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DETECTION OF OLIGONUCLEOTIDE DUPLEX FORMS BY ION-SPRAY MASS SPECTROMETRY

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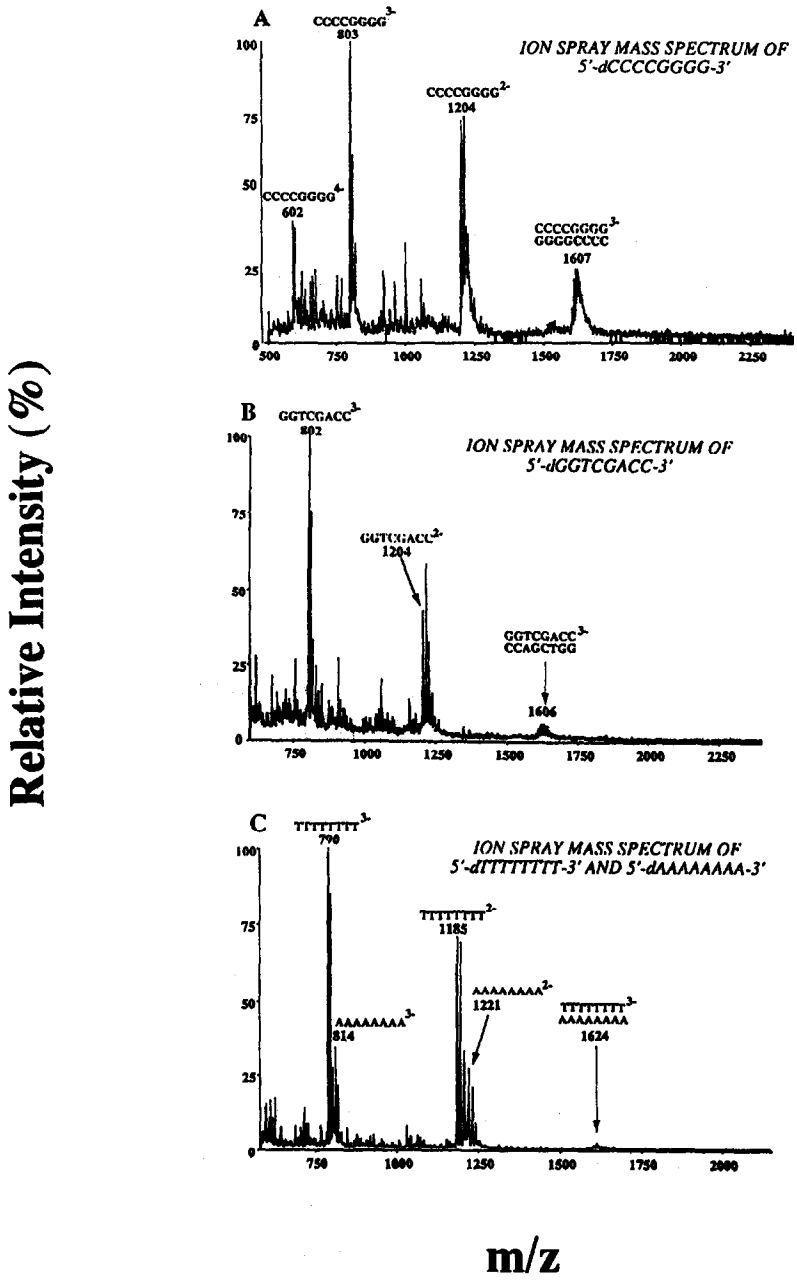
Abstract -- Three representative examples of duplex DNA, each eight base pairs in length, have been examined by ion-spray mass spectrometry. The ability to detect base-paired duplex ions in each case further indicates the use of this technique for monitoring noncovalent interactions in biological systems.

Newly developed techniques of electrospray[1] and ion-spray (pneumatically-assisted electrospray)[2] mass spectrometry (MS) permit the formation of gas phase ions directly from solution at atmospheric pressure via protonation and ion evaporation, thus providing a rapid and relatively simple method for the molecular weight (MW) determination of proteins and other macromolecules. Multiple charging of many high MW species produces a family of molecular ions whose mass-to-charge ratio lies in the 1,000-2,000 dalton (Da) mass range.

Recently we[3] and others[4] have shown that ion-spray MS permits the observation of enzyme-substrate, enzyme-product, receptor-ligand and other specific, noncovalent, protein association complexes of biological interest using conditions that closely resemble the physiological state. Noncovalent base-pairing of complementary oligonucleotides, which results in double and even triple-stranded biopolymers, is an important form of molecular recognition in the biological world. Here we report the successful detection of three representative examples of ionized duplex DNA by ion-spray MS. In one case, dissociation of the $[(dA)_8/(dT)_8]^{3-}$ duplex ion into its component octanucleotides under mild conditions and at low collision energies by tandem mass spectrometry (MS/MS) further confirms that base-pair binding in the gas-phase ion is truly noncovalent. These findings illustrate potential new applications of ion-spray MS in chemistry and biology.

FIGURE 1
ION-SPRAY MASS SPECTRA OF OCTANUCLEOTIDES



Samples[5] of the self-complementary octanucleotide 5'-dCCCCGGGG-3' (MW 2411.3 Da), known to form a miniature double-helix with three hydrogen bonds per base pair,[6] were annealed (95°C, 5 min, 10mM NH_4OAc, pH 7.5) and cooled slowly to rt. The mass spectrum in Figure 1A was obtained under conditions which maximized ion evaporation and minimized dissociation of noncovalent complexes.[7] Besides a series of multiply-charged single-stranded octamer ions, an ion current signal at *m/z* 1607 was observed, whose MW (4824 Da) corresponded with the duplex form of dC_4G_4 in the 3^- charge state (calc. MW 4822 Da) . A similar spectrum was obtained at pH 6. The origin of significant levels of single-stranded octamer in the spectrum is unknown, but may be due to dissociation of electrostatically destabilized higher charge states of the duplex. Consistent with this idea, no ion current signal was detected at *m/z* 963 for the 5^- duplex ion.

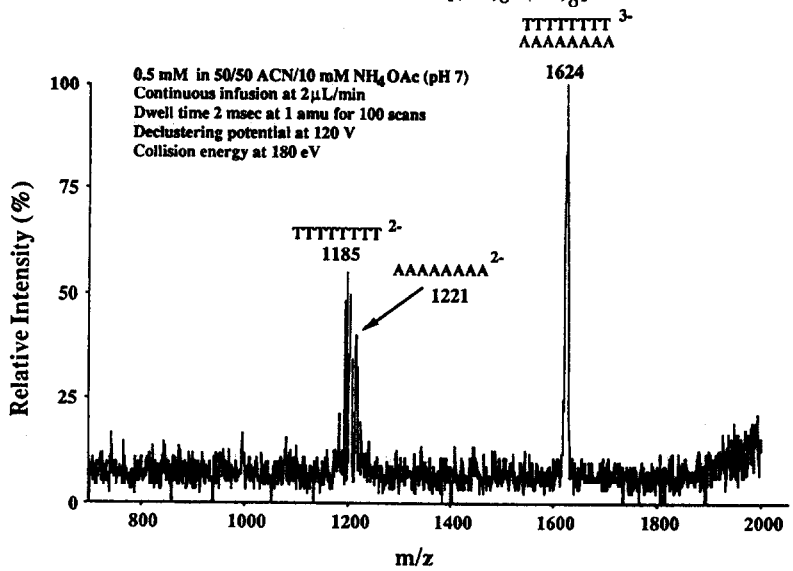
The ion-spray mass spectrum of 5'-dGGTCGACC-3' (MW 2409.3 Da), another self-complementary octanucleotide corresponding to the *Sal I* restriction endonuclease site, was obtained (1:1 acetonitrile:10 mM NH_4OAc, pH 7) as shown in Figure 1B. In addition to multiply-charged, single-stranded forms of the oligonucleotide, an ion current signal corresponding to $[duplex\text{-}dGGTCGACC]^{3-}$ was observed at *m/z* 1606, corresponding to a duplex MW of 4821±3 Da (calc. MW 4819 Da).

Unlike G-C base pairing, each A-T interaction in DNA results in only two hydrogen bonds. Therefore relatively weaker noncovalent association complexes might be expected in ion-spray mass spectra of A/T rich DNA duplexes. To test this hypothesis, equimolar samples of 5'-dTTTTTTTT-3' (MW 2371.3 Da) and 5'-dAAAAAAAA-3' (MW 2443.4 Da) were mixed and annealed (95°C, 5 min, 10 mM NH_4OAc, pH 7.0) before mass spectrometric analysis. The resulting spectrum (Figure 1C) revealed a significantly weaker signal for the 3^- duplex ion at *m/z* 1624.

To confirm that interstrand binding in the observed duplex oligonucleotide ions is noncovalent, tandem mass spectrometry (MS/MS) was performed with the ion-spray interface (Figure 2).[8] When the gas-phase ion at *m/z* 1624 corresponding to $[(dA)_8/(dT)_8]^{3-}$ was subjected to mild collisions with argon gas in the central collision cell of the tandem triple quadrupole mass spectrometer, the duplex oligonucleotide ion dissociated, as expected, into $(dA)_8^{2-}$ and $(dT)_8^{2-}$ ions observed at *m/z* 1221 and 1185, respectively. Singly-charged $(dA)_8^-$ (which is outside the observable *m/z* range of the instrument) and $(dT)_8^-$ ions were not detected. Note that little or no fragmentation of covalent bonds was evident under these experimental conditions.

Potential applications of this technique to triple-helical and circular DNA complexes, as well as protein-DNA and drug-DNA complexes, will be the subject of future reports from our laboratories.[9]

FIGURE 2
MS/MS SPECTRUM OF[$(dA)_8$/ $(dT)_8$] $^{3-}$



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7. Recorded on a Sciex TAGA 6000E, upgraded to an API III tandem triple quadrupole mass spectrometer by continuous infusion (1 mg/mL of oligonucleotide in 10mM aqueous NH_4OAc buffer) at 2μL/min through the ion-spray interface. The spectrum is an average of 100 scans from m/z 500 to 2400 (scan rate = 2 sec/scan, declustering energy 60 V).

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