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Investigation of Quadruplex Oligonucleotide−Drug Interactions by Electrospray Ionization Mass Spectrometry

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Selectivity, binding stoichiometry, and mode of binding of Tel01, distamycin A, and diethylthiocarbocyanine iodide (DTC) to the parallel stranded G4-quadruplex $[d(T_2G_5T)]_4$ were investigated by ESI-MS. The first drug/quadruplex complexes observed by ESI-MS are described. Tel01, distamycin A, and DTC all form complexes with quadruplex DNA, but only Tel01 is completely selective for quadruplex versus duplex oligonucleotide under the conditions employed. Previous solution determinations of the binding mode of Tel01 and distamycin A to quadruplex oligonucleotides indicate that Tel01 interacts through end-stacking with guanine tetrads of quadruplex DNA, while distamycin A interacts by binding to quadruplex grooves. When these two different drug/quadruplex complexes are subjected to collisionally activated dissociation in a mass spectrometer, the observed fragmentation patterns are distinct. Tel01/quadruplex complexes undergo facile loss of drug and dissociation to single-strand oligonucleotide ions, while distamycin/quadruplex complexes fragment into single-strand oligonucleotide ions in which the drug molecule is retained. Dissociation patterns for DTC/quadruplex complexes are similar to those of distamycin; therefore, it is concluded that DTC interacts with $[d(T_2G_5T)]_4$ through groove-binding. These ESI-MS results are applicable to both the identification and characterization of G-quadruplex interactive agents and may also be useful in probing unusual DNA structures.

Noncovalent interactions between nucleic acids and small molecules have important biological consequences, and electrospray ionization mass spectrometry (ESI-MS) has emerged as a useful tool in their investigation.[1,2] Stoichiometry and selectivity of the binding of small molecules to oligonucleotides is easily obtained with minimal sample consumption. Most studies have focused on the complexation of drugs with either duplex DNA or RNA, with only two reports of ESI-MS of higher order DNA structures,[3,4] and none on quadruplex DNA/drug complexation. The biological roles of alternative DNA structures, such as quadruplex DNA, are still under investigation; however, these nucleic acid structures are being pursued as current targets in drug design.[5,6] In particular, the putative involvement of G-quadruplex DNA in telomere maintenance[7] may be a relevant antitumor target. Stabilization of G-quadruplex DNA with quadruplex-interactive ligands leads to in vitro inhibition of telomerase, an enzyme expressed in many tumor cell lines that is responsible for the de novo synthesis of telomeric DNA.[8] Small molecules that bind to G-quadruplex DNA might also find utility as probes for these DNA structures and their functional significance.

Many short oligonucleotides with the ability to form G-quadruplex DNA have been structurally characterized.[9,10] G-quadruplex DNA is composed generally of three or more consecutive core tetrads, assembled from the guanine bases of up to four DNA strands (Figure 1). The central cavity of the guanine tetrad forms a binding site for metal ions, which are generally required for quadruplex formation. There are three main types of quadruplexes in vitro: G4, composed of four parallel DNA strands; G4′, composed of one DNA strand folded intramolecularly; and G′2, composed of two DNA strands in various orientations (Figure 1). These different quadruplex types have distinct groove geometries associated with the core tetrads, and the selective targeting of specific topological forms of quadruplex DNA has been proposed.[5,6,8,11,12] The parallel stranded G4-DNA has four grooves of identical medium width, while the other quadruplex types have grooves of differing width depending on topology.

The success of G-quadruplex interactive agents as potential drugs or biological probes for G-quadruplex structures depends on their selectivity for G-quadruplex DNA over other DNA struc-

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(1) Hofstadler, S. A.; Griffey, R. H. *Chem. Rev.* **2001**, *101*, 377−390.
(2) Beck, J. L.; Colgrave, M. L.; Ralph, S. F.; Sheil, M. M. *Mass Spectrom. Rev.* **2001**, *20*, 61.
(3) Goodlett, D. R.; Camp, D. G.; Hardin, C. C.; Corregan, M.; Smith, R. D. *Biol. Mass Spectrom.* **1993**, *22*, 181.
(4) Griffith, M. C.; Risen, L. M.; Greig, M. J.; Lesnik, E. A.; Sprankle, K. G.; Griffey, R. H.; Kiely, J. S.; Freier, S. M. *J. Am. Chem. Soc.* **1995**, *117*, 831.
(5) Kerwin, S. M. *Curr. Pharm. Des.*, **2000**, *6*, 441.
(6) Arthanari, H.; Bolton, P. H. *Chem., Biol.* **2001**, *8*, 221.
(7) Wellinger, R. J.; Sen, D. *Eur. J. Cancer* **1997**, *33*, 735.
(8) Mergny, J.-L.; Mailliet, P.; Lavelle, F.; Riou, J.-F.; Laoui, A.; Helene, C. *Anti-Cancer Drug Des.* **1999**, *14*, 327.
(9) For a recent review of G-quadruplex structures, see: Simonsson, T. *Biol. Chem.* **2001**, *382*, 621.
(10) Gilbert, D. E.; Feigon, J. *Curr. Opin. Struct. Biol.* **1999**, *9*, 305.
(11) Chen, Q.; Kuntz, I. D.; Shafer, R. H. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 2635.
(12) Neidle, S.; Harrison, J. R.; Reszka, A. P.; Read, M. A. *Pharmacol., Therap.* **2000**, *85*, 133.

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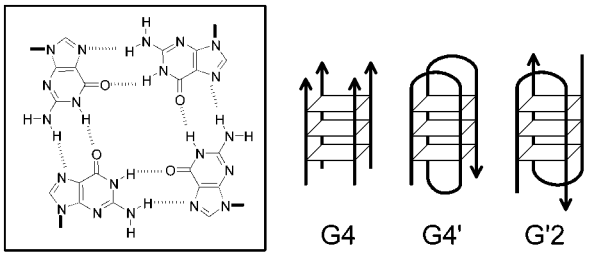

Figure 1. Structure of G-tetrad and different topological forms of quadruplex DNA.

tures. Consequently, drug design has focused on the issue of selectivity. The recently reported *N*,*N*′-bis(2-morpholinylpropyl)-3,4,9,10-perylenetetracarboxylic acid diimide (Tel01) (Figure 2) selectively binds and stabilizes G4-DNA $[d(TAGGGTTA)]_4$ and G4′-DNA $[d(TTAGGG)_4]$ by stacking on the faces of the terminal G-tetrads.[13] This end-stack mode of binding is common to related quadruplex-binding telomerase inhibitors, in which 1:1 and 2:1 complexes are typically observed.[14,15] At neutral pH, the selectivity of Tel01 for quadruplex DNA over duplex DNA is $\sim 10^3$. Diethylthiocarbocyanine iodide (DTC) (Figure 2) also binds G4-DNA $[d(TAGGGTTA)]_4$ and G4′-DNA $[d(TTAGGG)_4]$ as 1:1 and 2:1 complexes and inhibits telomerase.[16] However, unlike Tel01, DTC presumably binds quadruplex DNA through groove interactions, analogous to the binding of diethyloxadicarbocyanine (DODC) to G′2-DNA sequences.[11] DTC binds duplex DNA as well, although changes in its absorption spectra upon binding are different from those observed upon binding to quadruplex sequences. Recent NMR titrations have demonstrated that the well-known duplex DNA minor groove-binding ligand distamycin A (Dist. A) (Figure 2) reversibly binds the parallel stranded quadruplex $[d(TG_4T)]_4$ as a short-lived 2:1 complex.[17] At higher drug/DNA ratios, a 4:1 complex forms, and the NMR results are consistent with two opposite quadruplex grooves being occupied simultaneously by distamycin dimers.

In this report, we utilize ESI-MS to investigate the selectivity, binding stoichiometry, and mode of binding of Tel01, Dist. A, and DTC to the parallel stranded G4-quadruplex $[d(T_2G_5T)]_4$. Specifically, the ability of ESI-MS to distinguish selectivity between quadruplex and duplex DNA binding, as well as to differentiate between different modes of binding is of interest. The quadruplex $[d(T_2G_5T)]_4$ differs little from the well-characterized quadruplex $[d(TG_4T)]_4$[18] and, as such, is assumed to possess similar structural features. Since the different binding modes of Tel01 and Dist. A to quadruplex DNA have been elucidated in solution, results for these ligands are compared to those obtained for DTC.

EXPERIMENTAL SECTION

Chemicals. Dist. A was purchased from Sigma (St. Louis, MO). DTC was obtained from Aldrich (Milwaukee, WI). Both were used without further purification. Tel01 was prepared as previously described.[13] Self-complementary duplex oligodeoxynucleotide D2 [5′-GCGAATTCGC-3′], purified by RP-HPLC in an ammonium acetate buffer, was obtained from TriLink BioTechnologies (San Diego, CA). Quadruplex-forming oligodeoxynucleotide [5′-TTGGGGGT-3′] was synthesized on an Expedite Synthesizer (Applied Biosystems, Foster City, CA), purified by RP-HPLC using an ammonium acetate buffer/acetonitrile gradient, concentrated, and dialyzed in water to remove excess salt. Formation of the quadruplex Q1 $[d(T_2G_5T)]_4$ was facilitated by successive freeze−thaw cycles at 0 °C and confirmed by nondenaturing gel electrophoresis.

Procedures and Instrumentation. Stock solutions of Dist. A were prepared in distilled water and stored at 4 °C. DTC was dissolved in methanol and kept in the dark at room temperature. Tel01 was dissolved in 1:1 acetonitrile/water and stored at room temperature. The concentration of quadruplex Q1 solutions was determined spectroscopically, and stock solutions were diluted to 25 μM in 3:1 water/methanol, in the presence or absence of drug, just prior to analysis. Stock duplex D2 solutions were prepared in 1.0 M ammonium acetate and diluted to 25 μM in water and methanol, in the presence or absence of drug, to give a resulting 3:1 55 mM ammonium acetate/methanol solution.

Mass spectra were obtained with a ThermoFinnigan LCQ Duo (San Jose, CA) in the negative ion mode with the heated capillary normally set at 85 °C. Typical conditions utilized a source voltage of −3 to −4 kV, nitrogen sheath gas set at 20−25 arbitrary units, an ionization time of 500−1000 ms, and acquisition of 10−20 scans, each consisting of 10 microscans. The base pressure in the trap was $\sim 1 \times 10^5$ Torr. Solutions of oligonucleotide or drug/oligonucleotide were admitted by direct infusion with a Hamilton syringe pump (Holliston, MA) at 5−10 μL/min. Collisional activated dissociation (CAD)[19] experiments of selected drug/quadruplex complexes were performed. The desired complex was isolated in the trap by resonance ejection and subjected to collisional energy (reported as a percentage of 5 V_{0-p}) to produce fragmentation of the complex.

RESULTS AND DISCUSSION

ESI-MS Detection of Quadruplex $[d(T_2G_5T)]_4$. Optimal detection of quadruplex Q1 is dependent on both solution and interface conditions. Identification of quadruplex versus single-strand oligonucleotide peaks is based on the odd-charge states of Q1 and spacing of Na^+ adducts (Figure 3).[20] The observed charge states and number of adducted Na^+ atoms are similar to those reported for $d(CGCG_4GCG)_4$, the only previously reported G-quadruplex oligonucleotide analyzed by ESI-MS.[3] Peaks corresponding to quadruplex charge states in the absence of any adducted Na^+ atoms are not observed, in agreement with the requirement of cations for formation of quadruplex in solution. For example, the first and smallest peak evident for the $Q1^{-7}$ charge-state distribution (shown in Figure 3, inset) corresponds to quadruplex Q1 with two Na^+ atoms attached. The most intense

(13) Kerwin, S. M.; Chen, G.; Kern, J. T.; Thomas, P. W. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 447.
(14) Federoff, O. Y.; Salazar, M.; Han, H.; Chemeris, V. V.; Kerwin, S. M.; Hurley, L. H. *Biochemistry* **1998**, *37*, 12367.
(15) Read, M. A.; Neidle, S. *Biochemistry* **2000**, *39*, 13422.
(16) Kerwin, S. M.; Sun, D.; Kern, J. T.; Rangan, A.; Thomas, P. W. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 2411.
(17) Randazzo, A.; Galeone, A.; Mayol, L. *Chem. Commun.* **2001**, 1030.
(18) Phillips, K.; Dauter, Z.; Murchie, A. I. H.; Lilley, D. M. J.; Luisi, B. *J. Mol. Biol.* **1997**, *273*, 171.
(19) Busch, K. L.; Glish, G. L.; McLuckey, S. A. *Mass Spectrometry/ Mass Spectrometry: Techniques and Applications of Tandem Mass Spectrometry*; VCH: New York, 1988.
(20) Smith, R. D.; Loo, J. A.; Edmonds, C. G.; Barinaga, C. J.; Udseth, H. R. *Anal. Chem.* **1990**, *62*, 882.

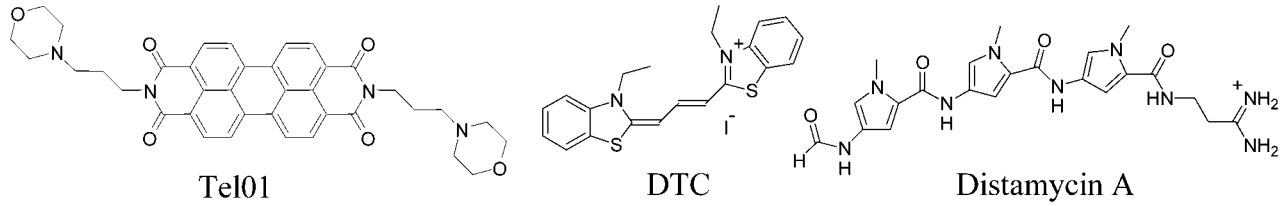

Figure 2. Structures of quadruplex DNA-binding drugs.

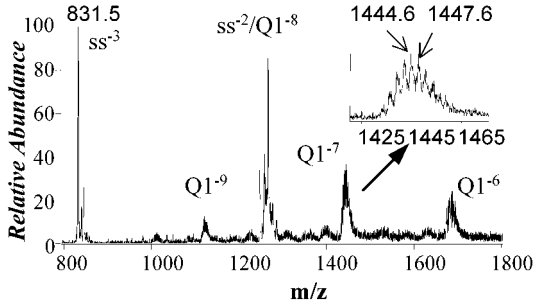

Figure 3. ESI mass spectrum of quadruplex Q1. Charge states are given near the corresponding peaks of interest. Quadruplex charge states are labeled Q1; single-strand charge states are labeled ss.

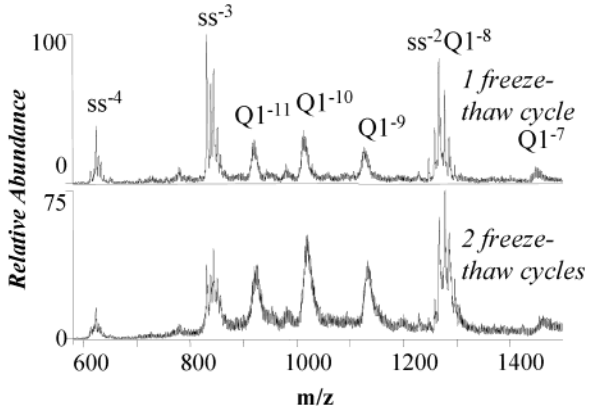

Figure 4. ESI-MS spectra of Q1 after one and two freeze−thaw cycles.

peaks correspond to $[Q1 + 6Na^+ - 13H]^{-7}$ and $[Q1 + 7Na^+ - 14H]^{-7}$ quadruplex ions. In contrast, the main single-strand oligonucleotide ion, ss^{-3} at m/z 831, corresponds to the oligonucleotide ion without any Na^+ atoms attached, although satellite Na^+ adducts are also present. It is likely that at least some of the cations associated with the quadruplex ions reside in the G-tetrad central cavities and remain associated with the quadruplex as it is transported to the gas phase during electrospray ionization. The proportion of quadruplex versus single-strand ions detected by ESI-MS of Q1 increases with successive freeze−thaw cycles of Q1 samples, in parallel with the formation of Q1 $[d(T_2G_5T)]_4$ in solution (Figure 4). However, the distribution and intensity of charge states is highly dependent on interface conditions. Even when mild ESI interface conditions are employed (i.e., low capillary temperature, voltages, and gas flow), some dissociation of quadruplex into single-strand oligonucleotide ions is observed in samples determined to be >95% quadruplex by nondenaturing gel electrophoresis. Additionally, as the temperature of the interface is increased from 85 to 150 °C, the corresponding quadruplex Q1 peaks disappear, leaving only single-stranded oligonucleotide ions (Figure 5).

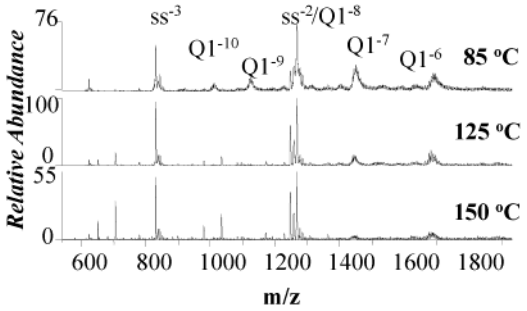

Figure 5. ESI-MS spectra of Q1 acquired at different heated capillary temperatures.

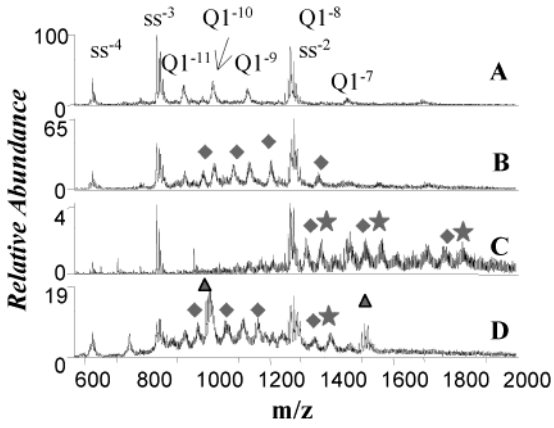

Figure 6. ESI-MS of drug/Q1 complexes. Spectrum A is for a solution containing quadruplex Q1 alone, B is for a solution containing Tel01 and Q1, C is for a solution containing DTC and Q1, and D is for a solution containing Dist. A and Q1: 1:1 drug/Q1 complexes are denoted by diamonds, 2:1 by stars, and 1:1 Dist. A/ss by triangles.

Drug/Quadruplex Oligonucleotide Complexation. The parallel stranded G4-quadruplex Q1 possesses four medium-width grooves in which drug complexation may occur, as well as a 3′- and 5′-terminal G-tetrad capable of stacking interactions with appropriate ligands. Tel01, DTC, and Dist. A all exhibit binding to Q1 at 1:1 drug/Q1 solution concentrations, although the distributions of the observed complex drug/Q1 stoichiometries are different (Figure 6). Both DTC and Dist. A form 1:1 and 2:1 complexes with Q1, even at equimolar concentrations of drug/Q1. As the concentration of drug is increased, 2:1 complexes become more abundant but the spectra are increasingly broad (data not shown). It is not clear whether the 2:1 complexes reflect the cooperative binding of two Dist. A or DTC molecules in one groove, separate binding events of a drug molecule to opposite grooves, or both; however, it is most likely that groove-binding is operative in both cases (vide infra). The presence of Dist. A/single-strand (1:1) complexes in the spectrum obtained for the solution containing Dist. A and Q1 (Figure 6D) may reflect some binding of Dist. A to single-strand oligonucleotide in solution. It may also

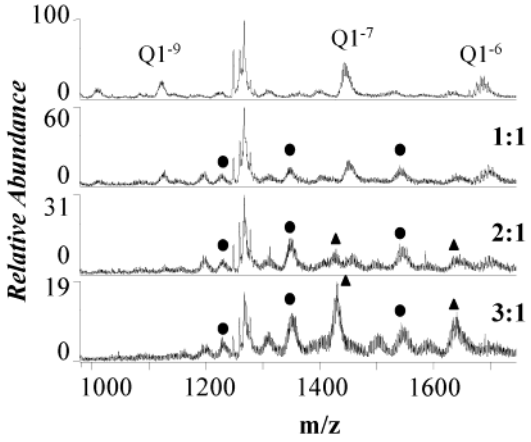

Figure 7. Concentration-dependent binding of Tel01 to Q1. Ratio of Tel01/Q1 is shown on the right side. Top spectrum is of Q1 alone. 1:1 Tel01/Q1 complexes are denoted by circles and 2:1 by triangles.

be that although some dissociation of the Dist. A/Q1 complexes occurs during the electrospray process, the electrostatic interactions between groove-bound Dist. A and oligonucleotide strand are strong enough that retention of the drug, and subsequent detection of Dist. A/single strand complex, occurs.

Tel01 binds to Q1 in a concentration-dependent manner, forming 1:1 complexes initially, followed by 2:1 complexes at higher Tel01/Q1 ratios (Figure 7). The clear concentration dependence of binding indicates that the drug/quadruplex complexes are specific and not the result of nonspecific association. Furthermore, the diminished quantity of 2:1 Tel01/Q1 complexes relative to the amounts seen for the analogous DTC and Dist. A solutions implies that both Tel01 drug molecules are binding separately to different sites, unlike the possible cooperative binding exhibited by Dist. A and DTC.

Drug Selectivity for Quadruplex versus Duplex Oligonucleotides. Duplex oligonucleotide/Dist. A complexes have been well-studied by ESI-MS.[1,2] The preference of Dist. A for binding AT base pairs in solution is mirrored in gas-phase determinations of binding affinity,[21] and fragmentation patterns of duplex oligonucleotide/Dist. A complexes indicate that minor groove-binding is maintained in the gas phase.[22] The spectra shown in Figure 8 summarize results for the selectivity of drug binding to the duplex oligonucleotide D2. As expected, ESI-MS spectra of equimolar solutions of Dist. A and D2, d(GCGAATTCGC)$_2$, exhibit 1:1 and 2:1 complexes (Figure 8D). Similar results are obtained for the binding of DTC to D2, although the intensities of the DTC/D2 complexes are less than those of Dist. A/D2 complexes at similar concentration ratios (Figure 8C,D). However, Tel01 shows no binding to duplex D2 at equimolar concentrations (Figure 8B). Addition of further equivalents of Tel01 does not induce any binding (data not shown). This behavior is consistent with a G-tetrad end-stacking mode of binding for Tel01/quadruplex interactions. An equivalent G-tetrad end-stacking association between the duplex and Tel01 is not possible. Under these conditions, in which the drug/oligonucleotide solutions are essentially neutral, only Tel01 displays complete selectivity for quadruplex versus duplex DNA.

(21) Gale, D. C.; Smith, R. D. *J. Am. Soc. Mass Spectrom.* **1995**, *6*, 1154.
(22) Wan, K. X.; Gross, M. L.; Shibue, T. *J. Am. Soc. Mass Spectrom.* **2000**, *11*, 450.

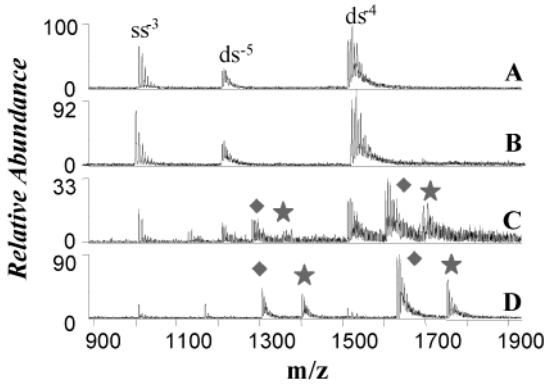

Figure 8. ESI-MS of drug/D2 complexes. Spectrum A is for a solution containing duplex D2 alone, B is for a solution containing Tel01 and D2, C is for a solution containing DTC and D2, and D is for a solution containing Dist. A and D2. All drug/D2 concentration ratios are 1:1. 1:1 drug/D2 complexes are denoted by diamonds and 2:1 by stars.

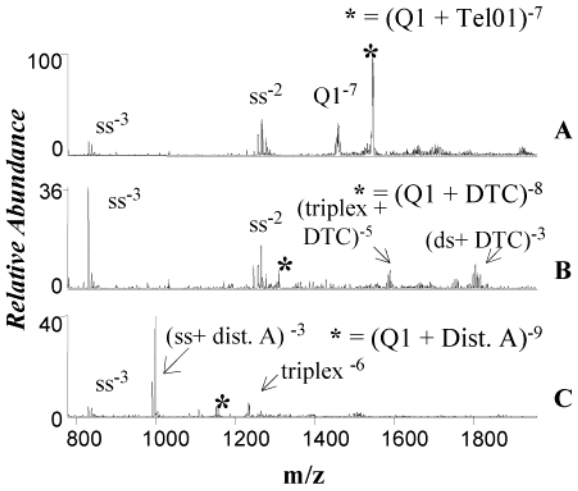

Figure 9. CAD of drug/Q1 complexes. Parent ions are denoted by an asterisk.

Distinguishing Mode of Binding in Drug/Quadruplex Complexes. Since the proposed binding modes for these different quadruplex ligands vary, we were interested in determining whether the gas-phase dissociation behavior of the corresponding complexes might allow differentiation of binding modes. CAD applied to the drug/Q1 complexes revealed unique fragmentation patterns. The dissociation of all Tel01/Q1 complexes generated fragments corresponding to quadruplex and single-strand charge states of Q1 without any bound drug. An example for the complex $[Q1 + Tel01 + 10Na^+ - 17H]^{-7}$ at 15% CAD voltage is shown in Figure 9A. As the complex dissociates, either Tel01 no longer has any strong affinity for the fragments generated or the stacking interactions between Tel01 and the guanine tetrad are not as strong as the cumulative hydrogen-bonding interactions stabilizing the quadruplex, leading to initial loss of Tel01. In either case, this facile loss of the drug is consistent with the proposed end stack binding mode of Tel01. It is also interesting that at this activation energy (15% CAD, 750 mV) the Tel01/Q1 complex parent ion is still the most intense in the spectrum. This is not the case for DTC and Dist. A complexes with Q1 at similar activation energies, indicating that Tel01 has a greater affinity for Q1.

Complexes of DTC and Dist. A with Q1 demonstrate different fragmentation patterns upon CAD. The complex [Q1 + DTC +

$7Na^+ - 15H]^{-8}$ at 15% CAD voltage produces primarily single-strand oligonucleotide fragments without drug, as well as double-strand and triplex fragments containing bound DTC (Figure 9B). DTC demonstrates some solution binding affinity for duplex and triplex oligonucleotides so its retention with these fragments is not surprising. In the case of Dist. A/Q1 complexes, single-strand oligonucleotide fragments containing bound distamycin and triplex fragments *without* drug are obtained upon 16% CAD voltage. These fragmentation patterns for complexes of DTC and Dist. A with Q1 are more consistent with stronger interactions between the drug and the oligonucleotide strands. In the gas phase, the electrostatic groove interactions between both Dist. A and DTC with an oligonucleotide strand of Q1 are stronger than the hydrogen bonds stabilizing the quadruplex, leading to retention of the drug/oligonucleotide strand interaction during dissociation. Retention of drug with an oligonucleotide fragment has previously been observed upon fragmentation of complexes between duplex oligonucleotides and minor groove-binding ligands such as Dist. A.[22] Covalent cleavage of these oligonucleotide fragments also occurs for longer sequences. No covalent cleavage of drug/Q1 complex fragments is observed here; however, the Q1 sequence is only eight base pairs long. Based upon previous ESI-MS findings for Dist. A/duplex complexes and NMR solution results for Dist. A/quadruplex interaction,[17] we conclude that the CAD patterns shown in Figure 9 for Dist. A and DTC are indicative of quadruplex groove-binding, in contrast to the end-stacking exhibited by Tel01.

(23) Mergny, J.-L.; Lacroix, L.; Teupade-Fichou, M.-P.; Hounsou, C.; Guittat, L.; Hoarau, M.; Arimondo, P. B.; Vigneron, J.-P.; Lehn, J.-M.; Riou, J.-F.; Garestier, T.; Helene, C. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 3062.

CONCLUSION

The information gained from these ESI-MS studies of drug/quadruplex complexes is applicable to both the identification and characterization of G-quadruplex interactive agents and, conversely, to different DNA structures. The advantages of ESI-MS in this context are obvious: it is versatile, requires minimal sample, and yields information that is often more difficult to obtain by other methods.[23] Selectivity, or lack thereof, for the quadruplex oligonucleotide $[d(T_2G_5T)]_4$, as well as binding stoichiometry and information on the mode of binding was obtained for the G-quadruplex interactive agents Tel01, DTC, and Dist. A. Collisional activated dissociation provides at least an initial assessment of the operative binding mode for a particular drug to G-quadruplex DNA. We are currently investigating other G-quadruplex ligands as well as the ability to distinguish between different G-quadruplex structural types. This information may lead to the identification of the most promising candidates for selective interaction with G-quadruplex DNA.

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