and BaOR are 63-78, 260-280 and 635-670 cm^{-1}, respectively (Tables I-III). This similarity in spin-orbit constants suggests that the $\tilde{A}$ states (and probably the $\tilde{B}$ and $\tilde{X}$ states) have similar atomic parentage to the fluorides.

The location of the $\tilde{B}$ and $\tilde{A}$ states relative to each other and the $\tilde{X}$ state also suggests close similarity with the MF molecules. The band origins ($v = 0$) for $\tilde{B}^2\Sigma^+$, $\tilde{A}^2\Pi_{3/2}$ and $\tilde{A}^2\Pi_{1/2}$ states are 18833,[47] 16566,[44] and 16493 cm^{-1}; 17271,[48] 15372,[45] and 15091 cm^{-1}; 14040,[46] 12262,[46] and 11630 cm^{-1} for CaF, SrF, and BaF, respectively. In general, the larger the alkyl group the more red shifted are the $\tilde{A}$-$\tilde{X}$ and $\tilde{B}$-$\tilde{X}$ transitions (Tables I-III).

The splitting between the $\tilde{A}$ and $\tilde{B}$ states is a ligand field separation between $d\sigma$ ($p\sigma$) and $d\pi$ ($p\pi$) orbitals. The various ligands can be arranged in a spectrochemical series[49] $F^- > OH^- > NH_2^- > O^-R > NCO^- > Cl^- > Br^- > I^-$ for the alkaline earths. The order is determined by the $\tilde{B}$-$\tilde{A}$ energy separation and reflects the strength of the ligand field interaction. The location of NH_2^- is from the work of Wormsbecher et al.[14] and our $SrNH_2$ observations.[50] Note that $SrNH_2$ has C_{2v} symmetry so that $\tilde{A}^2\Pi$ further splits into $^2B_1$ and $^2B_2$ states. The NCO^- splitting is from our own recent discovery of the CaOCN and SrOCN molecules.[3]

(47) Dulick, M.; Bernath, P. F.; Field, R. W. *Can. J. Phys.* **1980**, *58*, 703-712.
(48) Steimle, T. C.; Domaille, P. J.; Harris, D. O. *J. Mol. Spectrosc.* **1977**, *68*, 134-145.
(49) Cotton, F. A.; Wilkinson, G. *Advanced Inorganic Chemistry*, 4th ed.; Wiley: New York, 1980; p 663.

Conclusion

A large number of new Ca-, Sr-, and Ba-containing free radicals have been discovered and characterized by laser spectroscopy. The alkaline earth monoalkoxides were produced in the gas phase by the reaction of the metal vapor with the appropriate alcohol. The vibrational and electronic structure of the akaline earth monoalkoxides has been explained in terms of an M^+ ion perturbed by an O^-R ligand.

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(50) Brazier, C. R; Bernath, P. F., unpublished results.

The Use of a Stationary Cationic Surfactant as a Selective Matrix in ^{252}Cf-Plasma Desorption Mass Spectrometry

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Abstract: Mylar films impregnated with a cationic surfactant have been tested as an anion exchange matrix for ^{252}Cf-PDMS studies. The purpose of this matrix was to reduce the intermolecular binding forces and the effects of matrix impurities which hinder desorption-ionization of biopolymers bearing multiple anionic groups. The matrix selectively adsorbed the anionic moiety from aqueous solutions of an inorganic salt and several simple nucleic acid fragments. Significant enhancements in the yields were observed.

In many of the particle induced emission mass spectrometric methods (^{252}Cf-PDMS, SIMS, LDMS, FABMS) ionization-desorption occurs from a solid matrix. The disadvantage of ionizing molecules from the solid phase rather than the liquid or gas phase is the complexity of interactions that may attenuate or quench molecular ion formation. Impurities in the matrix or strong interactions between highly polar species have been identified as probable causes.[1] In FABMS when a liquid matrix is used these problems seem to be less severe. It has been proposed that the solvent used in these studies may modify the strong interactions among solute molecules, making it easier to desorb a molecule from the solute-solvent cluster because the binding energy between molecules is reduced. This suggests that molecular ion yields in the solid phase are attenuated when the energy binding a molecule to its surrounding matrix exceeds the energy that is available in the desorption process.[1] In an effort to reduce the strong interactions between molecules in the solid phase we first reported on the use of Nafion as an ion-containing polymer matrix that could selectively bind large organic cations, effectively reducing the interactions.[2] The selectivity of the sulfonic acid groups of Nafion for binding cations was demonstrated by incorporating only Cs^+ and not I^- when the film was exposed to an aqueous solution of CsI. Molecular ions of large organic cations, including Bleomycin-Cu^{2+} complex, adsorbed onto the film have been produced.

The advantages of Nafion are that it is a relatively low equivalent molecular weight polymer (1100 ew) that can be dissolved in an organic solvent system[3] but which will not redissolve after drying if exposed to aqueous solutions. Thin films of Nafion (<100 μg/cm) can be cast on a foil backing, either by electrospray[4] or spin coating.[5] The films are then used as a substrate to selectively adsorb desired cations from a solution. The films contain a high surface concentration of the adsorbed cations due to the low equivalent molecular weight of the polymer. Typical ion exchange resins are not useful because the particulates produce films too thick for ^{252}Cf fission fragments to penetrate and because the equivalent molecular weight is very high, resulting in a low surface concentration of the ions of interest. Furthermore, these films tend to redissolve when exposed to a solution.

We have sought to develop an anion exchange matrix similar to the Nafion that would reduce the intermolecular binding forces and the effects of matrix impurities which were felt to hinder desorption-ionization in ^{252}Cf-PDMS, particularly in the analysis

(1) Macfarlane, R. D. *Acc. Chem. Res.* **1982**, *15*, 268-275.
(2) Jordan, E. A.; Macfarlane, R. D.; Martin, C. R.; McNeal, C. J. *Int. J. Mass Spectrom. Ion Phys.* **1983**, *53*, 345-348.
(3) Martin, C. R.; Rhoades, T. A.; Ferguson, J. A. *Anal. Chem.* **1982**, *54*, 1639-1641.
(4) McNeal, C. J.; Macfarlane, R. D.; Thurston, E. L. *Anal. Chem.* **1979**, *51*, 2036-2039.
(5) Meyerhofer, D. *J. Appl. Phys.* **1978**, *49*, 3993-3997.

0002-7863/86/1508-2132$01.50/0

of biopolymers bearing multiple anionic functionalities. This includes polyphosphorylated nucleic acids[6] and polysulfonated polysaccharides. Successful results have been obtained with FABMS with a liquid matrix for both classes of compounds.[7,8]

In casting about for an alternate surface, we became aware that organic polymer surfaces impregnated with cationic surfactants have been shown to bind heparin, a polysulfonated polysaccharide which prevents blood coagulation, when the polymers are used in surgical applications, for example, as vascular grafts and prosthetic heart valves.[9] The cationic surfactants are typically quaternary amines bound to the surface which complex with the anionic sulfate groups of heparin. The mechanism for the binding of the surfactant to the substrate involves the interaction of the hydrophobic end of the surfactant with the polymer surface. A variety of polymers have been tested, including polypropylene, polyethylene, polycarbonate, Teflon, and Mylar.[10] Thin Mylar that has been aluminized on one side to make it conducting is routinely used in ^{252}Cf-PDMS experiments as a foil backing for molecules deposited by the electrospray method on the aluminum surface. Macfarlane has recently demonstrated that molecules could be selectively adsorbed from a solution onto the Mylar surface by virtue of their interactions with the functional groups of this polymer.[11] The film is still conducting so that an acceleration voltage can be applied to the foil. These observations promoted the idea of using Mylar films impregnated with a surfactant as a stationary matrix for the selective adsorption of anionic biopolymers from a solution.

In the experiments described in this study tridodecylmethylammonium chloride (TDMAC) was chosen as the cationic surfactant. This molecule has been widely used to produce heparinized polymer surfaces.[10] In this paper, we have identified the TDMAC mass spectrum and demonstrated the selectivity of the TDMAC–Mylar film. Spectra obtained from electrosprayed films of several simple nucleic acid fragments were compared with spectra obtained when a solution of the same molecule was adsorbed on the TDMAC–Mylar surface.

Experimental Section

TDMAC was obtained in the solid form from Polysciences, Inc. (Warrington PA). A 0.1 M solutin in 50:50 toluene:petroleum ether was used to coat the Mylar. Although other solvents could be used, we chose this one for the reason that it has been widely used in previous experiments because it causes swelling of the polymer substrate and the fact that the solvent can be easily removed.[10,12] A 25-μL droplet of the solution was placed on the Mylar surface of a 1.25-μm aluminized Mylar foil, Atlan-Tol Industries, Inc. (West Warwick, RI), for 1–3 min; the excess was removed by spinning the film at 3000 rpm for 1 min. Aqueous solutions of CsI and two ribonucleotide fragments (Sigma Chemical Co.) were prepared with HPLC H_2O (Burdick and Jackson). Solution concentrations were ranged from 10^{-2} to 10^{-3} M. The pH of the nucleotide solutions was 4.5. A drop of the solution of interest (typically 100 μL) was placed on the TDMAC–Mylar film for 5 min, wetting the entire surface. The sample was then briefly immersed in a beaker of HPLC H_2O. The samples were dried by spinning at 3000 rpm for 1 min. Electrosprayed films were prepared as previously described.[4] Samples to be electrosprayed were dissolved in a mixture of H_2O/methanol/2-propanol (Burdick and Jackson). Films were approximately 20 $\mu g/cm^2$. A description of the ^{252}Cf-PD mass spectrometer and data acquisition and analysis programs appears in a previous publication.[13] Mass calibrations were obtained by precisely measuring the time-of-flight of H^+ and $C_3H_2^+$ ions in the positive ion spectra and H^- and C_2H^- ions in the negative ion mode. The acceleration voltage was ±10 kV. A +3 kV postacceleration grid was used in conjunction with an 50 Ω impedence matched CEMA assembly (FTD 2003), Galileo Electro–Optics Corp. (Sturbridge, MA), to detect the secondary ions. The fission fragment flux was 2000 s^{-1}. The efficiency for transmission and detection was 32%. Data were accumulated for 1000 s in each spectrum. Unless otherwise indicated the data were plotted in 2.50 ns time bins.

Results

The positive ion ^{252}Cf-PD mass spectrum of tridodecylmethylammonium chloride is shown in Figure 1. Abundant quaternary ammonium cations, e.g., tridodecylmethylammonium ($TDMA^+$) ions, were formed. Integrating over the area of the peak gave a yield of approximately 0.5 ions/incident fission fragment. An extensive fragmentation pattern was produced containing structurally significant fragment ions. Successive losses of C_nH_{2n+2}, from n = 1 to 11, from each dodecyl chain was observed. The two most prominent fragment ions were imminium ions, $R_2N{=}CH_2^+$ (m/z 367) and $(CH_3)RN{=}CH_2^+$ (m/z 213), R = dodecyl. Fragment ions formed by loss of C_nH_{2n+2} from the latter species could be identified up to n = 8. Beyond that, the ions were not discernable from low molecular weight positive ions associated with the background of any ^{252}Cf-PD mass spectrum. An additional feature of this spectrum was the loss of H_2 from each of the fragment ions. This detail can be seen in the enlargements of the $TDMA^+$ cation and the three primary fragmentation ranges. Below m/z 80, numerous hydrocarbon peaks were present. In contrast to most other ^{252}Cf-PD mass spectra, virtually no sodium ions were observed. Typically the H^+ and Na^+ ions are used to generate the mass calibration curve. In this case the $C_3H_2^+$ ion was used to generate the mass calibration curve in place of Na^+ because of the absence of any appreciable Na^+ ion intensity. Above m/z 600, clusters of $TDMA^+$ were formed, for example, $(2TDMA^+ + Cl^-)^+$ and $(3TDMA^+ + 2Cl^-)^+$. Very little fragmentation of these ions was observed.

The negative ion spectrum of TDMAC (Figure 2a) was characterized by an intense chloride ion peak and a molecular ion formed by chloride attachment, $(TDMAC + Cl)^-$ at m/z 608. As in the positive ion spectrum, a series of cluster ions were formed of the variety $(nTDMAC + (n-1)Cl)^-$. The integrated intensity of the $(TDMAC + Cl)^-$ ion was very close to the value obtained for that of the $TDMA^+$ ion. It appears to be lower on the basis of the intensity axis (1×10^4 ions vs. 5×10^4 ions) because the $(TDMAC + Cl)^-$ ion is much broader. Negative fragment ions were of low intensity and irregular in pattern and, therefore, of little use in structurally characterizing the molecule.

This same film was removed from the mass spectrometer and exposed to a 1×10^{-2} M solution of aqueous CsI, as described in the Experimental Section. The negative ion spectrum in Figure 2b clearly showed that I^- displaced the Cl^- ion with almost complete efficiency. The molecular ion adduct, $(TDMA^+ + 2I^-)^-$ at m/z 791, was formed by I^- attachment to the iodinated neutral salt. The chloride ion intensity has been diminished by more than a factor of 30. An unexpected observation was the formation of I_2^- (m/z 254) and I_3^- (m/z 381) ions. No similar chloride ion clusters were noted. The negative ion spectrum of electrosprayed CsI (Figure 2c) contained far less iodide ion; iodine-containing clusters formed with Cs^+ and Na^+ were prominent. Comparison of the positive ion spectra of electrosprayed and exchanged CsI in Figure 3 verified that the I^- ion and not the Cs^+ ion was selectively adsorbed onto the TDMAC matrix. The intensity of the $TDMA^+$ peaks remained unchanged. The only evidence of iodide incorporation in the positive ion mode was the appearance of the iodinated cluster ion, $(2TDMA^+ + I^-)^+$, at m/z 1201 in addition to the $(2TDMA^+ + Cl^-)^+$ ion at m/z 1110 (Figure 4). The latter was of significantly lower intensity and did not reflect the Cl^-/I^- ion ratio. Loss of the neutral salt, dodecyl chloride or iodide, was identified as a primary fragment ion in both spectra in this figure. This ion must necessarily involve loss of the halide since it appeared at the same mass in both spectra. The ion at m/z 663 in Figure 4b appeared 538 u lower than the base peak. This peak is not observed in Figure 4a and, therefore, probably did not involve loss of the halide. No peak 538 u lower than the

(6) McNeal, C. J.; Narang, S. A.; Macfarlane, R. D.; Hsuing, H. M.; Brousseau, R. *Proc. Natl. Acad. Sci. U.S.A.* **1980**, *77*, 735–739.
(7) Grotjahn, L.; Frank, R.; Blocker, H. *Nucl. Acids Res.* **1982**, *10*, 4671–4678.
(8) Carr, S. A.; Reinhold, V. N. *J. Carbohydr. Chem.* **1984**, *3*, 381–401.
(9) Salzman, E.; Silane, M.; Lindon, J.; Brier-Russell, D.; Dincer, A.; Rosenberg, R.; Labarre, D.; Merrill, E. In "Chemistry and Biology of Heparin"; Lundbald, R. L., Brown, W. V., Mann, K. G., Roberts; H. R., Eds.; Elsevier/North Holland: New York, 1980; pp 435–448.
(10) Grode, G. A.; Anderson, S. J.; Grotta, H. M.; Falb, R. D. *Trans. Am. Soc. Artic. Intern. Organs* **1969**, *15*, 1–6.
(11) Macfarlane, R. D.; McNeal, C. J.; Martin, C. R. *Anal. Chem.*, submitted for publication.
(12) Leininger, R. T.; Crowley, J. P.; Falb, R. D.; Grode, G. A. *Trans Am. Soc. Artif. Intern. Organs* **1972**, *18*, 312–315.
(13) Macfarlane, R. D. *Anal. Chem.* **1983**, *55*, 1247A–1254A.

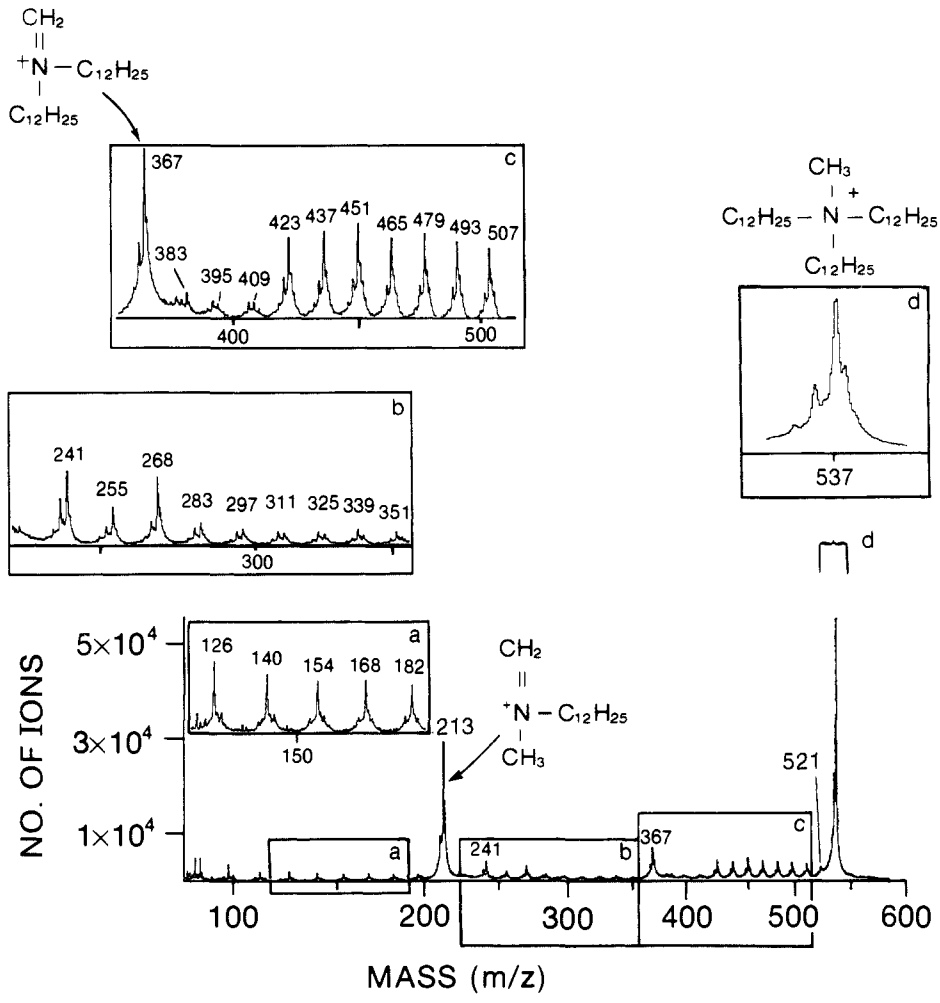

Figure 1. ^{252}Cf-PD positive ion mass spectrum of TDMAC. The three major fragmentation regions and the molecular ion are shown in the insets in which the x axis has been magnified. Unless otherwise indicated data in all figures are plotted in 2.50 ns time bins.

m/z 1110 peak in Figure 4a was observed. This ion may somehow involve loss of $TDMA^+$ and H as indicated in the figure; however, there was no other supporting evidence to confirm this assignment.

The purpose of using an anion exchange matrix was to find a surface that would improve the desorption ionization efficiency for polyanionic biopolymers, specifically nucleic acids. The next test of the TDMAC film was therefore to determine if a simple nucleic acid fragment could be selectively adsorbed then desorbed and ionized. The ammonium salt of the ribonucleoside monophosphate, adenylyl(3′→5′)cytidine (ApC), was chosen as the model compound. Films were prepared in the standard way by electrospraying a 1×10^{-2} M solution. The positive and negative ion spectra are plotted in Figures 5 and 6a, respectively. The identified positive ions appeared to all by associated with the free acid of ApC and not the ammonium salt even though the ammonium ion is observed in the spectrum. The peak at m/z 635 may be $(M_{NH4} + 2Na - H)^+$, the natriated ammonium salt; however, we would also have expected to find $(M_{NH4} + Na)^+$ at m/z 612, which we do not observe, if this were the case. The base fragments $(C + 2H)^+$ and $(A + 2H)^+$ were the most intense positive ion fragments. Nucleoside and nucleotide fragment ions have also been identified in the figure. The negative ion spectrum of electrosprayed ApC (Figure 6a) contained an intense $(M - H)^-$ peak and peaks of lower intensity due to the mononucleotide fragments. The adenine base peak $(A)^-$ at m/z 134 was prominent, but there was no evidence of the cytidine base fragment ion. We have not identified the peak at m/z 217.

An aqueous solution of ApC having the same concentration was adsorbed onto a TDMAC film. The resulting negative ion spectrum of ApC–TDMAC is shown in Figure 6b. The negative molecular ion and mononucleotide sequence ion yields increased over a factor of 5. The phosphoryl ion peak (m/z 79) was comparable to the chloride and iodide intensities, indicating that there was a quantitative displacement of Cl^- by ApC^-. Several ions have diminished intensities, including the unknown ion at m/z 217 and the CN^- ion. An unusual feature of the electrosprayed ApC spectrum was the appearance of the m/z 2^- and 4^+ peaks. The presence and intensity of these peaks are puzzling; they did not appear in any of the other spectra presented herein. Many low intensity peaks between the mononucleotide fragments and the molecular ion in the TDMAC spectrum were not able to be identified. In the higher mass range the $(TDMAC + Cl^-)^-$ peak intensity was reduced to a level barely above the background. The ApC analogue $(TDMA^+ + 2ApC^-)^-$ was observed in low abundance.

The positive ion ApC–TDMAC spectrum was essentially identical with the TDMAC positive ion spectrum (Figure 1) in the mass range below m/z 600. The $(2TDMA^+ + Cl^-)^+$ peak intensity was reduced by approximately a factor of 3; a new peak at m/z 1645 was apparent but of much lower intensity than the former ion, corresponding to $(2TDMA^+ + ApC^-)^+$. Ions of $(2TDMA^+ + ApC^-)^+ \cdot TDMAC$ and $(2TDMA^+ + ApC^-)^+ \cdot 2TDMAC$ corresponding to the addition of neutral TDMAC molecules to the TDMA–ApC salt were also identified in the spectrum.

To test whether polyanionic ions could be produced, we chose the ammonium salt of the trinucleoside diphosphate, ApApC (Figure 7), as the simplest case. This compound contained an additional adenosine monophosphate unit compared to ApC. A 2×10^{-3} M solution was electrosprayed. The positive and negative ion spectra are shown in Figure 8. As in the ApC spectra, no ions were identified that were attributable to the ammonium form of the nucleotide; all identified peaks were of the free acid. MH^+, MNa^+, $(M - H)^-$, and $(MNa - 2H)^-$ were the most prominent

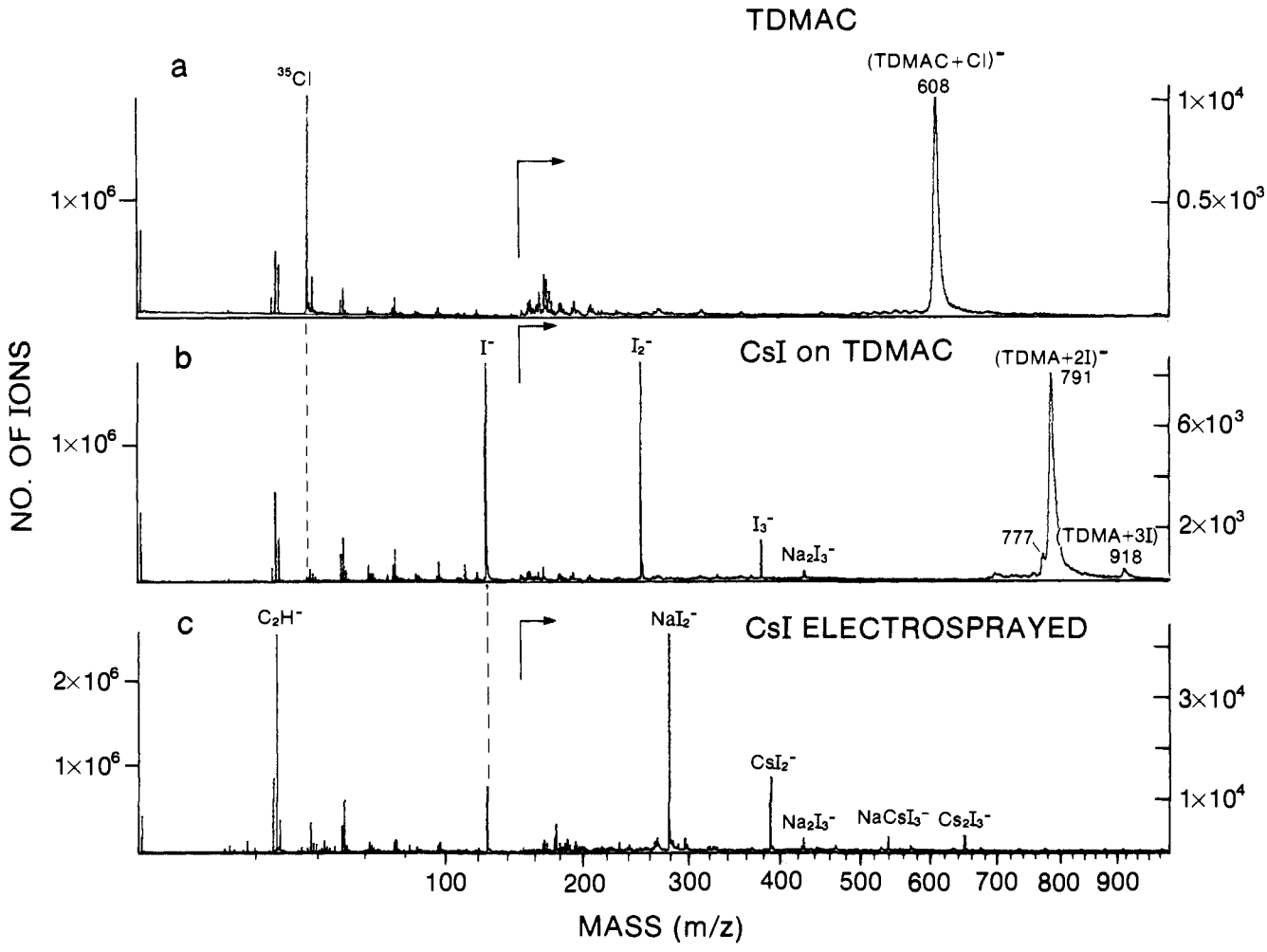

Figure 2. ^{252}Cf-PD negative ion mass spectra of (a) 0.1 M TDMAC on Mylar, (b) the same film after exposure to 1 × 10^{-2} M CsI, and (c) electrosprayed CsI. Data are plotted in 3.75 ns rather than in 2.50 ns time bins. The intensity of the data to the right of the arrow should be read from the right ordinate.

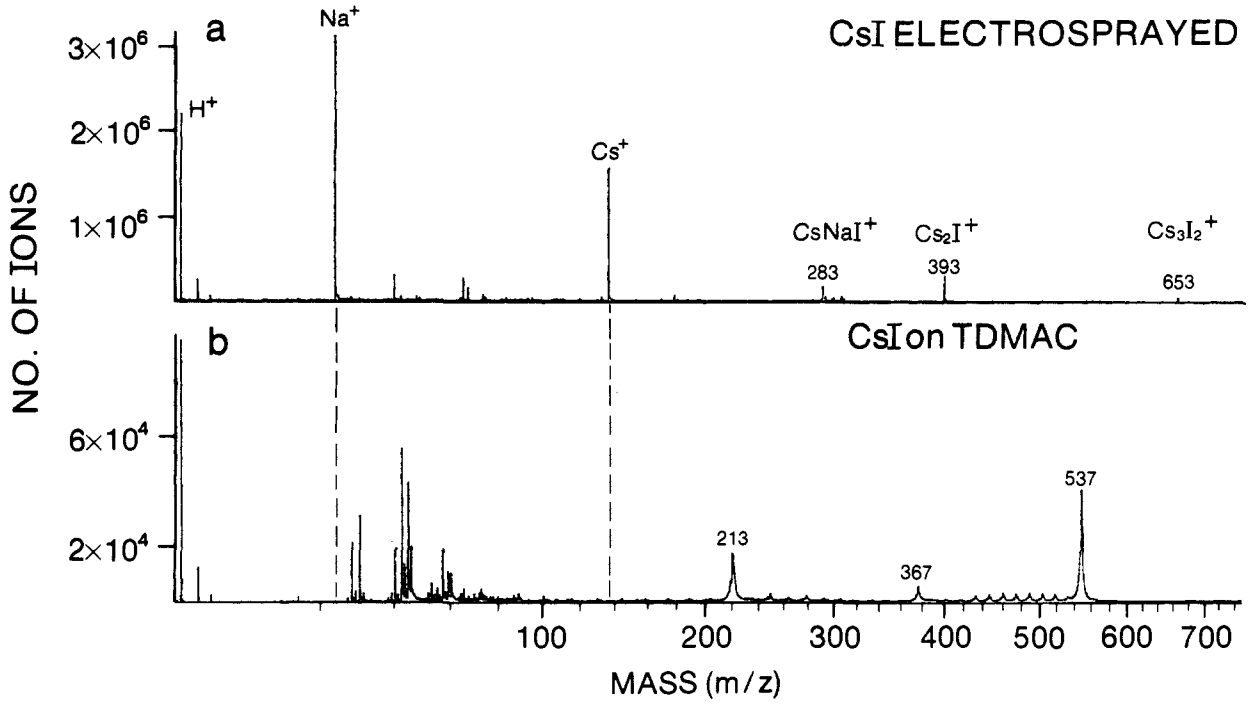

Figure 3. ^{252}Cf-PD positive ion mass spectra of (a) electrosprayed CsI and (b) 1 × 10^{-2} M CsI on TDMAC–Mylar. The peaks above m/z 200 are identified in Figure 1.

ions in the upper mass range. The four sequence ions Ap$^-$ (m/z 346), pC$^-$ (m/z 322), ApAp$^-$ (m/z 675), and pApC$^-$ (m/z 651) were more intense than the nucleotide fragment ions in the ApC spectrum. The negative molecular ion yields of ApC and ApApC

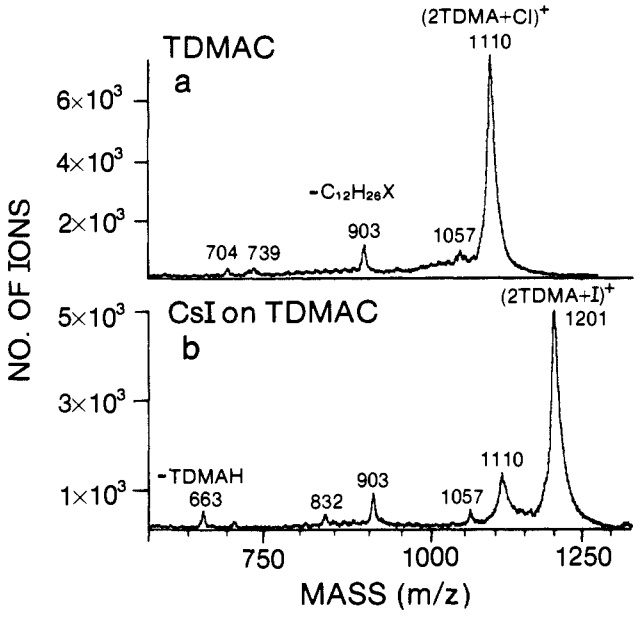

Figure 4. ^{252}Cf-PD positive ion mass spectrum of (a) 0.1 M TDMAC on Mylar in the higher mass range and (b) the same film after exposure to 1 × 10^{-2} M CsI.

were almost the same, while the positive molecular ion yield of ApApC was reduced nearly a factor of 2 compared to that of ApC. Some peaks in the range between the mono- and dinucleotide negative ion fragments were not identified, for example, m/z 408, 426, 571, 594, and 632; other features were similar to the ApC spectra. The peaks at m/z 522$^+$, 536$^+$, and 551$^+$ are common contaminants. The peak at m/z 789 was formed by loss of the cytidine base. A similar ion was also observed in the ApC spectrum at m/z 462. The peak at m/z 654 is the protonated dinucleotide sequence isomer (pApC + 2H)$^+$; m/z 676 may be a composite of (pApC + H + Na)$^+$ and the protonated sequence ion, (ApAp + 2H)$^+$. Both experimental masses are 1 u higher and lower (respectively) than the calculated masses. This error probably reflected the interference from the adjacent peak and the broad, complex structure of the peak.

The negative ion spectra of electrosprayed ApApC and ApApC–TDMAC, which was prepared from an aqueous 1 × 10^{-3} M solution, were very similar qualitatively in the mass range m/z 100–1000 (Figure 9). The enhancement in the yield was not as great as that observed for ApC. An additional ion corresponding to the expected nucleotide salt of TDMA$^+$, (TDMA$^+$ + ApApC^{2-})$^-$, was present in the ApApC–TDMAC spectrum. No mono- or dinucleotide fragments containing TDMA were observed. As in the ApC spectrum no appreciable intensity of chloride ions was detected; the PO_3^- ion was of comparable intensity to the same ion in the ApC spectrum. The positive ion ApApC–TDMAC spectrum was unchanged from that of the bare TDMAC film except for an additional ion corresponding to (3TDMA$^+$ + ApApC^{2-})$^+$.

Discussion

The adsorption of cationic surfactants on polymer surfaces has been studied extensively because of their industrial applications. Measurement of ζ potentials of polymer fibers such as polyester, of which Mylar is a variant, established that the polymer surfaces are negatively charged.[14] The study of ion adsorption by ^{252}Cf-PDMS on the Mylar film used in these experiments also corroborated these findings.[11] Cationic surfactants such as TDMAC will therefore be adsorbed on the Mylar foil with the long hydrocarbon chains pointing outward in the first monolayer. After the first layer, clustering of the surfactant on the polymer surface will occur at higher concentrations.[14,15] This model of the adsorption is consistent with the observations of TDMAC ion clusters in the ^{252}Cf-PD mass spectrum. If the TDMAC clusters were not observed in the film, the adsorbtion of the desired anionic component in the aqueous solution was negligible. This further supported the idea that multilayers of TDMAC on the Mylar are required so that the positively charged head groups will be pointing outward from the Mylar surface making them accessible anion-exchange sites.

A number of methods have been used to characterize surfactant structure and composition, including spectrophotometry titrations, chromatography, and mass spectrometry.[16] Of the latter method field desorption, laser desorption, fast atom bombardment, and thermal desorption have been successfully used.[16-18] Since extensive purification is not required for industrial purposes, the surfactants generally are a mixture comprised of compounds bearing hydrocarbon chains of varying lengths. Tandem mass spectrometry (MS/MS) has, therefore, been particularly useful for providing a direct mixture analysis. Combined with FAB and collision-activated dissociation (CAD), Lyon et al. have been able to determine the length and number of alkyl chains as well as other useful structural features.[17]

The first step in this study involved establishing the mass spectrum of the bare TDMAC films. The results demonstrated that ^{252}Cf-PDMS could provide useful structural information for this class of compounds. The positive ion ^{252}Cf-PD mass spectrum of TDMAC (Figure 1) bears far more resemblance to the CAD spectra of quarternary ammonium compounds obtained by Lyon rather than the FAB spectrum itself. In the positive FAB spectra the quarternary ammonium ion was the most abundant species; cluster ions were also observed. In the negative FAB spectrum peaks were observed due to the negatively charged counterions and counterion attachment of the neutral salt. Little fragmentation was observed in either mode. In addition to these types of ions, the positive ion spectrum obtained by ^{252}Cf-PDMS contained the same type of fragment ions observed in the CAD spectra of the molecular ion of a quaternary ammonium ion. In both the ^{252}Cf-PD and CAD mass spectra a series of nested fragment ions of the type C_nH_{2n+1} separated by 14 u extending from one carbon (n = 1) to the entire length of the chain (n = 12 for dodecyl) can be identified. Minor peaks two mass units lower are characteristic of both spectra. Whether the peak 2 u lower is due to loss of H_2 or the presence of an unsaturated homologue could not be determined unequivocably by ^{252}Cf-PDMS alone. In the studies described by Lyon et al. confirmatory ^{13}C and ^{1}H NMR ruled out the presence of an additional component. If the peak 2 u lower were due to the presence of an unsaturated homologue, it would also occur in more than one chain producing peaks 4 and 6 u lower additionally; alternatively, loss of H_2 could occur from more than one chain. Close examination of Figure 2 reveals a second peak of very low abundance 4 u lower than the main mass for each peak. We believe the sample to be free of other components, and since loss of H_2 was commonly observed in the CAD spectra, we have indicated the peak was due to the loss of H_2.

In the second phase of these experiments the chloride ion was displaced by the desired anionic component. The positive ion TDMAC spectrum was changed only in the type of cluster ion formed. Even though the chloride ion intensity is attenuated a factor of 30 when CsI was adsorbed, the cluster ion containing chloride (Figure 4) was only reduced a factor of 6. This suggested that interstitial chloride ions, possibly at the center of an aggregated structure, were hidden from the surface and, therefore, were not exchanged. This and the presence of the cluster ions confirmed that there was multilayer coverage of the TDMAC on the Mylar. The integrated intensity of cluster ions containing a

(14) Ginn, M. E. In "Cationic Surfactants"; Jungermann, E., Ed.: Marcel Dekker, Inc.: New York, 1970; pp 369–386.

(15) Robb, I. D. In "Anionic Surfactants—Physical Chemistry of Surfactant Action"; Lucassen-Reynders, E. H., Ed.; Marcel Dekker, Inc.: New York, 1981; pp 109–142.

(16) Llenado, R. A.; Neubecker, T. A. *Anal. Chem.* **1983**, *55*, 93R–102R.

(17) Lyon, P. A.; Crow, F. W.; Tomer, K. B.; Gross, M. L. *Anal. Chem.* **1984**, *56*, 2278–2204.

(18) Yergey, A. L.; Cotter, R. J. *Biomed. Mass Spectrom.* **1982**, *9*, 286–292.

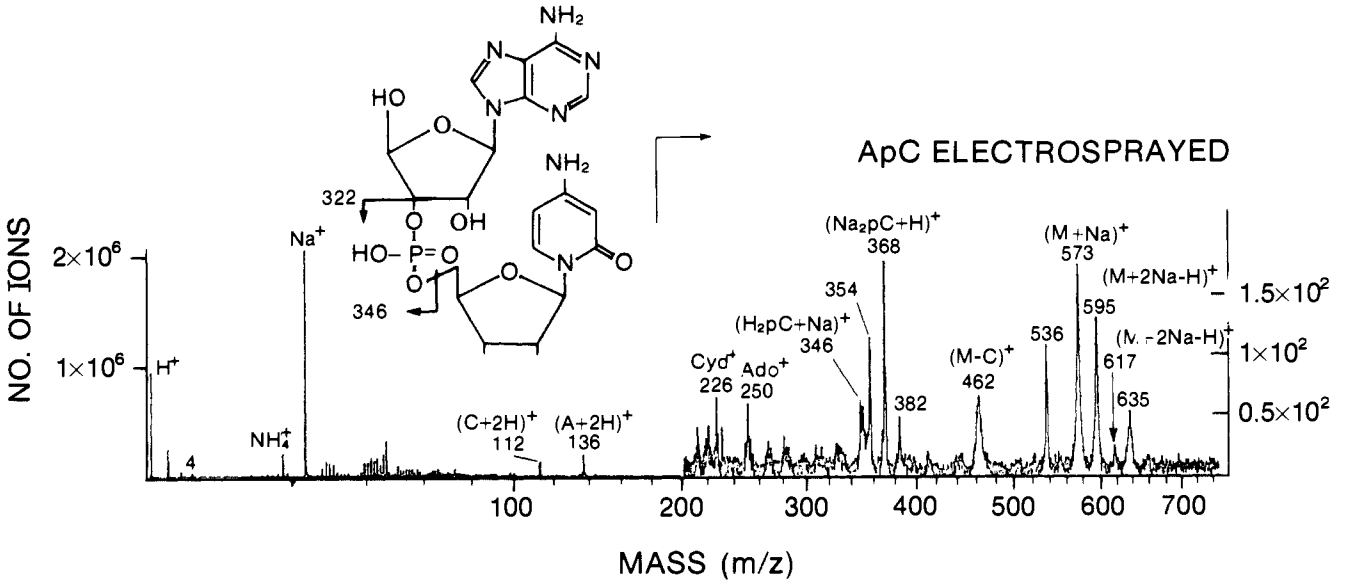

Figure 5. ^{252}Cf-PD positive ion mass spectrum of electrosprayed ApC. The structure of the free acid form of the molecule is shown in the inset. The average molecular weight of the free acid is 572.3 u.

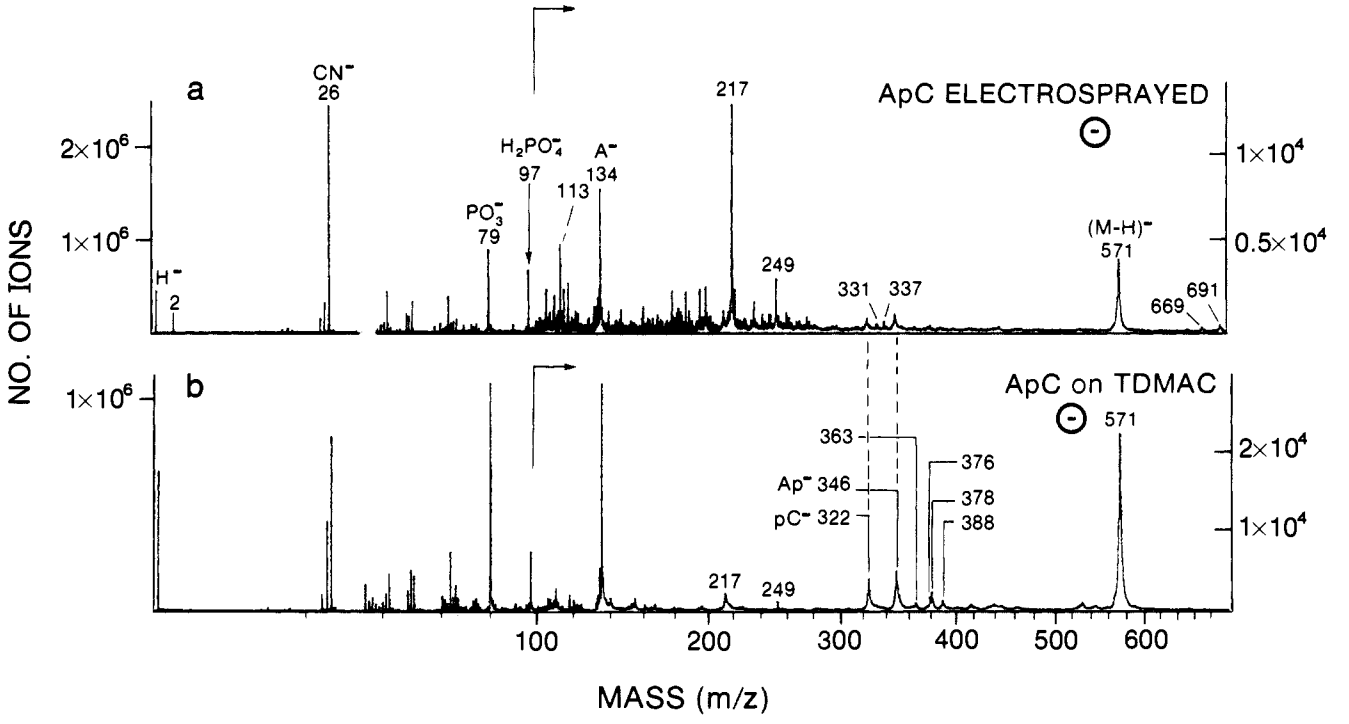

Figure 6. ^{252}Cf-PD negative ion mass spectra of (a) electrosprayed ApC and (b) 1×10^{-2} M ApC on TDMAC–Mylar.

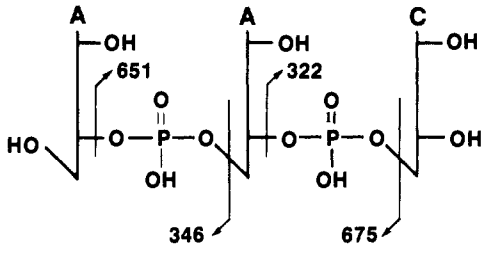

Figure 7. Shorthand structure of the free acid form of the trinucleoside diphosphate ApApC. Average masses of the four-sequence ion fragments are given. The average mass of the intact molecule is 901.6 u.

halide ion was two to four times less than the halide ion intensity. The $TDMA^+$ integrated intensity was equal to or greater than the halide ion abundance.

When ApC was used instead of CsI, there was still virtually quantitative displacement of Cl^-. The ApC^- integrated peak intensity was equivalent to that of chloride ion. A large enhancement in the yield of ApC molecular and fragment ions was observed compared to the electrosprayed sample. The enhancement for the trinucleoside diphosphate, ApApC, was not as dramatic. The yield of the $(M - H)^-$ ion at m/z 900 was only doubled. If the TDMA salt, $(TDMA^+ + ApApC^{2-})^-$, ion intensity is included, the enhancement was a factor of 3. The concentration of the ApApC solution used in the TDMAC experiment was 1×10^{-3} M, whereas the electrosprayed solution concentration was 2×10^{-3} M. An additional factor was that no attempt was made to minimize the concentration of the solution introduced onto the TDMAC–Mylar films in these preliminary experiments. Furthermore, we did not know the actual amount of ApC bound to the TDMAC surface since excess solution was removed by rinsing. The cationic surface sites should act to concentrate the desired anionic component on the surface. Optimally, only enough of the desired component to form a monolayer on the TDMAC may be required. With use of the standard method of preparing films

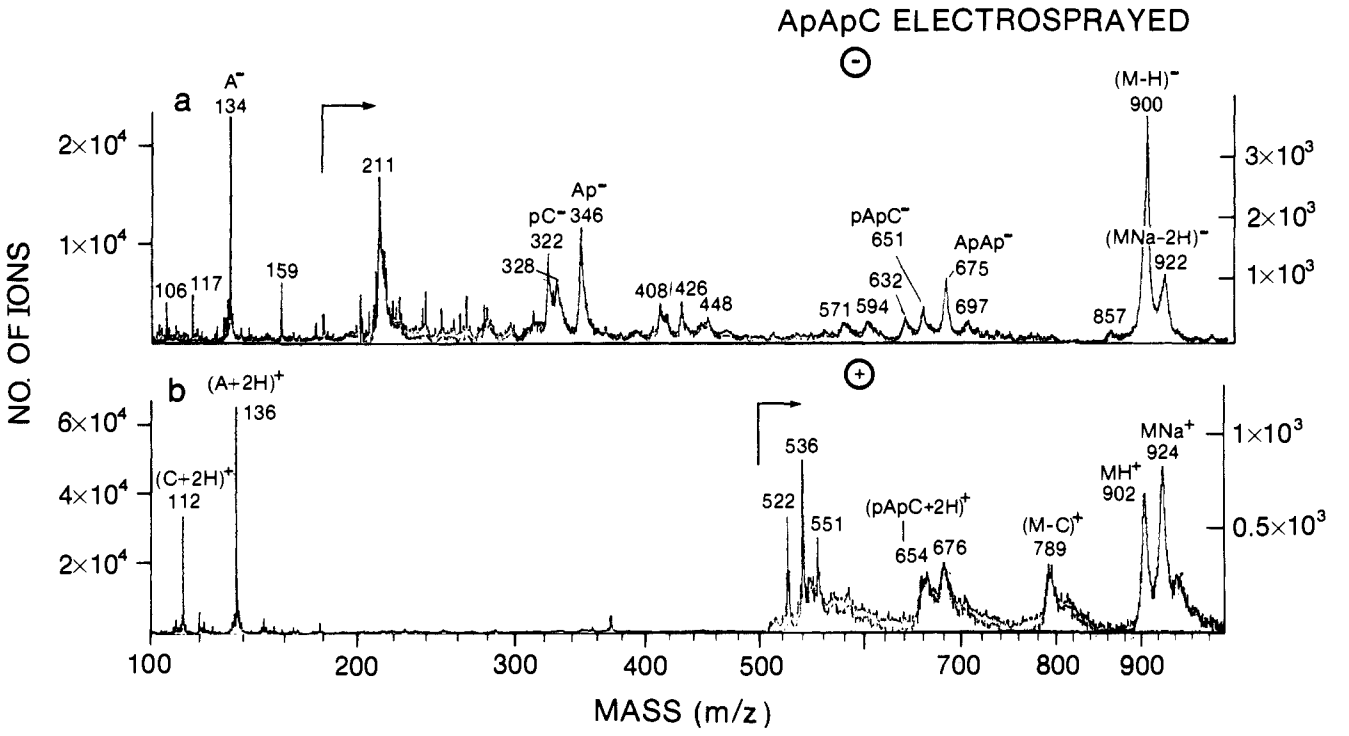

Figure 8. (a) ^{252}Cf-PD negative and (b) positive mass spectra of electrosprayed ApApC. Dinucleoside monophosphate sequence ion fragments $pApC^-$ and $ApAp^-$ contain one proionated phosphate group in their structure.

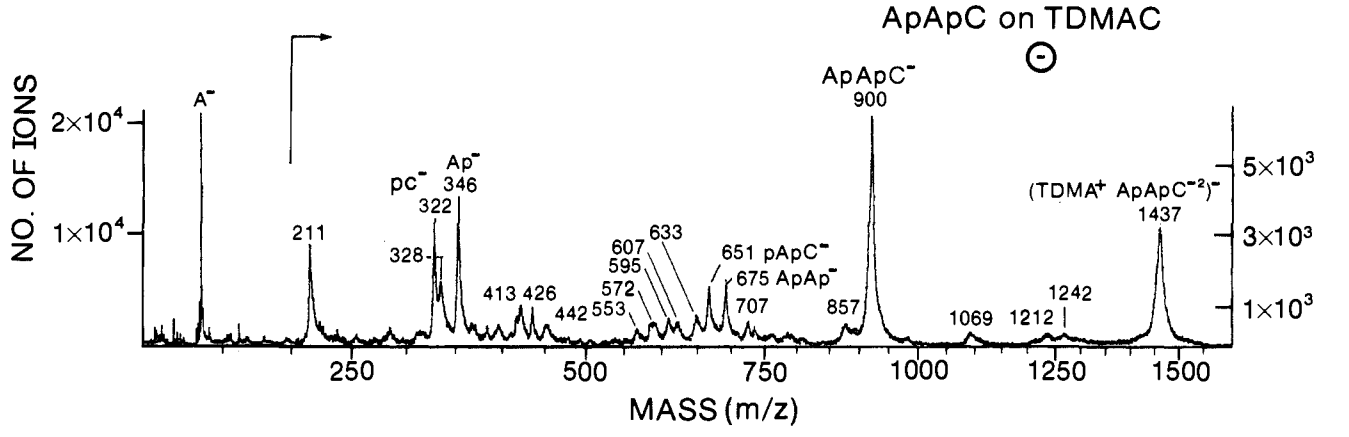

Figure 9. ^{252}Cf-PD negative ion mass spectrum of 1×10^{-3} M ApApC adsorbed on TDMAC–Mylar. The $ApApC^-$ ion contains one deproionated phosphate group.

by electrospray, it is difficult to achieve complete surface coverage with only a monolayer. The amount of material required to completely cover the surface by this method was at least an order of magnitude more than that calculated for complete monolayer coverage.[4] Another disadvantage of the electrospray method, of particular importance to the analysis of biopolymers, is the requirement that only a narrow range of organic solvents can be used to produce the electrospray. Oligonucleotides become increasingly insoluble in the required solvent system as the number of nucleotide units increases. The ability to use aqueous solutions to cast the films on the TDMAC surface becomes a critical factor. Even if there were no gain in sensitivity, this advantage alone would merit the use of this approach over the electrospray method.

The primary fragmentation model of the nucleotides reported here resulting in the production of the nucleotide sequence ions was not affected by the adsorption onto TDMAC. The major sequence ions produced are the result of fragmentation according to the pattern we established for chemically protected oligonucleotides,[19,20] The same type of ions are also observed in FAB mass spectra of nucleic acid fragments.[7,21] In the ApC negative ion spectra (Figure 6) the two sequence ions were more prominent for the ApC–TDMAC film than the electrosprayed ApC film. The only usual fragment ion that was absent from both spectra was the cytosine base fragment. The same ion is also absent in FAB spectra of ApC as well as several other cytosine-containing dinucleoside monophosphates.[21] The positive cytosine base fragment is observed by both methods.

Additional ions between the mononucleotide sequence ions and the molecular ion peak were present in the ApC–TDMAC spectrum but not in the spectrum of the electrosprayed film. These ions were of much lower abundance than the sequence ions but, nevertheless, would add a degree of ambiguity in the analysis of an unknown sequence. The structures of these ions were not identified. As in the spectra of the chemically protected oligonucleotides, the analysis of other sequence isomers should provide information on the structure of these ions in lieu of elemental

(19) McNeal, C. J.; Ogilvie, K. K.; Theriault, N. Y.; Nemer, M. J. *J. Am. Chem. Soc.* **1982**, *104*, 972–975.

(20) McNeal, C. J.; Ogilvie, K. K.; Theriault, N. Y.; Nemer, M. J. *J. Am. Chem. Soc.* **1982**, *104*, 976–980.

(21) Eagles, J.; Javanaud, G.; Self, R. *Biomed. Mass Spectrom.* **1984**, 41–46.

composition data.[19,20] There were not many more unidentifiable peaks in the ApApC–TDMAC spectrum than in the spectrum of the electrosprayed ApApC film. An additional type of fragment ion was produced for the trinucleoside diphosphate corresponding to elimination of adenine from the internal ribose group and cleavage after either the 5'- or 3'-phosphate group producing a mixture of the 5'- or 3'-monophosphate anions of 2,3-dihydroxy-3,4-dihydrofuran or perhaps preferentially one isomer over the other at m/z 211$^-$. Supporting evidence for the structure was that it was not present in the ApC spectrum and must, therefore, be a structural feature unique to ApApC or an impurity. Secondly, it must fragment to result in an ion bearing a single negative charge and not containing sodium so that it appeared in spectra of both the ApApC–TDMAC and electrosprayed ApApC films. Ions of this structure are reported in FAB mass spectra of oligonucleotides.[22]

There was a decrease in the m/z 217$^-$ peak intensity in the ApC spectrum (Figure 6) by a large factor. One plausible reason for this decrease would be if the ion contained sodium, for example, $(X + Na - 2H)^-$ or $(2X^- + Na)^-$. In the former case X must have a mass of 196. There is no positive ion in the spectrum of the electrosprayed sample that could be correlated with this mass. In the latter case, X has a mass of 97 which corresponds to the mass of the $H_2PO_4^-$ ion. The argument against this hypothesis is that we would have also expected to form $(2PO_3^- + Na)^-$ ions with approximately the same yeild or even a mixed cluster, but neither type of ion was observed. Furthermore, this peak was not present in the ApApC spectrum. Possibly this was a buffer contaminant in the sample. It is plausible that the yield of ApC was lower in the electrosprayed film because of the presence of contaminants or alkali metal ion salts. Ion yields could be diminished as a result of quenching or because the extraneous material comprises a fraction of the surface area. Eagles et al.[21] reported that the FAB spectrum of ApC was of low intensity compared to other dinucleoside monophosphates. No reason is proposed for this observation. It may be that impurities were present in this particular preparation which resulted in unfavorable conditions for desorption–ionization. Similarly, in the electrosprayed ApApC positive ion spectra (Figure 7b), common contaminating ions producing peaks at m/z 522$^+$, 536$^+$, and 551$^+$ may be partially responsible for the attenuated yield compared to the ApApC–TDMAC spectrum.

A final aspect of the ApApC–TDMAC spectrum that merits noting is the difference in peak widths of various ions. For example, in Figures 8a and 9 the adenine base fragment, m/z 134, and the phosphorylated-ribose fragment, m/z 211, are both composed of elements that do not contain significant isotopic abundance peaks aside from carbon. Both are low molecular weight peaks; yet, the peak widths were strikingly different.

This demonstrated an aspect of a time-of-flight mass analysis that often leads to the incorrect assumption that it is an inherently low resolution method. Chait and Field have shown that the broad peak shapes observed in ^{252}Cf-PDMS are the result of fragmentation of the ion after it is accelerated, either in the acceleration region or in the field-free drift region.[23] By using appropriate retarding potentials they were able to greatly enhance the mass resolution. We have observed that ions of the sugar moiety, either lacking in the phosphate group, the nucleobase, or both, are characteristically broad. We use this distinguishing feature to assist in the identification of the ion. If the biopolymer was a completely unknown structure, high resolution measurements would be essential in establishing the identity of this ion. But, since we know this molecule to contain sugars, phosphates, and nucleobases exclusively, even with low resolution, peak assignments can be made with a high degree of confidence. If an ambiguity does arise, the spectrum of a different structural analogue is compared as was the case when we first examined mass spectra of protected oligonucleotides.[19,20]

(22) Panico, M.; Sindona, G.; Uccella, N. *J. Am. Chem. Soc.* **1983**, *105*, 5607–5610.
(23) Chait, B. T.; Field, F. H. *Int. J. Mass Spectrom. Ion Phys.* **1981**, *41*, 17–29.

Conclusion

We have demonstrated the feasibility of using a stationary cationic surfactant as a matrix for the selective adsorption of inorganic and organic anions to be studied by mass spectrometry. This film meets the necessary established criteria: (1) it is a soluble molecule so that thin films can be prepared which (2) do not redissolve when exposed to aqueous solutions, and (3) it has a low equivalent molecular weight resulting in a sufficiently high concentration of available surface sites. In all cases studied an enhancement of the yield, relative to an electrosprayed film, was observed. We propose that this enhancement is in part a result of separating the nucleotide ions from each other by formation of the TDMA–anion complex thereby attenuating the strong intermolecular forces in polyanionic matrices. In addition, nucleotide negative molecular ion yields may have been enhanced by converting the free acid or ammonium salt to the $TDMA^+$ salt; this bulky cation should result in an increased distance between the two oppositely charged centers. This would have the effect of making it easier to separate the two charges, resulting in the formation of $TDMA^+$ and nucleotide$^-$ molecular ions. The preferential use of triethylammonium ions as the counterion in nucleotide salts studied by FABMS suggests[7] that a variety of bulky cationic groups may be useful in this regard. A potential disadvantage of the TDMAC is its relatively high molecular weight. This is especially relevant for polysulfonated polysaccharides since each sugar residue may contain multiple anionic groups, each binding a $TDMA^+$ ion. While this is useful in assessing the total number of charges, it results in excessively high molecular weight ions. It may be possible to reduce the chain length or decrease the number of dodecyl chains and still retain the useful properties of TDMAC. Experiments are in progress to determine the optimal parameters for the preparation of the TDMAC films and to quantitate the binding capacity by radiolabeling of the adsorbed anion. Well-established principles of surfactant chemistry can be used in establishing these conditions, rather than the, at times, seemingly capricious parameters required to produce electrosprayed films.

It may be possible to use very dilute solutions and utilize the TDMAC film to concentrate the desired anionic component on the surface. This factor and the ability to work with aqueous solutions of the biopolymers rather than the organic mixtures imposed by the electrospray method will allow physiological conditions to be mimicked more easily. Experiments are in progress to study the adsorption of organic cations onto anionic surfactants applying the same strategy described in this study. The diversity of available surfacts should allow us to design matrices that will enhance the molecular ion yields of large polyvalent biopolymers.

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