Influence of Substrate Properties on the Topochemical Polymerization of Diacetylene Monolayers

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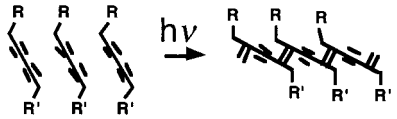
The influence of the underlying substrate on the UV initiated polymerization of diacetylene lipid monolayers was investigated using absorption spectroscopy and Brewster angle microscopy (BAM). The lipid/substrate affinity was tuned through choice of lipid headgroup as well as substrate properties. Lipids with positively charged headgroups, which readily polymerized in both blue (6 °C) and red (16 °C) polymer forms at the air/water interface, failed to polymerize when transferred to glass or hydrophobic glass (headgroups facing ambient) at either temperature. BAM analysis revealed that the diacetylene film was disordered on hydrophobic glass, which likely impeded the topochemical polymerization. On glass, however, BAM showed a highly crystalline film identical to that seen at the air/water interface, suggesting that strong interactions between the positively charged lipid headgroups and the glass inhibited polymerization in this case. In agreement, when the lipid/substrate interactions were reduced, either by introducing a cadmium arachidate bilayer between the diacetylene film and the glass or by substituting mica for glass, a limited polymerization occurred, forming the red film exclusively. As a further test, monolayers of acidic diacetylene lipids were deposited on glass. In this case polymerization was possible in both blue and red forms but diminished as the transferred film was aged. These results suggest that a strong lipid/substrate affinity may impede the topochemical polymerization, possibly by restricting the mobility of the lipids. By investigating polymerization as a function of substrate and headgroup chemistry, several factors influencing the lability of diacetylene films toward topochemical polymerization are presented.

Introduction

Polydiacetylene (PD) single crystals have received considerable attention over the past two decades, as they are model materials for investigating the physics of one-dimensional optical and electrical phenomena in organic polymers.[1] Highly conjugated thin films, prepared from lipids containing a diacetylene moiety in the hydrophobic tail region, hold promise in numerous applications ranging from biosensors to nonlinear optics.[2−4] PD coated coverslips are also of particular use for monitoring the axial response in confocal microscopy given the large one- and two-photon cross sections of the highly oriented two-dimensional crystals.[5]

Under appropriate conditions diacetylene lipid films can be polymerized by a UV initiated topochemical reaction to form extended linear polymers according to



where R represents the lipid tail facing the ambient and R′ contains the lipid headgroup, which is in contact with the hydrophilic support (or water, in the case of Langmuir monolayers). UV irradiation of diacetylene lipid monolayers often leads to the formation of a nonfluorescent blue polymer that can be converted into a highly fluorescent red form upon further UV irradiation or by imparting a stress (mechanical, thermal) to the polymer backbone. This chromatic transition is generally attributed to a reduction of the effective conjugation length of the polymer backbone.[6−8]

The topochemical reaction leading to diacetylene polymerization is very sensitive to the surrounding environment and packing of the lipids. If the reactive diacetylene moieties in the lipid tails are not oriented properly with respect to their neighbors, polymerization may be inhibited. Furthermore, as polymerization may transform the crystal lattice,[1] a certain degree of mobility of the lipids is deemed necessary.[9,10] Hence, PD films are often prepared as well ordered films at the air/water interface where lipid packing and mobility can be controlled, polymerized, and then transferred to solid substrates via the Langmuir−Blodgett (LB) or Langmuir−Schaefer (LS) methods.[11] Attempts at a direct polymerization of single monolayers of diacetylenes on solid substrates are often unsuccessful,[12] result in a reduced polymerization rate,[13] or yield films whose properties differ

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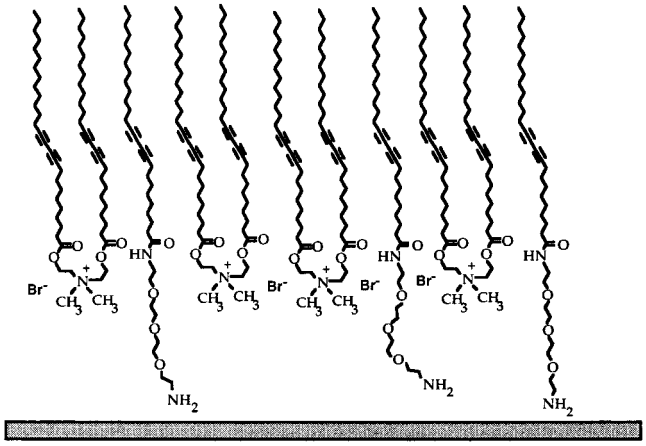

Figure 1. Idealized schematic of the mixed Bronco/EtO-HCA monolayer supported on a glass substrate.

from those of the counterparts polymerized at the air/water interface.[9]

Here we report on the influence of the underlying support on the topochemical polymerization of diacetylene monolayer films using absorption spectroscopy and Brewster angle microscopy (BAM). Polymerization at the air/water interface is compared with polymerization of films transferred by the Langmuir−Blodgett technique to either glass, hydrophobic glass (OTS-glass), cadmium arachidate (CdC_{20}) bilayers on glass, or cleaved mica. The effect of the diacetylene lipid headgroup chemistry on polymerization has also been investigated by using either acidic monolayers of 10,12-pentacosadiynoic acid (PCA) or basic monolayers comprised of a mixture of dimethylbis(2-(hexacosane-10,12-diynoyloxy)ethyl)ammonium bromide (Bronco) containing 10% hexacosa-10,12-diynoic acid (2-{2-[2-(2-aminoethoxy)ethoxy]ethoxy}ethyl)amide (EtO-HCA).

The motivation for this research stems from previous studies that reported Bronco monolayers do not polymerize or exhibit X-ray diffraction peaks when transferred on glass.[12,14] The Bronco/EtO-HCA mixture was investigated here in hopes that the ethylene oxide spacers in the EtO-HCA headgroup might act to support the transferred film, as illustrated in Figure 1, thus reducing the glass substrate's influence. As this mixed film also failed to polymerize on glass, several substrate modifications were investigated to determine which surface properties were hindering polymerization. Depending on the substrate, varying degrees of polymerization were observed according to the trend (glass = OTS-glass) < (CdC_{20} bilayer on glass)< (mica) ≪ (water), where no polymerization occurred on either glass or OTS-glass and extensive polymerization resulted at the air/water interface. On the OTS substrate polymerization was likely hindered by a disordering of the film, which was transferred with the alkyl tails in contact with the substrate. On the hydrophilic substrates this polymerization trend is explained in terms of a progressive reduction in the lipid/substrate affinity (and concomitant increase in lipid mobility). Further support for this hypothesis is found in the polymerization behavior of the acidic diacetylene PCA on glass, for which polymerization occurred but diminished significantly as the transferred film was aged.

Experimental Section

Chemicals. Octadecyltrichlorosilane (OTS) and 10,12-pentacosadiynoic acid (PCA) were purchased from ABCR (Karlsruhe, Germany). Diacetylenes with basic headgroups, dimethylbis(2-(hexacosane-10,12-diynoyloxy)ethyl)ammonium bromide (Bronco) and hexacosa-10,12-diynoic acid (2-{2-[2-(2-aminoethoxy)ethoxy]-ethoxy}ethyl)amide (EtO-HCA), were a gift from Biocircuits Corp. (Sunnyvale, USA). Arachidic acid and cadmium chloride were purchased from Sigma. All lipids were dissolved in chloroform (Sigma, HPLC grade) to form 1 mg/mL spreading solutions. Subphases were either pure Milli-Q water (R = 18 MΩ cm) or a 2×10^{-4} M solution of $CdCl_2$. The $CdCl_2$ solutions, used to stabilize and improve transfer of acidic monolayers, were passed through a 0.22 μm filter (PVDF membrane, Millipore, USA) prior to use.

Film Preparation and Polymerization. Monolayer films were prepared by conventional dropwise spreading, allowed to incubate for 5 min, and then compressed at 20 Å^2/(molecule min) to the desired surface pressure for either film transfer or polymerization at the air/water interface. Langmuir and Langmuir−Blodgett monolayers were polymerized with a Hg lamp (Camag, 17.6 W, 254 nm) positioned 10 cm above the films.

Bronco/EtO-HCA. At the air/water interface, these mixed films were polymerized under either constant pressure (30 mN/m) or constant area (53 Å^2/molecule at 16 °C). The absence of barrier feedback in the latter method permitted comparison of films polymerized at the air/water interface with films polymerized on solid substrates. Depending on subphase temperature, either blue or red films were formed. Temperatures of 16−20 °C resulted in red films after 2−5 min of UV exposure. Blue films formed at 6 °C for UV exposures between 5 and 30 s. This kinetics is slower but on the order of the one observed for a pentacosadiynoic amide derivative.[15] Continued UV exposure, or LB or LS transfer, turned the 6 °C film red.

PCA. Monolayers were prepared either on Milli-Q water or $CdCl_2$ subphases at 16 °C. Compared to those on H_2O, monolayers on $CdCl_2$ were more condensed and stable. PCA was held at 10 mN/m during transfer from H_2O subphases. On $CdCl_2$ subphases PCA was held at 30 mN/m during transfer.

Reflectivity Trough. A custom built PTFE trough (18 × 56 cm^2), with single barrier compression, was used to monitor polymerization at the air/water interface. The trough was enclosed in a dark cabinet and thermoregulated within ±0.5 °C using a circulating water bath. Reflectivity at normal incidence was measured differentially between the monolayer covered interface and the monolayer free interface behind the compression barrier.[16]

Substrates and Film Transfer. Manufacturer precleaned glass slides and coverslips (Marienfeld, Germany) were oxidized in a chromic acid solution (Chromerge, Manostat) at 50 °C for 30 min, rinsed extensively with Milli-Q water, and then dried in an oven at 70 °C. OTS silanized glass substrates were prepared by immersing acid cleaned slides in a 0.5 mM solution of OTS in bicyclohexyl (Aldrich) for 12 h followed by rinsing with chloroform and then 5 min of ultrasonication in methanol followed by rinsing under Milli-Q water. Water contact angles on the OTS-glass were typically 105−107°. Mica substrates (30 μm thick) were cleaved with adhesive tape just prior to film transfer. Single monolayers of the diacetylene films were transferred to both hydrophilic (glass, mica, arachidic acid bilayer) and hydrophobic (OTS) substrates by vertical (LB) deposition at a rate of 1 mm/min. To prevent possible shedding of the diacetylene monolayer on OTS upon retraction of the slide from the subphase, a capping arachidic acid layer was transferred to the slide on the upstroke. The transfer ratios for the various film/substrate combinations are listed in Table 1. Absorption spectra of the transferred films were obtained using a custom built spectrometer in which a monolayer free section of the substrate was used as reference.

Brewster Angle Microscopy. The film morphology at the air/water and air/glass interfaces was visualized using two custom built Brewster angle microscopes. At the air/glass interface (α_{BAM} = 56.3) a 632 nm He−Ne laser was used whereas at the air/water interface (α_{BAM} = 53.1) a 514 nm Ar−Kr laser (Coherent

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Table 1. Monolayer Transfer Ratios

monolayer	π_{xfer} (mN/m)	substrate	transfer ratio[a]
Bronco/EtO-HCA (monomer) 6−18 °C	30	glass	0.99
Bronco/EtO-HCA (red polymer)	14	glass	1.02
Bronco/EtO-HCA (monomer)	30	OTS-glass	1.01
CdC_{20} (capping layer)	30	Bronco/EtO-HCA on OTS	0.88
CdC_{20} bilayer		glass	
layer 1	30		1.06
layer 2	30		0.99
Bronco/EtO-HCA	30	CdC_{20} bilayer	0.94
Bronco/EtO-HCA	30	mica	0.7−0.8
PCA (H_2O)	10	glass	0.92
PCA ($CdCl_2$)	30	glass	0.96

[a] Transfer ratios calculated from ΔA (to keep π constant) relative to the surface area of the substrate.

Innova 70 Spectrum) was employed. Reflected light was passed through an analyzer and then collected on a CCD camera. In this technique p-polarized light impinges the interface at the Brewster angle, given by $\alpha = \arctan n_2/n_1$, where n_1 is the refractive index of air and n_2 that of water or glass. Under these conditions, no light is reflected from the interface. If a monolayer film with an index of refraction n_3 is introduced to the interface, the Brewster angle is no longer satisfied and light will be reflected.[17,18] The intensity of the reflected light is greatest if the film is highly oriented (i.e., if all the lipid dipoles are aligned with the incident laser through a uniform arrangement or tilt of the lipid tails). Slight differences in the dipole orientation provide significant contrast and make this technique very sensitive to the phase behavior and ordering of lipid monolayers. For diacetylene monolayers this technique has the further advantage that it can image both the nonfluorescent (monomer or blue polymer) diacetylene films and the fluorescent (red polymer) films. Images presented here are unfiltered single frames that have been corrected for the angular distortion inherent to this technique.

Fluorescence Microscopy. A Leica (Wetzlar, Germany) confocal microscope equipped with a 40× (NA = 1) oil immersion objective was used to image the red polymerized films. Fluorescence was excited at either 488 or 568 nm using a linearly polarized Ar−Kr laser and passed through a 600 nm long pass filter.

Results

Isotherms and Microscopy at the Air/Water Interface. Prior to investigating film polymerization on solid substrates, the phase behavior and polymerization of the lipids were first characterized at the air/water interface. The surface pressure versus molecular area (π−A) isotherms for the PCA and Bronco/EtO-HCA films are presented in Figure 2. PCA (often called PDA) is perhaps the most well studied diacetylene lipid (e.g., refs 19−23) and was employed here only as a control for the polymerization behavior of the Bronco/EtO-HCA monolayers. The PCA isotherms in Figure 2 are very similar to those of Tomioka et al.;[21] however, a collapse at 11−12 mN/m for PCA on H_2O has also been reported by Goettgens et al.[19] and Sasaki et al.[23] Early collapse was likely avoided here due to the relatively fast compression rate used. As a precaution against early film collapse, PCA monolayers on H_2O were only compressed to 10 mN/m for transfer to solid substrates. On $CdCl_2$ subphases PCA was transferred at 30 mN/m. The transfer ratios are given in Table 1.

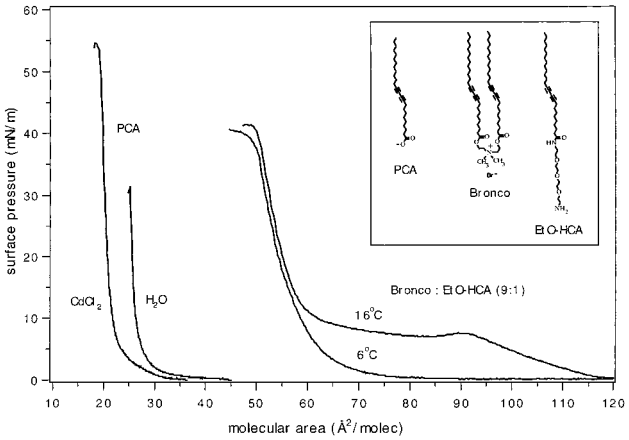

Figure 2. π−A isotherms and structures of the investigated diacetylene lipids. The Bronco/EtO-HCA isotherm was strongly dependent on subphase temperature, as evident in the 16 and 6 °C isotherms, both on water. The PCA isotherms were recorded on either water or $CdCl_2$ subphases at 16 °C.

In contrast to PCA, the Bronco/EtO-HCA mixture displayed an expanded to condensed (LE−LC) phase transition for subphase temperatures above 12 °C. The "overshoot" prior to the onset of the LE−LC transition at ∼91 Å²/molecule for the 16 °C isotherm in Figure 2 is typical for Bronco as well as a few other diacetylenes and even some saturated lipids.[24−27] This overshoot disappears as the compression rate is decreased and most likely represents an energy barrier for nucleating the condensed phase—indeed, BAM images (not shown) did not show any crystals prior to this point. By lowering the subphase temperature to 6 °C, the LE−LC phase transition could be completely suppressed, yet the extrapolated limiting molecular area remained rather invariant of subphase temperature (∼58 versus ∼60 Å²/molecule at 6 and 16 °C, respectively). This suppression of the LE−LC transition and the approximate invariance of the limiting molecular area with temperature have also been reported for pure Bronco films, which have limiting molecular areas near 65 Å²/molecule.[14,28] Our incentive for investigating polymerization at these two temperatures is that unique, temperature-dependent crystal structures are expected,[14] whose polymerization may be influenced to different degrees upon transfer to a solid support.

BAM analysis confirmed the dependence of crystal structure on temperature as seen in Figure 3. The large, highly anisotropic crystals seen in panels A and B are typical of the morphology observed above 12 °C. UV polymerization at a constant surface pressure of 30 mN/m (panel C) did not alter the general morphology or anisotropy of these crystals; however, a ∼10-fold increase in contrast resulted. Although a rather large (up to 25%) decrease in molecular area (leading to an increased packing density of lipid tails) accompanied polymerization, this increased intensity in BAM, as will be shown next, arises from the polymer backbones of this red film. In

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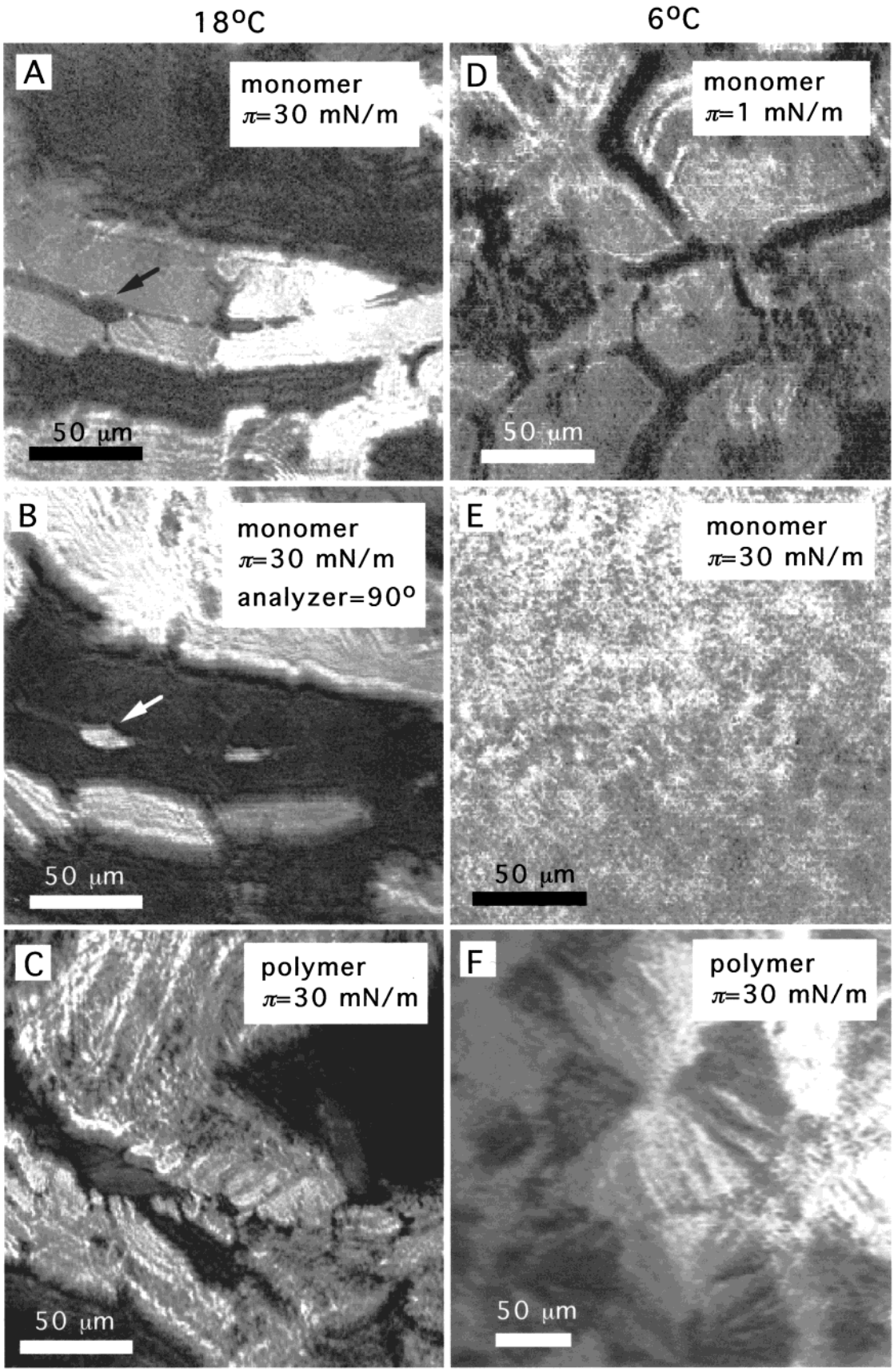

Figure 3. BAM images of Bronco/EtO-HCA films prepared at 18 °C (A−C) and 6 °C (D−F). At 18 °C the film is composed of highly anisotropic crystals, as seen in parts A and B, which show the same area of the film (see arrow for reference) under 0° and 90° analyzer rotations. The crystal morphology and anisotropy are retained following UV polymerization at 30 mN/m at the air/water interface (C). Due to the increased intensity of the polymerized film, the integration time in part C was 1/10 of that in parts A and B. Films prepared at 6 °C consisted of large isotropic domains (D) that disappeared upon film compression (E). Polymerization, however, revealed the presence of ordered domains (F). The film in part F was polymerized at 30 mN/m at the air/water interface and then transferred to glass.

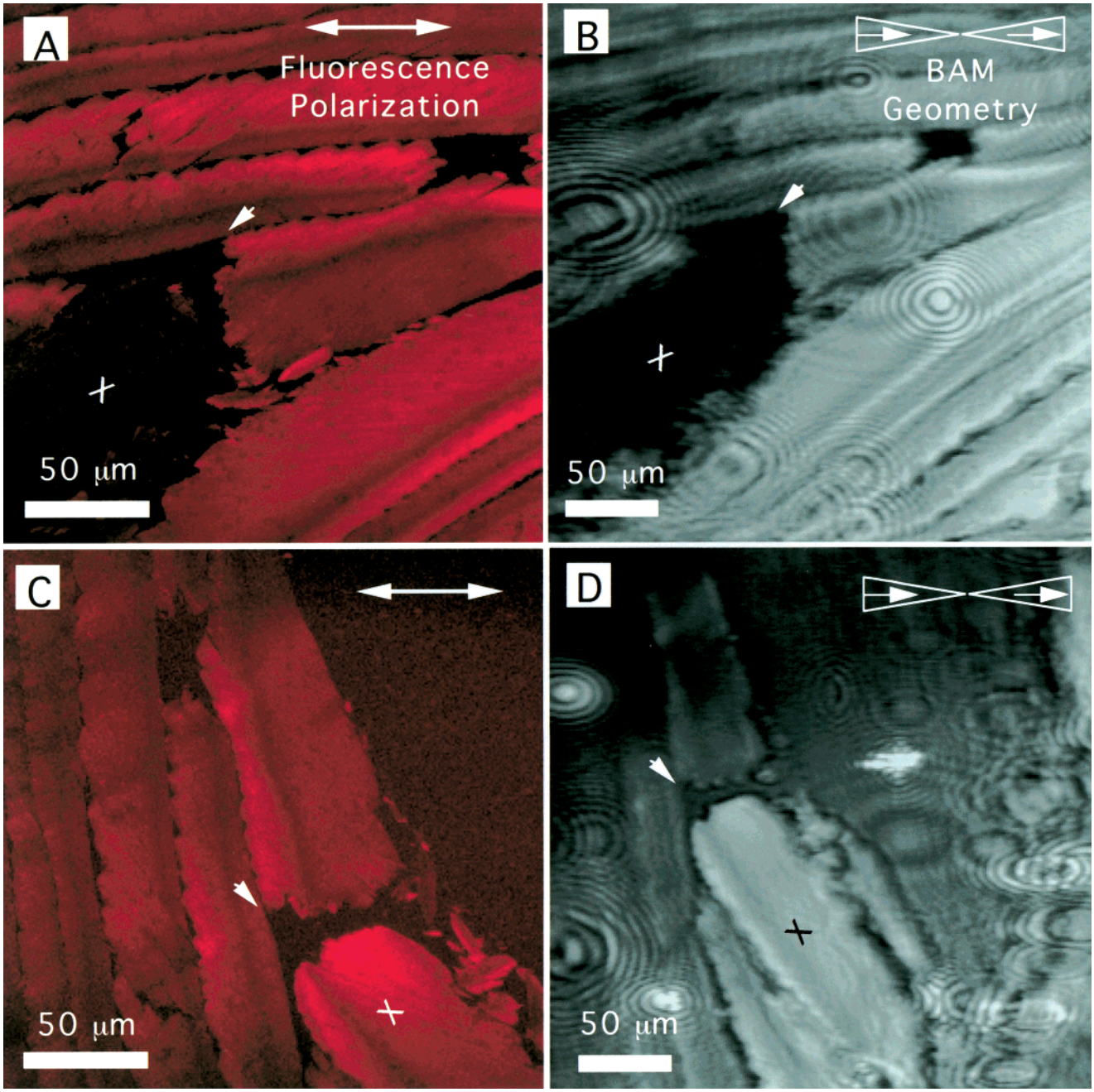

Figure 4. Fluorescence (A and C) and BAM (B and D) images of a Bronco/EtO-HCA film polymerized at 30 mN/m and 18 °C and then transferred to glass. The sample has been rotated 90° in parts C and D to illustrate the identical reversal of contrast in both microscopes (the X marks the same crystal in all images). For polymerized films the contrast in BAM arises from the polydiacetylene backbones that are aligned with the fluorescence absorption dipole moment.

contrast to the oriented crystals seen at 18 °C, the 6 °C film was composed of isotropic domains that appeared to coalesce upon compression, as seen in panels D and E. Polymerization also markedly increased the contrast (panel F) and resulted in a visibly blue film that turned red upon continued UV exposure.

To determine the origins of BAM contrast before and after polymerization, we transferred monolayers polymerized at 18 °C and 30 mN/m at the air/water interface to glass substrates and imaged the highly oriented single crystals using both fluorescence and Brewster angle microscopes, as shown in Figure 4. By evaluating the same area of the sample with both BAM and fluorescence microscopy, it is noted that the contrast is identical (panels A and B) and rotating the sample 90° (panels C and D) reverses the contrast in both microscopes. This comparison demonstrates that the BAM contrast in the polymerized film is indeed due to the conjugated backbone, which is aligned with the fluorescence absorption dipole moment. This was further confirmed through a selective photobleaching experiment, shown in Figure 5. Panel A shows a fluorescence image of the polymerized crystals. The crystals whose polymer backbones are aligned with the polarization of the excitation laser beam are brightest and consequently are the first to photobleach when the laser intensity is increased (panel B). In accordance with the assumption that the contrast in BAM arises from the electron dense polymer backbones, the BAM image in panel C mirrors the loss of contrast seen in the photobleached image. Whether photobleaching cleaves and thus reduces the conjugation length (and fluorescence) of the polymer backbone is uncertain.

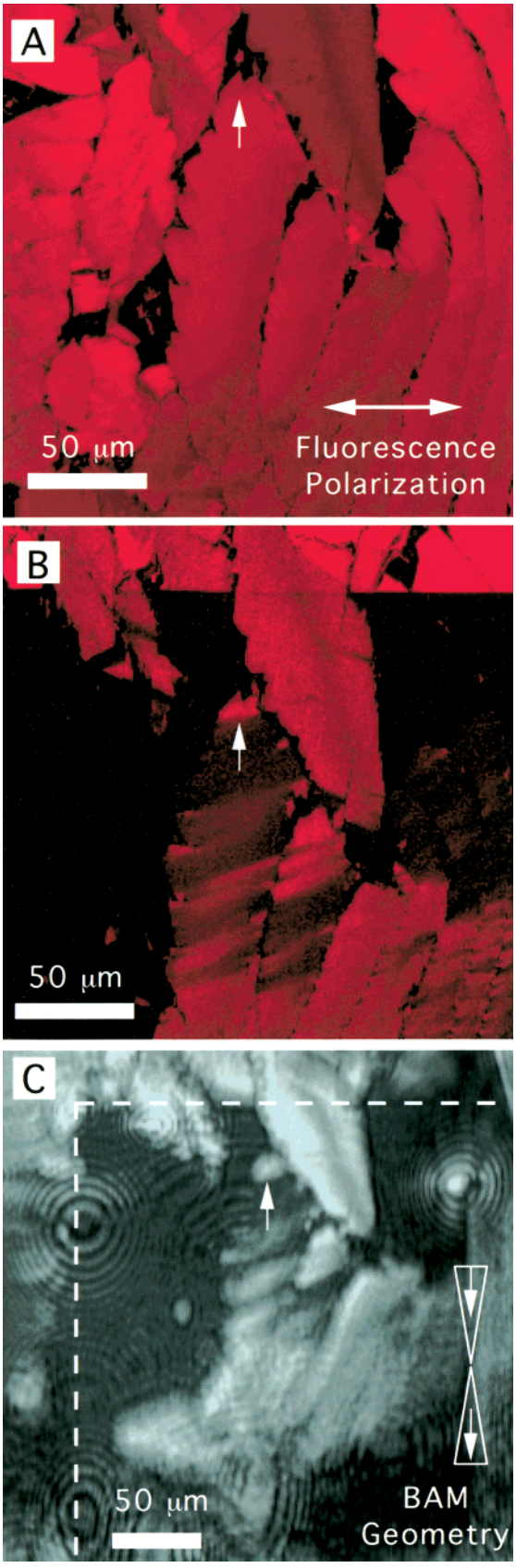

Figure 5. Selective photobleaching of red films of Bronco/EtO-HCA on glass. (A) Fluorescence image prior to photobleaching. (B) Selective photobleaching of those crystals whose absorption dipoles were aligned with the fluorescence polarization. (C) BAM image of the photobleached region (enclosed by dashed lines) that mirrors the loss of contrast in the fluorescence image, implicating the aligned polymer backbones as the source of contrast in both microscopes.

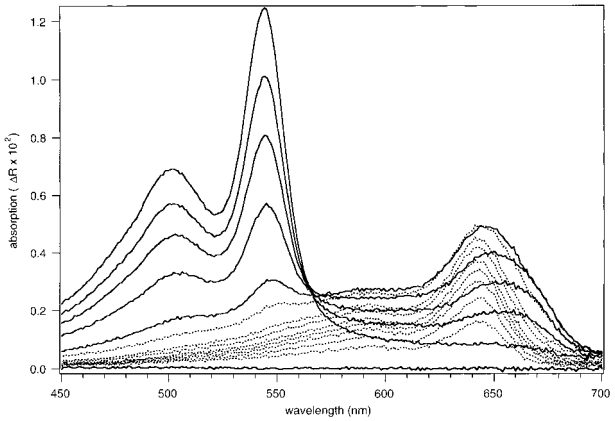

Figure 6. UV induced blue to red transition for a Bronco/EtO-HCA film polymerized at the air/water interface at 30 mN/m and 6 °C. For the first eight spectra (dashed lines) the blue film peak at 640 nm is increasing. The film was irradiated for 10 s between spectra which were recorded ~10 min apart. For the remaining five spectra (solid lines) the peak at 640 nm decreases and the red film peaks at 500 and 545 nm increase; the UV exposure between these spectra was 2 min.

Reflection Spectra at the Air/Water Interface. The Bronco/EtO-HCA polymerization at the air/water interface was further characterized by temporally monitoring the color transition. The series of reflectivity spectra shown in Figure 6 were obtained at 30 mN/m on a 6 °C subphase. A visibly blue film formed, which turned red after ~30 s of UV irradiation. The peak at 640 nm is associated with the blue film, and the peaks at 540 and 500 nm correspond to the red film. On 16−20 °C subphases visibly red films formed immediately after UV irradiation and displayed the characteristic peaks at 540 and 500 nm.

The blue to red transition, common to many diacetylenes, had not been previously reported for Bronco or its mixtures. This transition did not lead to any noticeable morphology changes as observed with BAM; however, both Langmuir−Blodgett and Langmuir−Schaeffer transfers of the blue film to either glass or hydrophobic OTS-glass turned the film red (i.e., mechanochromism). In contrast, blue films of PCA at the air/water interface can be readily transferred without a chromatic change.[3] The sensitivity of the Bronco/EtO-HCA film used here to undergo the blue to red transition may arise in part from the presence of two diacetylene groups per Bronco molecule (one per hydrocarbon chain), allowing a single Bronco molecule to be involved in two different polymer backbones. In accordance, both scanning force microscopy[24] and X-ray diffraction studies[12,28] of polymerized Bronco predict a molecular lattice in which parallel polymer backbones are linked to each other via the Bronco headgroup. This linking of polymer backbones in a 2-D network may impart an intrinsic stress in the polymer, thus priming the chromatic transition in Bronco even more so than for single chain diacetylene lipids. The influence of 10% of the single chain EtO-HCA diacetylene on the Bronco crystal lattice is, however, not known.

Polymerization of Transferred Films. *Bronco/EtO-HCA on Glass and OTS-glass.* Transfer of the highly crystalline (18 °C) Bronco/EtO-HCA in monomeric form to either glass or OTS-glass substrates resulted in transfer ratios near unity, as seen in Table 1. However, UV exposure of the transferred films did not initiate polymerization, as determined by the absence of the characteristic peaks in the absorption spectra. To ascertain whether the substrates were grossly disrupting the crystal structure and long range order, transferred films were

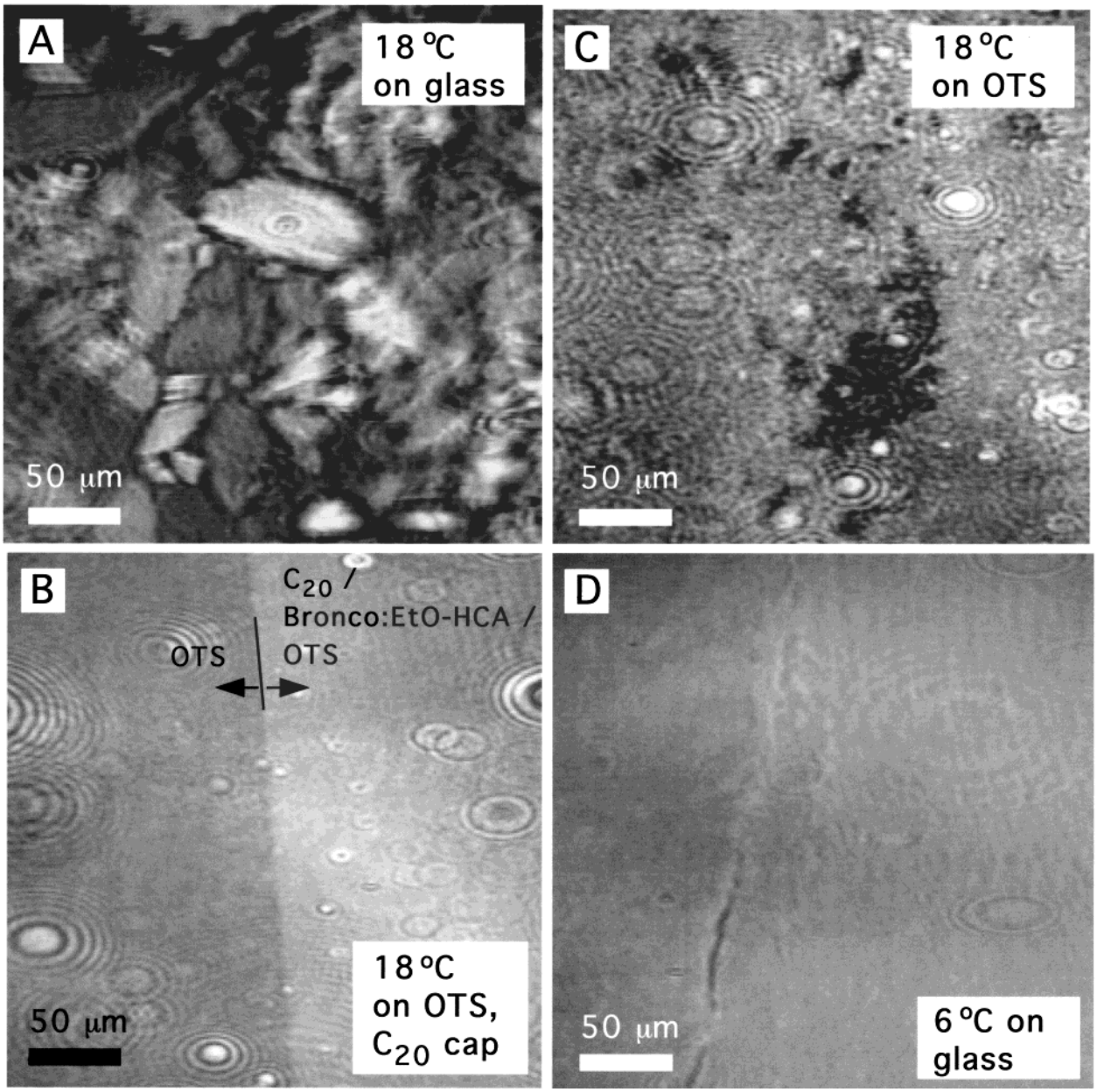

Figure 7. BAM images of transferred Bronco/EtO-HCA (monomeric) monolayers. (A) Preservation of crystal structure and anisotropy following film transfer to hydrophilic glass at 30 mN/m, 18 °C. (B) Arachidic acid (C_{20}) capped diacetylene film on OTS-glass. Image taken at the border of film transfer. (C) In the absence of the capping C_{20} monolayer the diacetylene film restructures upon withdrawal from the subphase. (D) Absence of structure in films transferred at 6 °C to glass.

imaged with BAM (Figure 7). As seen in panel *A*, the morphology and anisotropy of the unpolymerized 18 °C film on the glass substrate are essentially the same as those at the air/water interface (Figure 3A and B). When the monomeric film was transferred in a "heads-up" configuration to OTS-glass (Figure 7B), the diacetylene monolayer crystal structure was not apparent. Although the BAM laser must first penetrate the capping arachidic acid (C_{20}) layer employed in this case, the electron dense diacetylene crystals (when preserved upon transfer) would still likely contribute to the contrast in the BAM image.[29] The Bronco/EtO-HCA films on OTS that were not capped with C_{20} upon withdrawal from the subphase (Figure 7C) also failed to show any crystallinity. Moreover, the presence of large patches in the film suggested that the film was partially shed on the upstroke through the clean air/water interface, verifying that the capping C_{20} layer was necessary.

The polymerization of the Bronco/EtO-HCA 6 °C film on glass was also attempted, since, at the air/water interface, this film clearly exhibited a temperature dependent morphology (compare Figure 3C and F). The transfer ratio was again near unity (Table 1), and with the exception of some large cracks, BAM images (Figure 7D) showed a very uniform film. However, as with the films transferred at 18 °C, polymerization of the 6 °C film was also inhibited on glass. The inability to polymerize directly on glass is likely the result of strong electrostatic coupling between the negatively charged glass and the basic headgroups of the diacetylene lipids. To reduce the lipid/substrate affinity, which may render the lipids incapable of restructuring and hence polymerizing, several substrate modifications were investigated, as next outlined.

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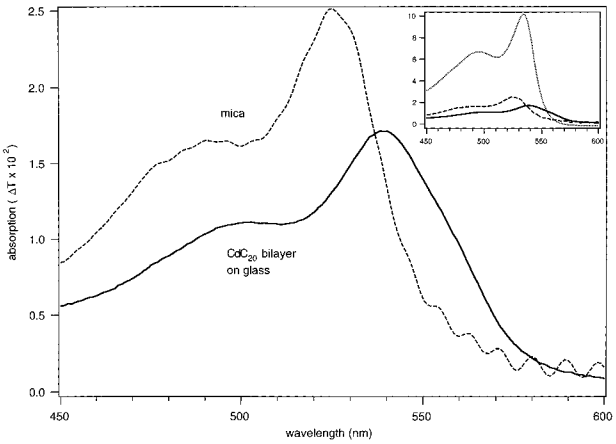

Figure 8. Post-transfer polymerization spectra of Bronco/EtO-HCA monolayers on CdC_{20} bilayer coated glass (solid line) and mica (dashed line). These spectra were taken after 10 min of irradiation, when maximum peak intensities were observed. Comparison of these films with a film fully polymerized at the air/water interface (at constant area of 53 Å^2/molecule) and then transferred to glass is shown in the inset.

Bronco/EtO-HCA on CdC_{20} Bilayers. In contrast to the hydrophobic OTS modified glass, the cadmium arachidate (CdC_{20}) bilayer coated glass presents a hydrophilic surface onto which the diacetylene monolayer is transferred in a "tails-up" orientation. A transfer ratio of 0.94 (Table 1) is indicative of a favorable coupling between the arachidic acid headgroups and the basic headgroups of the diacetylene film. In contrast to the coupling with the glass surface, however, the CdC_{20} bilayer is not a rigid substrate, which may facilitate the necessary restructuring of the adjacent diacetylene film during UV irradiation.

Exposing the CdC_{20} supported diacetylene film to UV resulted in a limited polymerization, as evidenced by the appearance of peaks at 539 and 502 nm in the absorption spectrum, shown in Figure 8. No peaks at longer wavelengths, indicative of a blue polymer film, were observed. The red peaks grew in intensity with UV exposure and reached a maximum within 10 min of irradiation. The reduced intensity of the peaks for this transferred film compared to a film first polymerized at the air/water interface at a constant area of 53 Å^2/molecule and then transferred (see inset of Figure 8) suggested that the extent of polymerization was still restricted on the CdC_{20} substrate compared to the air/water interface.

Bronco/EtO-HCA on Mica. To further probe the influence of the substrate on the polymerization, the diacetylene film was transferred directly on freshly cleaved mica. This substrate was chosen because it presents an atomically smooth, low friction hydrophilic surface that, to a first approximation, closely mimics the air/water interface. Transfer ratios of only 0.7−0.8 on this substrate, compared to transfers near unity on glass and CdC_{20} bilayers, indicate a reduced affinity of the Bronco and EtO-HCA lipids for mica. As with the CdC_{20} substrate, a limited polymerization following UV irradiation was also observed on the mica substrate (dashed spectrum in Figure 8). Only red film peaks were observed, at 525 and 492 nm, the intensity of which remained constant after 10 min of irradiation.

Unlike the case for glass substrates, the absorption spectra on mica were rather noisy and were low pass filtered. This noise is an interference effect as light penetrates the mica and is reflected at various depths from the cleavage planes. Nonetheless, it can be concluded from the relative peak intensities that polymerization occurred to a greater extent on mica than on the CdC_{20} bilayer, especially considering the reduced transfer ratios on mica.

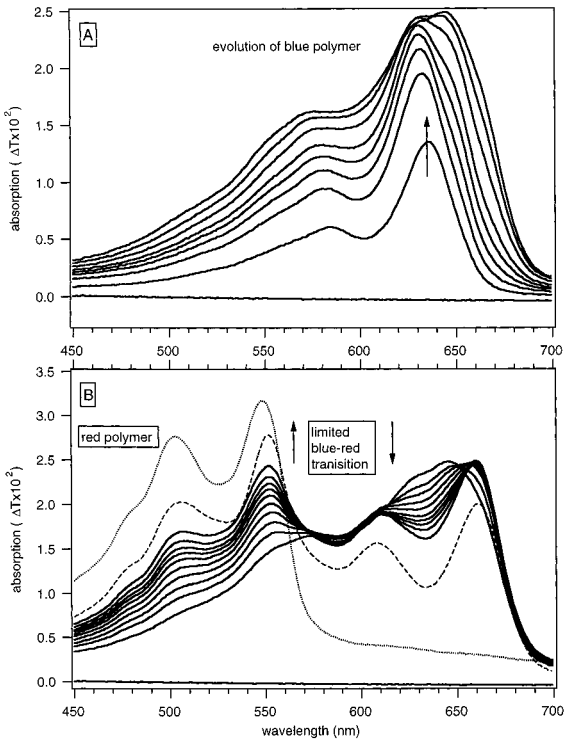

Figure 9. Polymerization of a PCA monolayer transferred to glass at 10 mN/m from H_2O. (A) Formation of the blue polymer film upon intermittent irradiation (2.5 min of irradiation between spectra). (B) A limited blue−red transition results from additional intermittent irradiation; however, a complete transition was not possible, even after 3 h of continuous irradiation (dashed line). In contrast, a freshly transferred monolayer continuously irradiated for 10 min forms the red film (dotted line).

PCA on Glass. An alternative to modifying the substrate as a means of tuning the lipid/substrate affinity is to change the headgroup chemistry of the lipid. To these ends, the polymerization behavior of an acidic diacetylene, PCA, was investigated on glass substrates. Since PCA is a single chain lipid with different phase behavior and packing density than those of the Bronco/EtO-HCA monolayers, this control primarily tests whether the glass substrates employed here are solely responsible for inhibiting topochemical polymerization.

PCA monolayers were transferred either at 10 mN/m from H_2O or at 30 mN/m from $CdCl_2$ subphases. The transfer ratios are given in Table 1. Films transferred at both pressures from the respective subphases could be polymerized in blue and, depending on the age of the film, red forms on the glass substrates. The absorption spectra for PCA monolayers transferred at 10 mN/m are given in Figure 9. Figure 9A illustrates the evolution of the blue polymer film. The spectra were recorded every 10 min, between which times the film was irradiated for 2.5 min. Continued irradiation resulted in a limited blue−red transition, as illustrated in Figure 9B. Complete conversion to the red form was, however, not possible. Even after several hours of continuous UV exposure (dashed line in Figure 9B) the transition was not complete. Similarly, films aged for 12 h after transfer could not be converted into purely red films after extended UV exposure. In contrast, freshly transferred films, irradiated continuously for just 10 min, formed the red polymer film (dotted line in Figure 9B). Clearly, the UV-induced restructuring of

the polymeric film, manifested as the blue−red transition, is hindered as the film ages on the glass support. This may be due to altered lipid mobility and conformation as the film "settles" onto the glass. A draining of the interfacial water layers that were transferred with the film onto the glass may also be occurring.

Discussion

The hindered polymerization of the positively charged Bronco/EtO-HCA diacetylene monolayers on glass may arise from an inability of the lipids to restructure during polymerization, which, at the air/water interface, resulted in large (up to 25%) decreases in the molecular area. It is likely that strong electrostatic interactions between the positively charged headgroups and the negatively charged glass effectively pin the monolayer on the substrate, hindering polymerization. This would also explain why limited polymerization occurred when a CdC_{20} bilayer was present between the diacetylene lipids and the glass. Similarly, polymerization directly on mica was likely favored over that on glass (and CdC_{20} bilayers), since mica presents an atomically flat, low friction surface that fosters lipid mobility. Indeed, fundamentally different lipid spreading behaviors on glass compared to mica attest to the strong influence of the underlying substrate on the dynamics of the supported lipid film.[30]

Although it may be argued that the presence of lubricating water layers on the hydrophilic substrates should render the transferred diacetylene monolayers sufficiently mobile for topochemical polymerization, this is not always the case. Studies of DMPC bilayers on MgF_2 substrates by Rädler et al. indicated that the lower leaflet is strongly coupled to this substrate surface, leading to a 3-fold reduction in lipid mobility compared to that for a free DMPC membrane.[31] In fact, they predicted an immobilization of up to 30% of the substrate-adjacent headgroups. Hetzer et al. also concluded that strong coupling of DPPC to silica, across an ultrathin water film, resulted in a 2-fold reduction in DPPC diffusion in the leaflet adjacent to the silica compared to the upper leaflet.[32] Considering these lipid diffusion studies with the substrate dependent polymerization results presented here, it is reasonable to assume that the positively charged Bronco and EtO-HCA headgroups are rendered sufficiently immobile on glass as to impede the necessary monolayer restructuring that accompanies topochemical polymerization. Thus, the trend of increasing polymerization (glass < CdC_{20} bilayer < mica ≪ water) may be interpreted as the result of a decreased diacetylene lipid affinity for (and increased mobility on) the underlying supports.

The polymerization of acidic PCA diacetylene monolayers directly on glass further demonstrated that the properties of the diacetylene lipid, as well as the underlying substrate, play a decisive role in determining whether topochemical polymerization can occur. Moreover, the diminished polymerization of the transferred PCA monolayers as the films were aged on the glass substrates underscores the sensitive nature of topochemical polymerization. In this case, minor changes in lipid−lipid and lipid−substrate interactions as the transferred film "relaxes" on the substrate are most likely responsible for the diminished blue−red transition. Future experiments will compare lipid polymerization kinetics as a function of time after film transfer as well as substrate properties.

Conclusions

Using absorption spectroscopy and Brewster angle microscopy (BAM), we have investigated the topochemical polymerization of diacetylene lipid monolayers both at the air/water interface and on hydrophilic and hydrophobic substrates. A general trend of increased polymerization as the lipid/substrate affinity was reduced (by varying the substrate properties or lipid headgroup) suggested that strong interactions between the monolayer and the underlying support may impede polymerization, most likely by restricting the mobility of the lipids which must have enough freedom to reorient during the polymerization process. Although a substrate (or transfer) induced disordering of the diacetylene monolayer structure can also inhibit polymerization, as was probably the case on the hydrophobic substrate, this did not appear to play a role on hydrophilic glass where large intact diacetylene crystals were observed with BAM. It was further demonstrated by combined Brewster angle and fluorescent microscopy analysis that the BAM contrast in UV irradiated films arises from the highly oriented polymer backbones, whereas it is attributed to lipid chain tilt prior to polymerization. A concomitant loss of contrast in BAM images upon selective photobleaching of the polydiacetylene crystals further supported this conclusion.

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