Orientation and Organization in Functionalized Phospholipid Monolayers at Gold Surfaces As Measured by Polarization Modulation Fourier Transform Infrared Spectroscopy

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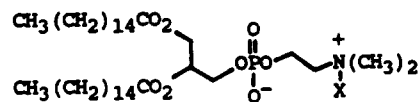
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Polarization modulation Fourier transform infrared reflectance (PM-FTIR) spectroscopy has been employed to study the pressure-dependent molecular structure of functionalized phospholipid monolayers at the gold–air interface. "Head-down" monolayers of dipalmitoylphosphatidylcholine (DPPC) and the anthraquinone- and anthracene-functionalized analogues (DPPC–AQ and DPPC–AN) have been prepared on vapor-deposited polycrystalline gold surfaces using Langmuir–Blodgett techniques. PM-FTIR spectra of monolayers deposited with varying molecular densities reveal differing degrees of order within the aliphatic chains and slight changes in orientation of the aromatic rings. The molecular order within the alkyl chains can be estimated from relative peak frequencies and intensities in the CH stretching region and follows the trend DPPC > DPPC–AQ > DPPC–AN. More expanded films of all three amphiphiles are found to possess more disordered alkyl chains. The relative intensities of the carbonyl and ring stretch bands of DPPC–AQ have been employed to estimate the molecular orientation of the anthraquinone moieties within the monolayer. The PM-FTIR spectroscopic results are used to refine the conclusions of previous film balance, electrochemical, and ellipsometric studies of the functionalized phospholipids within the free and supported monolayers.
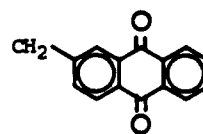
I. Introduction

Amphiphilic assemblies have long attracted the attention of scientists due to their importance in biological processes and technology.[1] Such systems are becoming more prevalent in electrochemical studies because of the desire to understand the importance of macromolecular architecture and molecular orientation on electrochemical processes at electrode surfaces. Self-assembled alkyl thiols on Au,[2] self-assembled bilayers on metal oxides,[3] Langmuir–Blodgett monolayers and multilayers on electrodes,[4] adsorbed layers on glassy carbon,[5] and drop-coated multilayers[6] on electrodes are a few of the examples of structured, amphiphilic assemblies employed in electrochemical studies. These studies have shown to varying degrees the importance of amphiphile mobility, supramolecular architecture, and ionic environment to the electrochemical performance, although the im-

SCHEME I. Phospholipids Employed in This Study

portance of molecular orientation within the electroactive assembly has yet to be determined.

Functionalized phospholipids[7,8] are ideal building blocks for the preparation of amphiphilic assemblies with a specific function, since phospholipids readily form a variety of amphiphilic structures—micelles, inverted micelles, monolayers, bilayers, and lamellae.[1] Quinone-functionalized phospholipids are interesting examples of this class in that they can be readily reduced by solution reagents[7] and solid electrodes[4e,5] and that their assemblies can be viewed as simple models for membrane-bound quinone assemblies found within photosynthetic reaction centers and the

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inner mitochondrial membrane.[9] DPPC–AQ (Scheme I) can be incorporated into phospholipid monolayers[4e] and bilayers (liposomes)[7] or adsorbed onto glassy carbon[5] to yield electroactive assemblies. Previous work[4e] with DPPC and DPPC–AQ monolayers at air–water, gold–air, and gold–water interfaces suggests that, as the film density increased, the DPPC and DPPC–AQ monolayers adopted similar film structures in which the bulky AQ group reoriented to permit a more compact molecular configuration. However, the previously employed Langmuir trough, ellipsometric, and electrochemical results have provided only inferential views of the molecular-level events occurring within the monolayers; a more direct probe of molecular structure is required.

Spectroscopy is a challenging, but powerful, probe of the structure and dynamics of amphiphiles at surfaces.[10-14] Various types of electronic and vibrational spectroscopies have been employed in this regard, with infrared reflectance measurements achieving widespread application[11,12] due to its ability to provide a direct measure of the orientation and order within the aliphatic chains of amphiphiles at the surface. Additionally, amphiphiles possessing a functional group with a characteristic IR signature such as a carbonyl can be readily observed. Infrared reflectance measurements are thus ideally suited to study DPPC–AQ monolayers. We have recently reported the implementation of a polarization modulation method of obtaining the differential reflectance spectra from monolayers and thin films at metal surfaces.[12h] This technique of polarization modulation Fourier transform infrared reflectance (PM-FTIR) spectroscopy[12] allows one to obtain spectra from the monolayers on the surface without the need of a reference sample.

We report herein the application of PM-FTIR differential reflectance spectroscopy to the study of anthraquinone- and anthracene-functionalized phospholipid monolayers at the gold–air interface. Relative band intensities of the CH_2 and CH_3 stretching modes provide quantitative measures of the relative order of the alkyl chains within the monolayers. These measurements reveal the similarities and differences in amphiphile packing within monolayers of varying molecular densities of the simple phos-

Figure 1. The π–A isotherm for (a) DPPC, (b) DPPC–AQ, and (c) DPPC–AN at the air–water (0.2 M KCl + 0.05 M phosphate, pH = 7.1) interface.

Figure 2. The cyclic voltammetric response of a Au/DPPC–AQ monolayer (π_{trans} = 30 mN/m) in 0.2 M KCl/H_2O at 100 mV/s where S = 10.8 $\mu A/cm^2$. The dashed line illustrates the background used to measure the surface density.

pholipid DPPC and the two functionalized analogues DPPC–AQ and DPPC–AN (Scheme I). The intensities of the head group carbonyl and ring-breathing modes of the DPPC–AQ monolayers at varying molecular densities serve as measures of the orientations of the AQ group. This molecular-level view afforded by PM-FTIR spectroscopy is then used to refine our previous views[4e] of the orientations and reorientations of the functionalized phospholipids within the Langmuir and Langmuir–Blodgett monolayers.

II. Experimental Section

L-α-Dipalmitoylphosphatidylcholine (DPPC) was purchased from Sigma Chemical Co. and used as received. The anthraquinone (DPPC–AQ) and anthracene (DPPC–AN) analogues were synthesized and purified as previously described.[7c] Proton NMR spectroscopy, thin layer chromatography, and microanalysis were utilized to confirm their purity. KCl (99.96+%, Mallinckrodt) and K_2HPO_4 (99.93+%, Mallinckrodt) were used as received. Distilled, deionized water was obtained from a Millipore system. Double-coated, double-polished polycrystalline gold on Si(100) wafers were obtained from Polishing Corp. of America (Santa Clara, CA). Rectangular plates were cut from the 3-in disks and cleaned repeatedly with H_2O_2/H_2SO_4.[4e] At least three cleanings were required to convert an "as-received" surface into a "clean" one as judged by IR spectroscopy.

Monolayers were spread at the air–water interface on a SVK Langmuir trough by carefully placing a $CHCl_3$ solution of the phospholipid onto the subphase (0.2 M KCl, 0.05 M phosphate, pH = 7.1) surface of a fully expanded trough. Lateral pressure–mean molecular area (π–A) isotherms were obtained upon slow compression (≤ 2 $Å^2$/molecule·min) of the monolayer. "Head-down" Langmuir–Blodgett films were prepared by slow emersion of the gold plate from the subphase. For transfer pressures (π_{trans}) of at least 10 mN/m, a transfer ratio of 1.0 was

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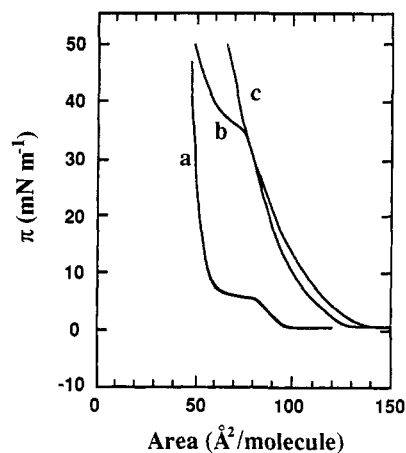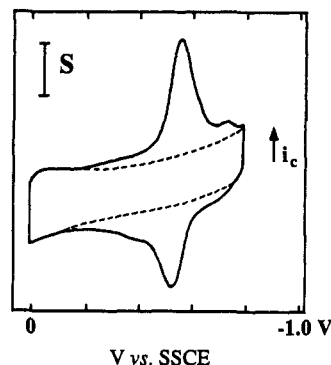
TABLE I: Vibrational Assignments for the Infrared-Active Modes of the DPPC–AQ Monolayer

freq, cm^{-1} ^{a}	assignment	
2965	$\nu_a(CH_3)$	asym CH$_3$ stretch (DPPC)^b
2925	$\nu_a(CH_2)$	asym CH$_2$ stretch (DPPC)
2880	$\nu_s^{FR}(CH_3)$	sym CH$_3$ stretch (DPPC)^c
2855	$\nu_s(CH_2)$	sym CH$_2$ stretch (DPPC)
1740	$\nu_{C=O}(CO_2R)$	C=O stretch (DPPC)
1678	$\nu_{C=O}(AQ)$ B$_{1u}$	B$_{1u}$ C=O stretch (AQ)^d
1593	$\nu_{C=C}(AQ)$ B$_{1u}$	B$_{1u}$ ring stretch (AQ)
1468	$\delta(CH_2)$	CH$_2$ bind (DPPC)
1381	$\delta_s(CH_3)$	CH$_3$ deformation (DPPC)
1330	$\nu_{C-C}(AQ)$ B$_{2u}$	B$_{2u}$ ring stretch (AQ)
1300	$\nu_{C-C}(AQ)$ B$_{1u}$	B$_{1u}$ ring stretch (AQ)
1190	ν_{O-P-O}	phosphate stretch (DPPC)

^{a} Band positions were obtained from a 2-cm^{-1} resolution spectrum. Variations in frequency are observed for different films and phases (cf. Table II). ^{b} DPPC assignments are from ref 13. ^{c} Part of a Fermi resonance doublet. ^{d} AQ assignments are from ref 17. B$_{1u}$ modes are defined as those with a dipole moment change along the CO symmetry axis and B$_{2u}$ modes as those with a dipole moment change perpendicular to that axis.

obtained. Lower π_{trans} led to transfer ratios approaching 1.5, indicating nonideal film transfer. Such films were not employed in this study.

PM-FTIR spectroscopic experiments were performed on a locally modified^{12h,i} Nicolet 740 spectrometer. The angle of incidence for the differential reflectance measurements was 76° from the surface normal. Signal-to-noise ratios of 50–100 for larger peaks and at least 15–20 for smaller peaks were obtained following subtraction of a slowly varying background from the wavelength dependence of the photoelastic modulator.^{12i} FTIR normal incidence transmission spectra of similar quality were obtained from a DPPC–AQ monolayer deposited onto a CaF$_2$ substrate.

III. Results

Phospholipid Monolayers. Monolayers of the phospholipids shown in Scheme I can be prepared at the air–water interface and on gold surfaces using a Langmuir trough. The π–A isotherm of DPPC shown in Figure 1 exhibits the expected E, LE, LC, and SC phases and transitions between these states.^{15,16} DPPC–AQ exhibits a larger lift-off area (130 Å^2/molecule), a higher pressure in the plateau, but a similar area in the SC state ($\approx$47 Å^2/molecule). The DPPC–AN isotherm differs considerably from those of DPPC and DPPC–AQ. The two functionalized phospholipids have similar lift-off areas, but DPPC–AN exhibits no plateau and reaches the collapse point at a much larger area ($\approx$66 Å^2/molecule). Interpretation of the isotherms is provided below.

Emersion of the gold plate through the Langmuir monolayers yields "head-down" Langmuir–Blodgett films. For $\pi_{trans} \geq 10$ mN/m, transfer ratios of 1.0 ± 0.1 are observed, indicating monolayer deposition. The gold-supported monolayers are electroactive in water as evidenced by the cyclic voltammetric response shown in Figure 2. Integration of the well-defined anodic and cathodic peaks indicates a surface density of 1.8 × 10^{-10} mol cm^{-2} of DPPC–AQ, which is in excellent agreement with the amount transferred from the trough (2.1 × 10^{-10} mol cm^{-2}).

Spectroscopy of the Supported Monolayers. PM-FTIR differential reflectance spectra were obtained for blank gold and monolayer-coated gold surfaces. Typical IR spectra in the CH stretching region are shown in Figures 3 for DPPC, DPPC–AQ, and DPPC–AN samples. Note the characteristic^{11} symmetric CH$_3$ and CH$_2$ (2880 and 2855 cm^{-1}, respectively) and asymmetric CH$_3$ and CH$_2$ (2965 and 2925 cm^{-1}, respectively) bands. Mode assignments and representative frequencies for these CH stretching bands are provided in Table I. As discussed below, the positions
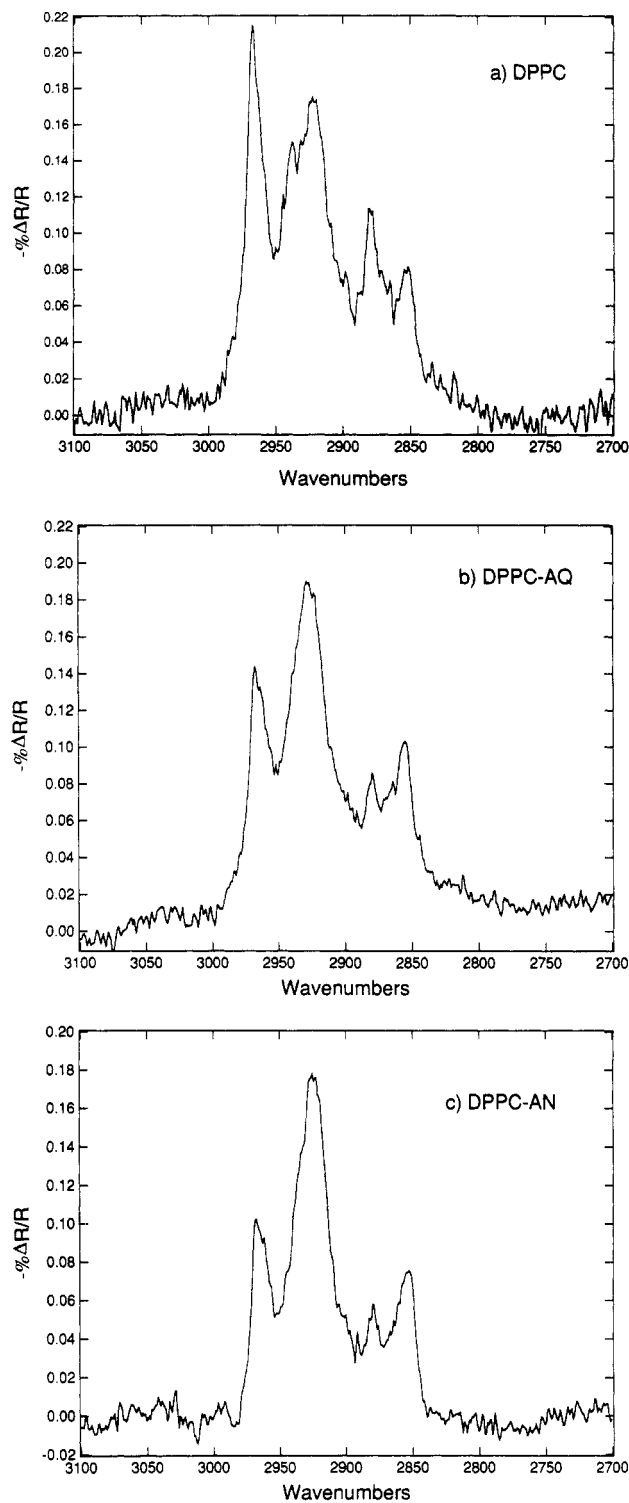
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(16) The gaseous phase is not observed in Figure 1, since the extremely large, initial spreading area (>400 Å^2/molecule) makes transferring condensed films ($\approx$50 Å^2/molecule) difficult.

Figure 3. PM-FTIR spectra (CH stretching region) of a single monolayer (π_{trans} = 40 mN/m) on polycrystalline gold: (a) DPPC; (b) DPPC–AQ; (c) DPPC–AN.

and relative intensities of the CH bands depend on the conformational disorder and orientation of the alkyl chains within the monolayers. These properties of the monolayers and the resultant changes in the IR spectra are controllable through the π_{trans} of the monolayer, as illustrated by the results in Table II and Figure 4. Notable among these data is the substantial change in the relative intensities of the CH$_3$ and CH$_2$ peaks with increasing π_{trans}. For example, the intensity ratio for the asymmetric stretches (I_{2965}/I_{2925}) of DPPC increases from 0.95 to 1.28 upon increasing π_{trans} from 10 to 40 mN/m. Figure 4 illustrates these changes in the I_{2965}/I_{2925} ratio for the three phospholipid monolayers. Note that in order to avoid any intensity changes due to differences in the surface coverage, we will employ ratios of band intensities when

TABLE II: Infrared Frequencies (cm⁻¹) for the CH Stretching Modes of the Phospholipid Monolayers and Related Systems

film (phase)[a]	$\nu_a(CH_3)$	$\nu_a(CH_2)$	$\nu_s^{FR}(CH_3)$	$\nu_s(CH_2)$
DPPC				
(LC)	2966	2922	2879	2853
(SC)	2967	2919	2879	2851
DPPC-AQ				
(LE)	2963	2928	2881	2857
(LE/LC)	2966	2927	2879	2856
(LC-SC)	2968	2924	2880	2855
DPPC-AN				
(LE)	2964	2927	2880	2857
(LC)	2968	2926	2880	2855
PE[b]				
(L)		2928		2856
(S)		2920		2850
$C_{15}H_{31}SH^c$				
(L)		2924		2855
(S)		2918		2851
(/Au)	2965	2918	2879	2850

[a]SC, LC, and LE refer to the physical states of the films based on previous[4e] Langmuir trough results; cf. Figure 1. [b]Solid (S) and liquid (L) polyethylene.[18] [c]Liquid, KBr, and adsorbed on gold samples.[11e]
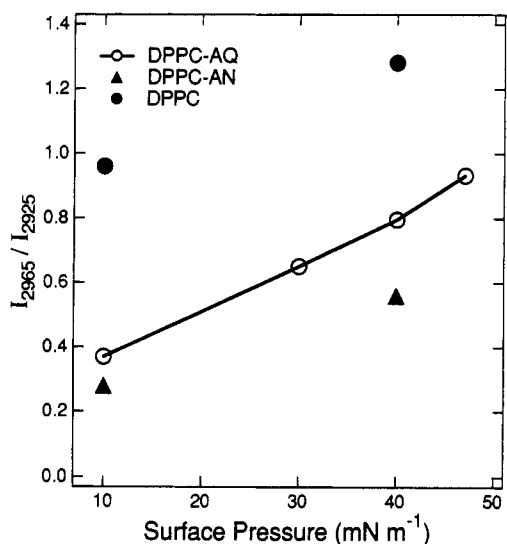

Figure 4. Intensity ratios of the asymmetric CH_3 and CH_2 stretching bands (I_{2965}/I_{2925}) of DPPC, DPPC-AQ, and DPPC-AN as a function of transfer pressure. As discussed in the text, a larger I_{2965}/I_{2925} ratio is indicative of greater conformational order.

comparing the methyl and methylene CH stretching modes for the three monolayers.

The mid-IR spectra of DPPC, DPPC-AQ and DPPC-AN films are shown in Figure 5. Mode assignments for the prominent peaks shown in Figure 5 are provided in Table I. In the DPPC-AQ spectrum (Figure 5b), four skeletal modes of the AQ head group can be seen at 1678, 1593, 1330, and 1300 cm⁻¹. These modes can be assigned by comparison to the IR absorption spectrum of anthraquinone.[17] The 1678-cm⁻¹ band is the AQ carbonyl stretching vibration with the transition dipole moment oriented along the C=O bond axis. Following the Mulliken convention,[17] vibrations of this symmetry are labeled B_{1u}. The 1593- and 1300-cm⁻¹ bands are due to skeletal vibrations that are likewise of B_{1u} symmetry, while the 1330-cm⁻¹ band is B_{2u} (along the long axis of AQ). As discussed below, the relative intensities of the B_{1u} and B_{2u} modes depend on the orientation of the AQ ring with respect to the surface normal. Figure 6 plots the intensities of the IR bands at 1740 and 1330 cm⁻¹ relative to the 1678-cm⁻¹ carbonyl band as a function of surface pressure. Comparisons of the band intensities to normal incidence transmission experi-
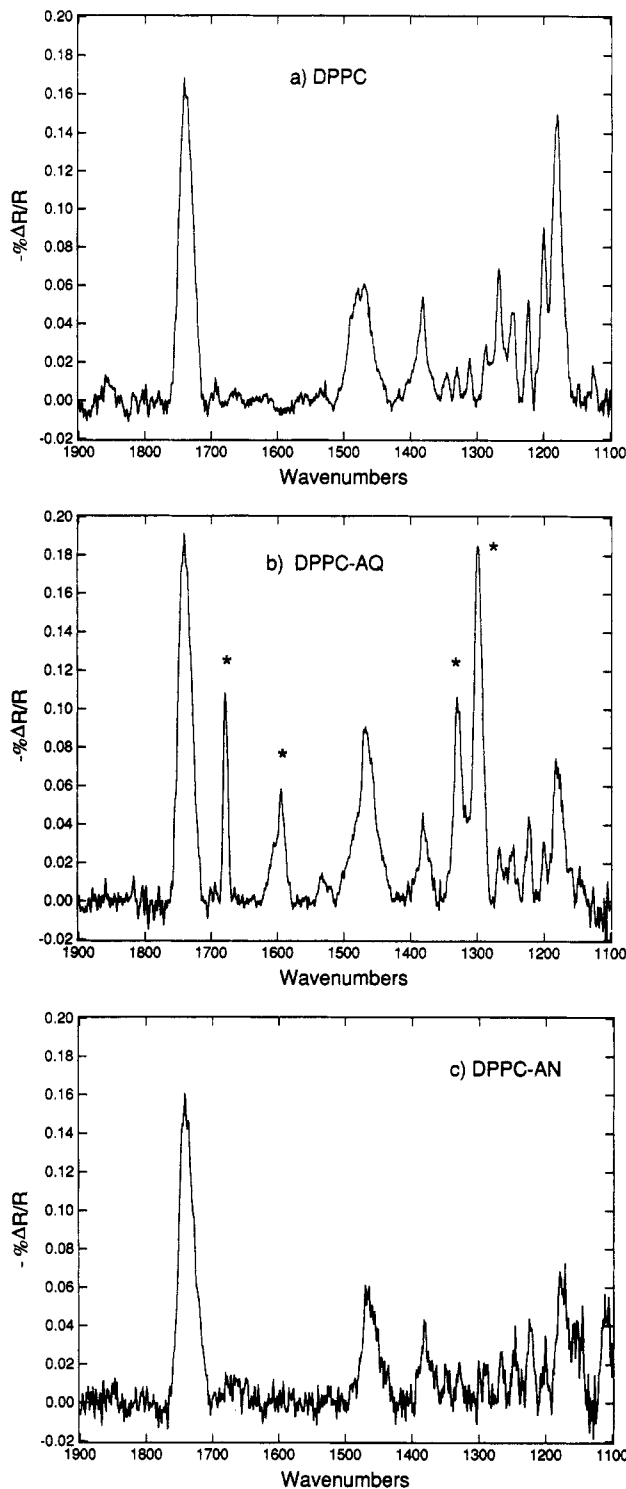
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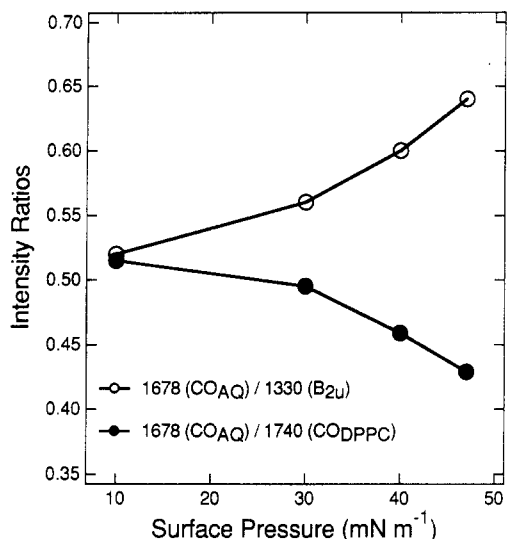

Figure 5. PM-FTIR spectra (mid-IR region) of a single monolayer (π_{trans} = 40 mN/m) on polycrystalline gold: (a) DPPC; (b) DPPC-AQ; (c) DPPC-AN. Bands marked with an asterisk in the DPPC-AQ spectrum are skeletal modes of AQ (cf. Table I).

ments of DPPC-AQ films on CaF_2 substrates will be used to estimate the AQ head group orientation. In contrast to the DPPC-AQ films, the mid-IR spectra of DPPC-AN monolayers reveal no sizable bands attributable to the AN head group. Apparently, either these vibrations have small oscillator strengths or the AN is oriented parallel to the surface so that no bands are observed.

IV. Discussion

Basic View of Phospholipid Monolayer Structure. DPPC is a representative phospholipid prevalent in biological membranes and commonly employed in the preparation of amphiphilic monolayers

Figure 6. Intensity ratios of the C=O bands and ring modes in the mid-IR region as a function of transfer pressure. The solid circles are the I_{1678}/I_{1740} ratio of the CO stretching bands from the anthraquinone and phospholipid moieties of the DPPC-AQ molecule. The open circles are the intensity I_{1678}/I_{1330} ratio of two anthraquinone modes at 1678 (B_{1u} symmetry) and 1330 cm^{-1} (B_{2u} symmetry) from the DPPC-AQ monolayer. Changes in these two intensity ratios indicate slight changes in the average orientation of the phospholipid head group and anthraquinone portions of the molecule with changing surface pressure.

and bilayers.[1] The structural similarity between DPPC and the functionalized phospholipids DPPC-AQ and DPPC-AN shown in Scheme I is evident. However, their behavior at the air–water interface reveals important differences in the inter- and intramolecular forces that shape the resultant supramolecular assemblies. The generally accepted view[15] of the DPPC monolayer structure as a function of surface pressure is one in which the amphiphiles move through five states on the water surface: (i) at a molecular area greater than 300 Å^2/molecule, the molecules lie disordered on the surface in the gaseous (G) state,[16] (ii) at a molecular area of 100–300 Å^2/molecule, the molecules begin to interact minimally with their alkyl chains disordered in the expanded (E) state, (iii) at a molecular area of 60–100 Å^2/molecule, the phospholipids interact increasingly in the liquid expanded (LE) and mixed LE/LC phases, (iv) at a molecular area of 50–60 Å^2/molecule, these interactions lead to a reduction in the disorder within the alkyl chains and form a liquid condensed (LC) phase, and (v) finally, at a molecular area of approximately 47 Å^2/molecule, the molecules pack with their alkyl chains in the all-trans, ordered form in a solid condensed (SC) phase. In the two condensed forms of the monolayer (LC and SC), the alkyl chains are oriented at approximately 30–40° from the surface normal,[13a] while the hydrophilic phosphocholine head group penetrates the aqueous surface.[15b]

The analogous view of the structure and dynamics of DPPC–AQ and DPPC–AN monolayers as a function of surface pressure has been presented previously[4e] and is based on Langmuir trough, ellipsometric, and electrochemical experiments. The gross similarities between the DPPC and DPPC–AQ isotherms suggest similar structure and dynamics within the monolayers. The greater lift-off area (the E–LE transition is observed at 130 Å^2/molecule) and significantly greater lateral pressure (34 versus 5 mN/m) required to maintain the LE/LC phase indicate that the bulky AQ group interferes with the packing of the amphiphiles. However, at high lateral pressures ($\approx$50 mN/m), DPPC–AQ adopts a molecular area similar to that of DPPC, indicating that the head group is extended into the aqueous phase and the tilted alkyl chains determine the molecular area of the SC phase. The DPPC–AN isotherm is dramatically different, indicating that DPPC–AN does not reorient as DPPC and DPPC–AQ do. The hydrophobic AN head group does not extend into the aqueous phase upon compression but remains bent back within the hydrophobic portion of the membrane.

This view of the monolayers has been supported by several ancillary experiments. When incorporated into DPPC liposomes, which possess an average amphiphile area of approximately 75 Å^2/molecule, the AQ group of DPPC–AQ is bent back into the hydrophobic portion of the membrane.[7c] Ellipsometry experiments reveal[4e] that the thickness of a condensed DPPC–AQ monolayer on gold is approximately 9 Å greater (36 versus 27 Å) than the corresponding DPPC monolayer. This clearly indicates that the AQ group is extended in the LC and SC phases of transferred monolayers. Voltammetry of DPPC–AQ monolayers of varying molecular densities in various electrolyte solutions is also best explained[4e] with the molecular and supramolecular conformations described above. The goal of the IR measurements is to determine more precisely the conformational state of the alkyl chains and the orientation of the functional head groups within the transferred monolayers as a function of surface pressure.

Spectroscopic Results—CH Stretching Region. The infrared-active CH stretching modes of aliphatic molecules have been used extensively to ascertain the molecular structure of long alkyl chains in organized monolayers. For IR radiation reflected from a gold substrate at a high angle of incidence, the only significant component of the electric field intensity is perpendicular to the surface.[11a] Previous groups have used this "surface selection rule" to measure the average orientation of ordered alkyl chains in organized monolayers through comparisons of the surface CH stretching spectrum to that of solid samples.[11] In addition to orientation measurements, the frequencies and relative intensities of the CH_2 and CH_3 stretching bands from long alkyl chains have been employed to ascertain the degree of conformational order in a wide variety of organized monolayers and phospholipid assemblies.[11,13] For example, if the monolayer consists of alkyl chains in a solidlike (all-trans) configuration, the antisymmetric methylene stretching band will occur at 2918 cm^{-1}, as compared to 2925 cm^{-1} for films with a more disordered, liquidlike structure. As seen in Table II, the data for the phospholipids show consistent trends with changing π_{trans}. The DPPC monolayer exhibits intermediate crystalline–liquid character at $\pi_{trans} = 10$ mN/m (LC phase) and distinct crystalline character at $\pi_{trans} = 40$ mN/m (SC phase). The DPPC–AQ and DPPC–AN monolayers show distinct liquid character in expanded (LE) phases and increasing crystalline–liquid character in more condensed (LE/LC and LC–SC) phases, indicating disorder within the alkyl chains even for $\pi_{trans} = 45$ mN/m. A completely condensed DPPC–AQ monolayer (SC phase) would require $\pi_{trans} \approx 50$ mN/m, a pressure too close to film collapse to permit ideal transfers.

Upon compression, the relative intensities of bands in the CH stretching spectra of the DPPC, DPPC–AQ, and DPPC–AN monolayers changed significantly due to a combination of orientation and conformation effects. These changes can be represented by the intensity ratio of the asymmetric methyl and methylene modes at approximately 2965 and 2925 cm^{-1}, respectively. This ratio, I_{2965}/I_{2925}, is plotted for the three films in Figure 4 as a function of transfer pressure and increases as the films become more compressed. The most tightly packed film, DPPC at 40 mN/m, exhibits a ratio of 1.28. Various methods have been employed to ascertain the orientation of alkyl chains in compressed films of DPPC,[13a] resulting in a picture of the DPPC alkyl chains in an all-trans configuration with an average tilt angle of 35° from the surface normal (note that the frequency of 2919 cm^{-1} observed for the antisymmetric methylene stretch in this film agrees with the structural interpretation).

For the DPPC–AQ and DPPC–AN films, the I_{2965}/I_{2925} ratio is significantly less than that observed for the DPPC film at all pressures. This is due to a combination of changes in conformation and orientation that result in the reorientation of some methylene groups in the alkyl chains such that their CH stretching transition dipole moments align more closely with the surface normal than in the all-trans case. For both DPPC–AQ and DPPC–AN monolayers, the I_{2965}/I_{2925} ratio increases as a function of surface pressure, but neither film ever achieves as ordered an alkyl chain structure as in the DPPC monolayer. In addition, both the I_{2965}/I_{2925} ratio and the $\nu_a(CH_2)$ frequencies demonstrate that the
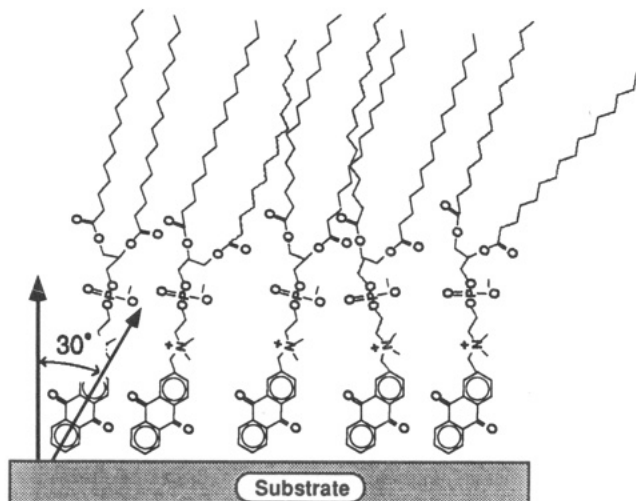
Figure 7. Schematic representation of the DPPC–AQ monolayer transferred to a gold substrate. The spectroscopic results indicate that the AQ head group is oriented with its long axis 30° from the surface normal and its molecular plane perpendicular to the surface.

DPPC–AQ film is consistently more ordered than the DPPC–AN film, in agreement with the π–A curves.

Spectroscopic Results—Mid-IR Region. The mid-IR spectrum of the DPPC–AQ monolayers contains bands from both the DPPC and the AQ head group portions of the amphiphiles. Changes are observed in the relative intensities of these bands as a function of surface pressure, indicating that the relative orientations of the two portions of the amphiphiles vary as the films are compressed. The reorientation of the AQ head group is an important question and has been invoked previously to explain the π–A and ellipsometric results.[4e]

An estimate of the average orientation of the AQ head group is available from a comparison of the band intensities measured for two experimental geometries: (i) grazing incidence reflectance from the gold surface, and (ii) normal incidence transmission through a CaF_2 substrate. The ratio of these two band intensities will be proportional to $\langle \tan^2 \theta \rangle$, where θ is the angle that the transition dipole moment makes with the surface normal. In order to make this comparison, the grazing incidence spectrum must first be normalized by the enhancement factor for the IR field intensity at the surface; we calculate an enhancement factor of 3.4 in agreement with other groups.[11a,g] Using the B_{2u} band at 1330 cm^{-1}, the long axis of the AQ head group in a DPPC–AQ film at 40 mN/m was found to have an average orientation of 30 ± 5° from the surface normal. Given this average orientation for the long axis, the short axis of the head group can take on values ranging from 60 to 90° from the surface normal (at 60° the AQ molecular plane is perpendicular to the surface and at 90° the short axis is parallel to the surface). Using the B_{1u} band at 1678 cm^{-1} (the B_{1u} band at 1300 cm^{-1} was not used due to spectral overlap with another band of the phospholipid), the short axis of the head group was found to have an average orientation of 59 ± 5° from the surface normal. This implies that the AQ head group is oriented on average with its molecular plane per-pendicular to the surface and its long axis tilted 30° from the surface normal.

The pressure dependence of the AQ orientation can be monitored by the changes in the intensity of the B_{1u} mode at 1678 cm^{-1}. Figure 6 plots the ratio of this band to the bands at 1740 (the DPPC carbonyl stretch) and 1330 cm^{-1} (the AQ B_{2u} ring mode). These ratios vary slightly by 10–20% with surface pressures from 10 to 40 mN/m, translating into relatively small (≤5°) changes in the tilt angle of the AQ group. Thus, the orientation of the AQ group within the transferred monolayer is only mildly dependent on the lateral density of the amphiphiles.

V. Summary

With the orientation and conformation information obtained from the PM-FTIR data, we can refine our picture of the pressure dependence of the AQ-functionalized phospholipid monolayers. The π–A curves suggest that compression of DPPC–AQ monolayers at the air–water interface into the LE/LC phase results in the AQ head group reorienting from the hydrophobic region of the monolayer to an extended form. Ellipsometric measurements of DPPC–AQ monolayers transferred to Au surfaces reveal the extended conformation in condensed phases but fail to reveal any dramatic change in film thickness with decreasing molecular area. The FTIR measurements require a molecular picture of the DPPC–AQ monolayer in which the molecular plane of the AQ head group is perpendicular to the surface and the head group's long axis is tilted at about 30° from the surface normal for all molecular densities. This refined view of the DPPC–AQ monolayers is depicted in Figure 7. It must be emphasized that a molecular picture in which the AQ group is either parallel or perpendicular to the metal surface can be ruled out since that orientation is incompatible with the observation of both the B_{1u} and B_{2u} bands in the IR spectrum. This new insight into the structure of the transferred films affirms our belief[4e] that DPPC–AQ monolayers at the air–water interface transfer to Au surfaces in an extended conformation, regardless of their conformation at the air–water interface.

A second observation from the PM-FTIR measurements is that, at all surface pressures, the alkyl chains of DPPC–AQ never achieve the orientation and conformation observed in the DPPC monolayer. The presence of the head group disrupts the packing of the DPPC–AQ molecules, apparently even in the condensed phase. This picture of a more disordered monolayer even at high surface pressures has important implications for the ion transport properties of the functionalized phospholipid monolayers during electrochemical charge-transfer processes.[4e]