Table XIV. Structural Paramters of FN=Y Type Compounds

	Y =			
	O[a]	NF(cis)[b]	CF_2[c]	N_2[d]
F–N (pm)	151.6	138.4 (1.0)	138.9 (2)	144.4 (1.0)
(FNY (deg))	109.9	114.5 (0.5)	107.9 (2)	103.8 (0.5)

[a] Reference 22, r_e values. [b] Reference 23. [c] Reference 24, $r_\alpha{}^0$ values. [d] This work.

Conclusion

We have succeeded in recording the microwave and IR spectra of gaseous triazadienyl fluoride with its ^{15}N-enriched isotopomers. The analysis of these spectra has established the molecular geometry (C_s symmetry) as well as the harmonic force field. The data have been interpreted on the basis of extended ab initio calculations, partially involving configuration interaction. The reason for the explosive nature of FN_3 is now well understood.

Acknowledgment. We thank the Deutsche Forschungsgemeinschaft and the Verband der Chemischen Industrie e.V.-Fonds der Chemie for financial support.

Registry No. FN_3, 14986-60-8; $F^{15}NNN$, 111999-52-1; $FN^{15}NN$, 111999-53-2; $FNN^{15}N$, 111999-54-3; N_2, 7727-37-9; $^{15}N_2$, 14390-96-6; HN_3, 7782-79-8; $Na^{15}NNN$, 111999-55-4; $NaN^{15}NN$, 111999-56-5.

In Situ FT-IR Investigation of Phospholipid Monolayer Phase Transitions at the Air–Water Interface

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Abstract: The liquid-expanded to liquid-condensed phase transition of 1,2-dipalmitoyl-*sn-glycero*-3-phosphocholine (DPPC) monolayers at the air–water (A/W) interface has been investigated in situ with external reflectance Fourier transform infrared spectroscopy. Infrared spectra have also been obtained for monolayer films of 1,2-dimyristoyl-*sn-glycero*-3-phosphocholine (DMPC, a liquid-expanded film) and 1,2-distearoyl-*sn-glycero*-3-phosphocholine (DSPC, a liquid-condensed film). All three phospholipid monolayer films were monitored in the conformation-sensitive C–H stretching region as a function of molecular area at the air–water interface. The measured frequencies of the symmetric and antisymmetric CH_2 stretching bands for the DPPC monolayer in the high molecular area, expanded phase (2853 and 2924 cm^{-1}, respectively) are comparable to those of bulk DPPC multilayer dispersions above its main thermotropic phase transition and indicate a fluid and disordered conformation in the hydrocarbon chains. The measured frequencies for the low molecular area, condensed phase of DPPC (2849 and 2919 cm^{-1}) indicate a rigid, mostly all-trans hydrocarbon chain conformation for the condensed phase monolayer, similar to the low-temperature, gel phase bulk DPPC dispersion. A continuous decrease in frequency with decreasing molecular area is observed throughout the transition region for the DPPC monolayer. These results demonstrate that the DPPC monolayer transition is (a) heterogeneous and biphasic in character, with coexistence of fluid and solid phases, and (b) involves a conformational change in the hydrocarbon chains of the monolayer as the average molecular area is decreased in the transition region. These trends are supported by the data for the DMPC and DSPC monolayer films. Molecular orientations relative to the substrate are also discussed in light of band intensities in both the parallel and perpendicular polarized spectra.

Monomolecular films composed of synthetic phospholipids have been widely utilized as model systems in order to study the physical properties of biological membranes.[1-3] The usefulness of these monolayer systems (which are usually constructed via Langmuir–Blodgett techniques) stems from the fact that the experimenter is allowed a wide lattitude in choosing the composition and physical state of the molecules in the monolayers; as a result, a large variety of model systems can be investigated. Recent biophysical applications of these monolayer techniques have included antibody–hapten binding in model phospholipid membranes,[4-6] protein surface activity,[7] and the insertion of signal sequence peptides into phospholipid monolayers.[8,9]

Unfortunately, while phospholipid monolayers at the A/W interface have been extensively studied as models for membrane bilayers and interfacial phenomena, there is virtually no information available concerning the detailed physical structure of these systems.[10] The lack of information concerning the molecular-level structure of monolayers at the A/W interface can be traced to the inability of most spectroscopic techniques to study a low surface area, flat water interface with sufficient sensitivity to produce spectra with reasonable signal-to-noise ratios.[10] The spectroscopic techniques in use consisted mainly of visible absorption and, of late, fluorescence methods.[2,11] Conformationally sensitive vibrational spectroscopic techniques were limited to transferred films on reflective metal substrates.[9,12,13] Recently, however, we have used external reflection Fourier transform infrared spectroscopy to measure, in situ, the vibrational spectra of insoluble mono-

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molecular films that were spread at the A/W phase boundary.[14-16] We have shown that H_2O possesses sufficient reflectivity throughout the mid-infrared region of the electromagnetic spectrum to enable us to obtain good signal-to-noise ratio spectra of spread monolayer films from the single-pass external reflection technique.[14] In addition, the optical physics of the experiment allow us to obtain both the parallel and perpendicular polarized spectra of the monolayer which are necessary for orientational studies,[15] unlike the more traditional case of reflection off polished metallic surfaces, in which only parallel polarized vibrations are observed.[13]

In the present paper, we directly address the questions of conformation and orientation of monomolecular surface films of synthetic phospholipids as a function of the monolayer surface pressure by means of the external reflection FT-IR technique. In particular, we have applied this method to study the so-called liquid-expanded to liquid-condensed (LE/LC) monolayer phase transition that occurs in the surface pressure-molecular area isotherms of 1,2-dipalmitoyl-*sn-glycero*-3-phosphocholine (DPPC). This particular transition has become the focus of renewed interest from the standpoint of its surface chemistry[17] as well as its biophysical applications.[18]

Spectra of 1,2-distearoyl-*sn-glycero*-3-phosphocholine (DSPC) and 1,2-dimyristoyl-*sn-glycero*-3-phosphocholine (DMPC) monolayers are also examined as a function of surface pressure. These lipids are known to exist in the liquid-expanded (DMPC) and liquid-condensed (DSPC) monolayer states at 22 °C.[1,19] Their spectra provide additional information about the two limiting case monolayer states of the LE/LC phase transition.

Experimental Methods

Materials. 1,2-Dipalmitoyl-*sn-glycero*-3-phosphocholine (DPPC), 1,2-dimyristoyl-*sn-glycero*-3-phosphocholine (DMPC), and 1,2-distearoyl-*sn-glycero*-3-phosphocholine (DSPC) were obtained from Avanti Polar Lipids, Inc. (Birmingham, AL) at 99% stated purity. Each lipid was further purified by dissolving it in a minimum of dry, glass-distilled chloroform (1 part) and precipitating in dry, glass-distilled acetone (19 parts). The precipitate was allowed to cure for 1 h under refrigeration before collecting by centrifuging and decanting. The procedure was repeated three times and the resulting solid was evaporated to dryness.

Monolayer Films. The phospholipid monolayers were formed on a Langmuir trough measuring 28.3 × 12.6 × 1.3 cm milled from solid Teflon. The Teflon trough was attached to a hollow aluminum base plate through which a thermostated water solution could flow. The accuracy of the measured temperature of the subphase in this configuration is estimated at ±0.2 °C. The phospholipid monolayer was spread from a 1-mg/mL solution in *n*-hexane/EtOH (9:1 v/v) onto the surface of the A/W interface. The Teflon trough was cleaned before each run by soaking overnight in chromic acid solution (Chromerge, Fischer Scientific) and was filled with triply deionized water (MilliQ, minimum resistivity of 15 MΩ/cm, Millipore Corp., Bedford, MA). Individual points on the pressure-area isotherm were obtained by adding 2-5-μL aliquots of solution per point and allowing 10 min evaporation time before recording both the change in weight of the Wilhelmy plate and the monolayer spectrum. The area per molecule was varied by the successive addition of solution. Changes in surface tension were monitored by the weight difference on a filter paper Wilhelmy plate (GVWP, 0.22 μm, Millipore Corp., Bedford, MA) which was suspended in the trough from a Cahn-27 electrical balance.[20] Each plate was washed successively in 6 M acetic acid and 5 M NH_3OH and then rinsed and soaked in triply deionized H_2O to remove any surface contaminant. A fresh plate was used for each experiment. A glass box surrounding the trough and optics eliminated perturbing air currents and contaminating organic materials.

Spectroscopy. Single reflectance FT-IR spectra were obtained with use of a modified Digilab FTS-20 vacuum Fourier transform infrared spectrometer (Digilab, Inc., Cambridge, MA) with the optical path diagrammed in Figure 1. The optical adaptations were designed to bring the modulated infrared radiation from the interferometer out onto the bench and to reflect the focused beam (~1 cm diameter incident spot) off the A/W interface. The incoming radiation was polarized with an Al wire grid polarizer on KRS-5 (Cambridge Physical Sciences). The angle of incidence was 28 ± 5° normal to the surface of the trough which is in the optimum range for observing both polarizations reflected from a H_2O substrate.[15] Typically, the FT-IR spectra were collected by co-adding 4096 scans at 8-cm^{-1} resolution with triangular apodization and one level of zero filling with a narrow-band liquid-N_2-cooled HgCdTe detector. Reflection-absorption spectra were obtained by ratioing each single beam monolayer spectrum against the single beam reflectance spectrum of pure water. Frequencies of the C-H stretching bands were calculated by using a center-of-gravity algorithm and are accurate to better than ±0.1 cm^{-1}. Detailed data reduction procedures for infrared phospholipid spectra have been previously described.[21,22]

Transmission spectra of bulk DPPC in aqueous suspension were obtained on a Digilab FTS-10 Fourier transform infrared spectrometer. Samples were prepared by hydrating the bulk phospholipid above its main phase transition temperature and vortexing. The sample was then cooled to room temperature. This cycle was repeated several times. The DPPC samples were prepared with use of a demountable transmission cell with CaF_2 windows and 6-μm Mylar spacers (Harrick Scientific, Ossining, NY).

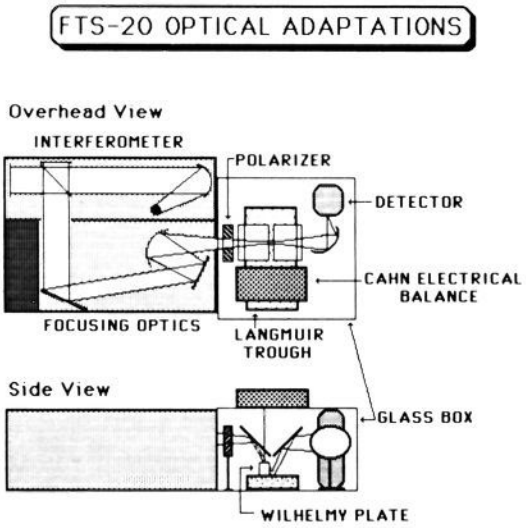

Figure 1. The modified optical design of the Fourier transform infrared spectrometer used in the experiments described in this paper. The modulated radiation from the interferometer is brought to a focus at the surface of a custom-built manual Langmuir-Blodgett film balance.

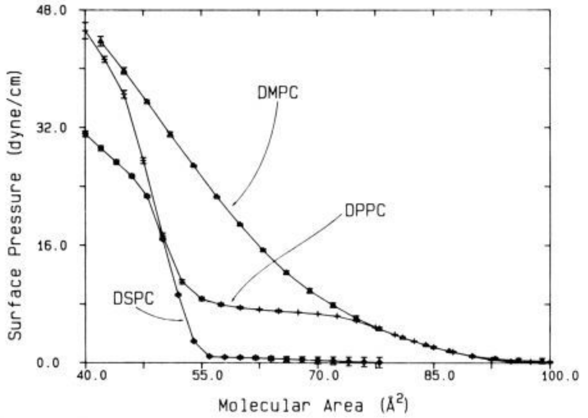

Figure 2. Mean values for the surface pressure-molecular area isotherms of three phosphatidylcholine lipids (DMPC, DPPC, and DSPC) obtained at 22 °C. Each curve was calculated from a minimum of three data sets; the standard deviations of each of the data points are also shown. These pressure-area isotherms were obtained on the film balance pictured in Figure 1.

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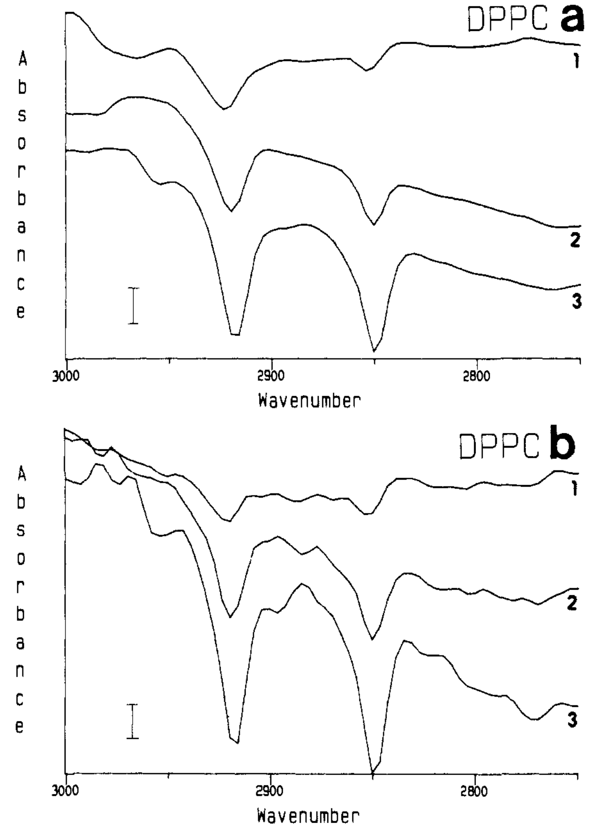

Figure 3. Representative infrared reflection spectra of DPPC monolayers shown for the hydrocarbon stretching region between 3000 and 2750 cm^{-1}. The spectra are shown for both (a) parallel and (b) perpendicular polarization. The molecular areas of the lipids in the spectra shown here are (1) 97 Å^2 (LE phase), (2) 60 Å^2 (LE/LC phase), and (3) 43 Å^2 (LC phase). The spectra are plotted in absorbance units (i.e., $-\log [R/R_0]$, where R is the single beam reflectance spectrum of the DPPC monolayer and R_0 is the single beam reflectance spectrum of pure H_2O). Each spectrum is the result of 4096 scans at 8-cm^{-1} resolution, triangular apodization, and one level of zero filling. The spectra presented here have not been smoothed. The insert marks in a and b indicate 0.001 absorbance unit.

Results and Discussion

Surface Chemistry. The mean surface pressure–molecular area isotherms for monolayers of DPPC, DSPC, and DMPC at the A/W interface obtained on our Langmuir–Blodgett trough are shown in Figure 2 at 22 °C. The precision and reproducibility of the aliquot addition method and the purity of the lipid are illustrated by the small standard deviation error bars on each isotherm. Three phases are readily distinguishable for DPPC: the liquid-expanded (LE) region ($> \sim 80$ Å^2), the main transition (LE/LC) region (from ~ 55 to 70 Å^2), and the liquid-condensed (LC) phase ($< \sim 50$ Å^2). The main phase transition is characterized by a plateau region on the curve whose slope increases with temperature. A temperature of 22 °C was chosen for subsequent spectroscopic studies because the DPPC isotherm is flatter and broader in the LE/LC region than at higher temperatures and exhibits little pressure change over a wider region of molecular area. Our pressure–area curves are in good agreement with the literature.[1,17,19,23]

External Reflection Spectroscopy. The polarized single external-reflection spectra are shown in Figure 3 for the acyl chain stretching region of DPPC monolayers in the LE, LE/LC, and LC phases. This region was chosen for investigation since the CH_2 symmetric and antisymmetric stretching vibrations have been shown to be conformation sensitive[24-28] and have been used to determine structure and phase behavior in various hydrocarbon systems, including phospholipids. Four bands are usually resolved in the C–H stretching region. They are the methylene CH_2 symmetric and antisymmetric stretching vibrations at about 2850 and 2920 cm^{-1}, respectively, as well as the less intense symmetric and asymmetric terminal methyl CH_3 stretching bands at about 2872 and 2956 cm^{-1}.

Figure 3 illustrates two important differences between the external-reflection infrared spectra of monolayers on water versus that of a monolayer on a reflective metal. The most noticable difference is that when plotted in the absorbance mode (i.e., $-\log [R/R_0]$, where R is the single beam reflectance spectrum of the monolayer and R_0 is the single beam reflectance spectrum of the H_2O), the absorption bands of the monolayer on water are negative. The basis for the negative absorbances in these monolayer spectra have previously been explored.[14-16] Second, due to the optical constants of H_2O, mean square electric fields are present in all three geometric orientations upon external reflection of infrared radiation off H_2O.[15] This enables us to measure both the parallel and perpendicular polarized spectra of the monolayer on H_2O, unlike the case for metals, where only the parallel polarized spectrum can be measured.[13,29] Data in both polarizations are required for orientational studies.

DPPC Monolayer. Typical infrared spectra in the C–H stretching region for a monolayer film of DPPC at the A/W interface are shown in Figure 3 as a function of molecular area and polarization. The spectra in the LE region were obtained at an average molecular area of 90 Å^2, while the LE/LC and LC spectra were taken at points along the pressure–area curve that correspond to 60 and 45 Å^2, respectively. Two major bands are evident in the spectra shown in Figure 3 at $\sim$2850 and $\sim$2920 cm^{-1}. These can be readily assigned to the symmetric and antisymmetric CH_2 stretching vibrations of the phospholipid acyl chains, as described above. The less intense terminal methyl stretches cannot be distinguished above the noise level. The intensity of the CH_2 stretching bands increases on going from the LE to LC phase at the A/W interface. This increase is due in part to an increase in the surface density of the sample, but it may also be indicative of orientational changes of the sample relative to the substrate (vide infra).

The monolayer phases of DPPC have frequently been compared with the thermotropic polymorphism of bulk phase DPPC, which has been extensively studied.[30] In aqueous suspension in the bulk phase below its characteristic phase transition temperature ($T_m \sim 41.5$ °C), the acyl chains of DPPC exist in a regular lattice structure characterized by an extended, mostly all-trans hydrocarbon chain.[31] As the temperature increases and approaches T_m, gauche conformers are introduced into the rigid lattice and the regular packing is disrupted. Above T_m in the so-called liquid-crystalline phase, large numbers of gauche conformers occur, resulting in a fluid acyl chain structure.[31]

Previous FT-IR studies of bulk DPPC in aqueous suspension have demonstrated that the frequencies of the CH_2 stretching bands may be empirically correlated with the molecular conformation of the phospholipid hydrocarbon chains (see e.g., ref 21 and 22 and references cited therein). Figure 4 is an example of the use of the antisymmetric CH_2 stretching band to follow the thermotropic phase transition of bulk DPPC. The low-temperature frequency ($\sim$2919 cm^{-1}) remains relatively constant throughout

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Table I. Average Wavenumber and Standard Deviation for Each Lipid Monolayer and Isotherm Phase

lipid	phase	mol. area (Å^2)	antisymmetric CH_2		symmetric CH_2	
			parallel	perpendicular	parallel	perpendicular
DMPC		60–90	2924.5 ± 2.4	2924.4 ± 1.6	2853.0 ± 1.4	2853.9 ± 1.5
		50	2922.1 ± 1.6	2922.7 ± 1.0	2853.2 ± 1.5	2853.5 ± 0.6
DPPC	LE	85–100	2924.7 ± 0.6	2923.4 ± 0.6	2853.7 ± 1.0	2853.5 ± 1.2
	LE/LC	55–65	2922.0 ± 1.5	2920.8 ± 1.4	2851.1 ± 1.4	2851.1 ± 1.6
	LC	40–48	2919.0 ± 0.6	2918.8 ± 0.9	2849.9 ± 0.5	2849.8 ± 0.5
DSPC		60–90	2919.2 ± 0.7	2918.6 ± 0.8	2849.8 ± 0.4	2849.7 ± 0.7
		47–53	2919.0 ± 1.2	2918.6 ± 0.5	2849.8 ± 0.8	2849.7 ± 0.7

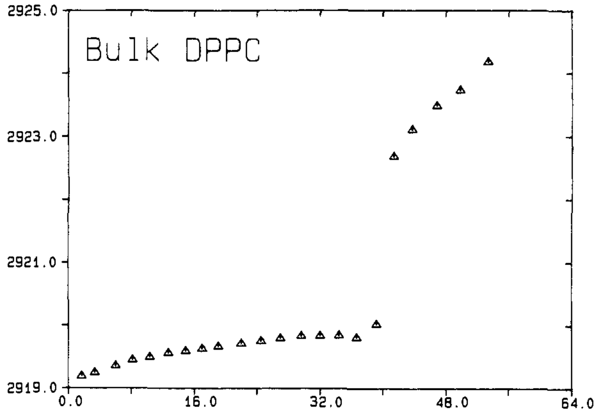

Figure 4. The frequency of the antisymmetric CH_2 stretching band at 2920 cm^{-1} plotted against temperature for an aqueous dispersion of bulk DPPC multilayer vesicles.

the gel phase but is sharply discontinuous at T_m and increases to a value of ~2924 cm^{-1} in the liquid-crystalline state.

In analogous fashion to the thermotropic phase transition of bulk phase DPPC, we may also follow the surface pressure–molecular area phase transition of the DPPC monolayer film at the A/W interface by using the frequencies of the CH_2 stretching bands. This is illustrated in Figure 5 where the frequency shift of the antisymmetric CH_2 stretching band for a monomolecular film of DPPC is plotted as a function of molecular area and polarization. The data presented in Figure 5 show that at high molecular areas in the LE phase, the measured frequencies (~2924 cm^{-1}) correspond to those obtained in the highly fluid, disordered liquid-crystalline state of bulk DPPC suspensions. Conversely, the measured frequencies of the low molecular area, LC phase (~2919 cm^{-1}) agree with those of the rigid, mostly all-trans gel phase of bulk DPPC.

Examination of the frequency versus molecular area data shown in Figure 5 suggests that the trend is for a continuous decrease in frequency with decreasing molecular area, unlike the discontinuous break at T_m in the thermotropic phase transition data for bulk DPPC (Figure 4). An analysis of the wavenumber versus molecular area data shown in Figure 5 using a least-squares correlation approach[32] results in a linear correlation coefficient $r = 0.91$ for parallel polarization and $r = 0.88$ for perpendicular polarization. Such a correlation coefficient does not prove a completely linear relationship between the variables; however, it does indicate that $(100\ r^2)\%$ or between 77 and 83% of the variation in the wavenumber data is accounted for by a linear difference in the molecular area variable.[32]

This pattern in the wavenumber versus molecular area curve (i.e., a continuous, if not exactly linear, decrease in frequency with decreasing molecular area) is also seen in the data for the symmetric CH_2 stretching band at ~2850 cm^{-1} for both parallel and perpendicular polarizations (data not shown). Table I summarizes the mean values and standard deviations for the frequencies of the CH_2 stretching bands of DPPC in each monolayer phase. The means corresponding to the separate monolayer phases (i.e., LE, LE/LC, and LC) listed in Table I were subjected to a statistical t test analysis[32] which confirmed that the mean values of the frequency in the three DPPC monolayer phases are significantly different from one another at the 95% confidence limit. This statistical difference is reproducible for both parallel and perpendicular polarizations of the antisymmetric CH_2 stretching band as well as for the parallel and perpendicular polarizations of the symmetric CH_2 stretch.

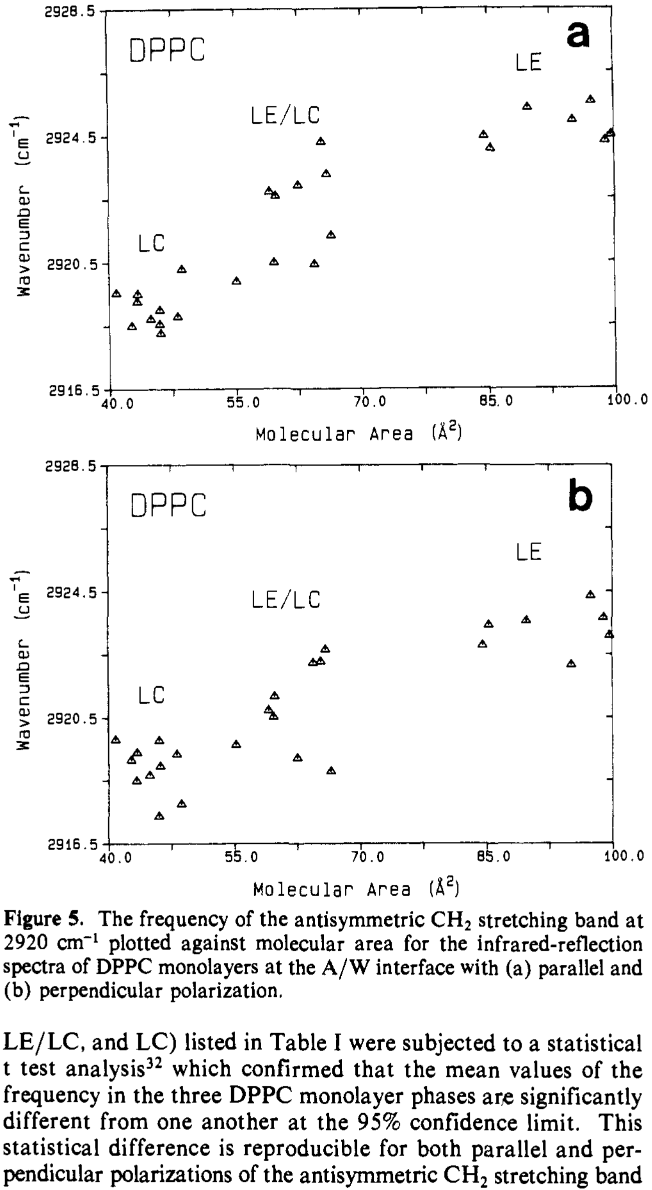

Figure 5. The frequency of the antisymmetric CH_2 stretching band at 2920 cm^{-1} plotted against molecular area for the infrared-reflection spectra of DPPC monolayers at the A/W interface with (a) parallel and (b) perpendicular polarization.

While the differences in frequency are small, they are large enough to be related to real conformational differences among the three monolayer phases based on the thermotropic phase transition data of bulk DPPC (Figure 4 and ref 21 and 22). As seen in bulk DPPC suspensions, a solid–liquid phase transition involves approximately a 4 cm^{-1} frequency shift, comparable to the ~4.5 cm^{-1} difference observed between the LC and LE phases in the DPPC monolayer.

A comparison of the scatter in the data plotted in Figure 5 with the measured standard deviations in Table I shows a common tendency among the measured frequencies; that is, the mean frequencies of the CH_2 stretching bands in the LC and LE phases tend to have the smallest standard deviations, with the mean standard deviations (±) of these two phases showing little or no

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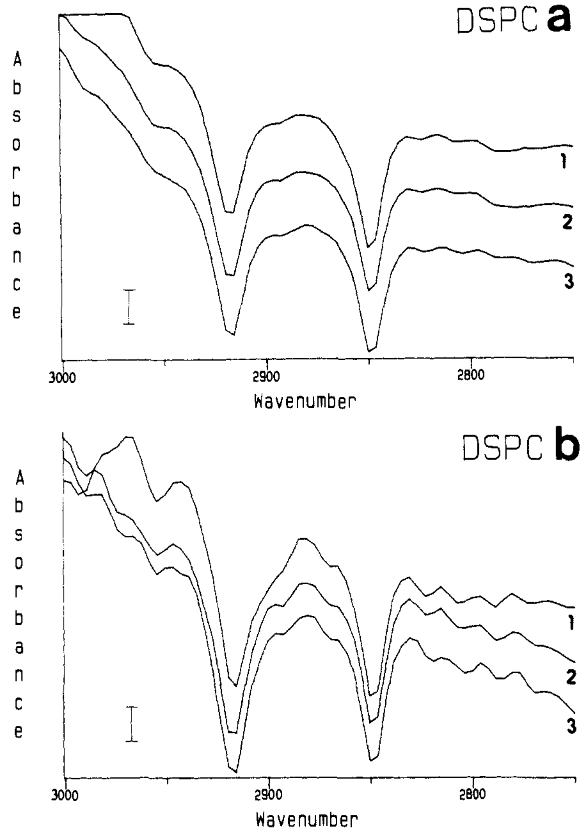

Figure 6. Representative infrared reflection spectra of DSPC monolayers shown for the hydrocarbon stretching region between 3000 and 2750 cm^{-1}. The spectra are shown for both (a) parallel and (b) perpendicular polarization. The molecular areas of the lipids in the spectra shown here are (1) 70 Å^2, (2) 61 Å^2, and (3) 53 Å^2. Each spectrum is the result of 1024 scans. Other conditions as described in Figure 3. The insert marks in a and b indicate 0.001 absorbance unit.

overlap. However, the mean frequencies of the CH$_2$ stretching bands for the DPPC molecules in the LE/LC main transition region always have the largest standard deviation. In addition, the scatter of the frequency data in this transition region is always large enough to encompass the range for both the LC and LE phases.

The results presented in Figure 5 and Table I support the following interpretation of the monolayer phase transition for DPPC. In the LE phase, the DPPC monolayer is homogeneous in nature with the acyl chains in a highly disordered, fluid conformation as determined by the average frequency of the CH$_2$ stretching bands. The LC phase is also homogeneous, but with a highly ordered, mostly all-trans acyl chain conformation. In the intermediate LE/LC transition region, the DPPC monolayer is heterogeneous and biphasic in character, with coexistence of fluid and solid phases as shown by the continuous decrease in the CH$_2$ frequency as well as the large standard deviation of the mean for the CH$_2$ frequency in the LE/LC region which overlaps the two limiting phases.

Recent epifluorescence and electron microscopy studies have also supported the notion of a fluid–solid coexistence region for the LE/LC transition.[33-36] Our results demonstrate that the LE/LC transition involves a conformational change in the hydrocarbon chains of the DPPC monolayer as the average molecular area is decreased in the coexistence region.

DSPC Monolayer. In order to better understand the character of each phase of the pressure–area curve of the DPPC monolayers at the A/W interface, spectra are presented for phospholipid monolayers of known surface characteristics. It has long been established that a monomolecular film of DSPC at 22 °C behaves as a liquid–condensed surface film where the acyl chains are ordered and oriented mostly perpendicular to the surface.[19,23]

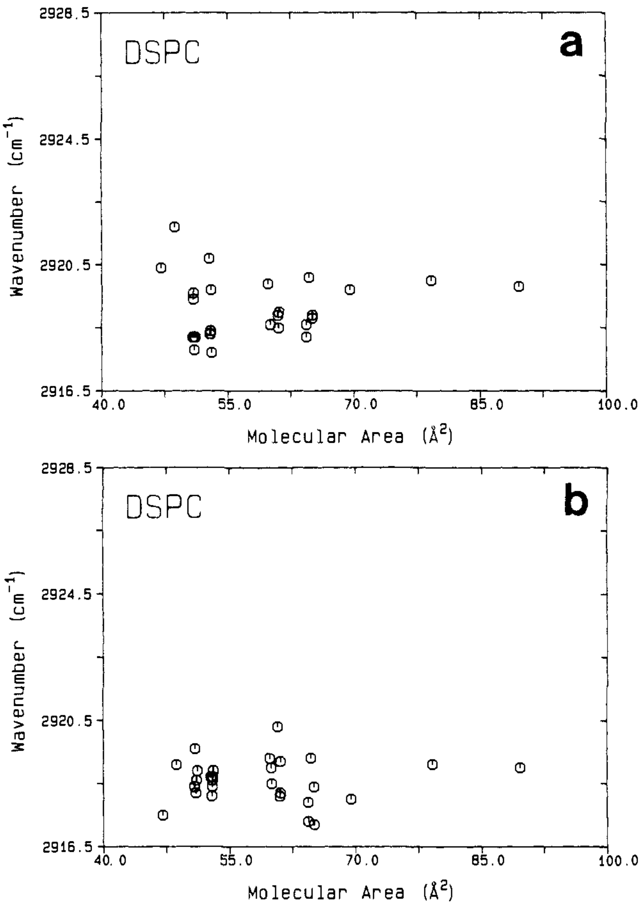

Figure 7. The frequency of the antisymmetric CH$_2$ stretching band at 2920 cm^{-1} plotted against molecular area for the infrared reflection spectra of DSPC monolayers at the A/W interface with (a) parallel and (b) perpendicular polarization.

Infrared spectra of DSPC monolayers were taken at various points along the pressure–area curve. Representative spectra are shown in Figure 6 in the acyl chain C–H stretching region for perpendicular and parallel polarizations at molecular areas of 70, 61, and 53 Å^2. Even at low surface densities, two strong bands are evident for both polarizations at 2919 and 2850 cm^{-1}, the antisymmetric and symmetric CH$_2$ stretching bands, respectively. The frequencies of both bands remain relatively constant throughout the 90–47 Å^2 molecular area range as illustrated for the antisymmetric CH$_2$ stretch in Figure 7. The frequency of the symmetric CH$_2$ stretching band follows the same pattern (data not shown). Calculation of the mean frequency in the region before the rise on the pressure–area curve (>55 Å^2) yields a frequency of 2919 cm^{-1}, the same value as in the region after the surface pressure increases (<55 Å^2) (Table I) indicating no statistical difference between these regions. The constant nature of the frequency is in good agreement with surface chemistry data that the nature of the DSPC film properties do not change throughout its pressure–area curve.[19]

As seen in Table I, the average frequency for each band in the DSPC monolayer spectrum closely agrees with the average frequency of the corresponding bands in the LC phase of the DPPC monolayer spectrum. The infrared spectral data for DSPC and the LC phase of the DPPC monolayer show that the hydrocarbon chains for these lipids exist in an ordered, mostly all-trans conformation.

The intensity of the bands in the C–H stretching region for the DSPC monolayer in both polarizations is independent of the molecular area of the lipid (Figure 6), unlike the case for the

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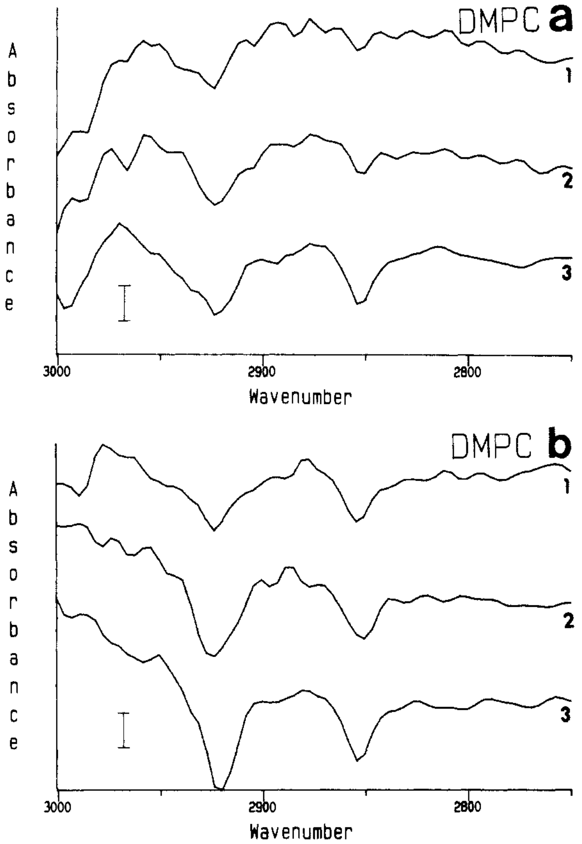

Figure 8. Representative infrared reflection spectra of DMPC monolayers shown for the hydrocarbon stretching region between 3000 and 2750 cm^{-1}. The spectra are shown for both (a) parallel and (b) perpendicular polarization. The molecular areas of the lipids in the spectra shown here are (1) 73 Å^2, (2) 59 Å^2, and 50 Å^2. Each spectrum is the result of 1024 scans. Other conditions as described in Figure 3. The insert marks in a and b indicate 0.001 absorbance unit.

DPPC monolayer, where the intensity of the bands grew with increasing surface coverage (Figure 3). A reasonable explanation for these data is that the individual DSPC molecules do not exist as a random, homogeneous film at low surface coverage. Rather, due to the increased attractive van der Waals interactions of the long (C_{18}) hydrocarbon chain, they are present in the form of domains. These attractive van der Waals interactions are known to dominate the hydrophobic effect in phospholipids[37] and are used in statistical mechanical models of monolayer phase transitions.[38,39] These results suggest that the domains of DSPC, and not the individual lipid molecules, grow in number and/or size until they coalesce with decreasing molecular area resulting in an increase in surface pressure. Previous studies have shown that stearic acid exists as domains or patches in organic solution and the A/W interface.[23]

DMPC Monolayer. DMPC is known to be a liquid-expanded film at 22 °C where the acyl chains are highly fluid and disordered and can therefore be compared with the LE phase of DPPC.[19,23,37]

Figure 8 illustrates the methylene absorption bands in the parallel and perpendicular polarized spectra of a DMPC monolayer at molecular areas of 73, 59 and 50 Å^2. The intensities of the bands in the C–H stretching region are weaker and less clearly defined than those for DPPC and DSPC. Consequently, increased uncertainty in band frequency at such low intensities is reflected in increased scatter when the frequency of the antisymmetric CH_2 stretching band is plotted as a function of molecular area (Figure 9). The large scatter makes it difficult to define with precision any trends in frequency over the range of the pressure–area curve, but the pattern and average frequencies (Table I) remain constant for both the symmetric and antisymmetric stretching bands in both parallel and perpendicular polarizations. Despite the large standard deviation, the average frequency is clearly elevated in contrast to that of the condensed DSPC film and corresponds well with the average frequency of DPPC in the LE phase, indicating that the hydrocarbon chains of the DMPC film remain in a highly disordered conformation[1,19,23] throughout the range of surface pressures studied.

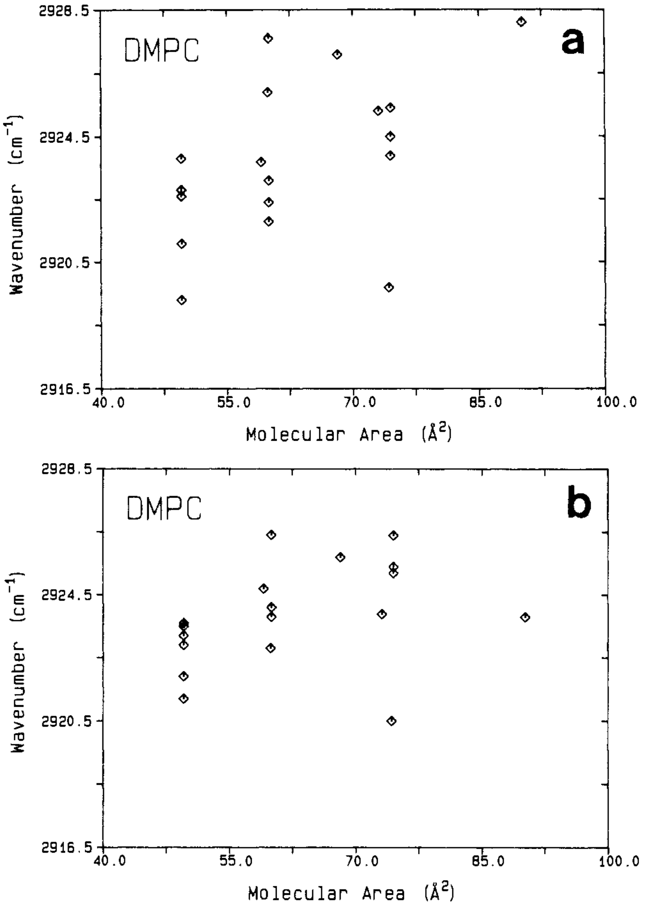

Figure 9. The frequency of the antisymmetric CH_2 stretching band at 2920 cm^{-1} plotted against molecular area for the infrared reflection spectra of DMPC monolayers at the A/W interface with (a) parallel and (b) perpendicular polarization.

Conclusions

External-reflection infrared spectra of phospholipid monolayers on an aqueous substrate as a function of molecular area are obtainable in situ in both polarizations. The C–H stretching frequencies, which are sensitive to conformational changes in the lipid acyl chains, were monitored over the range of the pressure–area curves for DPPC, DSPC, and DMPC monolayers.

The antisymmetric and symmetric CH_2 stretching frequencies were observed for the DMPC monolayer and the LE phase of the DPPC monolayer at 2924 and 2853 cm^{-1}, respectively. These frequencies are similar to those of bulk DPPC multilayer dispersions above the melting point, T_m, and reflect a fluid and disordered conformation opf the hydrocarbon chains. Frequencies for these same bands in the DSPC monolayer and the LC phase of the DPPC monolayer coincided with the gel phase frequencies of bulk DPPC at 2919 and 2849 cm^{-1}. The lower frequencies indicate a more ordered, all-trans conformation of the acyl chain.

Figure 5 illustrates the continuous transition of the antisymmetric stretch throughout the LE, the LE/LC, and the LC phases of the pressure–area curve of DPPC monolayers. Coupled with the uncertainty in the mean frequency of the LE/LC phase which encompasses the range of the other two phases, this continuous transition suggests a biphasic transition of fluid and solid states in a heterogeneous mixture. The frequency shift in the LE/LC phase of DPPC demonstrates a conformational change of the

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hydrocarbon chain is involved in the phase transition.

Finally, further examination of the data reveals that the bands of the perpendicular polarized spectra of DMPC are more intense than those in the parallel spectra. Band intensity depends upon the magnitude of the interaction of the bond dipole with the electric field which is a function of the angle of incidence and the optical constants of the reflective substrate (in this case, water). Qualitatively the difference in polarized spectra for DMPC translates into a more random, liquid-like arrangement of the flexible hydrophobic chains. DMPC monolayer spectra contrast with those of DSPC where the methylene stretch intensities are stronger and independent of polarization. The similarity in polarized intensities of DSPC suggests that these molecules are standing nearly upright and are essentially close-packed and well-oriented at the A/W interface, which are established characteristics of condensed films.[23] The exact orientations may be calculated from the band intensities in each polarization. The angles of the hydrocarbon chains relative to the substrate are currently under investigation in this laboratory.[40]

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Microwave, Infrared, and Raman Spectra, Conformational Stability, Structure, Dipole Moment, and Vibrational Assignment for Cyclopropyl Isocyanate

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Abstract: The microwave spectrum of cyclopropyl isocyanate, c-C_3H_5NCO, has been recorded from 18.5 to 40.0 GHz. Two sets of *a*-type, R-branch transitions have been observed and assigned, on the basis of the rigid rotor model, to the trans and the cis conformers, which have the isocyanate moiety oriented in an s-trans or s-cis fashion, respectively, relative to the three-membered ring. In addition to the ground vibrational state transitions, lines due to two excited states of the CNC bend and four excited states of the asymmetric torsion for the trans conformer, as well as one torsional excited state for the cis form, have been assigned. The B and C rotational constants for the ground vibrational state of the trans and cis conformers are the following: $B = 1784.303 \pm 0.003$, $C = 1716.133 \pm 0.003$ and $B = 2186.797 \pm 0.010$, $C = 2106.242 \pm 0.010$ MHz, respectively. The values of the A rotational constants for both conformers were not well determined because the measured transitions are not very sensitive to these constants. From the relative intensity measurements in the microwave spectrum, the torsional frequency is estimated to be 24 cm^{-1} for the trans conformer and 14 cm^{-1} for the cis form. The dipole moment components determined from the Stark effect are $|\mu_a| = 2.56 \pm 0.02$, $|\mu_c| = 0.71 \pm 0.03$, and $|\mu_t| = 2.65 \pm 0.02$ D for the trans conformer and $|\mu_a| = 2.720 \pm 0.004$, $|\mu_b| = 0.17 \pm 0.01$, and $|\mu_t| = 2.726 \pm 0.001$ D for the cis conformer. With reasonably assumed structural parameters for the cyclopropyl moiety and bond distances for the isocyanate group, estimates of the differences between the cis and trans conformers of the title molecule for the following parameters were made: r(C–N), ∠CNC, and ∠CCN. The infrared (3300 to 30 cm^{-1}) and Raman (3300 to 10 cm^{-1}) spectra have been recorded for the gas and the solid states. Additionally, the Raman spectrum of the liquid has been recorded and qualitative depolarization values have been obtained. Based on the band contours, depolarization values, and group frequencies, the normal vibrational modes have been assigned. From a temperature study of the Raman lines of an assigned conformer pair in the liquid phase, the value of ΔH between conformers has been determined to be 39 ± 5 cm^{-1}, with the trans form being more stable. However, from the Raman spectrum of the solid the cis conformer has been determined to be the preferred form in the crystal. These results are compared with the corresponding quantities in some similar molecules.

The changes in the cyclopropane ring structure upon substitution have been investigated recently for compounds with various unsaturated and saturated substituents. From the microwave study[1] of cyanocyclopropane and cyclopropylacetylene, the ring C_1C_2 bond adjacent to the point of substitution was determined to be about 0.025 Å larger than the C_2C_3 bond opposite to the substituted carbon. In isocyanocyclopropane, however, the C_1C_2 distance determined from the microwave study[2] was found to be only 0.008 Å larger than the C_2C_3 distance. These differences in the ring CC distances were explained on the basis of π conjugative interactions of the substituents with the cyclopropane ring. The smaller difference between the two ring CC distances in isocyanocyclopropane is significant in view of the fact that the atom attached to the cyclopropane ring is N in isocyanocyclopropane and C in the former two compounds.

We have recently investigated the microwave spectrum of cyclopropyl isothiocyanate[3] and determined that the molecule exists as an equilibrium mixture of the trans and cis conformers at ambient temperature. Only one torsional excited state was observed and assigned for each of the two conformers in the microwave spectrum suggesting, perhaps, that a small energy barrier exists between the two conformers and that most of the molecules are freely rotating at room temperature. The structural parameters

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† Taken in part from the thesis of R. J. Berry which will be submitted to the Department of Chemistry in partial fulfillment of the Ph.D. degree.

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