Lateral Diffusion Study of Amphiphiles in Air–Water Monolayer Films of Polymerizable Surfactants

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ABSTRACT: The behavior of the chromophore *N*-[(1-pyrenylsulfonyl)dipalmitoyl]-L-α-phosphatidylethanolamine (pyrene-DPPE) embedded in unpolymerized and polymerized air–water monolayers of 10,12-nonacosadiynoic acid (diacetylene 16-8), octadecyl methacrylate (ODMA) and (octadecyldimethylammonio)-ethyl methacrylate bromide (ODAEMA), has been investigated by using surface pressure–area isotherm measurements, and steady-state and time-resolved fluorescence spectroscopy. Steady-state fluorescence measurements revealed pyrene-DPPE to be homogeneously distributed in the diacetylene 16–8 monolayer only in the liquid-expanded (LE) phase on an alkaline subphase. The lateral diffusion coefficients determined for the unpolymerized diacetylene 16–8 monolayer from steady-state measurements are in good agreement with literature values. Polymerization of the diacetylene 16–8 monolayer resulted in the formation of a liquid-condensed phase film and aggregation of pyrene-DPPE in the monolayer film, thus preventing a fluorescence quenching study. Pyrene-DPPE was found to be aggregated in both the monomer and polymer monolayers of ODMA. A derivative of ODMA incorporating a quaternary ammonium group (ODAEMA) was synthesized. ODAEMA showed LE isothermal behavior and gave a homogeneous distribution of pyrene-DPPE in the monomer monolayer film. The lateral diffusion coefficients obtained in the unpolymerized ODAEMA monolayer film were found to decrease with increasing surface pressure, reflecting a decrease in monolayer fluidity with compression. In the polymerized film of ODAEMA, nonexponential fluorescence decay behavior of pyrene-DPPE was observed. The fluidity of the polymerized ODAEMA monolayer is estimated to be at least 1 order of magnitude less than that for the corresponding monomer monolayer.

1. Introduction

In recent years there has been considerable interest in monolayers, bilayers, and Langmuir–Blodgett (LB) multilayers both as biomembrane models and because of their potential technological applications in the field of microelectronics.[1–20] A concern common to both areas is that of film stability, and because of this, a great deal of attention has been focused on stabilizing organized systems of amphiphilic molecules via polymerization.[11–20] Higher mechanical and/or better chemical and thermal stability can be achieved by polymerization of the reactive groups within the amphiphile.

A wide variety of polymerizable amphiphiles have been synthesized and extensively investigated by different methods at the air–water interface and in LB films.[11,20] Polymerizable groups can be located in the head group and/or the alkyl chains of amphiphilic molecules, and include diene, diyne (diacetylene), acrylate, and methacrylate groups which can be polymerized upon exposure to UV light or by radical initiators. The position of the polymerizable group within the amphiphiles influences the phase transition and fluidity characteristics of the monomeric and polymerized systems.[13,14,20]

We have been particularly interested in the characterization of air–water monolayers by the measurement of the mutual lateral diffusion coefficient of suitable probe–quencher pairs.[8–10] The fluorescence quenching technique employed is an alternative to the methods of fluorescence recovery after photobleaching (FRAP)[20–23] and pyrene excimer formation.[24–26] The advantages of the fluorescence quenching approach have been described in recent papers.[8–10]

The fluorescence quenching approach we have used is, however, dependent on there being a homogeneous distribution of probe and quencher within the matrix monolayer under investigation. Previous experience[8–10] has led us to formulate some qualitative empirical rules for predicting the miscibility of amphiphiles in monolayers and has suggested that complete miscibility of probe, quencher, and matrix is likely to be the exception rather than the rule. Accordingly, we have investigated the monolayer properties of three polymerizable amphiphiles in order to determine the mixing properties of the probe–quencher–matrix systems and to determine the extent to which the fluorescence quenching technique can be applied to yield reliable lateral diffusion coefficient data. Diyne and methacrylate amphiphiles, with polymerizable groups in the alkyl chain and the polar head group of the amphiphile, respectively, were employed as the monolayer matrices. Steady-state and time-resolved fluorescence spectroscopy were used to study the effect of polymerization on the homogeneity of distribution of the lipoidal probe pyrene-DPPE in the monolayer film.

2. Experimental Section

2.1. Materials. The chromophore *N*-[(1-pyrenylsulfonyl)-dipalmitoyl]-L-α-phosphatidylethanolamine (pyrene-DPPE) and the quencher 4-(*N*,*N*-dimethyl-*N*-hexadecylammonio)-2,2,6,6-tetramethylpiperidinyl-1-oxyl iodide (CAT-16) were both purchased from Molecular Probes Inc. The alkylamine quencher, octadecyldimethylamine (ODDMA), was obtained from Pfaltz and Bauer Inc. All were used without further purification.

The structures of the three different polymerizable amphiphilic compounds investigated are shown in Figure 1.

10,12-Nonacosadiynoic acid (diacetylene 16–8) was obtained from Tokyo Kasei and was purified by recrystallization from diethyl ether (Ajax; AR grade) immediately before use to remove any polymer residue.

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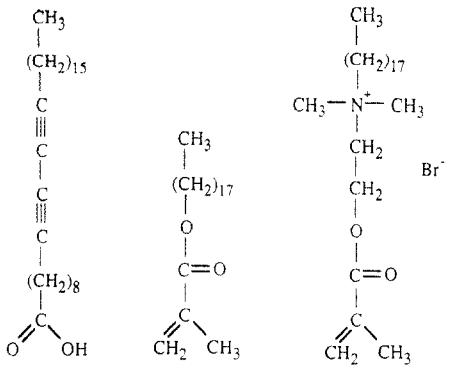

Figure 1. Structures of the three polymerizable amphiphilic molecules. (1) 10,12-nonacosadiynoic acid (diacetylene 16–8); (2) octadecyl methacrylate (ODMA); (3) (octadecyldimethylammonio)ethyl methacrylate bromide (ODAEMA).

Octadecyl methacrylate (ODMA) was purchased from Tokyo Kasei and was used as received. Poly(octadecyl methacrylate) (PODMA) was obtained from Monomer-Polymer and Dajac Laboratories, Inc. The PODMA sample had a weight average molecular weight of 175 000 and a number average molecular weight of 47 800.[27] PODMA was purified by repeated precipitation from the toluene solution by the addition of excess methanol. The precipitated polymer was freeze dried for 12 h, followed by vacuum drying at room temperature for 72 h to remove residual methanol.

The monomer (octadecyldimethylammonio)ethyl methacrylate bromide (ODAEMA) was prepared by a modification of the method of Nagai *et al.*[28] (*N,N*-Dimethylamino)ethyl methacrylate (Aldrich Chemical Co.) (2.9 g, 0.010 mol) was quaternized by reacting with freshly distilled 1-bromooctadecane (Fluka) (7.3 g, 0.011 mol), in acetone (20 mL), at room temperature for 7 days in the presence of 2000 ppm *p*-methoxyphenol (inhibitor). After removal of acetone, anhydrous ether was added, and the precipitated solid was filtered and then repeatedly recrystallized from ethyl acetate. The purified product was obtained as a white powder, and its structure was established by ^{1}H NMR. ^{1}H NMR spectra were recorded as solutions in $CDCl_3$ on a Bruker AC 200 spectrometer operating at 200 MHz with use of the solvent proton signal as reference. The resonances were assigned as follows: δ 6.08 (m, 1H, proton *trans* to a methyl group), 5.61 (m, 1H, proton *cis* to methyl group), 4.61 (m, 2H, N–CH_2–CH_2–O), 4.09 (m, 2H, N–CH_2–CH_2–O), 3.55 (m, 2H, $CH_3(CH_2)_{15}CH_2CH_2$–N), 3.46 (m, 6H, N–CH_3), 1.91 (bs, 3H, OC(O)C(CH_2)CH_3), 1.65 (m, 2H, $CH_3(CH_2)_{15}CH_2CH_2$–N), 1.10–1.30 (m, 30H, $CH_3(CH_2)_{15}CH_2CH_2$–N), 0.93 (bt, 3H, $CH_3(CH_2)_{15}CH_2CH_2$–N).

Polymerization of the monomer ODAEMA was accomplished by catalytic polymerization induced by 2,2′-azobis[isobutyronitrile] (AIBN). ODAEMA (1.2 g, 2.5 mmol) and AIBN (8.0 mg, 0.05 mmol, 2 mol %) were placed in a polymerization flask to which H_2O (90 mL) was added. The mixture was agitated to obtain a homogeneous solution. The flask was degassed by the freeze–thaw technique, sealed in a nitrogen atmosphere, and placed in a constant temperature bath at 70 °C for 17 h. The polymer was freeze dried for 24 h, followed by vacuum drying for 3 h to give a white powder (1.0 g). Polymerization of ODAEMA was determined by ^{1}H NMR spectra by following the decrease in intensity of the vinyl protons (δ 6.08 and 5.61 ppm), and the completion of polymerization was indicated by the disappearance of the vinyl proton peaks. Further evidence for polymerization was the line broadening of peaks observed in the ^{1}H NMR spectrum.

Sodium perchlorate (AR grade) was purchased from Merck and sodium hydroxide (AR grade) from Ajax Chemicals. All nonaqueous solvents were spectroscopic grade and were obtained from Ajax Chemicals or Merck. "Milli-Q" water was used to prepare the subphase (conductivity $< 1 \times 10^{-6}$ S cm^{-1}, surface tension = 72 mN m^{-1} at 25 °C). Chloroform was used as the spreading solution for all monolayer experiments.

2.2. Surface Pressure–Area Measurements. Surface pressure–area (π–A) measurements of the pure amphiphile monolayers were conducted on a 57.9 × 13.5 cm^2 poly(tetrafluoroethylene) (PTFE) Langmuir trough, with a PTFE barrier, driven at a compression rate of 0.03 nm^2 molecule^{-1} min^{-1}. A 59.7 × 16.5 cm^2 PTFE Langmuir trough with a compression rate of 0.05 nm^2 molecule^{-1} min^{-1} and a 47.0 × 15.0 cm^2 PTFE Langmuir trough (KSV-2200) (compression rate 0.05 nm^2 molecule^{-1} min^{-1}) equipped with a quartz window in the bottom were used for steady-state monolayer fluorescence experiments. The KSV-2200 trough was also used for time-resolved monolayer fluorescence experiments.

All surface pressure–area measurements were made by the Wilhelmy hanging plate method.[29] For π–A measurements of the pure amphiphiles and steady-state monolayer fluorescence measurements, a 4.3-cm mica plate suspended from a Shinkoh 2-g capacity strain gauge was used. The apparent changes in weight with monolayer compression were converted to voltages by the strain gauge and recorded on an Apple Macintosh PC. A 3.0-cm roughened platinum plate suspended from a Cahn microbalance was used in the time-resolved monolayer fluorescence measurements. The change in voltage from the microbalance was monitored by the KSV-2200 trough controller and recorded on an IBM PC with software from KSV, Helsinki.

Experiments were initiated by filling the trough with the appropriate subphase. Approximately 10^{17} molecules from 1 mM chloroform solutions mixed to the desired ratio were spread onto the subphase, using a 100-μL SGE syringe. The solvent was then allowed to evaporate for 10 min, after which the monolayer was compressed as desired.

2.3. Steady-State Monolayer Fluorescence Measurements. Steady-state fluorescence measurements were performed on two different experimental setups. The first setup, located at The University of Melbourne, employed a Perkin-Elmer LS-5 luminescence spectrophotometer, and details of the complete system have been given previously.[8-10] Briefly, two silica fiber optic bundles were used to transfer the exciting light and the fluorescence to and from the monolayer. Since the fluorescence signal from the monolayer was small, it was necessary to subtract the background signal due to scatter from the subphase of the exciting light and/or fluorescence from the PTFE. The background signal, monitored at the same emission wavelength as that of pyrene-DPPE, was recorded for 10 min, averaged, and then subtracted from the fluorescence signal when the monolayer was present. Fluorescence intensity curves as a function of monolayer compression were obtained using this experimental setup. Thus the fluorescence intensities at various surface pressures (I values) were obtained for each of the monolayers of different quencher concentration. The I_0 value at a given surface pressure was obtained from a monolayer containing only pyrene-DPPE in the matrix monolayers. Addition of the quenchers CAT-16 and ODDMA to the monolayer films of the matrices did not significantly affect the packing of the molecules, and in all cases, with the exception of the ODAEMA matrix, the π-A isotherms were within ±0.02 nm^2 molecule^{-1} of each other in both the absence and presence of quencher. Addition of CAT-16 quencher to the monolayer film of ODAEMA caused an increase in area, at a particular surface pressure, with the π–A isotherms differing in average area per molecule by ca. 0.07 nm^2 at the high end of the concentration range of CAT-16 (25 mol %). This resulted in a different surface concentration of pyrene-DPPE molecules for the reference monolayer and for monolayers containing quencher. To account for this variation in average area per molecule, and for the dependence of the fluorescence lifetime of pyrene-DPPE on the average area per molecule, it was necessary to apply a correction for the pyrene-DPPE/CAT-16/ODAEMA system in the following manner. All fluorescence intensities were scaled to constant surface concentration of pyrene-DPPE and then further corrected for the variation of fluorescence lifetime of pyrene-DPPE with the average area per molecule (see later).

In the second setup (University of Uppsala), a silica lens and mirrors were used to focus the excitation light from a pulsed (20-Hz) nitrogen laser (λ_{em} = 337 nm) (Laser Science, Inc., WSL-337ND) onto the monolayer. The exciting light then passed through a quartz window in the bottom of the trough and into a black box, which acted as a light sink, reducing the intensity of the scattered light. The emission was collected and transmitted

to an optical multichannel analyzer (OMA, EE & G Model 1460) by means of a silica optical fiber, positioned at a right angle to the surface. Typical exposure times were 20 s. Details are described elsewhere.[30]

2.4. Time-Resolved Monolayer Fluorescence Measurements. Fluorescence decay curves were measured by the time-correlated single-photon counting method.[31] The decay measurements were made at the University of Uppsala using frequency-doubled (320 nm) radiation from a DCM dye-laser (Spectra Physics Model SP 375 and 344S) synchronously pumped by a mode-locked Nd:YAG laser (Spectra Physics Model SP 3800). The exciting light was focused onto the monolayer by means of a lens and mirrors and passed through a quartz window in the bottom of the trough. The emission from the monolayer was focused by a fused silica lens onto a Hamamatsu (Model R15640) microchannel plate photomultiplier tube, having passed through a polarizer set so as to remove distortion of decay curves by rotational relaxation. The wavelength of observation (400 nm) was determined by an Oriel narrow-band pass filter and an Oriel low fluorescence cutoff filter. The electronics and analysis software have been described elsewhere.[30]

All time-resolved fluorescence measurements showed a background decay component attributable to fluorescence from the PTFE trough. This background decay was recorded under the same experimental conditions as those used to record the fluorescence decay when the monolayer was present. A photodiode (Elfa Ltd., Model BPW 34) connected to a voltage/frequency converter and counter monitored the exciting light and gated the detection system to ensure the background and monolayer decays were recorded with the same overall excitation intensity. Typical exposure times were 20 min. The background decay is short-lived and after 2 ns is less than 5% (in counts) of the monolayer decay. The background decay was subtracted channel by channel from the measured monolayer decays (i.e. background plus monolayer) to yield the true monolayer decay curves before the latter was analyzed. The quality of the fit and the estimated decay times obtained by subtracting the background decay from the measured monolayer fluorescence decay were very similar to those obtained by analyzing all but the first 2 ns of the data without the background subtraction.

As mentioned earlier, the average area per pyrene-DPPE molecule at a given surface pressure for the ODAEMA matrix differs for the monolayers with and without CAT-16 quencher. As the fluorescence lifetime of pyrene-DPPE was found to vary with average area per molecule, the unquenched lifetime needed for the analysis of any particular experiment was first corrected to the value appropriate for the average area per molecule involved.

2.5. Monolayer Polymerizations. Polymerization of the air–water monolayers was initiated by a low pressure mercury lamp (Model G24T7H, Ultra Violet Supplies, Victoria, Australia) giving an incident radiation of approximately 500 μW cm^{-2} at the air–water interface, as measured using a UVX radiometer (Model UVG-11, Ultraviolet Products Inc., San Gabriel, CA) equipped with a UVX-25 detector. All polymerizations were carried out in a nitrogen atmosphere to prevent ozonolysis of the polymer backbone.[32]

Results and Discussion

3.1. Diacetylene Amphiphile (1). A requirement for applying the fluorescence quenching approach is that the pyrene-labeled probe be homogeneously distributed in the host monolayer film.[8-10] The surface pressure–area (π–A) isotherm for the diacetylene 16–8 compound on a pure water subphase is shown in Figure 2. This π–A isotherm is less condensed than the π–A isotherms for the fully saturated equivalent due to increased steric interactions caused by the linear diacetylene section in the alkyl chain.[33,34] The limiting area per molecule of 0.25 nm^2 is in close agreement with values reported in the literature.[34-38] The π–A isotherm of diacetylene 16–8 was repeated on a water subphase containing 0.1 M $NaClO_4$, which has previously been found to produce stable monolayers for the CAT-16 quencher.[8-10] The π–A isotherm of diacetylene 16–8 measured on a 0.1 M $NaClO_4$ subphase was found to be identical to that measured on pure water. On the basis of previous work, the condensed nature of the π–A isotherm of unpolymerized diacetylene 16–8 suggests that pyrene-DPPE will not distribute homogeneously in this monolayer film.[8-10]

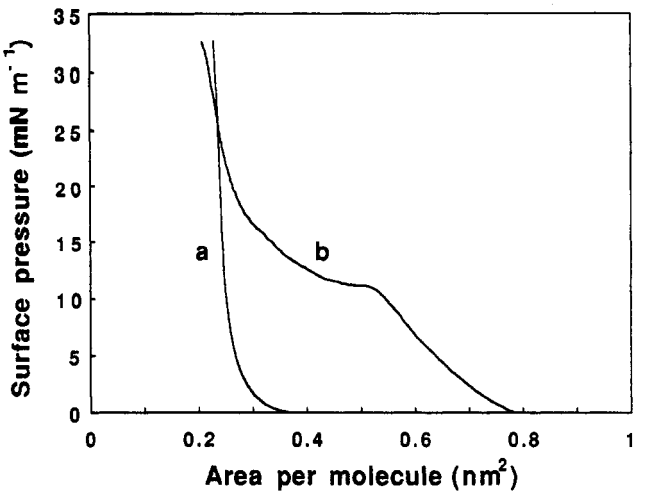

Figure 2. Surface pressure–area isotherms of diacetylene 16–8 in the monomeric form on different subphases: (a) pure H_2O or 0.1 M $NaClO_4$; (b) basic 0.1 M $NaClO_4$. Temperature = 22 ± 1 °C.

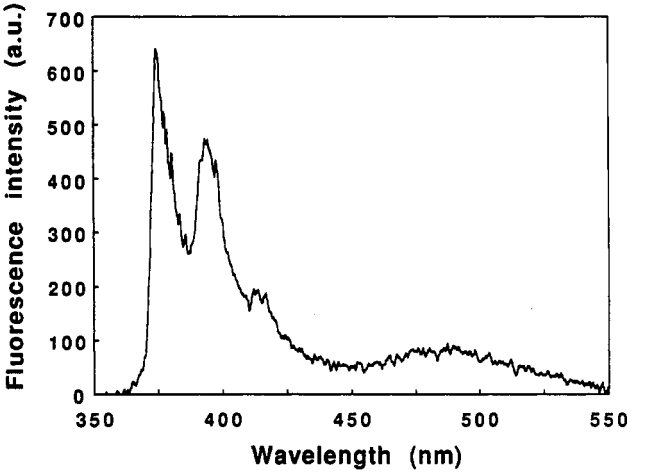

Figure 3. Steady-state fluorescence spectra of a 1 mol % pyrene-DPPE/diacetylene 16–8 monolayer at π = 10 mN m^{-1}. Excitation wavelength = 337 nm. Temperature = 21 ± 1 °C. Subphase: 0.1 M $NaClO_4$.

For the fluorescence measurements, 1 mol % of pyrene-DPPE was incorporated into the unpolymerized diacetylene 16–8 monolayer. The fluorescence spectra for 1 mol % pyrene-DPPE diluted into a diacetylene 16–8 monolayer on a 0.1 M $NaClO_4$ subphase, recorded at 10 mN m^{-1}, is shown in Figure 3. The fluorescence spectrum exhibits monomer peaks at 378, 397, and 417 (shoulder) nm and a broad band around 480 nm, which is characteristic of the formation of excimers of pyrene.[39] The excimer emission is indicative of aggregated pyrene-DPPE in the monolayer film, as homogeneously distributed pyrene-DPPE does not show the excimer band.[8-10]

In previous work with the ODDMA quencher, a basic (pH 12.5) 0.1 M $NaClO_4$ subphase was used to prevent dissolution of ODDMA in the subphase and to prevent protonation of the amino group and consequent loss of quenching capacity. The π–A isotherm for diacetylene 16–8 obtained on a basic (pH 12.5) 0.1 M $NaClO_4$ subphase is also shown in Figure 2. This π–A isotherm displays three distinct regions: (i) a liquid-expanded (LE) phase

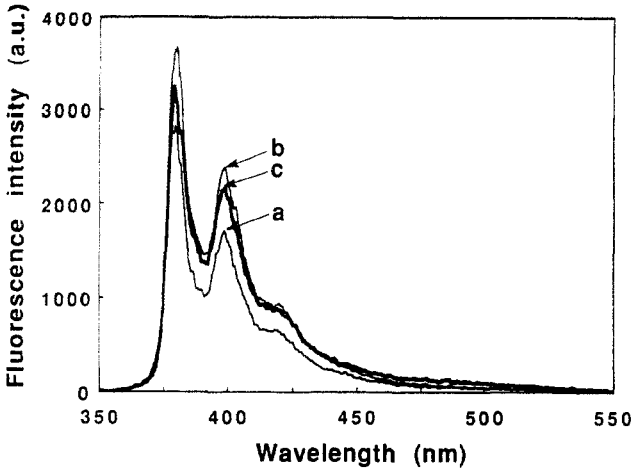

Figure 4. Steady-state fluorescence spectra of a 1 mol % pyrene-DPPE/diacetylene 16–8 monolayer at various surface pressures: (a) $\pi = 5$ mN m^{-1}; (b) $\pi = 15$ mN m^{-1}; (c) $\pi = 25$ mN m^{-1}. Excitation wavelength = 337 nm. All spectra were recorded at 21 ± 1 °C. Subphase: basic 0.1 M $NaClO_4$.

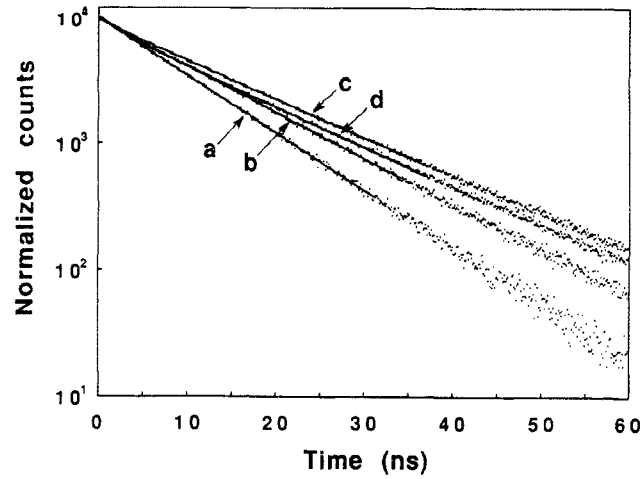

Figure 5. Fluorescence decays of a 1 mol % pyrene-DPPE/diacetylene 16–8 monolayer at various surface pressures: (a) π = 5 mN m^{-1}; (b) π = 10 mN m^{-1}; (c) π = 18 mN m^{-1}; (d) π = 25 mN m^{-1}. λ_{ex} = 320 nm and λ_{em} = 400 nm. Subphase: basic 0.1 M $NaClO_4$. Temperature = 21 ± 1 °C. All fluorescence decays have been normalized to the same maximum intensity.

at surface pressures below about 13 mN m^{-1}, (ii) a plateau region with compression, and (iii) a liquid-condensed (LC) phase of an unionized monolayer. Patil and co-workers[40,41] have also observed these phases in the π–A isotherms of saturated fatty acids on high alkaline (pH ~9–13) subphases. They have reported that ionization is essentially complete in the LE phase of these monolayers and that ionization is incomplete in the plateau region of the π–A isotherms. Their experimental results suggest that the increased surface potential which develops during compression decreases the surface pH and consequently the degree of ionization in the monolayer. This decrease in ionization during compression of the monolayer increases the relative amount of unionized fatty acid molecules, and the increasing condensing effect of these neutral molecules generates the plateau. The fatty acid molecules are also believed to form condensed acid-soaps, and at pH 10 and above, fatty acid monolayers generate a strong anionic field with counterions such as Na^+ and K^+ binding to the monolayer and condensing the film.[40,41]

The fluorescence spectra for a monolayer of 1 mol % pyrene-DPPE diluted into an unpolymerized diacetylene 16–8 monolayer on a basic 0.1 M $NaClO_4$ subphase, recorded at surface pressures of 5, 15, and 25 mN m^{-1}, are shown in Figure 4. The fluorescence spectrum at 5 mN m^{-1} (LE phase) shows, as expected, monomer emission only, indicating that pyrene-DPPE is homogeneously distributed in this region of the monolayer film. Further compression to 15 mN m^{-1} (plateau region) does not change the structure of the fluorescence spectrum but shows an increase in the fluorescence intensity. This increase in fluorescence intensity is due to an increase in the surface concentration and an increase in the lifetime of pyrene-DPPE upon compression.[42] In the LC phase, at a surface pressure of 25 mN m^{-1}, the fluorescence spectrum shows a decrease in the monomer emission intensity and a small relative increase in emission above 450 nm, suggestive of excimer fluorescence. These results are in agreement with previous studies which show pyrene-DPPE to be miscible in matrices which display LE isothermal behavior, but aggregated in the LC phase of monolayers.[8–10,42]

Time-resolved fluorescence measurements were undertaken to confirm the conclusion from the steady-state measurements that pyrene-DPPE is homogeneously distributed in the LE phase of the unpolymerized diacetylene 16–8 monolayer. Previous studies[8–10,42] have shown that where excimer emission is absent, an exponential fluorescence decay is observed for pyrene-DPPE in the host monolayer (in the absence of quencher) after subtraction of a short-lived component (due to fluorescence from the PTFE trough), from the observed decay. Figure 5 shows the fluorescence decay behavior of a 1 mol % pyrene-DPPE/diacetylene 16–8 monolayer on a basic 0.1 M $NaClO_4$ subphase at surface pressures of 5, 10, 18, and 25 mN m^{-1}. Even after subtraction of the fast background decay, the decays are best described by a double-exponential of the form

$$I(t) = A_1 \exp(-t/\tau_1) + A_2 \exp(-t/\tau_2) \quad (1)$$

where $I(t)$ denotes the fluorescence intensity at time t, A_1 and A_2 are the preexponential factors, and τ_1 and τ_2 are the fluorescence lifetimes. The results of the fitting are shown in Table I. Examination of Table I shows a short lifetime of about 2–3 ns and a longer lifetime between 9.5 and 13.7 ns, which increases as the surface pressure increases. The quantum yield of the fluorescence of the fast decay component increases steadily with increasing surface pressure, further suggesting that the nonexponentiality of the decays is real. In the light of previous experience this nonexponentiality is unlikely to be due to inadequate subtraction of the fast background decay.[8–10,42] The steady-state fluorescence spectra show no evidence of excimers in the LE phase of the diacetylene 16–8 monolayer, nor of any other emitter other than the

Table I. Decay Parameters for Pyrene-DPPE Diluted into Various Matrix Monolayers, Measured at Different Surface Pressures (λ_{ex} = 320 nm, λ_{em} = 400 nm; Temperature = 20 ± 2 °C)

matrix	π/mN m^{-1}	A_1[a]	τ_1/ns	A_2[a]	τ_2/ns	χ^2
diacetylene 16-8	5	0.06	2.9	0.94	9.5	1.2
	10	0.08	2.6	0.92	11.9	1.1
	18	0.12	3.0	0.88	14.2	1.0
	25	0.19	3.2	0.81	13.7	1.1
ODAEMA	5	1.00	5.3			1.3
	10	1.00	6.1			1.4
	15	1.00	7.0			1.3
	20	1.00	8.0			1.4
	25	1.00	8.7			1.3
poly-ODAEMA	5	0.17	5.7	0.83	18.0	1.1
	15	0.22	5.7	0.78	19.6	1.1
	25	0.25	6.2	0.75	20.6	1.2

[a] The sum of the fraction of the components has been normalized to unity.

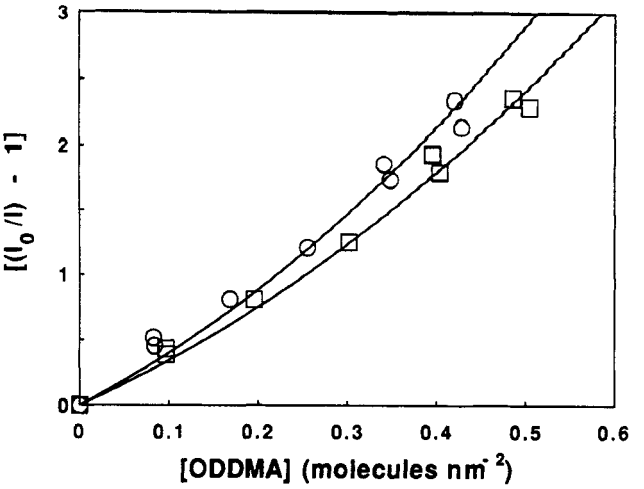

Figure 6. Stern–Volmer plots for the quenching of 1 mol % pyrene-DPPE by ODDMA in diacetylene 16–8 monolayers at various surface pressures: (○) 5 mN m^{-1}; (□) 10 mN m^{-1}. λ_{ex} = 350 nm and λ_{em} = 400 nm. Temperature = 22 ● 1 °C. Subphase: basic 0.1 M $NaClO_4$. The solid lines are the theoretical curves calculated using eq 4, with parameters given in the text.

monomer. The nonexponentiality of the decay curves may be due to the existence of pyrene-DPPE molecules in different environmental sites in the monolayer film and/or excitation energy trapping by some higher aggregated species of the pyrene chromophores (see section 3.3). It may be noted, however, that the contribution to the quantum yield of fluorescence from the shorter lived component is small (<5%). On this basis, and given the lack of steady-state spectroscopic evidence for aggregation of pyrene-DPPE in the LE phase of the diacetylene 16–8 monolayer, the longer decay times will be used in the analysis of the steady-state fluorescence quenching results for the diacetylene 16–8 monolayer. The presence of nonexponential decay curves in the absence of quencher prevents the use of time-resolved measurements to determine the lateral diffusion coefficients using the fluorescence quenching approach.

Stern–Volmer plots for the quenching of 1 mol % pyrene-DPPE by ODDMA in a diacetylene 16–8 monolayer, on a basic 0.1 M $NaClO_4$ subphase, at surface pressures of 5 and 10 mN m^{-1}, are shown in Figure 6. The plots show the expected upward curvature characteristic of quenching in a two-dimensional environment.[8-10,43,44] The solid curves in Figure 6 are the nonlinear least-squares (NLLS) best fit curves to the experimental steady-state quenching data (see below).

The fluorescence decay following a δ pulse excitation for a two-dimensional system is given by the expression[45,46]

$$\ln \frac{F(t)}{F(0)} = -k_0 t - \frac{1}{2}a^2 c_0 Q_2(ha, t/\tau_q) \qquad (2)$$

with

$$Q_2(ha, t/\tau_q) = \frac{[A_1(t/\tau_q)^\alpha + A_2\sqrt{t/\tau_q}]ha}{A_3(t/\tau_q)^\beta + A_4/\sqrt{t/\tau_q} + ha} \qquad (3)$$

where k_0 is the natural decay constant which is determined in a separate experiment, a is the probe–quencher encounter distance, c_0 is the quencher concentration (molecules/Å^2), $h = k_q a/2D$ and is thus a parameter that weights reaction against diffusion where k_q is the first-order quenching rate constant for the P*–Q pair, D is the mutual diffusion coefficient, and $\tau_q = a^2/D$, and α, β, and A_1–A_4 are parameters determined for different ranges of t/τ_q values.

Table II. Fluorescence Decay Lifetimes and Mutual Lateral Diffusion Coefficients of 1 mol % Pyrene-DPPE Diluted with Diacetylene 16-8 or ODAEMA for Different Surface Pressures (Temperature = 20 ± 2 °C)

		$10^7 D$/cm^2 s^{-1}			
		steady-state		time-resolved	
matrix	π/mN m^{-1}	ODDMA	CAT-16	CAT-16 (10%)	CAT-16 (20%)
diacetylene 16-8	5	3.2			
	10	2.0			
ODAEMA	5		7.3	4.2	4.0
	10		5.1		
	15		4.0	1.7	0.78
	20		3.5		
	25		2.7	0.68	0.17

The relation between the steady-state fluorescence intensity and quencher concentration for diffusion-controlled quenching in two dimensions has the same functional form as the expression in three dimensions[44] and is given by

$$\frac{I_0}{I} = \frac{a\tau_f}{[1 - b(\pi/a)^{1/2}\exp(b^2/a)\,\mathrm{erfc}(b/(a)^{1/2})]} \qquad (4)$$

where $a = 1/\tau_f + 2.28[Q]D$ and $b = 3.72D^{1/2}R[Q]$. I_0 and I refer to the steady-state fluorescence intensity in the absence and presence, respectively, of quencher molecules, [Q] is the quencher concentration (molecules/Å^2), R is the probe–quencher encounter distance, and τ_f is the fluorescence lifetime in the absence of quencher molecules. The function erfc(x) is the complementary error function as defined in standard mathematical tables. The numerical values in the above equation have been calculated for the time range $t/\tau_q \leq 5$,[8-10] and differ slightly from those reported by Owen,[44] whose approximation underestimated the value of Q_2 by more than 10% when $t/\tau_q \geq 1$.[45,46] This time range has been used by taking into account the approximate lifetime of pyrene-DPPE in the air–water monolayers.

The steady-state fluorescence quenching data of Figure 6 were NLLS fitted to eq 4. The NLLS fits require values for the encounter distance and the fluorescence lifetime of pyrene-DPPE in the monolayer film. The longer decay times of pyrene-DPPE in the unpolymerized diacetylene 16–8 monolayer at 5 mN m^{-1} (9.5 ns) and 10 mN m^{-1} (11.9 ns) were used, with a value of 0.8 nm for the encounter distance. The encounter distance was calculated as the sum of the molecular radii of pyrene-DPPE and ODDMA, in turn calculated from the limiting molecular areas obtained from the surface pressure–area isotherms. The expanded nature of the ODDMA and pyrene-DPPE monolayers introduces some uncertainty into the determination of the encounter distance. For example, a change in the encounter distance from 0.8 to 0.9 nm changes the lateral diffusion coefficient by about 20%. The values obtained for the lateral diffusion coefficients are 3.2 × 10^{-7} cm^2 s^{-1} at 5 mN m^{-1} and 2.0 × 10^{-7} cm^2 s^{-1} at 10 mN m^{-1} (see Table II). These values are in excellent agreement with those obtained by Meller *et al.* ((3.5 ± 0.5) × 10^{-7} cm^2 s^{-1} at 5 mN m^{-1} and (1.8 ± 0.3) × 10^{-7} cm^2 s^{-1} at 10 mN m^{-1}) for the lateral diffusion of *N*-(7-nitro-2,1,3-benzooxadiazol-4-yl)phosphatidylethanolamine (NBDPE) in the LE phase of monomeric diacetylene lipid monolayers, using the FRAP method.[20]

Stern–Volmer plots for the quenching of pyrene-DPPE by CAT-16 in a diacetylene 16–8 monolayer, on a basic 0.1 M $NaClO_4$ subphase, produced experimental data which show significant upward deviation from the calculated

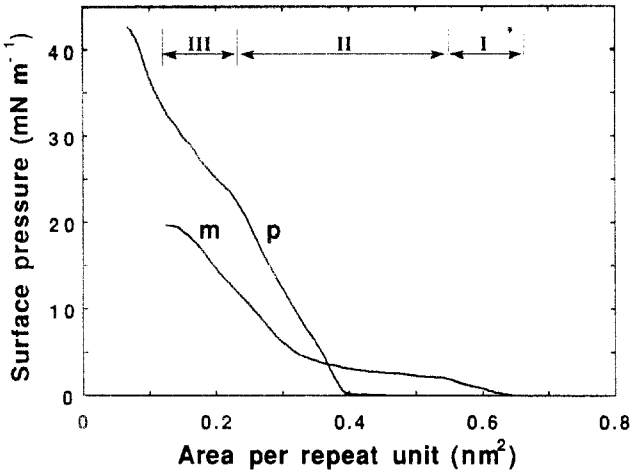

Figure 7. Surface pressure–area isotherms of ODMA in the monomeric (m) and polymeric (p) form. Subphase: 0.1 M $NaClO_4$. Temperature = 23 ± 1 °C.

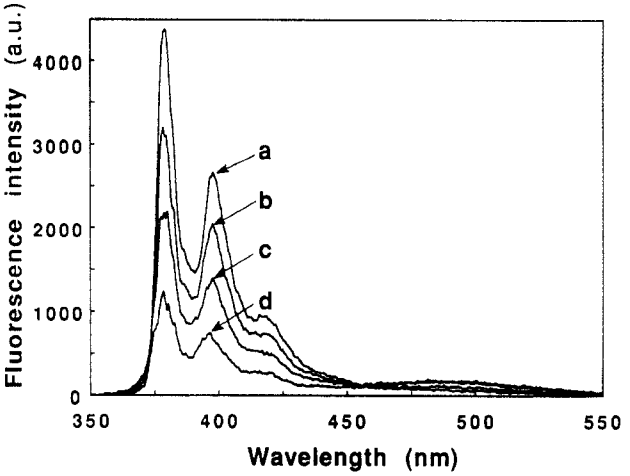

Figure 8. Steady-state fluorescence spectra of a 1 mol % pyrene-DPPE/ODMA monolayer at various surface pressures: (a) π = 1 mN m^{-1}; (b) π = 3 mN m^{-1}; (c) π = 5 mN m^{-1}; (d) π = 10 mN m^{-1}. Excitation wavelength = 337 nm. All spectra were recorded at 21 ± 1 °C. Subphase: 0.1 M $NaClO_4$.

curves (data not shown), indicating static quenching is influencing the emission intensity in the steady-state measurements. The model used for analysis of the steady-state data does not take into account static quenching, and therefore reliable lateral diffusion coefficients are not obtainable from the data.

As mentioned earlier, a requirement for applying the fluorescence quenching approach is that pyrene-DPPE be homogeneously distributed in the host monolayer film. Given that the time-resolved measurements for pyrene-DPPE diluted into diacetylene 16–8 (in the absence of quencher) on a basic 0.1 M $NaClO_4$ subphase are non-exponential, time-resolved measurements on monolayer films containing quencher for this system were not performed. Thus, it is not possible to rule out a contribution to the steady-state quenching data by static quenching.[8-10,47] The fits of the theoretical curves to the experimental data for the quenching of pyrene-DPPE by ODDMA in the unpolymerized diacetylene 16–8 monolayer film (Figure 6) are reasonable over the whole range of quencher concentrations, suggesting that any interference of the results by static quenching is small.[8-10,47]

Since diacetylenes react topochemically, polymerization can only occur if the amphiphiles are in the LC monolayer state.[33-38] Polymerization of the diacetylene 16–8 monolayer on a 0.1 M $NaClO_4$ subphase was achieved by irradiation of the unpolymerized monolayer at 20 mN m^{-1} for 30 min. The π–A isotherm for the polymerized monolayer was more condensed (data not shown) than the corresponding monomer π–A isotherm. As expected from the condensed nature of the π–A isotherm, the presence of 1 mol % pyrene-DPPE in this polymerized monolayer revealed pyrene-DPPE to be aggregated, with pyrene excimer emission being observed. This precludes the use of the fluorescence quenching approach to determine lateral diffusion coefficients for the polymerized monolayer. Nor can the influence of polymerization on molecular mobility in the diacetylene 16–8 monolayer be studied.

3.2. Methacrylate Amphiphile (2). In contrast to the diacetylene amphiphile (1), which is polymerizable in the alkyl portion of the chain, the methacrylate compound (2) can be polymerized in the head group region of the molecule.

The π–A isotherm of unpolymerized ODMA at 24 °C on 0.1 M $NaClO_4$, obtained by continuous compression, is shown in Figure 7. This π–A isotherm is identical to that obtained on pure water and corresponds closely to that reported by Naegele and Ringsdorf.[48] At areas per molecule between 0.55 and 0.65 nm^2 molecule^{-1} (region I), the monolayer film is in the LE state. Region II is characterized by a coexisting phase, and condensation to the LC state occurs. At areas per molecule below 0.23 nm^2 molecule^{-1} (region III), the formation of multilayers is believed to occur.[48] The fluorescence spectra of 1 mol % pyrene-DPPE diluted into an unpolymerized ODMA monolayer, at various surface pressures, are shown in Figure 8. The fluorescence spectra show the presence of pyrene excimers throughout the whole range of measured surface pressure (even in the LE phase), indicating pyrene-DPPE is aggregated in the monolayer film. The characteristics of the π–A isotherm of prepolymerized ODMA (PODMA) at the air–water interface are also shown in Figure 7. This π–A isotherm agrees well with those reported in the literature.[27] The fluorescence spectra of 1 mol % pyrene-DPPE embedded in PODMA also show the presence of pyrene excimers through the surface pressure range 0 (40 nm^2/monomer unit) to 26 mN m^{-1} (data not shown). The aggregation of pyrene-DPPE in both the unpolymerized and polymerized ODMA monolayer films precludes a diffusion study for these monolayers.

3.3. Quaternary Methacrylate Amphiphile (3). The quaternary methacrylate compound (3) is similar to compound 2, having the same polar head group and alkyl chain length, but contains a quaternary ammonium moiety at the 18-C atom position and two additional CH_2 units attached to this group. The aim of incorporating this quaternary ammonium group into the methacrylate amphiphile was to promote homogeneity of the pyrene-DPPE chromophore in a polymerizable methacrylate monolayer film in order to allow investigation of the fluidity of the film using the fluorescence quenching approach.

The π–A isotherm of amphiphile 3 on a 0.1 M $NaClO_4$ subphase is shown in Figure 9. In contrast to compound 2, amphiphile 3 displays a LE phase at all measured surface pressures. The shape of the π–A isotherm of amphiphile 3 on pure water is the same as that on 0.1 M $NaClO_4$, but the area per molecule occupied is about 10% smaller for the whole surface pressure range for the water subphase. The fluorescence spectra for a 1 mol % pyrene-DPPE/ODAEMA monolayer recorded at surface pressures of 5, 15, 25, and 35 mN m^{-1} are shown in Figure 10. Only monomer emission from excited pyrene-DPPE is seen and there is no change in the spectrum upon compression. In

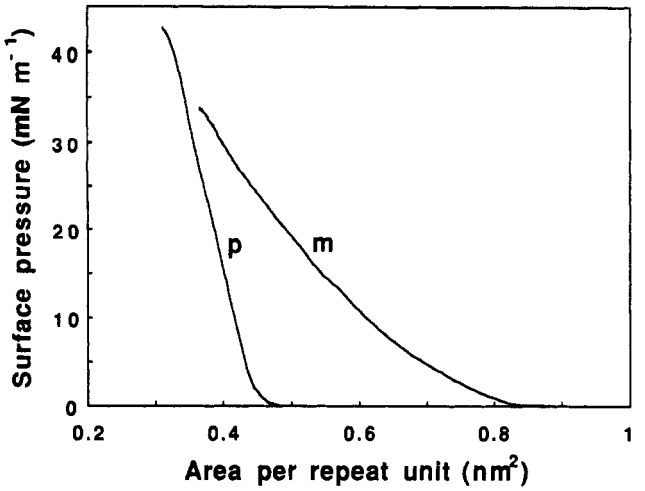

Figure 9. Surface pressure–area isotherms of ODAEMA in the monomeric (m) and polymeric (p) form. Subphase: 0.1 M $NaClO_4$. Temperature = 22 ± 1 °C.

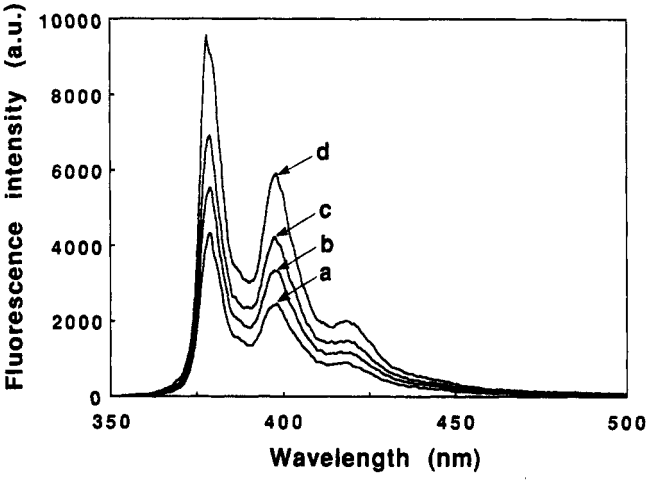

Figure 10. Steady-state fluorescence spectra of a 1 mol % pyrene-DPPE/ODAEMA monolayer at various surface pressures: (a) π = 5 mN m^{-1}; (b) π = 15 mN m^{-1}; (c) π = 25 mN m^{-1}; (d) π = 35 mN m^{-1}. Excitation wavelength = 337 nm. All spectra were recorded at 21 ± 1 °C. Subphase: 0.1 M $NaClO_4$.

addition, the monomer fluorescence intensity was found to be constant with time. Both the absence of pyrene excimer emission and the constant monomer emission from pyrene-DPPE with time (at a constant surface pressure) indicate that pyrene-DPPE is miscible in the monomer monolayer film of ODAEMA.[8-10,42] Additional evidence for this comes from the time-resolved fluorescence measurements. Figure 11 shows the fluorescence decay behavior of a 1 mol % pyrene-DPPE/ODAEMA monolayer at surface pressures of 5, 15, and 25 mN m^{-1}. The decays are best fitted by a single-exponential decay function, also indicating a homogeneous distribution of the pyrene chromophore within the monolayer film. The values of the fluorescence lifetimes at different surface pressures are given in Table I.

Stern–Volmer plots for the quenching of 1 mol % pyrene-DPPE by CAT-16 in an ODAEMA monolayer, at 5 and 25 mN m^{-1}, are shown in Figure 12. A value of 1.0 nm was used for the encounter distance (calculated as the sum of the molecular radii of pyrene-DPPE and CAT-16). The values of the lateral diffusion coefficients calculated are given in Table II. These diffusion coefficients are comparable with values reported in the literature for lipid molecules in various phospholipid and polymerizable matrix monolayers.[8-10,20,21,23,49] The lateral diffusion coefficient decreases with increasing surface pressure, reflecting a decrease in monolayer fluidity with compression.

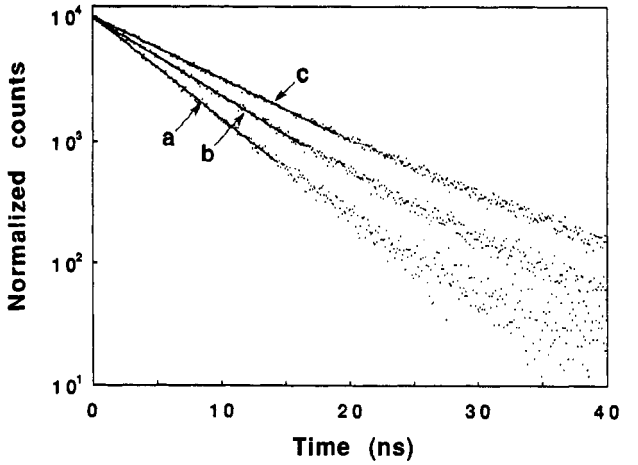

Figure 11. Fluorescence decays of a 1 mol % pyrene-DPPE/ODAEMA monolayer at various surface pressures: (a) π = 5 mN m^{-1}; (b) π = 15 mN m^{-1}; (c) π = 25 mN m^{-1}. λ_{ex} = 320 nm and λ_{em} = 400 nm. Subphase: 0.1 M $NaClO_4$. Temperature = 21 ± 1 °C. All fluorescence decays have been normalized to the same maximum intensity.

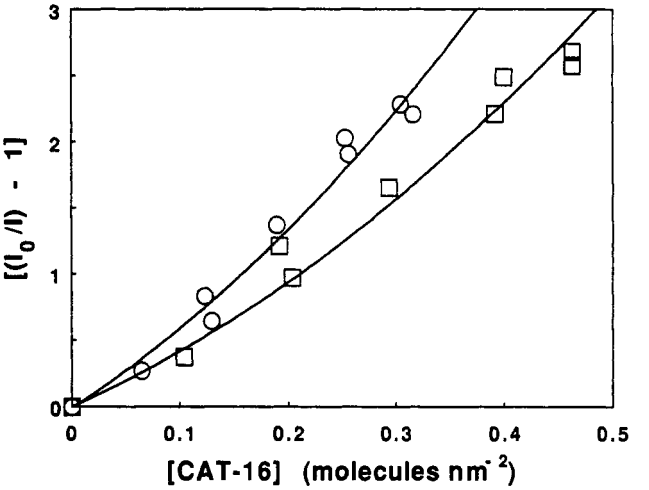

Figure 12. Stern–Volmer plots for the quenching of 1 mol % pyrene-DPPE by CAT-16 in ODAEMA monolayers at various surface pressures: (○) 5 mN m^{-1}; (□) 25 mN m^{-1}. λ_{ex} = 350 nm and λ_{em} = 400 nm. Temperature = 22 ± 1 °C. Subphase: 0.1 M $NaClO_4$. The solid lines are the theoretical curves calculated using eq 4, with parameters given in the text.

The fluorescence decay curves at 5 mN m^{-1} for 1 mol % pyrene-DPPE diluted into ODAEMA, in the presence of CAT-16, are shown in Figure 13. The quenched decay curves (b and c) are nonexponential, as expected from the theory of diffusional quenching in two dimensions. The fluorescence decay curves were NLLS fitted to eq 1 using a modified Levenberg–Marquardt algorithm,[50] which eliminates the need for partial derivatives. A value of 1.0 nm was used for the encounter distance. The reduced χ^2 value was used to judge the quality of the fit. The solid lines are the fitted curves, and the mutual lateral diffusion coefficients determined from the fittings are shown in Table II. The decay curves are not well described by the theory of diffusional quenching in two dimensions, and this can be seen by the quality of the fits. At a CAT-16 concentration of 10 mol %, the calculated curve fits the experimental data with a χ^2 value of about 2. The fits are even poorer at concentrations of 20 mol % CAT-16, yielding χ^2 values of about 5. The lateral diffusion coefficient values for the best NLLS fits to the time-resolved quenching data are given in Table II. The difference between the 10 mol % CAT-16 and 20 mol %

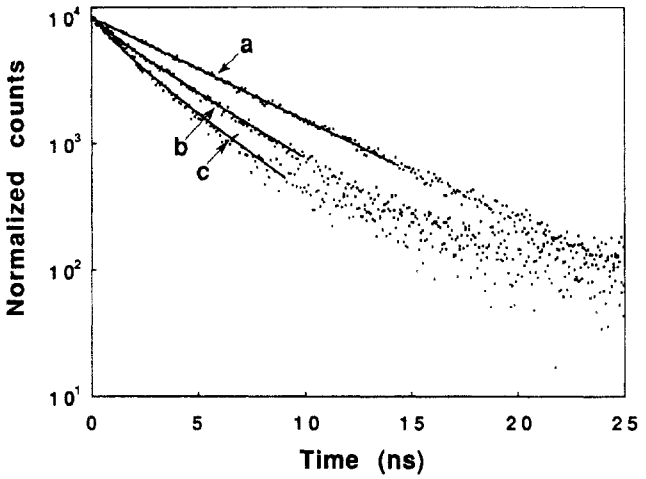

Figure 13. Fluorescence decays of a 1 mol % pyrene-DPPE/ODAEMA monolayer for various concentrations of CAT-16 at π = 5 mN m^{-1}: (a) 0 mol % CAT-16; (b) 10 mol % CAT-16; (c) 20 mol % CAT-16. All fluorescence decays have been normalized to the same maximum intensity. Subphase: 0.1 M $NaClO_4$. λ_{ex} = 320 nm and λ_{em} = 400 nm. Temperature = 21 ± 1 °C.

CAT-16 time-resolved values is greatest at higher surface pressures, where the difference in the average area per molecule occupied per pyrene-DPPE molecule is largest, for a given surface pressure (see section 2.4). As might be expected in view of the poor fit of the time-resolved data, the results for the time-resolved measurements are different (smaller) than those from the steady-state data. The fact that the steady-state data can be well fitted by the theory while the time-resolved data cannot, shows the lack of sensitivity of the former type of measurement to the presence of complicating effects. Steady-state measurements reflect the effect of diffusional quenching on the dominant fluorescent emission process. In one sense, steady-state measurements have the advantage of ignoring small numbers of molecules which decay rapidly in the absence of quencher. On the other hand, steady-state quenching data are susceptible to the occurrence of static quenching while time-resolved data are not.

In order to investigate the effect polymerization has on lateral mobility and miscibility of pyrene-DPPE, poly-ODAEMA was examined as a monolayer at the air–water interface using steady-state and time-resolved fluorescence methods. The polymerization of amphiphile 3 was performed in isotropic solution via the radical initiator AIBN and not by in situ UV irradiation. The π–A isotherm for poly-ODAEMA on 0.1 M $NaClO_4$ is shown in Figure 9. As a result of polymerization, the π–A isotherm shows a reduction in the average area per repeat unit and becomes more condensed. The polymerized monolayer is more stable, collapsing at a surface pressure of 43 mN m^{-1}, compared to 34 mN m^{-1} for the monomer monolayer. The fluorescence spectrum of 0.5 mol % pyrene-DPPE in poly-ODAEMA, like that of the monomeric ODAEMA monolayer, shows monomer emission only (data not shown). The presence of only monomer emission is somewhat surprising given the condensed nature of the π–A isotherm. The time-resolved fluorescence decays, however, are nonexponential (data not shown) and are best fitted to a double-exponential decay function. The results of the fittings for various surface pressures are shown in Table I. The contribution of the fast decay component to the quantum yield of fluorescence is given by the ratio of the product of the preexponential factor A_1 and the fluorescence lifetime τ_1 to the sum of the products of both preexponential factors and their respective fluorescence lifetimes. At the measured surface pressures, the contribution of the short decay component to the quantum yield of fluorescence is in the range 6–10%, and therefore may not be observed in the steady-state fluorescence spectrum but will result in nonexponential decay behavior of the temporal response of the emission.

Yamazaki *et al.*[51,52] have studied the molecular association of 16-(1-pyrenyl)hexadecanoic acid (PHA) embedded in stearic acid Langmuir–Blodgett (LB) films using steady-state and time-resolved fluorescence techniques. Both monomer and excimer fluorescence bands were observed for the films, even at a few mole percent of PHA. The nonexponential fluorescence decay behavior of the PHA chromophores observed for the monomer band was interpreted in terms of excitation energy trapping by some higher aggregated species of the pyrene chromophores. Rapid nonradiative processes, possibly associated with irreversible photochemistry in such aggregates, might cause them to act as excitation energy traps for neighboring chromophores, leading to nonexponential decay curves. On the basis of this hypothesis, the nonexponentiality of the decay curves observed for pyrene-DPPE in the polymerized ODAEMA film may, similarly, be due to excitation trapping by nonfluorescent dimers or aggregates. Alternatively, the occurrence of the nonexponential decays in the pyrene-DPPE/poly-ODAEMA system may be explained by the interaction between the pyrene-DPPE chromophores and the quaternary ammonium groups of the polymer film. Recently, Almgren *et al.*[53] studied systems containing pyrene and tetraalkylammonium halides (R_4NX where X = Cl^-, Br^-) using time-resolved and steady-state fluorescence quenching techniques and solubility measurements. It was found that the quenching of pyrene fluorescence by R_4NBr is a synergistic effect, requiring Br^-, and is enhanced by the simultaneous presence of the quaternary ammonium ion. The formation of a weak complex between pyrene and the quaternary ammonium ion was suggested. The quenching of excited pyrene by R_4NBr was found to be due to the inhomogeneously distributed Br^- ions, resulting in the nonexponential fluorescence decay behavior of pyrene. This weak interaction between pyrene and quaternary ammonium groups has also been observed by a number of other workers using ^1H NMR,[54] solubility measurements,[55] and the intensity ratio of the peaks III (383 nm) and I (372 nm) in the pyrene monomer fluorescence spectrum.[56–58] In addition, it has been found that the formation of pyrene–quaternary ammonium ion complexes reduced the efficiency of pyrene excimer formation.[53] This may account for the absence of excimer emission from the steady-state spectroscopic data for the pyrene-DPPE/poly-ODAEMA system. This then raises the question of why single-exponential fluorescence behavior is observed for pyrene-DPPE in the ODAEMA monolayer (in the absence of quencher) and nonexponential fluorescence behavior is observed for pyrene-DPPE in the polymerized ODAEMA film (in the absence of quencher). The nonexponential fluorescence behavior may be due to the polymerized film adopting different conformational and configurational properties compared to the unpolymerized film, facilitating the formation of pyrene–quaternary ammonium complexes. On the other hand, it is reasonable to assume that pyrene-DPPE is distributed nonuniformly in the polymerized film, emitting from different environmental sites within the monolayer, leading to the nonexponential fluorescence decay behavior observed.

It is worth noting that upon recording the fluorescence spectrum of pyrene-DPPE in the poly-ODAEMA monolayer, the emission intensity was observed to decrease with

increasing UV irradiation. This decrease in intensity can be explained by the low mobility of the pyrene-DPPE chromophores in the polymer film. It is likely that chromophores within the irradiated area are destroyed at a rate faster than they can be replenished by diffusion from outside the irradiated area. Some recovery of the emission intensity was monitored after blocking the beam for several minutes, indicating that diffusion is very slow. In this case, and on the basis of values obtained from other measurements employing the fluorescence quenching technique and the same experimental setup,[8-10,42] an upper limit of 10^{-8} cm^2 s^{-1} may be estimated for the lateral diffusion coefficient in the polymer monolayer film of ODAEMA. This suggests that there is a decrease of at least 1 order of magnitude in the lateral diffusion coefficient for the polymerized ODAEMA film, compared to the monomeric ODAEMA film. Values between 10^{-7} and 10^{-10} cm^2 s^{-1} have been reported for lateral diffusion coefficients of lipid probe molecules in various diene, diacetylene, and methacrylate polymer monolayers at the air–water interface.[20,21,49]

4. Conclusions

The nature of the π–A isotherm for diacetylene 16–8 makes it possible to undertake a fluorescence quenching study of diffusion only on a basic subphase and in the LE phase of the monolayer. The results obtained are, however, somewhat equivocal because of the complex nature of the fluorescence decay. The lateral diffusion coefficients determined from the steady-state measurements are consistent with values reported in the literature. The polymerized film of diacetylene 16–8 could not be studied because of aggregation of the pyrene-DPPE probe molecules.

The results for the unpolymerized methacrylate amphiphile do not fit the pattern of earlier work[8-10] in that the steady-state spectroscopic measurements show pyrene-DPPE to be aggregated even in the LE phase of the monolayer film. Pyrene-DPPE is also aggregated in a polymerized monolayer of the methacrylate amphiphile. The aggregation of pyrene-DPPE in both the unpolymerized and polymerized methacrylate monolayer films prevents a fluorescence quenching study on them.

For the quaternary methacrylate amphiphile in the unpolymerized monolayer, the surface pressure–area isotherm and steady-state and time-resolved measurements all provide evidence that pyrene-DPPE is homogeneously distributed in the monolayer film. The steady-state data using CAT-16 as quencher in the unpolymerized ODAEMA monolayer give lateral diffusion coefficients which are in agreement with values reported for lipid molecules in various matrix monolayers. The time-resolved data, however, are poorly fit by the model highlighting the insensitivity of the steady-state approach to complicating factors. Although the steady-state spectroscopic data show no evidence for a second emitter for pyrene-DPPE in a polymerized ODAEMA monolayer, the time-resolved data reveal nonexponential decays. The lateral diffusion coefficient for a polymerized monolayer film of ODAEMA is considerably less than that for an unpolymerized film.

In summary, the systems studied clearly demonstrate the difficulties involved in applying the bimolecular fluorescence quenching approach for the determination of lateral diffusion coefficients to air–water monolayers. At the same time they indicate the value of adequate photophysical characterization before a diffusion study is undertaken. The fluorescence quenching approach, although elegant in conception and free of the experimental problems associated with the FRAP and pyrene excimer techniques, requires initial conditions in the monolayer system that are not readily achieved in all systems. It should be noted, however, that the effects observed in this work are likely to be present in systems studied by the FRAP method and the probability of their occurrence may cast doubt on some FRAP work in which adequate photophysical characterization of the systems investigated has not been undertaken.

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References and Notes

(1) Fendler, J. H. *Membrane Mimetic Chemistry*; Wiley: New York, 1982.
(2) Roberts, G. G. *Adv. Phys.* **1985**, *34*, 475.
(3) Kuhn, H.; Möbius, D.; Bücher, H. *Physical Methods of Chemistry*; Weissberger, A., Rossiter, B., Eds.; Wiley: 1972; Vol. 1, p 577.
(4) Gaines, G. L. *Insoluble Monolayers at Liquid-Gas Interfaces*; Wiley: New York, 1966.
(5) Roberts, G. G. *Sens. Actuators* **1983**, *4*, 131.
(6) Swalen, I. D.; Allara, D. L.; Andrade, I. D.; Chandross, E. A.; Garoff, S.; Israelachvili, J.; McCarthy, T. I.; Murray, R.; Pease, R. F.; Rabolt, I. F.; Wynne, K. I.; Yu, H. *Langmuir* **1987**, *3*, 932.
(7) Sugi, M. *J. Mol. Electron.* **1985**, *1*, 3.
(8) Caruso, F.; Grieser, F.; Murphy, A.; Thistlethwaite, P. J.; Urquhart, R.; Almgren, M.; Wistus, E. *J. Am. Chem. Soc.* **1991**, *113*, 4838.
(9) Caruso, F.; Grieser, F.; Thistlethwaite, P. J.; Almgren, M.; *Biophys. J.*, in press.
(10) Caruso, F.; Grieser, F.; Thistlethwaite, P. J.; Almgren, M.; *Langmuir*, in press.
(11) Ringsdorf, H.; Schlarb, B.; Venzmer, J. *Angew. Chem., Int. Ed. Engl.* **1988**, *27*, 113 and references therein.
(12) Fendler, J. H. *Science* **1984**, *223*, 888.
(13) Bader, H.; Dorn, K.; Hupfer, B.; Ringsdorf, H. *Adv. Polym. Sci.* **1985**, *64*, 1.
(14) Gros, L.; Ringsdorf, H.; Schupp, H. *Angew. Chem., Ind. Ed. Engl.* **1981**, *20*, 305.
(15) Elbert, R.; Laschewsky, A.; Ringsdorf, H. *J. Am. Chem. Soc.* **1985**, *107*, 4134.
(16) Tieke, B.; Weiss, K. *Colloid Polym. Sci.* **1985**, *263*, 576.
(17) Laschewsky, A.; Ringsdorf, H.; Schmidt, G.; Schneider, J. *J. Am. Chem. Soc.* **1987**, *109*, 788.
(18) Biddle, M. B.; Lando, J. B.; Ringsdorf, H.; Schmidt, G.; Schneider, J. *Colloid Polym. Sci.* **1988**, *266*, 806.
(19) Duda, G.; Schouten, A. J.; Arndt, T.; Lieser, G.; Schmidt, G. F.; Bubeck, C.; Wegner, G. *Thin Solid Films* **1988**, *159*, 221.
(20) Meller, P.; Peters, R.; Ringsdorf, H. *Colloid Polym. Sci.* **1989**, *267*, 97.
(21) Kim, S.; Yu, Hyuk, *J. Phys. Chem.* **1992**, *96*, 4034.
(22) Teissie, J.; Tocanne, J.-F.; Baudras, A. *Eur. J. Biochem.* **1978**, *83*, 77.
(23) Peters, R.; Beck, K. *Proc. Natl. Acad. Sci. U.S.A.* **1983**, *80*, 7183.
(24) Loughran, T.; Hatlee, M. D.; Patterson, L. K.; Kozak, J. J. *J. Chem. Phys.* **1980**, *72*, 5791.
(25) Subramanian, R.; Patterson, L. K. *J. Am. Chem. Soc.* **1985**, *107*, 5820.
(26) Bohorquez, M.; Patterson, L. K. *J. Phys. Chem.* **1988**, *92*, 1835.
(27) Drummond, C. J. Elliot, P.; Furlong, D. N.; Barnes, G. T. *Thin Solid Films* **1992**, *210/211*, 69.
(28) Nagai, K.; Ohishi, Y.; Inaba, H.; Kudo, S. *J. Polym. Sci. Polym. Chem. Ed.* **1985**, *23*, 1221.
(29) Heimenz, P. C. *Principles of Colloid and Surface Chemistry*; Marcel Dekker, Inc.: New York and Basel, 1977; p 210.

(30) Wistus, E.; Mukhtar, E.; Almgren, M.; Lindquist, S.-E. *Langmuir* **1992**, *8*, 1366.
(31) O'Connor, D. V.; Phillips, D. *Time-Correlated Single Photon Counting*; Academic: London, 1984.
(32) Patel, G. N.; Lee, L. T. C. *J. Macromol. Sci. Phys.* **1983**, *22*, 259.
(33) Tieke, B.; Lieser, G. *J. Colloid Interface Sci.* **1982**, *88*, 471.
(34) Tieke, B. *Adv. Polym. Sci.* **1985**, *71*, 79.
(35) Ringsdorf, H.; Day, D. *J. Polym. Sci., Polym. Lett. Ed.* **1978**, *16*, 205.
(36) Day, D.; Hub, H. H.; Ringsdorf, H. *Isr. J. Chem.* **1979**, *18*, 325.
(37) Tieke, B.; Lieser, G. Weiss, K. *Thin Solid Films* **1983**, *99*, 95.
(38) Collins, M. A. *J. Poly. Sci Polym. Phys. Ed.* **1988**, *26*, 367.
(39) Birks, J. B. *Photophysics of Aromatic Molecules*; Wiley-Interscience: New York, 1970.
(40) Patil, G. S.; Mathews, R. H.; Cornwell, D. G. *J. Lipid Res.* **1972**, *13*, 574.
(41) Patil, G. S.; Mathews, R. H.; Cornwell, D. G. In *Monolayers*; Goddard, E. D., Ed.; Advances in Chemistry Series 144; American Chemical Society: Washington, DC, 1975; Chapter 5, p 44.
(42) Caruso, F.; Grieser; Thistlethwaite, P.; Almgren, M.; Wistus, E.; Mukhtar, E. *J. Phys. Chem.* **1993**, *97*, 7364.
(43) Kano, K.; Kawazumi, H.; Ogawa, T.; Sunamoto, J. *J. Phys. Chem.* **1981**, *85*, 2204.
(44) Owen, C. S. *J. Chem. Phys.* **1975**, *62*, 3204.
(45) Medhage, B.; Almgren, M. *J. Fluorescence* **1992**, *2*, 7.
(46) Medhage, B. Ph.D. Thesis, Uppsala University, Uppsala, Sweden, 1993.
(47) Weber, G. In *Flavins and Flavoproteins*; Slater, E. C., Ed.; Elsevier: Amsterdam, 1966; p 15.
(48) Naegele, D.; Ringsdorf, H. *J. Polym. Sci., Polym. Chem. Ed.* **1977**, *15*, 2821.
(49) Meller, P. Ph.D. Thesis, Unviersity of Mainz, Mainz, Germany, 1985.
(50) Bevington, P. R. *Data Reduction and Error Analysis for the Physical Sciences*; McGraw-Hill: New York, 1969.
(51) Yamazaki, I.; Tamai, N.; Yamazaki, T. *J. Phys. Chem.* **1987**, *91*, 3572.
(52) Yamazaki, T.; Tamai, N.; Yamazaki, I. *Chem. Phys. Lett.* **1986**, *124*, 326.
(53) Almgren, M.; Medhage, B.; Mukhtar, E. *J. Photochem. Photobiol.* **1991**, *59*, 323.
(54) Fendler, J. H.; Fendler, E. J.; Infante, G. A.; Pong-Su Shih; Patterson, L. K. *J. Am. Chem. Soc.* **1975**, *97*, 89.
(55) Almgren, M.; Grieser, F.; Thomas, J. K. *J. Am. Chem. Soc.* **1979**, *101*, 279.
(56) Lianos, P.; Viriot, M. L.; Zana, R. *J. Phys. Chem.* **1984**, *88*, 1098.
(57) Turro, N. J.; Kuo, P. L. *Langmuir* **1985**, *1*, 170.
(58) Kalyanasundaram, K.; Thomas, J. K. *J. Am. Chem. Soc.* **1977**, *99*, 2039.