Subsurface Formation of Amide in Polyethylene-*co*-Acrylic Acid Film: A Potentially Useful Reaction for Tethering Biomolecules to a Solid Support

Ping Zhang, Chaobin He, Richard D. Craven, Jeffrey A. Evans, and Newton C. Fawcett*

Department of Chemistry and Biochemistry, The University of Southern Mississippi, Hattiesburg, Mississippi 39406-5043

Yuliang Wu and Richard B. Timmons

Department of Chemistry, University of Texas at Arlington, Arlington, Texas, 76013

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ABSTRACT: Aqueous solutions of alkylamines and 95% ethyl alcohol will react with the acid chloride of polyethylene-*co*-acrylic acid to form amides and ester, respectively. Chemical and spectroscopic evidence, as well as contact angle measurements, shows that the reactions are due to formation of acid chloride groups below the polymer's surface. A film of $SOCl_2$-treated polyethylene-*co*-acrylic acid retains its reactivity to amines and alcohol even after long soaking in aqueous base. Alkylamines and ethyl alcohol readily penetrate into the polymer film where they react with interior acid chloride groups, while water and charged or large molecules do not. To explain the film's properties, a three-layer model of its morphology is proposed. Practical application of the acid chloride film to the tethering of aminoalkyl-5′-modified oligo-DNA is demonstrated. A site-specific reaction tethers the oligo-DNA to the film exclusively via its aminoalkyl tail. The reaction occurs spontaneously when the acid chloride film is allowed to soak in an aqueous solution of oligo-DNA at pH 11.5. Spectral and contact angle evidence and the reactions of model compounds indicate that the terminal amino group on the modified oligo-DNA reacts with an internal acid chloride group to form an amide bond. A highly active, surface-tethered oligo-DNA results.

Introduction

While searching for improved methods of covalently tethering biomolecules to solid supports, we were puzzled to find that films of polyethylene-*co*-acrylic acid (PEAA), after reaction with thionyl chloride, would yield amide on reaction with amines, even in aqueous solution and even if the films were washed in water after their reaction with thionyl chloride. Further investigation, however, revealed that what we had wrongly assumed to be simple surface chemistry was, in fact, occurring below the film's surface. Thus, while the aliphatic acid chloride groups on the surface were readily hydrolyzed, acid chloride groups just below the surface retained their reactivity, even after treatment of the film with aqueous alkali. These interior acid chloride groups reacted readily with alkylamine to form amide, not only when the amine was applied neat but also when applied as a dilute aqueous solution. It soon became apparent that both thionyl chloride and alkylamines could readily penetrate PEAA but water and charged species could not. Altering the nature of the film's surface by applying a few angstroms of very hydrophobic $-[CF_2]_n-$, laid down by pulsed plasma polymerization, made the film impenetrable by alkylamine, suggesting that these rather peculiar properties of PEAA film may be especially dependent on the film's morphology, not merely its native hydrophobic character.

In this paper we describe how films of the acid chloride of PEAA may be made, stored without protection from moisture, and then reacted with aqueous solutions of amines to form amides. We present chemical, spectroscopic, and other evidence that the reaction of thionyl chloride-reacted PEAA films with alkylamines occurs below the film's surface and that water and charged species do not readily penetrate the film. We propose a three-layer morphology model to explain the film's properties. We also suggest that the chemistry described can provide an exceedingly simple means of covalently tethering synthetic oligo nucleotides to a solid support with high retention of the oligo's bioactivity, and we describe an example tethering reaction.

Results and Discussion

Formation and Reactions of PEAA Acid Chloride. After a PEAA film was reacted with neat $SOCl_2$ for about 6 h at room temperature, an ATR-IR spectrum of the reacted film showed that the carboxylic acid's absorption band at 1706 cm^{-1} had almost disappeared and a new absorption band at 1799 cm^{-1} had appeared (Figure 1, spectrum 3). This new band was assigned to the C═O stretch in an acid chloride.[1]

When $SOCl_2$-reacted film was immersed in either neat *N*,*N*′-dimethyl-1,6-hexanediamine (DMHDA) or a 50% aqueous solution for ∼5 h, washed with water and 95% EtOH, and then dried in vacuo for an hour to remove nonbound DMHDA, the 1799 cm^{-1} peak characteristic of the acid chloride almost disappeared and a new peak characteristic of the amide asymmetric stretch appeared at 1644 cm^{-1} (Figure 1, spectrum 4). Thus, the reaction product of the $SOCl_2$-reacted film and DMHDA appeared to be the amide.[1] The results were the same regardless of whether neat, 50%, or 0.001 M, unbuffered, aqueous DMHDA was used. Since in aqueous solution one would expect hydrolysis to compete effectively with amide formation, these results were puzzling and were only explained after further investigation.

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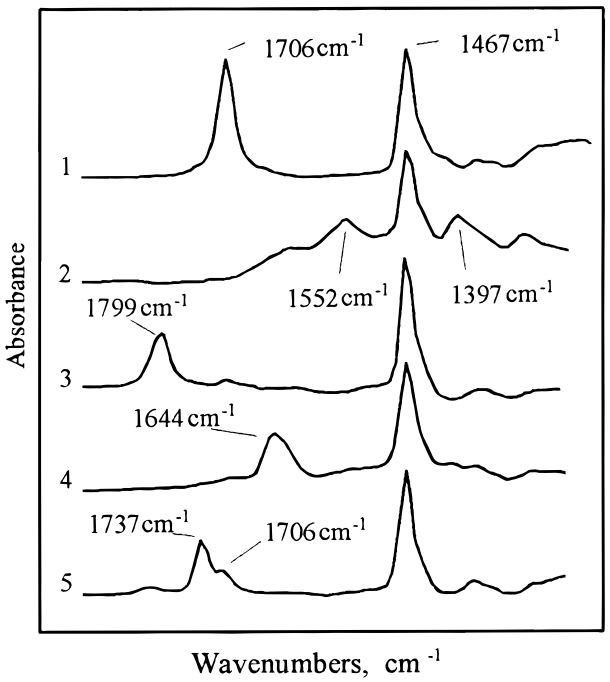

Figure 1. ATR-IR spectra of unreacted PEAA film and the same film after various treatments: (1) PEAA film, (2) PEAA film after 15 min in DMHDA, (3) PEAA film after 6 h in $SOCl_2$, (4) PEAA film reacted with $SOCl_2$ and then with DMHDA for 5 h, and (5) PEAA film reacted with $SOCl_2$ and then with EtOH for 5 h.

When the PEAA film in its original carboxylic acid form was immersed in pure DMHDA at room temperature for 15 min and nonbound DMHDA removed, as before, new bands emerged in the ATR-IR spectrum: one at 1552 cm^{-1} and the other at 1397 cm^{-1} (Figure 1, spectrum 2). The same result was obtained using a 50% aqueous DMHDA solution as a substitute for neat DMHDA. The DMHDA itself had no absorption bands near 1552 and 1397 cm^{-1}. These two bands, however, are characteristic of carboxylate.[1] When the films reacted with DMHDA were washed with 6 M HCl and 95% EtOH, the bands at 1552 and 1397 cm^{-1} disappeared, and the spectrum returned to its original carboxylic acid form. In another experiment, the PEAA film was reacted at 90 °C with 30% NaOH, a procedure known to increase the surface concentration of carboxylate groups as well as converting carboxylic acid to carboxylate.[2] The ATR-IR spectrum after treatment with hot base was very similar to that obtained by treatment of unreacted PEAA with DMHDA. Again, the spectrum reverted to that of the original carboxylic acid film after the sodium hydroxide-reacted film was immersed in 6 M HCl for a few minutes. These experiments demonstrate that only the carboxylate−*N*,*N*′-dimethylhexanediaminium ion pair is produced by direct reaction between unmodified PEAA film and DMHDA. The original PEAA film in its carboxylic acid form did not react with DMHDA to form amide or any other detectable covalent reaction product.

In another experiment, $SOCl_2$-reacted film was placed in 95% EtOH for 5 h. An ATR-IR spectrum then showed a new band centered at 1743 cm^{-1} while the band at 1799 cm^{-1}, characteristic of the acid chloride, was attenuated (Figure 1, spectrum 5). The band at 1743 cm^{-1} was assigned to the ester carbonyl stretch.[1]

These spectra show that the carboxylic acid groups in PEAA film are converted to acid chloride when PEAA

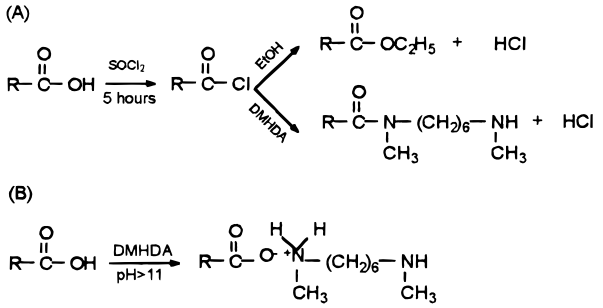

Figure 2. Reaction paths of (A) covalent bond and (B) ion-pair formation.

is reacted with $SOCl_2$. They also show that acid chloride film forms amide when placed in neat or unbuffered, aqueous DMHDA and an ethyl ester when placed in aqueous ethyl alcohol, but unreacted PEAA film only forms an ion pair when exposed to DMHDA. The ion-pair formation is reversible. Figure 2 summarizes the chemistry we observed. Figure 2A illustrates covalent bond formation while Figure 2B illustrates ion-pair formation.

When one considers that aliphatic acid chloride usually hydrolyzes rapidly and spontaneously on exposure to water, observation of identical chemistry, regardless of whether neat or aqueous amine was reacted, indicated that acid chloride groups lay beneath the film's surface and that both DMHDA and EtOH could penetrate below the surface to react with these subsurface acid chloride groups at a significantly faster rate than could water. Later, we found that after many hours in water the film's reactivity with DMHDA was unchanged. Apparently water is virtually excluded from the interior or, if not excluded, penetrates exceedingly slowly. These conclusions are consistent with the depth to which the evanescent wave in ATR-IR experiments is capable of penetrating (vide infra).

Resistance of the $SOCl_2$-Reacted PEAA Film to Hydrolysis. Several attempts were made to hydrolyze the $SOCl_2$-reacted PEAA films. In the first such experiment, the PEAA film was left in air without any humidity control for more than 67 h. The ATR-IR spectrum showed no change in the acid chloride C═O band at 1799 cm^{-1} (Figure 3), but a new, weak absorption centered at 1413 cm^{-1} appeared. It is not known to what this 1413 cm^{-1} band is attributable. The $SOCl_2$-reacted film was also immersed in 0.05 M NaOH solution for more than 17 h. Still there was no change in its ATR-IR spectrum (Figure 4), as the ratio of 1799 cm^{-1} band to 1467 cm^{-1} band remained constant as shown in Table 1. The acid chloride groups responsible for the 1799 cm^{-1} band in the ATR-IR spectrum were not hydrolyzed, though they would be expected to react readily with water upon contact. Apparently water did not reach these acid chloride groups or reached them too slowly for their reaction to be detected, even though the film was immersed in aqueous solution for 17 h.

Hydrolytic Stability of the Amide. The amide bonds formed within the film by reaction with DMHDA were unaffected by (1) 0.05 M aqueous NaOH for 19 h at room temperature, (2) 0.05 M aqueous NaCl for 43 h at room temperature, and (3) 1 M phosphate buffer (pH = 7.0) at 90 °C for up to 2.5 h.

Morphology of $SOCl_2$-Reacted PEAA Film. Penetration of the evanescent IR wave in ATR-IR spectroscopy depends on the waveguide used. For our apparatus,

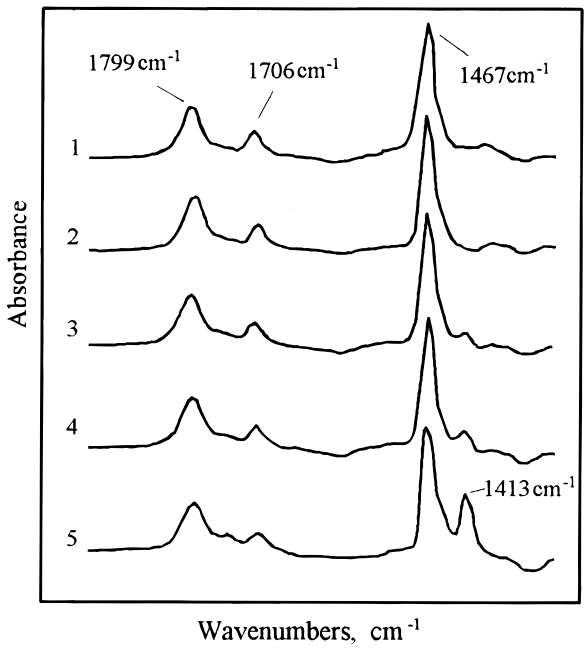

Figure 3. ATR-IR spectra of PEAA film reacted with $SOCl_2$ for 6 h and then left in air for 3.5, 15.5, 27.5, 42.5, and 67 h (spectra 1−5, respectively).

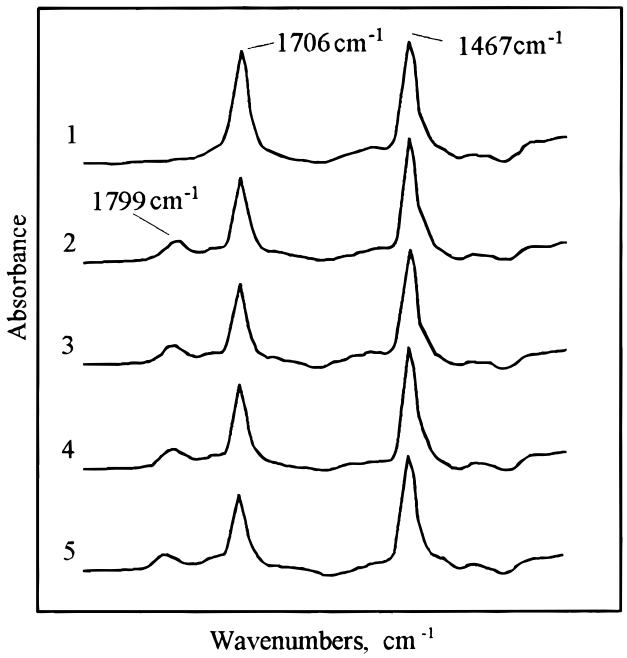

Figure 4. ATR-IR spectra: (1) new PEAA film, (2) of (1) after 15 min in $SOCl_2$, (3) of (2) after 1 min in 0.01 M NaOH, (4) of (2) after 4 h in 0.01 M NaOH, and (5) of (4) after 17 h in 0.05 M NaOH.

Table 1. Stability of the $SOCl_2$-Reacted PEAA Film

experiment[a]	2	3	4	5
ratio of 1799 cm^{-1} band to 1467 cm^{-1} band	0.12	0.12	0.11	0.12
ratio of 1706 cm^{-1} band to 1467 cm^{-1} band	0.63	0.66	0.61	0.61

[a] Experimental conditions are same as listed in Figure 3.

we calculated that the wave penetrates roughly 2.7 μm (27 000 Å) into a film in intimate contact with the waveguide's surface.[3] Thus, the spectroscopic technique we used is insensitive to slight chemical changes occurring only on the sample's outermost surface. It is unlikely that we would have detected reactions of less than 1% of the absorbing species. Consequently, even if the film were hydrolyzed to a depth of about 270 Å (1% of 27 000 Å), the ATR-IR spectrum would be little affected. Any acid chloride groups formed on the surface of the film must have been readily hydrolyzed, but because we cannot observe this surface hydrolysis using ATR-IR, we conclude that there is a substantial number of acid chloride groups remaining unhydrolyzed below the film's surface.

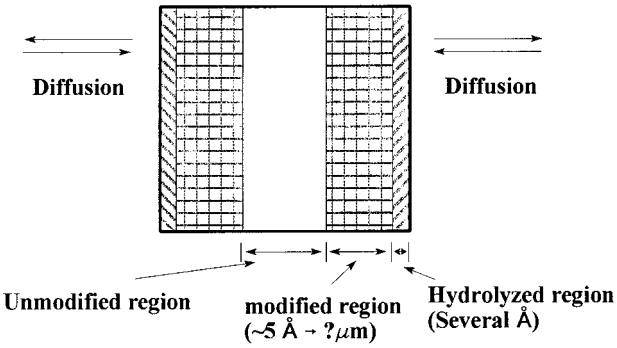

Figure 5. Proposed morphology of the $SOCl_2$-reacted PEAA film.

The penetration depth of ESCA is much less (20−100 Å) than that of ATR-IR. When the $SOCl_2$-reacted films were examined by soft X-ray ESCA, only a very weak chlorine peak was observed. We estimated that if all of the acid groups in the region accessible by ESCA were to be converted to acid chloride, one would expect <4 at. % chlorine. The experimental ESCA signal, however, is consistent with about 1 at. % chlorine. This indicates that the majority of the outermost acid chloride groups were hydrolyzed by moisture in the air or perhaps by a trace of H_2O in the hexane solvent used to remove unreacted $SOCl_2$ from the film specimens prepared for the ESCA experiments. The ESCA results, however, could have been influenced by a greater concentration of the film's polyethylene component near the surface.

When both the ATR-IR and ESCA results are considered, a picture of the $SOCl_2$-reacted film emerges in which acid chloride groups lying below the surface remain intact even after treatment with aqueous base, while the outermost acid chloride groups in the first few angstroms are hydrolyzed. The contact angle[4] of water on native PEAA film is fairly high, 71° and 56° for the advancing and receding angles, respectively, showing the film to be quite hydrophobic. This is consistent with the findings of Gagnon et al.,[2] who showed that in PEAA the carboxylic functional groups prefer a location in the polymer's interior to one on its surface. Contact angle measurement for water wetting of freshly prepared acid chloride film gave a values of 64° and 32° for the advancing and receding angles, respectively. After washing the surface with water, the contact angles increased back to 75° and 48°, values near those for unreacted PEAA film. It appears that hydrolysis of the outermost acid chloride groups on $SOCl_2$-reacted film returns the surface to its native hydrophobic character, forming a barrier to hydrolysis of deeper lying acid chloride groups. On the basis of these considerations, the morphology of $SOCl_2$-reacted PEAA film we propose is depicted in Figure 5. In the outermost few angstroms, the acid chloride groups are hydrolyzed. The acid chloride layer lies beneath the carboxylic acid layer, where it is protected from hydrolysis by the hydropho-

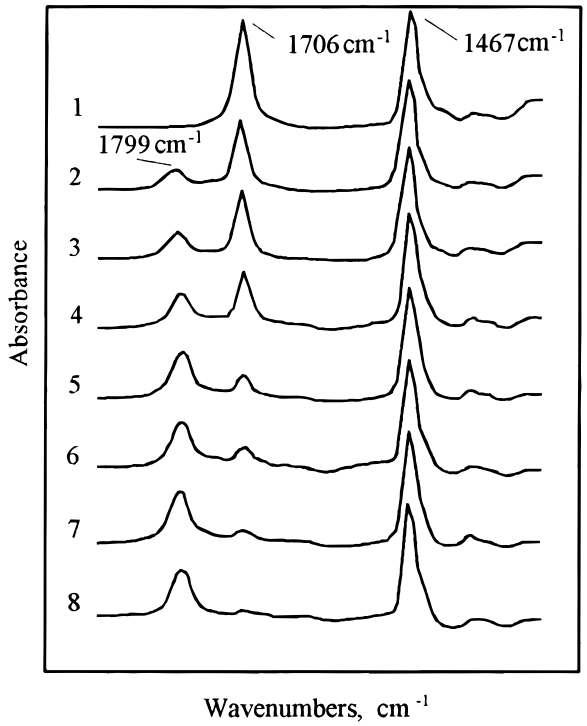

Figure 6. ATR-IR spectra showing the time dependency of the $SOCl_2$ treatment: (1) new PEAA film, (2) to (8) the film after 5 min, 30 min, 2 h, 6 h, 13 h, 18 h, and 48 h in $SOCl_2$ at room temperature, respectively.

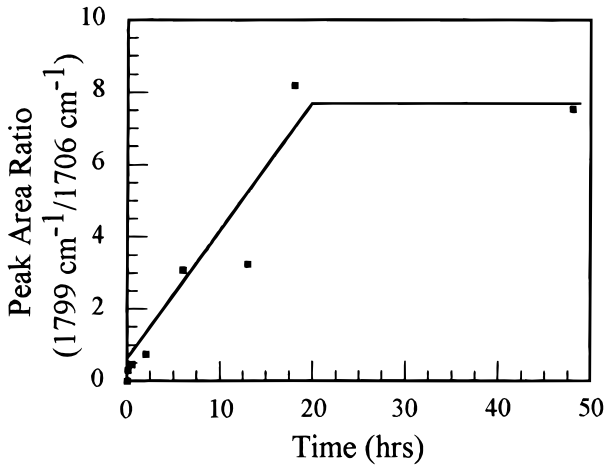

Figure 7. Area of the 1799 cm^{-1} peak divided by the area of the 1706 cm^{-1} peak in the ATR-IR spectra of Figure 6 versus reaction time with $SOCl_2$. Assuming the penetration depth of the ATR-IR evanescent wave to be about 2.7 μm, the graph suggests that the $SOCl_2$ reacts to a depth of $\sim$2 μm in 20 h at room temperature.

bic, and undoubtedly low dielectric, property of the film's surface. The third layer depicted in Figure 5 represents virgin PEAA not yet reacted with $SOCl_2$.

The thickness of the acid chloride layer, i.e., the second layer, can be controlled simply by changing the time of reaction with $SOCl_2$. We have studied the time dependency of this reaction by following changes in the ATR-IR spectrum of a film as a function of the immersion time in $SOCl_2$ (Figure 6). The peak areas at 1799 and 1706 cm^{-1} were measured, and the ratio of the area of the 1799 cm^{-1} peak to the area of the 1706 cm^{-1} peak was plotted as a function of time (Figure 7). It can be seen in Figures 6 and 7 that the intensity of acid chloride C═O absorption increases, while the intensity

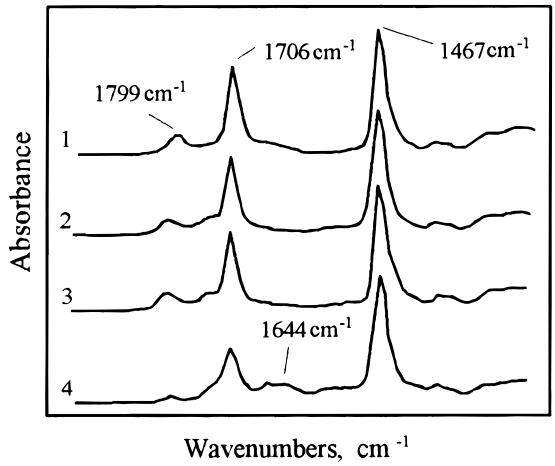

Figure 8. ATR-IR spectra showing the pH effect on the reaction between acid chloride of PEAA and DMHDA: (1) new PEAA film after 15 min in $SOCl_2$, (2) the $SOCl_2$-reacted PEAA film after 24 h in 0.001 M DMHDA (pH 8.3) at room temperature, (3) same as (2) except pH 10.0 for 5 h, and (4) same as (2) except pH 11.5 for 5 h.

of the carboxylic acid C═O absorption decreases with increasing reaction time. The 1706 cm^{-1} carboxylic acid peak can still be seen, although it is substantially diminished, even after the PEAA film had been immersed in $SOCl_2$ for 48 h (Figure 6, spectrum 8). This tiny, persistent peak may be contributed by a combination of carboxylic acid groups from surface hydrolysis plus residual unreacted carboxylic acid groups in the interior.

Influence of pH. The morphology of $SOCl_2$-reacted PEAA film should affect the film's reaction properties. A layered structure of the modified film requires that reactants penetrate the first layer before reaching and reacting with the acid chloride groups. Below its pK_a ($\sim$10) the positively charged protonated form of DMHDA will be in excess, while above its pK_a the free amine will dominate. Consequently, one does not expect DMHDA to react with $SOCl_2$-reacted film below its pK_a. Using ATR-IR spectra, we studied the reaction of DMHDA in 1 M sodium phosphate buffer with the $SOCl_2$-reacted PEAA film at three different pH values: 8.3, 10.0, and 11.5. The ATR-IR spectra (Figure 8) of films reacted at pH 8.3 and 10.0 did not show any obvious sign of amide absorption at 1644 cm^{-1}, nor did the acid chloride group's absorption band at 1799 cm^{-1} diminish. Reaction at pH 11.5, however, causes the amide band at 1644 cm^{-1} to appear and the acid chloride's absorption band at 1799 cm^{-1} to decrease proportionally. Secondary alkylamines have pK_a values above 10; thus, these results are consistent with protonated amine being unable to penetrate the film. Reaction at pH values above the amine's pK_a should be diffusion-controlled, and as such, the reaction rate would depend on the concentration of uncharged DMHDA. We did not investigate pH values above11.5 because of our intention to adapt subsurface amide formation to the tethering of DNA to PEAA film. It is known that DNA is slowly hydrolyzed by strong base.[5]

We attempted reaction of the $SOCl_2$-reacted film with the two-carbon, amino-terminated, alkyl phosphate $H_2NCH_2CH_2PO_4H_2$ at pH 11.5. There are only two methylene units in the molecule, and the length of this molecule is less than 10 Å. After $SOCl_2$-reacted film was put into 0.01 M $H_2NCH_2CH_2PO_4H_2$ in phosphate buffer

at pH 11.5 for more than 22 h at room temperature, we could not detect amide either by ATR-IR or in the ESCA spectrum (not shown here), nor did the ESCA spectrum show evidence of surface phosphorus. Unfortunately, neither of these spectroscopic techniques, at the surface concentrations involved, would be capable of detecting reacted aminoalkyl phosphate, so we cannot say definitively whether the two-carbon chain was sufficiently long to permit the amino group to reach far enough below the film's surface to react with internal acid chloride groups. After reaction, however, the contact angle for surface wetting was slightly reduced, suggesting that perhaps some of aminomethylene tails were able to reach acid chloride groups and react. The results unequivocally confirm, however, that the aminoalkyl phosphate could not penetrate into the interior, probably because of the negatively charged phosphate group. Had the molecule penetrated the film, amide formation would not be restricted to a single layer, and one would expect to observe amide formation in the ATR spectrum, as we had previously observed when DMHDA was used as the reactant.

Influence of Molecular Size on Film Penetration. Reactants too large to penetrate the first film layer should be incapable of reaching internal acid chloride sites. We attempted reaction with D-ribose. This compound has no charge on the molecules but has a bulky five-member ring and a short $HO-CH_2-$ arm. No ester formation was observed by ATR-IR spectroscopy after exposing $SOCl_2$-reacted PEAA film to 0.01 M D-ribose in pH 10.9 phosphate buffer at room temperature for more than 22 h. Reaction with EtOH to form the ethyl ester, however, was complete after 10 h. Apparently the five-member ring is too large to migrate into the film. Again, as was the case after attempted reaction with aminoalkyl phosphate, the contact angle for surface wetting was somewhat reduced by attempted reaction with ribose, suggesting that perhaps some of the two-carbon hydroxy side chains were capable of penetrating to subsurface acid chloride groups.

Because our interest is in immobilizing oligo-DNA on films, we also attempted reaction of the $SOCl_2$-reacted film with deoxyadenosine monophosphate (deoxy-AMP). This compound has an aromatic amine group that could react with acid chloride, but the organic, uncharged, portion of the molecule should be too large to diffuse far enough into the film to react. No spectroscopic evidence for reaction was obtained under the same reaction conditions used with ribose. This indicated that the DNA nucleotides will not react with internal acid chloride groups. Unmodified, oligo-DNA should also be incapable of reacting with the film, as was later shown to be the case.

Tethering of Oligo-DNA to $SOCl_2$-Reacted PEAA Films. *Strategy for Highly Site-Specific Tethering of Oligo-DNA to $SOCl_2$-Reacted PEAA Films.* Synthetic oligo-DNAs modified at the 5′-end with either C_6- or C_{12}-alkylamino tails are commercially available. Because of the structural similarity between these amino-terminated tails and DMHDA, if $SOCl_2$-reacted PEAA film were to be exposed to an aqueous solution of such a modified oligo-DNA, one would expect its amino tail to penetrate the film and react with subsurface acid chloride to form an amide bond, just as DMHDA did. Because of hydrophilicity, charge, and size, the DNA portion of the oligo should be constrained to remain in the aqueous phase on the surface of the film, where it should retain its normal activity in hybridizing to a complement strand of DNA. The subsurface amide bond holding the oligo to the film should be well protected from chemical attack and quite stable. We carried out just such an experiment, as described in Figure 9. We chose an oligo with a C_{12}-tail, rather than a C_6-tail, because the C_{12}-tail should be capable of greater penetration depth into the film.

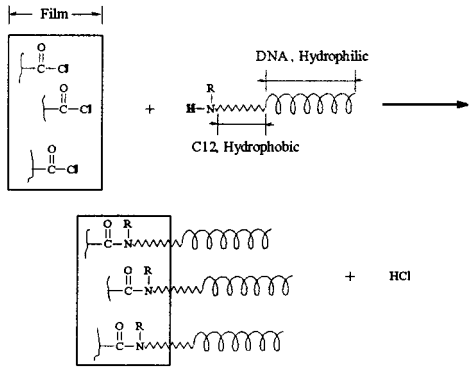

Figure 9. Illustration of highly site-specific tethering of oligo-DNA to $SOCl_2$-reacted PEAA films.

Tethering of 30-mer-DNA Probe. Thionyl chloride-reacted PEAA film was allowed to soak 5 h at room temperature in a pH 11.5, phosphate buffer solution of C_{12}-amine-modified oligo-DNA solution (1×10^{-3} M). After the soaking period, the film's ATR-IR spectrum showed only slight evidence of reaction of the C_{12} tail. The reason for this weak signal is very likely that the penetration depth of the evanescent IR wave, which is about 2.7 μm, is much greater than the penetration depth of the tails, which can be no more than about 20 Å; thus, the fraction of the total IR absorption affected by reaction of the film with the tails is very small. ESCA also failed to show clear evidence of oligo on the films surface. The presence of the tethered oligo is easily shown, however, by its specific association with complementary DNA to produce double-stranded DNA (i.e., hybridization). This was done using synthetic, complementary target, followed by staining with ethidium bromide. Ethidium bromide intercalates into double-stranded DNA, resulting in fluorescence under 260 nm irradiation. The fluorescence intensity increases with increasing length of the double-stranded segments. In our case the maximum length of the segment was 30-base pairs, assuming the tethered 30-mer-oligo to be 100% active. The fluorescence at 260 nm was recorded on film. Hybridization to tethered oligo resulted in bright orange fluorescence characteristic of the presence of double-stranded, i.e., hybridized, DNA (Figure 10).

Control hybridization experiments using noncomplementary DNA resulted in only negligible background fluorescence. Also, control experiments in which repeated attempts were made to react oligo-DNAs without the 5′-C_{12} tail to $SOCl_2$-reacted PEAA films failed to show the presence of surface oligo-DNA after hybridization attempts. Similarly, when PEAA films that had not been exposed to $SOCl_2$ were allowed to soak for 5 h in a buffered, pH 11.5 solution of the amino-C_{12}-modified oligo-DNA (1×10^{-3} M), no oligo-DNA attached to the film. This was shown by absence of fluorescence after attempted hybridizations followed by staining. The control experiments show that bonding of the oligo-DNA to the film does not occur unless the oligo has an alkylamine tail, and the film is in its acid chloride form.

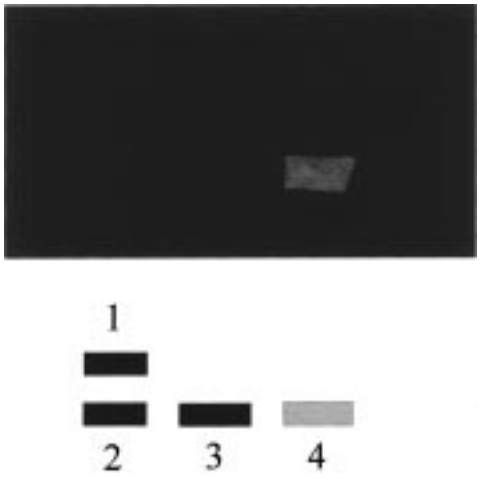

Figure 10. Photograph of the ethidium bromide stained films under 260 nm illumination: (1) PEAA film, not reacted with $SOCl_2$ soaked in 67-mer target hybridization solution (pH 11.5) for 6 h at room temperature; (2) PEAA film soaked in phosphate buffer solution only; (3) PEAA film after 15 min in $SOCl_2$, followed by hexane wash and reaction with amino-C_{12}-5′-modified 30-mer-oligo-DNA (0.5 mM) in 1 M pH 11.5 phosphate buffer for 5 h at room temperature; and (4) film of (3) after hybridization to complementary DNA for 6 h at room temperature in 1 M, pH 7, buffer. The boxes labeled 1−3 indicate the position of the nonfluorescing controls.

Furthermore, contact angle measurements and consideration of the ease with which aliphatic acid chloride is hydrolyzed both suggest that the surface of the acid chloride film is like that of unreacted PEAA film. Thus, it appears that the oligo-DNA bonds to the film internally with the same chemistry that DMHDA was shown, by spectroscopic evidence, to bond. Apparently the oligo-DNA-C_{12} tails are capable of sufficient penetration below the film surface to react with subsurface acid chloride groups.

A stability test for the immobilized DNA was carried out by exposing the DNA-bearing film to 0.5 M NaOH solution for 30 min at room temperature. The immobilized DNA retained its hybridization activity even after this alkaline treatment. As yet, no quantitative data exist for the surface concentration of oligo achieved or to show what percentage of the tethered DNA is active toward hybridization.

Oligo-DNA inactivation from insufficient specificity of reaction site occurs when conventional means are used to attach short oligo-DNA to a support.[6] The chemistry reported here, however, ensures that the oligo-DNA will be end-attached, which is ideal for retaining its hybridization activity. The strong fluorescence we obtained after ethidium bromide staining of the 30-mer hybrid is encouraging because the surface concentration of oligo-DNA on the film cannot be very great. The 10 mol % acrylic acid content of the PEAA can provide only a low concentration of reachable acid chloride groups below the film's surface. The strong fluorescence after hybridization suggests, therefore, that, of the oligo-DNA present, a high fraction is active.

Conclusions

Thionyl chloride penetrates and reacts with polyethylene-*co*-acrylic acid film to form acid chloride groups which lie below the film's surface. A three-layer model for the morphology of this modified PEAA film is proposed. The films's hydrophobic surface layer, probably with mostly inwardly oriented carboxylic acid groups, acts as a barrier to ions and to large or highly polar molecules, such as water. This barrier protects subsurface acid chloride groups and renders them resistant to hydrolysis. Small, relatively nonpolar molecules, like *N*-alkylamines, and ethyl alcohol diffuse into the film and react with subsurface acid chloride to form amides and ester, respectively. After treating a PEAA film with thionyl chloride, the film may be washed, dried, and stored until needed, without protection from atmospheric moisture. The modified film may be useful as a material for tethering biomolecules using very simple chemistry. Although both PEAA and $SOCl_2$-reacted PEAA film resist physical adsorption of DNA at pH values above the pK_a of carboxylic acid, tethering of DNA to the reacted film occurs spontaneously when the film is allowed to soak, at room temperature, in a basic aqueous solution of aminoalkyl-5′-substituted oligo-DNA. The tethering reaction ensures site-specific end attachment of the oligo, resulting in a bioactive oligo-DNA surface. The bonds holding the DNA to the film are protected from exposure to water and polar species and are stable to hybridization conditions. Reaction with amino groups at the end of C_{12}-alkyl tails indicates a significant acid chloride content persists in the film within ≤20 Å of the surface. The observation of decreased contact angle after exposing the $SOCl_2$-reacted PEAA film to β-aminoethyl phosphate may indicate that some of the amino phosphate compound was able to react with the film, suggesting that a few acid chloride groups may exist within about 4−5 Å of the film's surface. It seems likely that an acid chloride gradient exists within the film starting a few angstroms below the surface. The concentration of subsurface acid chloride was shown to depend on the reaction time between $SOCl_2$ and the PEAA film.

Experimental Section

Materials. Polyethylene-*co*-acrylic acid (PEAA, 20 w/w % acrylic acid), thionyl chloride, sodium hydroxide, sodium phosphate, ethidium bromide, hexane, *N*,*N*′-dimethyl-1,6-hexanediamine (DMHDA), 2-aminoethyl dihydrogen phosphate, and 95% ethyl alcohol were purchased from Aldrich and used without further purification. Polyethylene-*co*-acrylic acid thin film, ∼80−100 μm thick, was prepared by melting and compressing PEAA pellets between two glass plates. The synthetic oligo-DNA was purchased from Integrated DNA Technologies, Inc. (Coraville, IA), with the standard purification and used as received.

Apparatus and Procedures. The PEAA film was covered with pure $SOCl_2$ or 50% (v/v) $SOCl_2$/hexane solution from 5 min to several hours to convert the PEAA's carboxylic acid functional groups to acid chloride. This was followed by extensive washing with HPLC grade hexane, as supplied, and then vacuum-drying for 2 h at room temperature. The film was stored in air at room temperature until further use.

Thirty-mer DNA modified at the 5′-end with the addition of an amino-terminated C_{12}-alkyl tail was reacted with the $SOCl_2$-reacted film by immersing the film in a 1 M, sodium phosphate buffer (pH 11.5) solution of the oligo (0.5 mM). The reaction was allowed to proceed for 5 h at room temperature, after which the film was washed with the buffer to remove any unreacted oligo-DNA.

The presence of 30-mer DNA on the film was indicated by soaking the film for 6 h in a 4 μM solution of 67-mer DNA in 1 M sodium phosphate buffer (pH 7). The 67-mer contained a 30-mer tract complementary to the DNA on the film. After rinsing away excess complement DNA with buffer, the film was stained by soaking in a buffered, pH 7, ethidium bromide solution (0.6 μg/mL) for 10 min. The fluorescence of the film at 260 nm was observed and photographed against nonfluorescing controls (see Figure 10).

A Mattson Polaris FT-IR spectrophotometer and a Wilmad, 141-3-horizontal ATR apparatus equipped with a 45° reflection angle, zinc selenide waveguide was used to obtain reflectance IR spectra from 4000 to 400 cm^{-1}.

A Perkin-Elmer PHI 5000 series spectrometer equipped with an X-ray monochromator was used to get ESCA spectra. The X-ray source produced Al Kα at 1486.6 eV. A pass energy of 17.90 eV giving a resolution of 0.60 eV with $Ag(^3d_{5/2})$ was employed. A Rame-Hart goniometer (Rame-Hart, Inc., 43 Bloomfield Ave., Mountain Lakes, NJ, model 100-00-115) was used for static water contact angle measurements.

Pulsed plasma coating[7] of $SOCl_2$-reacted PEAA with $-[CF_2]_n-$ was done using hexafluoropropylene oxide (C_3F_6O) monomer purchased from PCR, Inc. (Gainsville, FL). A standard monomer pressure (0.43 Torr) and gas flow rate (9.6 cm^3 (STP)/min) were used for film deposition. The plasma polymerization was carried out at an RF peak power of 300 W under a pulsing condition of 10 ms on and 300 ms off.

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