Conformational Order of Phospholipids Incorporated into Human Erythrocytes: An FTIR Spectroscopy Study†

David J. Moore,‡,§ Richard H. Sills,‡ Neeta Patel,§ and Richard Mendelsohn*,§

Department of Pediatrics, The Children's Hospital of New Jersey, and New Jersey Medical School/University of Medicine and Dentistry of New Jersey, Newark, New Jersey 07107, and Department of Chemistry, Newark College of Arts and Sciences, Rutgers University, 73 Warren Street, Newark, New Jersey 07102

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ABSTRACT: Acyl chain perdeuterated dimyristoylphosphatidylcholine (DMPC-d_{54}) and dimyristoylphosphatidylserine (DMPS-d_{54}) were incorporated by incubation into human erythrocytes. Light microscopic analysis demonstrated that erythrocytes incubated with DMPC-d_{54} became echinocytic while those incubated with DMPS-d_{54} became stomatocytic. This indicates that DMPC-d_{54} was incorporated preferentially into the outer monolayer whereas DMPS-d_{54} was selectively incorporated into the inner monolayer. Fourier transform infrared (FTIR) spectroscopy was used to monitor the conformational order of the incorporated phospholipids. The asymmetric CD_2 stretching frequency of the inserted perdeuterated acyl chains was measured in both isolated membranes and intact erythrocytes as a function of temperature. DMPC-d_{54} incorporated into erythrocytes exhibited a cooperative phase transition at ∼19 °C, i.e., at the same temperature as pure vesicles. In contrast, DMPS-d_{54} incorporated into red cells exhibited no phase transition, but possessed conformational order similar to that of the liquid-crystalline state. These results suggest that DMPC-d_{54} persists in domains in the outer monolayer while DMPS-d_{54} is dispersed in the inner monolayer. These experiments are the first to demonstrate that FTIR spectroscopy can be utilized to monitor directly a specific species of lipid molecule from the entire phospholipid population.

Much of our current understanding of biological membranes is derived from studies of human erythrocytes. The asymmetric distribution of lipid classes across the human erythrocyte membrane is well established (Devaux & Zachowski, 1994; Schroit & Zwaal, 1991). This asymmetry is partially maintained by a Mg^{2+}- and ATP-dependent protein which specifically transfers aminophospholipids (i.e., PE[1] and PS) across the membrane bilayer (Devaux, 1992). The preservation of lipid asymmetry is important in the maintenance of the biconcave shape of the erythrocyte (Devaux, 1991) as well as other cellular functions (Zachowski, 1993; Connor et al., 1994).

Changes in the relative composition, or area, of the lipid monolayers of the membrane bilayer can alter the morphology of the human erythrocyte. Introducing exogenous lipids, specifically, into the inner or outer leaflet of the red cell membrane produces characteristic changes in morphology. Incubation of erythrocytes with phosphatidylcholine (PC) or phosphatidylserine (PS) results in echinocytosis or stomatocytosis, respectively (Ferrell et al., 1985a,b; Daleke & Huestis, 1989). This arises because PC is incorporated into, and remains in, the outer monolayer whereas PS is transported to the inner monolayer; the resulting expansion of either the inner or the outer monolayer produces the characteristic shape change.

The concept of domains in cell membranes has become central to the explanation and understanding of membrane structure, organization, and function (Welti & Glaser, 1994; Tocanne et al., 1994). Domains may consist of proteins or lipids and can be either lateral or transverse. A variety of mechanisms are postulated to play a role in the formation of membrane lipid domains including interactions between proteins, both cytoskeletal and integral, and membrane lipids. The presence of membrane lipid domains is postulated to be important for the function of a variety of membrane proteins (Glaser, 1993; Gascard et al., 1993). Several techniques have been applied to the study of lipid structure and domain formation in monolayers and bilayers including X-ray scattering (Möhwald, 1990), Brewster angle microscopy (Overbeck et al., 1994), and fluorescence microscopy (McConnell, 1991; Stine, 1994). Fluorescence studies of domains in pure lipid systems have produced remarkable images, depicting the form these domains can take (Rice & McConnell, 1989; Flörsheimer & Möhwald, 1989). More recently, microscopy with both fluorescently labeled lipids and proteins has been applied to the study of lipid and protein domains in erythrocyte membranes (Rodgers & Glaser, 1991, 1993). In general, hemoglobin quenching and the biconcave shape of the intact erythrocyte make fluorescence studies in red cells problematic, and, therefore, such studies are usually conducted on ghosts (Wang et al., 1993). Nonetheless, these experiments have demonstrated the presence of lipid and protein domains in the erythrocyte membrane.

FTIR spectroscopy is increasingly being applied to the qualitative and quantitative study of molecular conformational order in models for biological membranes (Mendelsohn & Senak, 1993; Mantsch & McElhaney, 1991). However,

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* To whom correspondence should be addressed.

‡ The Children's Hospital of New Jersey and New Jersey Medical School/University of Medicine and Dentistry of New Jersey.

§ Rutgers University.

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[1] Abbreviations: FTIR, Fourier transform infrared; PS, phosphatidylserine; PE, phosphatidylethanolamine; DMPS-d_{54}, acyl chain perdeuterated dimyristoylphosphatidylcholine; DMPC-d_{54}, acyl chain perdeuterated dimyristoylphosphatidylserine.

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only recently has FTIR spectroscopy been successfully used to measure conformational order in whole cell membranes. Experiments from this laboratory with live mycoplasma (Moore & Mendelsohn, 1994) and intact human erythrocytes (Moore et al., 1995) have demonstrated the utility of FTIR spectroscopy as a unique biophysical technique for obtaining molecular conformation information from live cells. In general, the extremely fast time scale of molecular vibrations (10^{-12} s) and the ability to monitor endogenous molecules, rather than chemically quite distinct probe molecules, are significant advantages of this technique.

Our prior study utilized the C−H stretching modes of the erythrocyte membrane acyl chains to evaluate conformational order in peroxidized cells (Moore et al., 1995). Such studies provide an overview of the conformational status of the entire membrane. Since the conformation-sensitive vibrational frequencies of each lipid class are quite similar, it was not possible to isolate the behavior of specific phospholipid components of the membrane. To address this issue, acyl chain perdeuterated phosphatidylserine (PS) and phosphatidylcholine (PC) were incorporated into the cell membranes, and the frequency of the asymmetric CD_2 stretching mode was monitored in the FTIR spectra. The distinctive infrared stretching modes of the CD_2 groups are readily distinguished from all other membrane vibrations and were used to monitor the conformational order of these individual phospholipids within the membrane.

MATERIALS AND METHODS

Materials. Acyl chain perdeuterated dimyristoylphosphatidylserine (DMPS-d_{54}) and dimyristoylphosphatidylcholine (DMPC-d_{54}) were purchased from Avanti Polar Lipids (Alabaster, AL). All other chemicals were of cell culture grade and purchased from Sigma Chemical Co. (St.Louis, MO).

Erythrocytes. Human erythrocytes were collected from healthy adult volunteers by venipuncture into heparin. Cells were washed 3 times with phosphate-buffered saline (PBS: 138 mM NaCl, 5 mM KCl, 6.1 mM Na_2HPO_4, 1.4 mM NaH_2PO_4, and 5 mM glucose, pH 7.4), and the buffy coat was removed.

Vesicle Preparation. DMPS-d_{54} and DMPC-d_{54} were suspended in PBS (1.5 mg/mL) and sonicated in a bath sonicator for 1 h. These solutions partially clarified.

Erythrocyte−Vesicle Incubations. Packed, washed erythrocytes (1 mL) were mixed with an equal volume of sonicated vesicles (1.5 mg/mL) to give a final cell concentration of 50%. For overnight incubation periods (14 h), the PBS was supplemented with additional glucose and penicillin. In all experiments, a parallel control incubation (minus only the lipid vesicles) accompanied the fusion experiment. The level of incorporation of exogenous phospholipids was determined either by gas chromatographic analysis of methyl esters prepared from phospholipids extracted from membranes or from the relative integrated intensities of the CD_2 and CH_2 stretching vibrations in the IR spectra of extracted lipids, first corrected for different extinction coefficients with standard samples. Incorporation levels ranged from about 1−3%.

Erythrocyte Morphology. Interference contrast microscopy (ICM) was used to examine the morphology of erythrocytes after incubation with DMPS-d_{54}, DMPC-d_{54}, or PBS only. At the end of the incubation period, an aliquot of cells was fixed in 1% glutaraldehyde in PBS. A further sample was fixed after the cells had been washed 2 times with 10 volumes of PBS to remove any unincorporated lipid. These fixed samples were then examined with ICM. The morphologic index was determined as previously described (Fuji et al., 1979; Daleke & Huestis, 1989). Discocytes were given a score of 0, stomatocytes were scored from −1 to −4, and echinocytes from +1 to +5. The morphologic index was determined from averaging 200 cells by a single blinded observer.

Methods and Sample Preparation for FTIR Spectroscopy. All spectra were acquired on a Mattson RS-1 spectrometer by co-addition of 1024 interferograms collected from 4000 to 400 cm^{-1} at 4 cm^{-1} resolution. The interferograms were apodized with a triangular function and Fourier-transformed with one level of zero-filling. To minimize water vapor absorption, the spectrometer was continually purged with N_2 gas. The CaF_2 windows enclosing all samples were held in a thermostated transmission cell (Harrick Scientific, Ossining, NY). Temperature was controlled with a circulating water bath (Fisher Scientific, Springfield, NJ).

Sonicated vesicle suspensions of pure DMPS-d_{54} and DMPC-d_{54} were placed between CaF_2 windows separated with a 25 μm spacer. Spectra were acquired from 10 to 40 °C.

After incubation, erythrocyte samples were spun down on a table-top centrifuge, and the supernatant was removed. The erythrocytes were then washed with 10 volumes of PBS and the cells separated from the vesicles by centrifugation for 3 min at 3000*g* (Daleke & Huestis, 1989). This washing process was then repeated, and, after removing the supernatant the second time, the erythrocyte pellet was concentrated by spinning for 5 min at 10000*g*. To test for the presence of vesicles, FTIR spectra were acquired from successive supernatants. Using the spectroscopy protocols described below, CD_2 stretching modes from perdeuterated lipid could be measured only in the initial supernatants. Experiments with cells incubated for short times (<1 min) with either DMPC-d_{54} or DMPS-d_{54}, and then washed, exhibited neither morphological changes nor CD_2 stretching vibrations. This implies that there was no sticking nor trapping of the vesicles. For FTIR spectroscopy, the final (vesicle-free) washed erythrocyte sample was placed between two CaF_2 windows separated with a 50 μm spacer. Spectra were acquired over the desired range of temperatures (10−32 °C for PC-enriched samples and 10−40 °C for PS-enriched samples) at 2−3° intervals for some experiments and at 1° intervals when detailed evaluation of phase transition temperatures was sought.

Isolated erythrocyte membranes ("ghosts") were prepared by a standard protocol (Steck & Kant, 1974). After incubation, washed erythrocytes were lysed in 40 volumes of ice-cold 5 mM phosphate buffer. This procedure was repeated 2 more times, yielding hemoglobin-free white ghosts. For FTIR spectroscopy, the membrane samples were concentrated on an ultracentrifuge and placed between CaF_2 windows separated with a 25 μm spacer. Spectra were acquired over temperature intervals and ranges as noted above.

Processing of FTIR Spectra. At the relatively low concentration of lipid used for the pure vesicle samples, it was necessary to use spectral subtraction of water to confirm

the presence of CD_2 stretching bands. Fourier second derivatives were computed from interferograms that had been truncated (break point 0.6). Peak positions were then calculated with a center-of-gravity routine. This choice of break point somewhat reduced the spectral noise without distorting the peaks. The symmetric and asymmetric CD_2 stretching vibrations modes appear at ~2090 and 2190 cm^{-1}, respectively. The broad contour from ~2000 to 2300 cm^{-1} present in all spectra arises from a mode of associated water molecules. The symmetric CD_2 stretching mode at ~2090 cm^{-1} is inherently less intense than the asymmetric mode and is overlapped by water vapor absorption. Therefore, the frequency of the asymmetric stretching mode was preferred as a measure of acyl chain conformational order in perdeuterated DMPS and DMPC, whether in pure vesicles or in lipids incorporated into the erythrocyte membrane.

The frequency of the symmetric CH_2 stretching vibration, arising from the native lipids of the red cell membrane, was measured in the second-derivative spectra of ghosts from erythrocytes with incorporated DMPS-d_{54} or DMPC-d_{54}.

RESULTS

The photographs in Figure 1 depict typical erythrocyte samples after 14 h of incubation at 37 °C. The cells in Figure 1A are control cells incubated in PBS alone. Erythrocytes incubated with DMPS-d_{54} are shown in Figure 1B; these cells are clearly stomatocytic, indicating that the DMPS has been incorporated into the inner monolayer of the cell membrane. The echinocytic cells in Figure 1C depict erythrocytes after incubation with DMPC-d_{54} vesicles. The echinocytosis indicates that the DMPC-d_{54} is located primarily in the outer monolayer of the cell membrane. The average morphological indices for cells with DMPS-d_{54} or DMPC-d_{54} incorporated were −1.7 and +2.8, respectively.

In Figure 2A−C the original, difference, and second-derivative FTIR spectra (taken at 12 °C) of erythrocytes incubated with DMPS-d_{54} for 14 h are displayed. Equivalent spectra of erythrocytes incubated with DMPC-d_{54} are displayed in Figure 3. The bands at ~2090 and 2195 cm^{-1} arise in each case from the symmetric and asymmetric CD_2 stretching vibrations, respectively. The difference spectra (Figures 2B and 3B) clearly reveal the presence of the CD_2 stretching modes, but the difficulty in selecting a reproducible, consistent subtraction factor led us to utilize the second-derivative spectra (Figures 2C and 3C) for precise frequency determinations.

The second-derivative spectra of the CH_2 and CD_2 stretching regions of ghosts isolated from erythrocytes fused with DMPS-d_{54} are displayed in Figure 4A and Figure 4B, respectively. The symmetric (~2850 cm^{-1}) and asymmetric (~2920 cm^{-1}) CH_2 stretching modes from the native lipids are the dominant features in the upper spectrum. In the lower spectrum, the CD_2 stretching modes from the exogenous lipid are the dominant features. Equivalent ghost spectra from DMPC-d_{54}-fused erythrocytes are displayed in Figure 5A,B. All data are for spectra taken at 12 °C.

The thermotropic behavior of the asymmetric CD_2 stretching frequency is plotted in Figure 6A,B,C for pure sonicated DMPS-d_{54} vesicles, ghosts from incorporated erythrocytes with DMPS-d_{54} incorporated, and intact red cells with DMPS-d_{54} incorporated, respectively. The gel−liquid-crystal phase transition of DMPS-d_{54} in pure vesicles is evident at

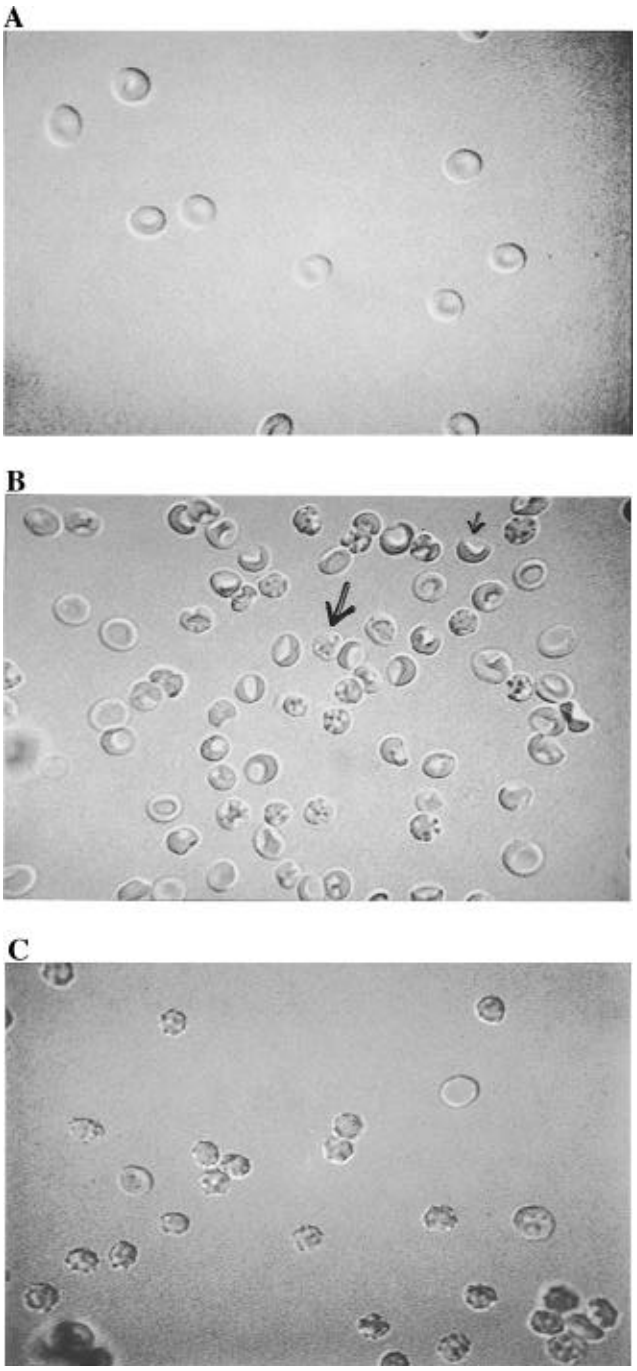

FIGURE 1: (A) Normal discocyte human erythrocytes. (B) Erythrocytes after 14 h of incubation with DMPS-d_{54}. A typical stage 1 cup-shaped stomatocyte is indicated by the small arrow; the larger arrow indicates a stage 3 stomatocyte with multiple invaginations. (C) Erythrocytes after 14 h of incubation with DMPC-d_{54}. The typical spikelike protrusions of echinocytes are clearly visible in almost all of the cells.

~34 °C. The phase transition is abolished for cells with the DMPS-d_{54} component incorporated into the inner erythrocyte monolayer (Figure 6C) and in ghosts derived from PS-incubated erythrocytes (Figure 6B). It is noted that the scatter in Figure 6C, which ranges from 0.75 to 1.5 cm^{-1}, arises from two sources. First, the weak signals limit the precision of the center of gravity algorithm. Second, there is sample to sample variability in the morphological indices as well as in the extent of incorporated phospholipids. Single experiments from samples with high morphological indices show somewhat better precision as judged by the constancy

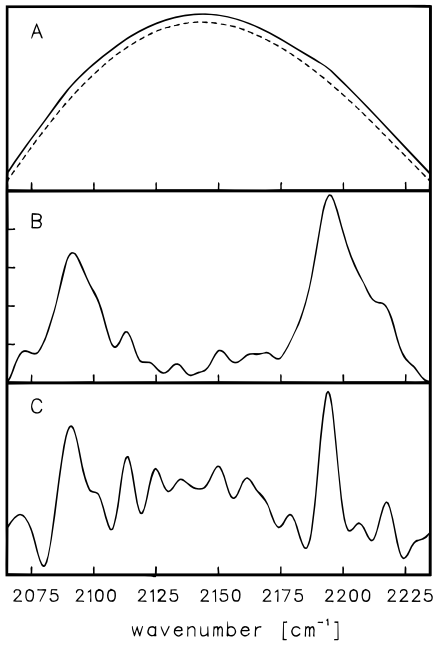

FIGURE 2: FTIR spectra (A) of erythrocytes with DMPS-d_{54} incorporated (—) and buffer (- - -). Below are the difference spectrum generated by subtraction of buffer (B) and the second-derivative spectrum (C). The original spectrum was acquired at 12 °C. In (B), the distance between tick marks on the ordinate scale represents 1 milliabsorbance unit (mAU).

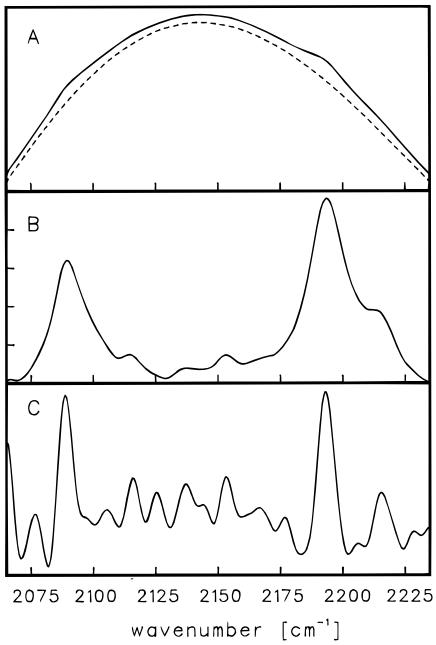

FIGURE 3: FTIR spectra (A) of erythrocytes with DMPC-d_{54} incorporated (—) and buffer (- - -). Below are the difference spectrum generated by subtraction of buffer (B) and the second-derivative spectrum (C). The original spectrum was acquired at 12 °C. In (B), the distance between tick marks on the ordinate scale represents 1 milliabsorbance unit (mAU).

of the measured frequency as the temperature is increased. Such a data set is shown in Figure 7, and provides further evidence for the abolition of the phase transition. In Figure 8A,B,C, the thermotropic responses of the asymmetric CD_2 stretching frequency are plotted for DMPC-d_{54} in pure vesicles, for ghosts from red cells with DMPC d_{54}-incorporated, and for intact erythrocytes with DMPC-d_{54} incorporated. The gel−liquid-crystal phase transition of

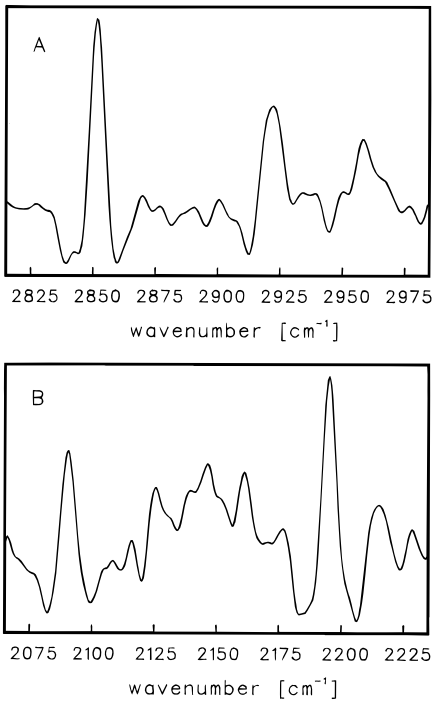

FIGURE 4: Second-derivative spectra of ghosts from erythrocytes with DMPS-d_{54} incorporated. (A) shows the symmetric and asymmetric CH_2 stretching modes at ∼2850 and 2920 cm^{-1}; (B) shows the symmetric and asymmetric CD_2 stretching modes at ∼2090 and 2190 cm^{-1}. The spectrum was acquired at 12 °C.

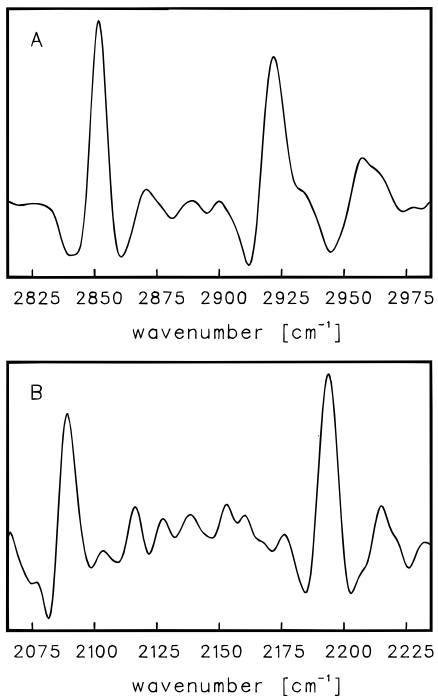

FIGURE 5: Second-derivative spectra of ghosts from erythrocytes with DMPC-d_{54} incorporated. (A) shows the symmetric and asymmetric CH_2 stretching modes at ∼2850 and 2920 cm^{-1}; (B) shows the symmetric and asymmetric CD_2 stretching modes at ∼2090 and 2190 cm^{-1}. The spectrum was acquired at 12 °C.

DMPC-d_{54} in pure vesicles is clearly evident at ∼19−20 °C, with a frequency increase of about 1.5 cm^{-1} at the transition In fused erythrocytes (Figure 8C), the transition is still detectable with about a 1 cm^{-1} increase in the frequency. As in the case of PS-fused cells, the scatter in the data points may arise from different morphological indices as well as

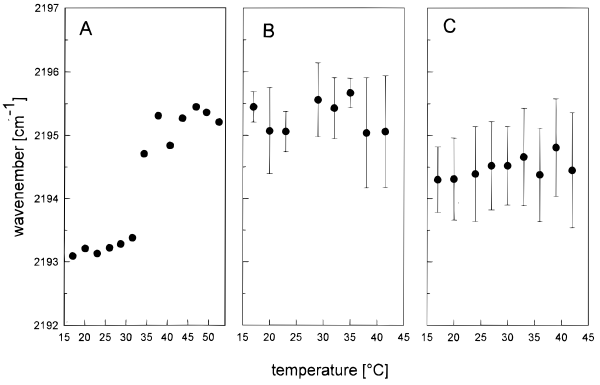

FIGURE 6: Thermotropic response of the asymmetric CD_2 stretching frequency of DMPS-d_{54} in pure vesicles (A), in ghosts from erythrocytes with DMPS-d_{54} incorporated (B), and in intact red cells with DMPS-d_{54} incorporated (C). In (B) and (C), the values are the mean and standard deviation of four separate experiments.

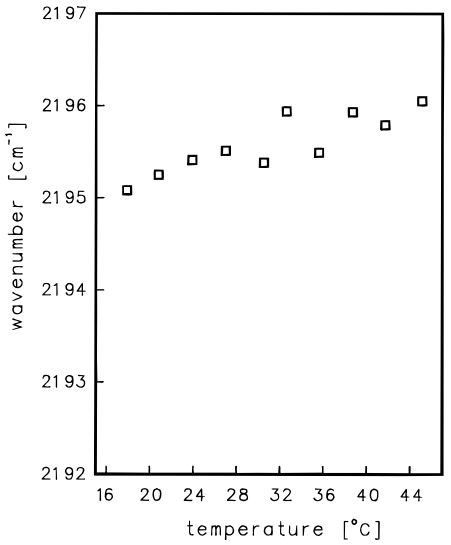

FIGURE 7: Thermotropic run depicting the variation of the asymmetric CD_2 stretching frequencies in a sample of intact red cells with DMPS-d_{54} incorporated. The sample has a relatively high morphological index.

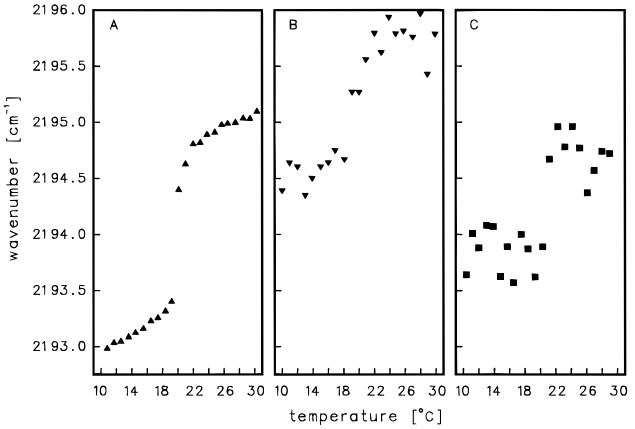

FIGURE 8: Thermotropic response of the asymmetric CD_2 stretching frequency of DMPC-d_{54} in pure vesicles (A), in ghosts from erythrocytes with DMPC-d_{54} incorporated (B); and in intact red cells with DMPC-d_{54} incorporated (C). In (B) and (C), the values are taken from five or six separate experiments and are averaged for a particular temperature.

weak signals. The CD_2 stretching frequencies from a single thermotropic experiment for a sample with a high morphologic index are shown in Figure 9. In this case, the full range

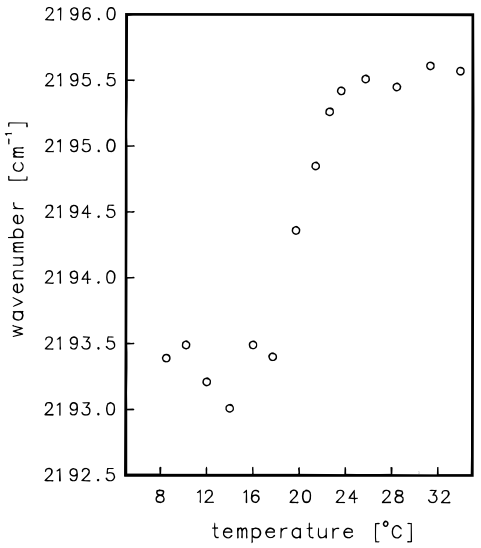

FIGURE 9: Thermotropic run depicting the variation of the asymmetric CD_2 stretching frequencies in a sample of intact red cells with DMPC-d_{54} incorporated. The sample has a relatively high morphological index.

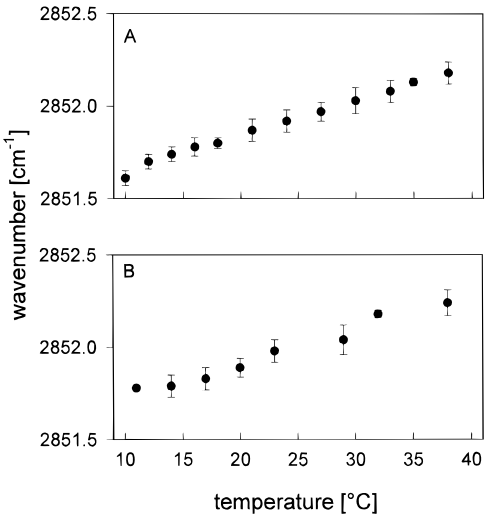

FIGURE 10: Thermotropic response of the symmetric CH_2 stretching frequency in ghosts from red cells with DMPC-d_{54} incorporated (A) and from red cells with DMPS-d_{54} incorporated (B).

of frequency shift was observed. The transition range appears to be slightly broadened, with a higher completion temperature, although this observation is near the limit of experimental precision. In ghosts derived from fused erythrocytes (Figure 8B), a transition of reduced magnitude (approximately a 1 cm^{-1} change in the frequency compared with 1.5 cm^{-1} for vesicles) is observed. The frequencies in this case are higher at all temperatures than those of the intact cells or vesicles. The origin of this observation is unclear, but may be related to the effects of altered refractive indices in going from relatively dilute vesicles or cells to more highly concentrated ghosts. In any case, the persistence of the transition is demonstrated.

Figure 10A and Figure 10B display the symmetric CH_2 stretching frequency in ghosts from erythrocytes which have incorporated DMPC-d_{54} or DMPS-d_{54}, respectively.

DISCUSSION

It has been demonstrated that normal erythrocytes can incorporate phospholipids with varying acyl chain length

(Daleke & Huestis, 1985; Ferrell et al., 1985a,b). Several groups have determined that PC preferentially incorporates into the outer monolayer, expanding it to cause the formation of echinocytes, which in turn provide a direct morphological marker for the specific incorporation. PS incorporates into the outer monolayer and is effectively translocated into the inner leaflet, expanding it, and resulting in the formation of stomatocytes. Our microscopic findings, rendered more quantitative by the use of the morphologic indices, confirm that perdeuterated DMPS and DMPC behaved comparably to proteated DMPC and DMPS. Samples incubated with DMPC-d_{54} consistently demonstrated echinocytosis and the expected positive values for the morphologic indices while samples incubated with DMPS-d_{54} consistently contained stomatocytes and negative morphologic indices. These findings provide a simple confirmation of the location of the incorporated deuterated phospholipids within the membrane. The morphologic data are also significant because they occur in clinical human disease as well as in experimental models (Bessis, 1973).

The lower sensitivity of FTIR compared with fluorescence techniques using specifically labeled molecules is such that the entire population of the phospholipid (1−3% of the total phospholipid) to be inserted had to be acyl chain perdeuterated to provide an adequate spectroscopic signature. However, in the current study, the ability of deuterated lipids to induce morphological changes equivalent to incorporated native (i.e., proteated) lipids was evident, thus removing possible ambiguities about the location of the probe species.

The important new aspect of this work is the application of FTIR spectroscopy to monitor the conformational order of the exogenous lipids in the living cell, through examination of the thermotropic behavior of the CD_2 stretching modes. Our prior studies have demonstrated the usefulness of FTIR spectroscopy in examining lipid extracts of erythrocyte membranes (Sills et al., 1994) as well as isolated membranes and intact erythrocytes (Moore et al., 1995). The earlier work involved examination of the CH_2 stretching vibrations which arise from a weighted average of the methylenes according to their relative proportions in the membrane. Examination of the thermotropic response of these CH_2 stretching vibrations (Figure 10) reveals that the average conformational state of all the acyl chains is somewhere between a fully ordered state (∼2849−2850 cm^{-1}) and a disordered state (∼2853−2854 cm^{-1}). The gradually increasing frequency as the temperature is increased from 10 to 40 °C represents the progressive occurrence of conformational disorder. Evidence for domains (possibly arising from specific lipid classes) is not seen in spite of the high precision (better than 0.1 cm^{-1}) of the data (Figure 10). This is not surprising, since the CH_2 stretching frequency arises from the more than 200 different phospholipids within the erythrocyte membrane (Kuypers et al., 1994). Thus, CH_2 stretching vibrations arising from a specific membrane phospholipid domain would be difficult to detect. Our current experimental approach utilizes the asymmetric CD_2 stretching vibrations which are located in an IR spectral region relatively free of interference from other vibrational modes. Thus, we are able to detect vibrations from the exogenous species even though the added phospholipid comprises only ∼1−3% of the total.

Deuterated molecules, widely used in vibrational and NMR spectroscopic investigations of lipid order and phase behavior, are nonperturbing probes. Deuterated lipids behave similarly to their proteated analogues except that gel−liquid-crystal transitions are shifted down in temperature by 3−5°. In terms of miscibility properties, it has been demonstrated that acyl chain perdeuterated 1,2-dipalmitoylphosphatidylcholine forms an ideal mixture with its fully proteated analogue (Klump et al., 1981). Thus, the similar polarity between the C−H and C−D bonds ensures that conformational information deduced from deuterated analogues can be safely assumed to be applicable for proteated species.

FTIR spectroscopy offers several advantages over alternative methods used to study membranes in intact cells. The elegant and widely used fluorescence methodologies offer the most sensitivity for direct visualization of domains. However, while the technique permits visualization of domains, it provides no information concerning the conformational order of the bilayer. Since the erythrocyte incorporates phospholipids asymmetrically, our IR method permits us to monitor the conformational order of a particular species in a specified monolayer. Substantial differences were noted between the DMPS-d_{54} incorporated into the inner monolayer and DMPC-d_{54} incorporated into the outer monolayer. The thermotropic gel→liquid crystal phase transition was completely abolished upon its insertion into the inner leaflet (Figures 6 and 7). The conformational order of the incorporated PS is similar to that of the L_α (liquid-crystal) state of this molecule, as judged by the similarity of the CD_2 stretching frequency (∼2195 cm^{-1}) in both cases. This suggests that the cooperative interactions between DMPS molecules necessary for observation of a phase transition are lost upon its incorporation and subsequent translocation into the inner leaflet of the erythrocyte membrane.

In contrast, the phase transition of DMPC-d_{54} (∼19 °C) is still detected (Figures 8 and 9) after its incorporation into the outer monolayer. As cooperative interactions between DMPC-d_{54} molecules are required to produce a transition, this observation reveals the persistence of domains of DMPC-d_{54}, at least at temperatures below 19 or 20 °C. Whether the domains persist at temperatures above the completion of the melt cannot be deduced from these measurements. A second unanswered question is whether this exogenous lipid behaves (conformationally) in a fashion similar to the remainder of the PC class. A combination of FTIR spectroscopy and fluorescence microscopy will be required to address this issue. However, it is relevant that a previous study of intact erythrocytes demonstrated that native PC exists in different domains in the cell membrane (Shukla & Hanahan, 1982).

It is noted that the wavenumber change during the melting of DMPC-d_{54} domains is on average greater in the vesicles than in the intact cells or ghosts, although particular samples with high levels of incorporation may show a large wavenumber increase (e.g., Figure 9). This suggests that, on average, not all of the molecules undergo the transition. An exact fraction cannot be computed since the wavenumber position of the CD_2 stretching frequency is not a linear function of the fraction of molecules in a particular conformational state.

Many studies have addressed issues concerning the origin of domains. In a recent elegant report, Rodgers and Glaser detected a high correlation between the location of band 3 and PC in erythrocyte ghosts. The domain size was dependent on ionic strength but not on temperature (Rodgers

& Glaser, 1993). The complementary nature of the current FTIR approach with that of fluorescence is clear. This FTIR experiment directly monitors the acyl chain conformation of a particular lipid species and suggests that the presence of band 3 does not greatly alter the conformational properties of the inserted PC.

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