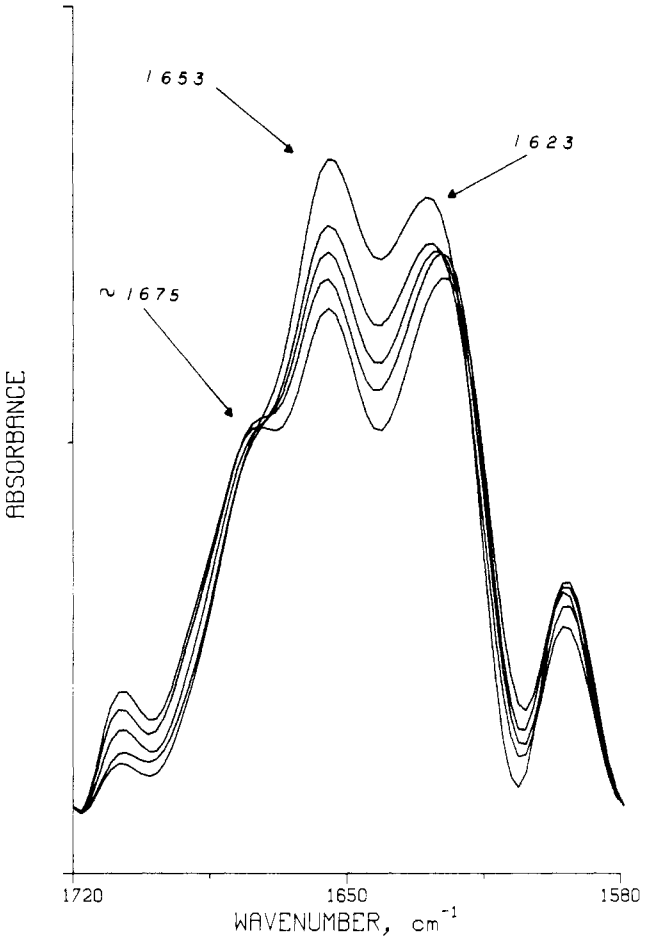

FIGURE 6: Data of Figure 5B, i.e., the 1600–1700-cm^{-1} spectral region for a 105:1 PS:glycophorin complex. However, a 30-cm^{-1}-wide Lorentzian band has been Fourier self-deconvolved from the data, and the bands have been reduced in width by a factor of 1.8.

random-coil structure and 10% β-sheet as found by Schulte & Marchesi (1979). When the temperature of the complex is raised, the shoulder near 1675 cm^{-1} broadens and merges with the main peak, suggesting a weakening and possible disruption of the β-sheet structure.

(B) PS/DPPC-d_{62}/Glycophorin. In order to directly compare the relative affinity of glycophorin for PS or PC, the ternary system PS/dipalmitoylphosphatidylcholine-d_{62}/glycophorin (50:50:1, mole ratios) was examined. The advantage of this system, in which the DPPC component has its acyl chains perdeuterated, lies in the fact that the C–D stretching modes absorb in a spectral region free from interference from the other components (Mendelsohn & Maisano, 1978). The effect of glycophorin on the melting of each lipid component may then be probed directly. The FT-IR parameter used to probe the deuterated chains of DPPC-d_{62} is the frequency of the symmetric CD_2 stretching modes near 2100 cm^{-1}. The procedure has been used previously (Dluhy et al., 1983a) for the ternary system DPPC/DMPC-d_{54}/glycophorin. The effect of 1 mol % glycophorin on each of the lipid components is shown in Figures 7 and 8 for DPPC-d_{62} and PS, respectively, where the temperature dependences of the CD_2 symmetric stretching mode (for perdeuterated DPPC) and the CH_2 symmetric stretching mode (for PS) are plotted. The appropriate control systems, a 1:1 binary mixture of the two lipids along with the pure lipid components, are included for comparison in each case. As shown in Figure 7, the melting of pure DPPC-d_{62} occurs rather sharply at 37 °C. Addition of 50 mol % PS broadens the melting, in a way analogous to that observed for PS/DPPC in the differential scanning calorimetry (DSC) studies of Stewart et al. (1979). Addition of glycophorin narrows the range of melting of the binary lipid system from 16 °C (17–33 °C) in PS/DPPC-d_{62} to 10 °C (21–31 °C) in the ternary case.

It is of particular interest that the onset of melting for the DPPC-d_{62} component is increased in temperature when glycophorin is added to a binary mixture of PS/DPPC-d_{62}. Finally, it is noted that in both the gel and liquid-crystalline phases, addition of a second lipid component followed by protein leads to progressively increasing disorder in DPPC-d_{62} as monitored by the increase in the CD_2 frequency.

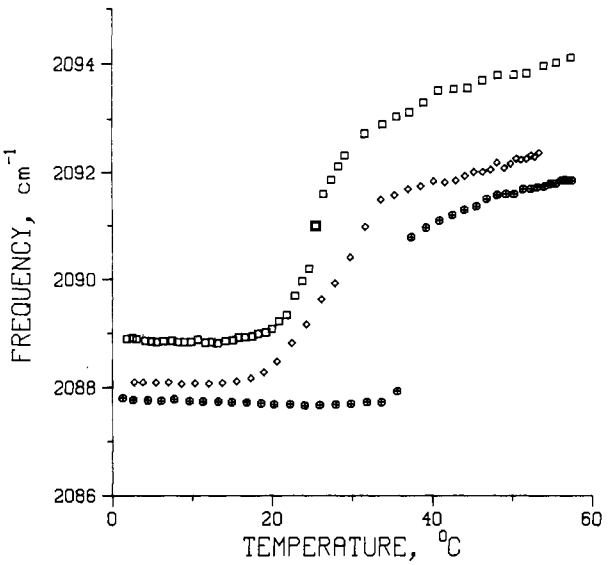

FIGURE 7: Plots of frequency vs. temperature for the symmetric CD_2 stretching mode of DPPC-d_{62} in multilamellar vesicles (⊕), in a 1:1 mole ratio vesicle complex with PS (◊), and in the ternary system DPPC-d_{62}/PS/glycophorin with a 50:50:1 mole ratio (□). Note the increase in the temperature at the onset of melting for the ternary complex compared with the binary lipid mixture.

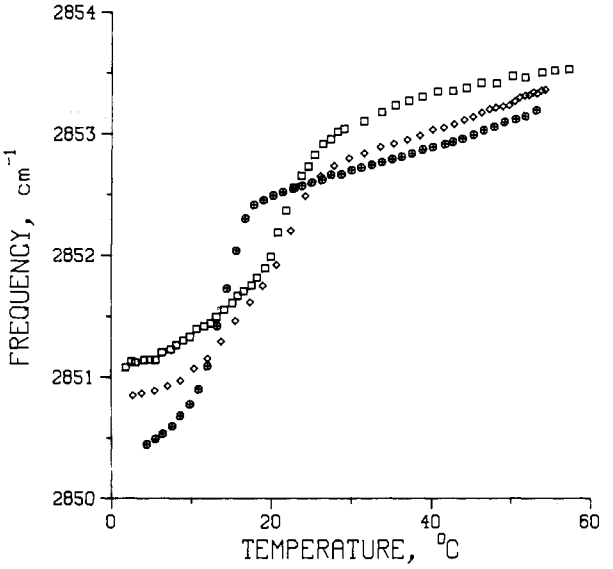

FIGURE 8: Plots of frequency vs. temperature for the symmetric CH_2 stretching vibrations of PS in pure PS vesicles (⊕), in a 1:1 mole ratio vesicle complex with DPPC-d_{62} (◊), and in the ternary system 50:50:1 DPPC-d_{62}/PS/glycophorin (□).

The melting of pure PS shown in Figure 8, as discussed previously, indicates a phase transition between 8 and 16 °C. In the PS/DPPC-d_{62} binary lipid mixture, the transition range is broadened to 15 °C and is observed from 9 to 24 °C. The onset temperature is not well-defined while a slight inflection in the data is seen at 20 °C. Addition of protein to the binary system produces subtle changes in the shape of the PS melting curve (Figure 8). The onset temperature, poorly defined in the binary lipid mixture, is no longer discernible. Instead, disorder is noncooperatively introduced into the acyl chains over the range 4–20 °C. At 20 °C, the inflection noted in the binary mixture is better delineated, so that a residual melting event occurs over the range 20–25 °C.

Discussion

The effects of 1 mol % glycophorin on the phase behavior of PS as observed in the current study are considerably more pronounced than those noted for DMPC (Mendelsohn et al., 1981). The effect of glycophorin on the latter was to slightly reduce T_m and to broaden the melting range. The lipid phase transition was still clearly defined. In contrast, as seen in the current work, glycophorin nearly abolishes the gel–liquid-crystal transition seen in pure PS, leaving a slight residual melting shifted to higher temperatures (transition range in pure PS, 8–16 °C; in PS/glycophorin, 14–22 °C). While the detection of this residual transition approaches the limit of experimental precision, it was observed with several different infrared spectral parameters as indicators, as well as in samples with different lipid:protein ratios (Figure 2). In addition, FT-IR data clearly show that glycophorin introduces static disorder into the gel phase of PS, a result of being unable to pack efficiently into an all-trans acyl chain environment.

The current data for the melting of the lipid acyl chains, coupled with the observed alterations in the C=O bands that reflect protein-induced changes in the PS interfacial regions, suggest, but do not prove, that glycophorin interacts more strongly (preferentially) with PS compared with PC. The results of the FT-IR experiments on the ternary system bear directly on this point. The observed changes in the melting profiles for DPPC-d_{62} (Figure 7) upon going from the binary mixtures of PS/DPPC-d_{62} to the ternary complex are consistent with the protein preferentially interacting with the PS component. When this interaction takes place, the residual bulk lipid becomes enriched in DPPC-d_{62}. As expected for a higher melting component, this lipid undergoes a phase transition whose melting range is reduced in magnitude but exhibits a higher onset temperature than in the unperturbed binary lipid mixture.

It is noted that calorimetric data (not shown) for the ternary system are consistent with this interpretation. The excess heat capacity curve shows a phase transition whose melting range is reduced from the binary mixture and which occurs at higher temperatures. The transition enthalpy is also reduced. A detailed discussion of the calorimetric data will be given elsewhere. Further evidence in support of the preferential interaction of glycophorin with the PS component comes from Raman spectroscopic studies of glycophorin/DPPC-d_{62} (Taraschi & Mendelsohn, 1980). In that system, the lipid melting range was broadened and reduced in temperature upon the addition of protein. In contrast, the current study shows that the DPPC-d_{62} in the ternary system melts with a narrower range and a higher onset temperature than in the binary lipid mixture. This is the result expected only if the DPPC-d_{62} is selectively excluded from the vicinity of glycophorin; i.e., a preferential interaction occurs with PS.

The current results are consistent with suggestions of van Zoelen et al. (1977) and Armitage et al. (1977) that, after lithium diiodosalicylate extraction of the protein from the erythrocyte membrane, some anionic lipid remains tightly bound to protein. Furthermore, van Zoelen et al. (1977) found that glycophorin treated with neuraminidase (thereby removing sialic acid) preferentially interacted with monomers of negatively charged lipid molecules. The current experiments demonstrate a preferential interaction with anionic lipids (PS) in a direct way by using a model membrane with a well-defined chemical composition and bilayer structure.

There have been two recent studies which bear on glycophorin interaction with lipid classes other than the PC's. Ong & Prestegard (1982) have used ^{13}C NMR to study the interaction of glycophorin with PC/PE mixtures in varying proportions. Some preferential interaction with PE was noted. The ability of glycophorin to control lipid polymorphism was

shown by Tarachi et al. (1983), who demonstrated that protein exerted a bilayer-stabilizing effect on cardiolipin.

The current study and related work referred to above demonstrate the need for examination of a variety of lipid classes as to their association with a particular membrane protein before attempting to construct general models for lipid/protein interactions. Results from experiments with neutral lipid classes cannot be assumed applicable to acidic systems. The FT-IR approach appears to be a useful method for mapping out particular preferences in a nonperturbative fashion.

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