Observation of Duplex DNA–Drug Noncovalent Complexes by Electrospray Ionization Mass Spectrometry

David C. Gale, David R. Goodlett,† Karen J. Light-Wahl, and Richard D. Smith*

Chemical Sciences Department
Pacific Northwest Laboratory
Richland, Washington 99352

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The interaction and noncovalent binding of small organic molecules to duplex DNA is of interest for use in antitumor, antiviral, and antibiotic applications.[1] Small molecule–oligonucleotide duplex complexes have been studied by several techniques including NMR,[2] X-ray crystallography,[3] gel footprinting,[4] and more recently FTIR[5] and linear dichroism.[6] The study of small molecule–oligonucleotide duplex complexes has been performed to elucidate basic structure–function relationships with the eventual goal of performing a rational design of sequence specific DNA-binding molecules.[1g,7] Minor groove binders are one group of molecules that have been shown to interact with duplex DNA.[8] Minor groove binders are typically crescent-shaped linear molecules that fit into the DNA duplex minor groove of four or five successive A, T base pairs.[7a,8] Distamycin A (Dm), the subject of this work, is a naturally occurring antibiotic.[8,9] Hydrogen bonding, van der Waals forces, and electrostatic interactions have been suggested to account for the sequence selectivity of this molecule.[2d,e,7] The oligonucleotide duplex utilized in this study, formed by the self-complementary sequence 5′-dCGCAAATTTGCG-3′, has been studied with and without minor groove binding molecules previously.[2a,d,e,3d,7,8,10] The oligonucleotide duplex forms due to hydrogen bond formation, hydrophobic interactions, and electrostatic forces in the presence of stabilizing counterions.[11] Results from NMR have shown that both 1:1 and 2:1 Dm/oligonucleotide duplex noncovalent complexes can be formed in solution, where Dm is noncovalently bound in the minor groove of the AATT or ATTT oligonucleotide duplex regions.[2d] For the 2:1 Dm/oligonucleotide duplex, the Dm is bound as a side-by-side antiparallel dimer in the minor groove of the duplex.[2d] Association constants for the binding of Dm to a 16 base pair oligonucleotide duplex and to a 1:1 Dm/16 base pair oligonucleotide duplex have been measured previously and are 1.3×10^7 and 7.9×10^8 M^{-1}, respectively.[12]

A number of examples for the ionization and detection of noncovalent complexes in solution by electrospray ionization mass spectrometry (ESI-MS) have been reported.[13] Recently, the specific multimeric association of oligonucleotides has been observed.[14] In this communication, we report the first use of ESI-MS for detection of a self-complementary Dickerson–Drew[15] type oligonucleotide duplex, 1:1 Dm/oligonucleotide duplex, and 2:1 Dm/oligonucleotide duplex noncovalent complexes.

Negative ion ESI mass spectra were acquired with a quadrupole mass spectrometer described previously.[16] The mass spectrometer used a heated metal capillary interface[17] without counter-current gas flow. Low capillary-skimmer potentials (ΔCS) and capillary temperatures were required to observe the noncovalent complexes. Experimental conditions for all reported data utilized a ΔCS of −100 V, inlet capillary heating of ~10 W (capillary surface temperature of ~75 °C), capillary length of 20 cm (15 cm heated), sample flow rate of 0.20 μL min^{-1},[18] an electrospray potential of −2.4 kV, and a coaxial sheath gas of SF_6.[19] The self complementary 12-base oligodeoxyribonucleotide, 5′-dCGCAAATTT-GCG-3′ (M_r = 3645.47), was prepared using phosphoramidite chemistry and purified by reversed-phase HPLC followed by Centricon 3 filtration. The concentration of oligonucleotide for all experiments was 20 μM (measured as single-stranded material). Dm (Sigma, St. Louis, MO, M_r = 481.5) was purified by HPLC. Oligonucleotide samples were annealed by heating to 95 °C for 5 min and cooling to room temperature over 3 h. Solution conditions for oligonucleotide duplex formation generally require salt and buffer concentrations that are not desirable for ESI-MS.[11] Several different salts (10 mM NaCl, KCl, Tris, and

* Author to whom correspondence should be addressed.
† Current address: Immunobiology Research Institute. Route 22 East, P.O. Box 999, Annandale, NJ 08801-0999.

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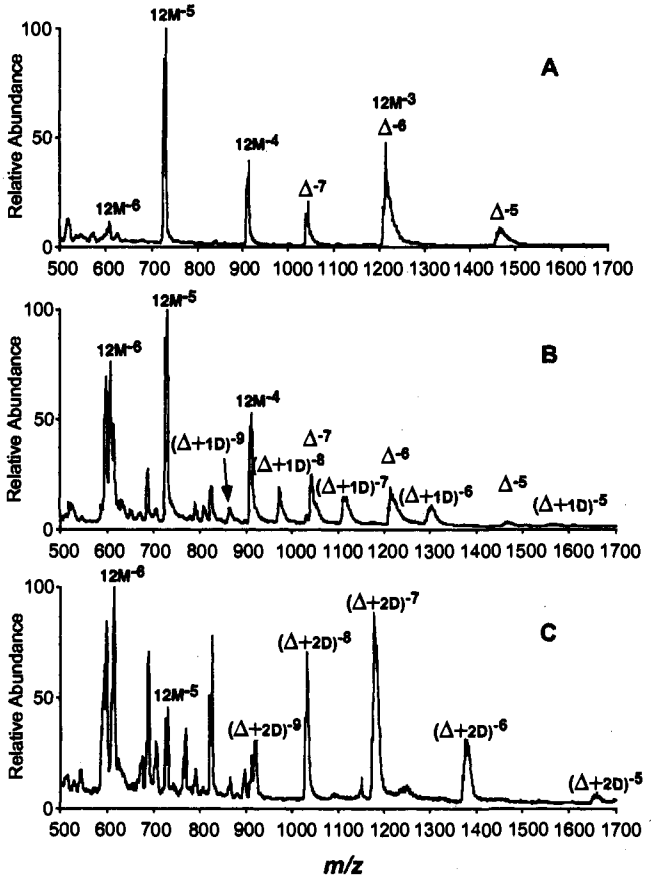

Figure 1. Negative ion electrospray mass spectra of annealed 20 μM (single-stranded concentration prior to annealing) oligonucleotide, 5′-dCGCAAATTTGCG-3′, in 10 mM ammonium acetate/10 mM ammonium citrate (pH ~8.3) with different concentrations of the minor groove binding molecule Dm. Peaks labeled 12M are assigned to single-stranded oligonucleotide, Δ to oligonucleotide duplex, (Δ+1D) to 1:1 Dm/oligonucleotide duplex, and (Δ+2D) to 2:1 Dm/oligonucleotide duplex. All spectra were acquired with a ΔCS of −100 V, capillary heating of ~10 W (capillary surface temperature of ~75 °C), a flow rate of 0.20 μL min^{-1}, and an electrospray potential of −2.4 kV: (A) no Dm, an average of 32 scans consuming 28 pmol of oligonucleotide; (B) 5 μM Dm, an average of 80 scans consuming 64 pmol of oligonucleotide; (C) 20 μM Dm, an average of 46 scans consuming 37 pmol of oligonucleotide.

$MgCl_2$) were evaluated as hybridizing buffers for compatibility with ESI-MS. Single-stranded 5′-dCGCAAATTTGCG-3′ was observed both with and without salt adducts under low energy interface conditions in the presence of these electrolytes. No oligonucleotide duplex ions were observed with these buffers by ESI-MS in our experiments. The most effective buffer for observation of the oligonucleotide noncovalent duplex by ESI-MS was 10 mM ammonium citrate/10 mM ammonium acetate (AC/AA), pH 8.3.

The ESI mass spectrum of the annealed self-complementary oligonucleotide in the AC/AA buffer is shown in Figure 1A. The concentration of oligonucleotide duplex is expected to be 10 μM. Both single-stranded and duplex oligonucleotide ions are observed in the spectra, with the duplex dominating at higher mass to charge (m/z), consistent with earlier results.[14] Single-stranded ions may be due to incomplete duplex formation or the breakup of duplex ions in the electrospray interface.[14b] The peaks at m/z 1040 (Δ^{-7}) and 1457 (Δ^{-5}) are odd charge states specific to the oligonucleotide duplex. No oligonucleotide duplex ions were observed from an annealed deionized water solution using the same mass spectrometric conditions (data not shown). As expected, counterions are required to stabilize the oligonucleotide duplex.[11] Peaks at m/z 910 and 1214 can arise from both single-stranded and duplex oligonucleotide ions, due to the same m/z of even charge states. However, an assignment based on the charge-state distribution can be made. The peak at m/z 910 is probably single-stranded oligonucleotide ions ($12M^{-4}$) on the basis of the intensity of $12M^{-5}$ ions, and the peak at m/z 1214 is attributed primarily to oligonucleotide duplex ions (Δ^{-6}) on the basis of the intensity of Δ^{-7} and Δ^{-5} ions.

Figure 1B shows the ESI spectra for an annealed 5 μM Dm/10 μM oligonucleotide duplex in AC/AA buffer solution. In comparing panels A and B of Figure 1, major new peaks are observed, representative of the 1:1 Dm/oligonucleotide duplex. These 1:1 Dm/oligonucleotide duplex ions are at m/z 862, 970, 1109, 1294, and 1553. After annealing, the concentration of 1:1 Dm/oligonucleotide duplex and oligonucleotide duplex should be equivalent if all of the Dm binds to an oligonucleotide duplex. As expected from the solution chemistry, ions representative of both the oligonucleotide duplex and the 1:1 Dm/oligonucleotide duplex are observed in approximately equal intensities.

At higher drug to oligonucleotide ratios, 2:1 minor groove binding molecule/oligonucleotide duplex noncovalent complexes have been observed.[2a,d,e,12]

Figure 1C shows the ESI spectra for an annealed 20 μM Dm–10 μM oligonucleotide duplex in AC/AA buffer solution. The Dm to oligonucleotide duplex ratio in solution is expected to be 2:1. A charge-state distribution for the 2:1 Dm/oligonucleotide duplex noncovalent complex (evident by the unique odd charge state peaks at m/z 1178 and 1649) is observed at higher m/z. Broad peaks for the oligonucleotide duplex, 1:1 Dm/oligonucleotide duplex, and 2:1 Dm/oligonucleotide duplex are due to adducted counterions. Single-stranded oligonucleotide and single-stranded oligonucleotide with Dm ions are observed at lower m/z that may arise from incomplete duplex formation or dissociation of higher charge state complexes in the ESI interface.[14b] Importantly, at this ratio of Dm to oligonucleotide duplex, no oligonucleotide duplex or 1:1 Dm/oligonucleotide complex is observed.

In summary, we have observed by ESI-MS the noncovalent complex formed between a minor groove binding molecule and a 12 base pair self-complementary oligonucleotide. When the ratio of Dm to oligonucleotide was varied, oligonucleotide duplex, 1:1 Dm/oligonucleotide duplex, and 2:1 Dm/oligonucleotide duplex noncovalent complexes were observed, consistent with NMR results for the same sequence and Dm to oligonucleotide duplex concentration ratios.[2d] These results indicate that ESI-MS is an effective analytical technique for the detection of specific drug–oligonucleotide duplex noncovalent complexes and that specific noncovalent complexes can be observed reflecting stoichiometry in solution. Additional experiments to determine if ESI-MS can provide information on the specificity and selectivity of additional minor groove binding and intercalating molecules are in progress.

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