Solution Composition and Thermal Denaturation for the Production of Single-Stranded PCR Amplicons: Piperidine-Induced Destabilization of the DNA Duplex?

John B. Mangrum,* Jason W. Flora,* and David C. Muddiman†,‡
Department of Chemistry, Virginia Commonwealth University, Richmond, Virginia, USA

Strategies to produce single-stranded PCR amplicons for detection by electrospray ionization Fourier transform ion cyclotron resonance mass spectrometry (ESI-FTICR-MS) were investigated using modified electrospray solutions and by thermally denaturing the duplex structures with a resistively heated electrospray ionization source. A synthetic 20-mer oligonucleotide annealed to its complementary strand was used as a model system for initial experiments. Electrospray solutions were altered by varying the relative proportion of aqueous phase in efforts to induce destabilization of the double helix. When the electrospray solution contains a 25% aqueous content, the 20-mer oligonucleotide is detected in its double-stranded form. Increasing the proportion of aqueous phase in the electrospray solution to 60% destabilized the double helix, resulting in the detection of only single-stranded species. This strategy was extended to an 82-bp polymerase chain reaction (PCR) product derived from the human tyrosine hydroxylase gene (HUMTH01). In efforts to destabilize the 82-bp PCR product, electrospray solutions reaching 70% aqueous content were necessary to promote the detection of only single-stranded amplicons. Implementation of the resistively heated transfer line and an electrospray solution in which the oligonucleotide is on the threshold of duplex stability allowed for double-stranded and single-stranded species to be generated from the same ESI solutions at both ambient and elevated transfer line temperatures, respectively, without disruption of the electrospray process. The volatile base piperidine, present at 20 mM concentrations in the electrospray solution, was found to play a critical role in the formation of single-stranded species at the higher aqueous percentages and a duplex destabilization mechanism has been proposed. (J Am Soc Mass Spectrom 2002, 13, 232–240) © 2002 American Society for Mass Spectrometry

Characterization of DNA sequence variations in the post-genome era will require approaches that surpass existing methodologies in sensitivity, accuracy, and throughput. Recently, electrospray ionization [1] Fourier transform ion cyclotron resonance mass spectrometry (ESI-FTICR-MS) [2] has emerged as an ideal approach for genotyping length polymorphisms, namely, short tandem repeats (STRs) [3, 4]. STRs are the most valuable form of sequence variation for use as genetic markers because they are highly polymorphic [5, 6], occur approximately every 10–20 kb in the human genome [7–9], and are relatively easy to isolate using the polymerase chain reaction (PCR) [7, 8, 10]. PCR amplicons generated from STR loci are typically observed as duplexes when using ESI-MS [3, 4, 11–23]. This soft ionization process enables retention of the Watson-Crick base pairing [24] of PCR amplicons.

ESI coupled with FTICR [25] has provided extraordinary accomplishments in the measurement of double-stranded PCR amplicons, with the largest detected being 500 bp in length [17]. In addition, single acquisition mass spectra have been obtained from 5 nM solutions of PCR amplicons [22]. The ultra-high mass accuracy of FTICR has been maximized with the use of a dual electrospray source [21, 26] allowing mass accuracy that routinely approaches the theoretical limit of $\pm$10 ppm for large biomolecules (>10 kDa) [27]. However, despite its powerful capabilities, the most challenging aspect of ESI-FTICR-MS is obtaining mass measurements with high enough mass accuracy to accurately genotype the length polymorphisms derived from complex STRs, which contain multiple repeat units, base insertions, and deletions [4, 28]. The difficulty of obtaining accurate mass measurements within duplex PCR amplicons arises from the fact that a base

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Address reprint requests to Dr. D. C. Muddiman, Department of Chemistry, Virginia Commonwealth University, 1001 West Main Street, P.O. Box 842006, Richmond, VA 23284, USA. E-mail: dcmuddim@vcu.edu

*Both researchers equally contriuted to these investigations.

†Affiliate appointment in Biochemistry and Molecular Biophysics.

‡Massey Cancer Center.

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substitution, such as an A → T transversion, cannot be resolved by mass because of the T → A transversion in the complementary strand resulting in a mass difference of zero. Therefore, the production of single-stranded (ss) PCR amplicons is essential for the accurate characterization of base substitutions housed within STRs [3, 16].

Strategies for the routine production of single-stranded PCR products for mass spectrometry have included nozzle skimmer dissociation [29], biotin-streptavidin chemistry [30–36], and the DNA repair enzyme, lambda exonuclease [3, 16]. It has been reported that the use of nozzle skimmer dissociation on duplex 16-mer oligonucleotides with a G–C content reaching 75% showed complete dissociation with no signs of covalent fragmentation [29]. Subsequently, in our laboratory, nozzle skimmer dissociation of a 20-mer with a G–C content of 70% promoted the formation of single-stranded amplicons; however, predominately duplex DNA remained in the spectrum and significant fragmentation was observed (data not shown). The production of single-stranded amplicons using biotin–streptavidin chemistry involves a biotinylated strand being bound to a magnetic bead and then denatured to remove the nonbiotinylated strand via strong base or thermal denaturation [30]. This method of producing single strands, although successful, has been shown to exhibit significant sample loss due to difficulties in removing the unbiotinylated strands [37]. Single-stranded PCR products have been successfully generated enzymatically in our lab via the use of lambda exonuclease which selectively digests the 5' phosphorylated strand of a duplex, leaving the complementary strand available for mass analysis [3, 16]. While this method has great applicability, it requires an additional enzymatic digestion step and incomplete phosphorylation of the primers renders the amplicon resistant to digestion by lambda exonuclease [3, 16]. However, this method is advantageous because it allows researchers the ability to select which strand is generated following the digestion process as well as reduce the complexity of the mixture (e.g., multiplex PCR).

Efforts are being made to generate single-stranded amplicons **on the fly** prior to entering the mass spectrometer. Williams and co-workers have shown that thermal denaturation of oligonucleotides in the transfer line can be achieved via the use of a resistively heated coiled wire [38]. Generating single-stranded amplicons in the transfer line prior to the entrance into the mass spectrometer is advantageous because it eliminates the probability of sample contamination by reducing sample handling, as well as reducing the time frame for which samples can be prepared and analyzed. Our lab has recently developed a method using flow injection of PCR amplicons that will allow the analysis of >2000 genotypes per day [3, 23] greatly enhancing the genotyping capabilities of ESI-FTICR-MS.

Herein, we explore solution compositions and thermal denaturation during the ESI process in efforts to control the structural stability of DNA double helices. Experimental conditions have been developed which can promote either single-stranded or double-stranded moieties prior to entrance into the FTICR-MS.

Experimental

Materials and Sample Preparation

Complementary synthetic 20-mer oligonucleotides, 5'-CAG CGT GCG CCA TCC TTC CC-3' and 5'-GGG AAG GAT GGC GCA CGC TG-3' were purchased from Midland Certified Reagent Company (Midland, TX). All oligonucleotides were desalted prior to ESI using the microdialysis apparatus previously described [18, 39] except with a molecular weight cut off (MWCO) of 18,000 Da for the dialysis membrane. Concentrations for the stock solutions of oligonucleotides were quantified using UV-Vis spectrophotometry at 260 nm prior to dilution. The complementary 20-mers were annealed in a 1:1 molar ratio at 90 °C for 2 min in their respective ESI solutions (vide infra) and slowly cooled to room temperature, with a final concentration of 4 μM duplex. The 20-mer oligonucleotide duplex (average MW = 12,237.9 Da) has an estimated melting temperature (T_m) of 71.6 °C which was calculated using eq 1 [40]:

$$T_m = 22 + 1.46I_n \text{ where } I_n = 2 \times (\#\text{ GC base pairs}) + (\#\text{ AT base pairs}) \quad (1)$$

The T_m is defined as the temperature at which one half of the duplex structure is in the denatured state. All other reagents were purchased from Sigma-Aldrich (St. Louis, MO); they were of the highest possible purity and used without purification.

PCR Amplification

The tetrameric repeating motif within HUMTH01 was amplified from K562 human genomic DNA (Promega, Madison, WI). This individual is homozygous for the 9.3 allele, thus yielding an 82 bp PCR amplicon with a theoretical average molecular weight of 50,535.47 Da for the double-stranded species. Each 50 μL reaction consisted of 1× Pfu buffer, 1.5 units of Pfu (Promega, Madison, WI), 200 μM of each dNTP (Promega), 0.5 μM of each primer (Midland Certified Reagent Co., Midland, TX) and 100 ng of template. Forward and reverse primer sequences along with themal cycler parameters are described in previously published work [3]. PCR amplicons were diluted with their respective electrospray solutions to give final concentrations of 2 μM.

Mass Spectrometry

ESI [1] offers a soft ionization method for transferring intact molecules from the solution phase to the gas phase without imparting fragmentation. Electrospray

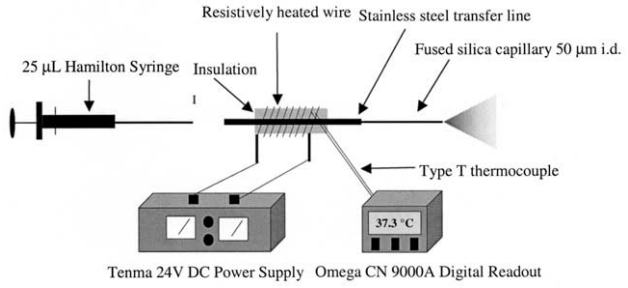


Figure 1. Schematic of ESI source with resistively heated transfer line.

ionization also has the ability to bestow multiple charge-states on large molecules resulting in *m/z* ratios that fall within the *m/z* range of most mass analyzers [1, 41]. The composition of ESI solutions can play an important role in charge-state distribution as well as ion intensities. ESI solutions will be delineated in the text as they were changed throughout the course of these experiments; however, imidazole was found to play an inconsequential role in duplex stability and therefore was not incorporated in later ESI solutions to simplify mechanistic arguments [42, 43]. All spectra were obtained using a modified IonSpec Corporation (Irvine, CA) FTICR mass spectrometer with a 4.7 tesla superconducting magnet (Cyromagnetics, Inc. Oak Ridge, TN). The ESI source (Analytica of Branford, Inc, Branford, CT) was modified with a heated metal capillary [44], applying a constant temperature (allowing routine detection of noncovalent complexes without exception) for all experiments, and a micro-electrospray emitter. Emitter tips were hand pulled from a fused-silica capillary of 50 μm to 10 μm i.d. [45]. All samples were infused using a Harvard Syringe pump PHD 2000 (South Natick, MA) at an infusion rate of 3.3 nL/s.

All mass spectra were single acquisitions using 512 k of data for 20-mer oligonucleotides and 32 k of data for the 82-bp HUMTH01 PCR amplicon acquired at a 500 kHz ADC rate. Acquisitions involved two 1-s hexapole accumulations followed by gated trapping [46]. The unapodized data were zero filled two times and Fourier transformed prior to spectral analysis.

Figure 1 shows a schematic of the heated transfer line used for the thermal denaturation experiments. A DC voltage was applied using a Tenma 24V DC power supply (Springboro, OH) resistively heating the monochrome wire coiled around high temperature insulation. Real time temperature measurements were acquired using a type T thermocouple attached between the transfer line and the insulation. Temperatures were displayed on an Omega CN9000A readout (Stamford, CT).

Results and Discussion

Figure 2a shows an ESI-FTICR mass spectrum of the double-stranded 20-mer (T_m = 71.6 °C) using our traditional oligonucleotide ESI solution, 60% acetonitrile, 20% isopropyl alcohol, 20% aqueous phase with final

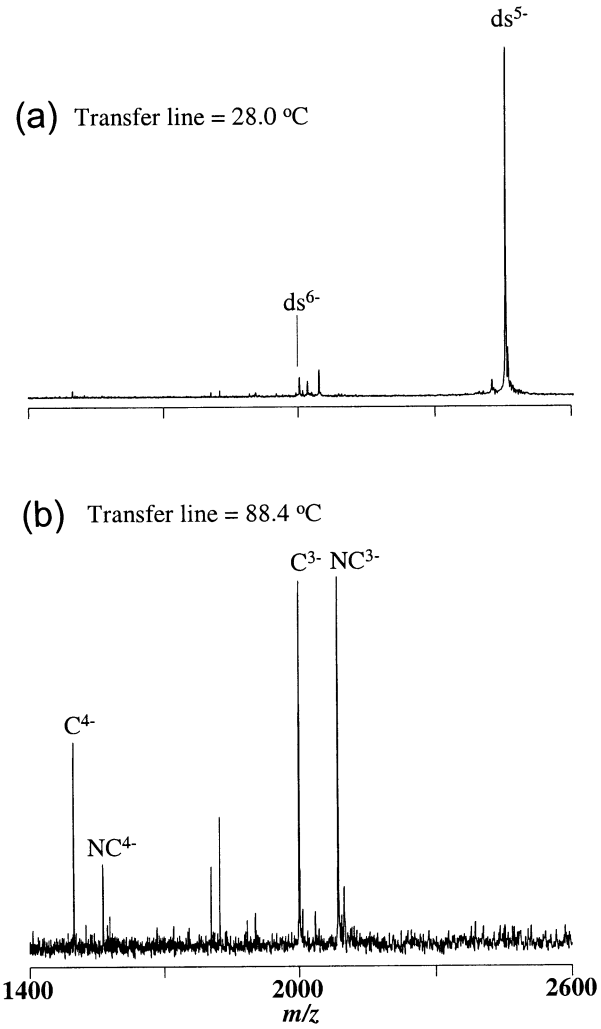


Figure 2. ESI-FTICR mass spectra of duplex 20-mer oligonucleotide using an ESI solution consisting of 60% acetonitrile, 20% isopropyl alcohol, 2 mM ammonium acetate with final concentration of 20 mM piperidine and imidazole. (**a**) Mass spectrum of the 20-mer duplex observed at ambient temperature (28 °C). (**b**) Mass Spectrum of the 20-mer duplex, illustrating that ssDNA is observed when temperature of the heated metal transfer line (88.4 °C) is raised significantly above its T_m. All charge-states, coding (C) and noncoding (NC) strands are labeled as well as double-stranded DNA (ds).

concentrations of 2 mM ammonium acetate and 20 mM piperidine and imidazole [3, 4, 16–23]. Figure 2b shows the result of using the resistively heated transfer line providing complete thermal denaturation of the duplex DNA (20-mer) as it was introduced into the orifice of the mass spectrometer. However, the elevated temperatures required to destabilize the hydrogen bonding between the nucleobases of the 20-mer resulted in a highly unstable electrospray, causing a significant reduction of absolute ion intensity and lack of reproducibility.

In efforts to circumvent the instability and low signal-to-noise ratio during the thermal denaturation, the aqueous character of the electrospray solutions was

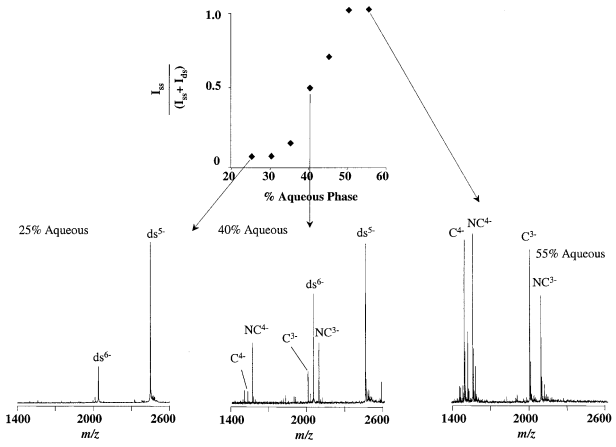


Figure 3. Plot of relative ion intensity of single-stranded 20-mer versus percent aqueous content. Representative spectra are shown using ESI solution with 25, 40, and 55% aqueous content. (I_{ss} = intensity of single-stranded species), (I_{ds} = intensity of double-stranded species).

increased. However, we observed that as the aqueous content of the ESI solutions was increased to higher proportions, the dsDNA became denatured and single-stranded DNA (ssDNA) was detected. Figure 3 shows ESI-FTICR mass spectra of the 20-mer DNA duplex at ambient temperature with various aqueous proportions including a plot of relative ion intensity of the single-stranded oligonucleotides (intensity of single stranded species, I_{ss}, divided by total intensity, $I_{ss} + I_{ds}$) versus the percent aqueous content. ESI solutions consisting of a 25% aqueous content resulted in retention of the duplex structure. The 20-mer oligonucleotide in an electrospray solution comprised of a 40% aqueous phase exists in both duplex and single-stranded forms. As the aqueous phase was increased to 50–60%, the 20-mer DNA duplex became destabilized and only single-stranded oligonucleotides are observed.

The signal-to-noise ratio of the single-stranded mass spectral data presented in Figures 2b and 3 appears to have been compromised by the higher aqueous content. This is attributed to the following: (1) The total ion intensity is distributed among four species (C^{4-}, NC^{4-}, C^{3-}, and NC^{3-}) at high aqueous content (55%) rather than two species (ds^{6-} and ds^{5-}) at low aqueous content (25%); (2) the surface tension is higher when one increases the aqueous content of the solution; however, we kept the heated metal capillary temperature constant which likely resulted in lower ion production; and (3) the FTICR signal scales linear with charge; thus, the lower charged (i.e., single-stranded species) induce less image current than the duplex species. The *average* charge-state for the duplex species also increases with increasing aqueous content (Figure 3). This is attributed to: (1) The higher aqueous content droplets will accommodate a higher charge density, therefore imparting more charge onto the oligonucleotide structure; and (2) the apparent pH of the solution increases slightly with increasing aqueous content. An increase in pH decreases the relative proportion of protonated piperidine which is known to reduce charge-states when in its protonated form [43].

The single-stranded products detected in Figure 3 show no evidence of fragmentation and represent individual intact strands. This is consistent with the work done by Gabelica et al. in which they used a higher aqueous solution for the nozzle skimmer dissociation of 16-mer duplexes [29]; compared to the data presented in Figure 3, their duplexes were destabilized to a greater extent because of the higher aqueous content allowing for the conversion to the single-stranded state using collisional heating in the nozzle-skimmer region. Our previous work with nozzle skimmer dissociation (vide supra) utilized a solution composition that was conducive to duplex stability (i.e., 20% aqueous content; c.f.g. Figures 2b and 3) and therefore resulted in higher amounts of covalent fragmentation.

Based on the initial findings with the 20-mer model system, an 82-bp PCR amplicon was electrosprayed using ESI solutions that were comprised of 50, 60, and 70% aqueous phase. Higher aqueous contents were chosen based on the number of base pairs and estimated T_m for the PCR amplicon for a specific ionic strength. The theoretical T_m for the amplicon was generated using eq 2 [40]:

$$T_m = 81.5 + [16.6(\log[X^+])] + [0.41(\%GC)] - \left[\frac{500}{\#\text{ of base pairs}}\right] \quad (2)$$

Eq 2 is only valid for monovalent cation concentrations, represented by $[X^+]$, in the range of 0.01–0.4 M and the DNA duplex of interest must contain a GC percentage between 30–75% [40]. The sodium ion concentration was assumed to be zero since all samples were thoroughly dialyzed to remove the adduction of salt (i.e., the sodium ion concentration would be a minor contributor to the overall ionic strength of the solution). However, the ESI solutions also contain ammonium ions and protonated piperidine (vide infra). The T_m was calculated using protonated piperidine concentrations as determined by α-values for a given pH (vide infra). Figure 4a shows an ESI-FTICR mass spectrum of a stable DNA duplex obtained from a 50% aqueous ESI solution (pH = 11.1; T_m = 58.5 °C). Figure 4b shows a mass spectrum of the 82-bp PCR amplicon obtained from an ESI solution containing 60% aqueous phase, resulting in only a marginal amount of single-stranded species (pH = 11.2; T_m = 57.6 °C). Figure 4c shows a mass spectrum of a completely denatured 82-bp PCR product obtained from an ESI solution with 70% aqueous phase (pH = 11.3; T_m = 56.9 °C). Thus, as we increased the aqueous character of the ESI solution, the pH also increased which decreased the amount of protonated piperidine and therefore decreased the ionic strength of the solution. However, over this narrow

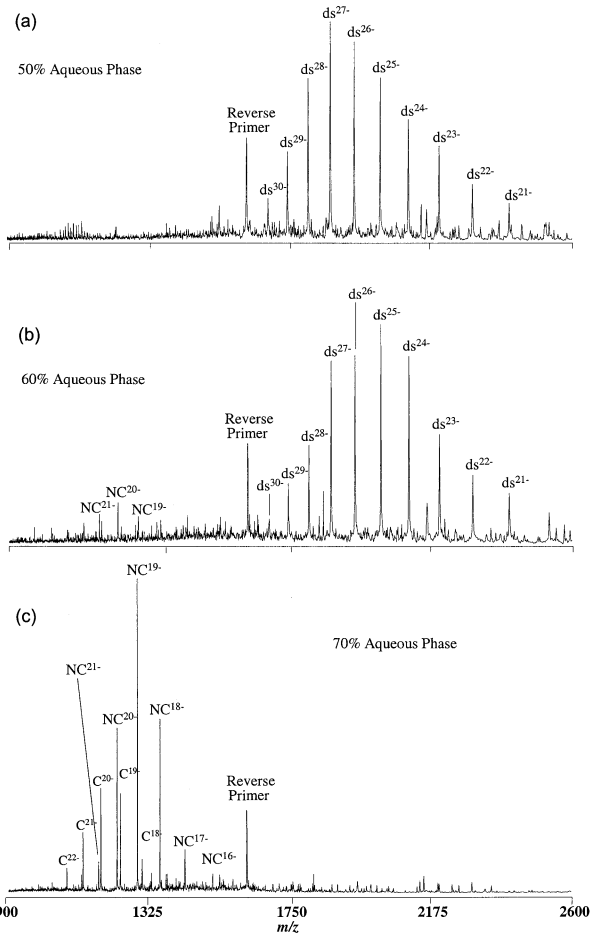


Figure 4. ESI-FTICR mass spectra of an 82-bp PCR amplicon at ambient temperature using ESI solutions of varying aqueous content. Solutions consisted of 20 mM piperidine, 20 mM imidazole, and 2 mM ammonium acetate with (**a**) 50% aqueous (pH = 11.1), (**b**) 60% aqueous (pH = 11.2), and (**c**) 70% aqueous (pH = 11.3).

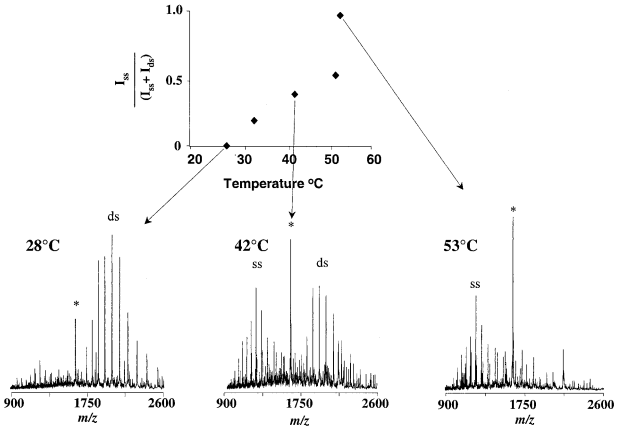


Figure 5. Plot of relative ion intensity of an 82-bp PCR product versus temperature of resistively heated transfer line. ESI-FTICR mass spectra obtained from a 60% aqueous ESI solution illustrating the production of single-stranded species with variable transfer line temperatures. (* = reverse primer).

range of ionic strengths, the melting temperature only changed by 1.6 °C suggesting that additional factors play a role in the DNA duplex stability such as the degree of hydration and the hydrogen bonding capability of neutral piperidine.

In efforts to thermally control the state of the species (ssDNA or dsDNA) observed in the mass spectrum, resistive heating of the transfer line using the 60% aqueous ESI solution (pH = 11.2; T_m = 57.6 °C) was chosen since it appears to be on the threshold of the 82-bp duplex stability (see Figure 4b). Figure 5shows the resulting mass spectra of the 82-bp PCR product in an ESI solution of 60% aqueous content while increasing the solution temperature via the resistively heated transfer line. This method allows one to control the state of the amplicon during the same ESI experiment. Figure 5 also shows a plot of the relative ion intensity of the single stranded state versus the temperature of the heated transfer line. At a transfer line temperature of 28 °C, the amplicon that is detected is primarily dsDNA. As the transfer line temperature is raised to 42 °C, the PCR amplicon is observed in both the double-stranded and single-stranded states. Increasing the temperature of the transfer line to 53 °C resulted in the production of single-stranded moieties with only minimal amounts of duplex remaining. The PCR amplicon destabilized at a temperature well below the estimated T_m (57.6 °C), which is consistent with our observation that with a higher aqueous phase in the electrospray solution, the T_m decreases (see Figures 3 and 4).

It was observed that with the increase in aqueous content of the ESI solutions, the apparent pH of each solution also increased. This indicated that the pH of the electrospray solution might play a crucial role in DNA duplex stability. Piperidine, a strong base (pKa = 11.1), raises the pH of the solutions as well as reduces the alkali metal adduction [43]. Based on an aqueous phase α-plot for piperidine, at pH values below its pKa (11.1), the protonated form of piperidine is predominant in the ESI solution. At pH values above the pKa, piperidine resides primarily in its neutral state. In accord with the α-plot, α_0 = 0.61 for the protonated form of piperidine and α_1 = 0.39 for neutral piperidine, for the 20% aqueous ESI solution (apparent pH = 10.9). The corresponding α values for the 55% aqueous ESI solution (apparent pH = 11.3) are α_0 = 0.43 and α_1 = 0.57. For the 20-mer oligonucleotide, these two aqueous percentages and their corresponding α values illustrate that the percentage of protonated **and** neutral piperidine in the solution seemed to play a decisive role as to whether the 20-mer resides in the double-stranded or single-stranded form.

Figure 6 shows the ESI-FTICR mass spectra of a 20-mer electrosprayed from various ESI solutions, none of which contained imidazole. Figure 6a shows an ESI-FTICR mass spectrum of the 20-mer electrosprayed from a 20% aqueous solution in which the double-stranded form is observed as expected for this pH. Figure 6b shows a mass spectrum where the ESI solu-

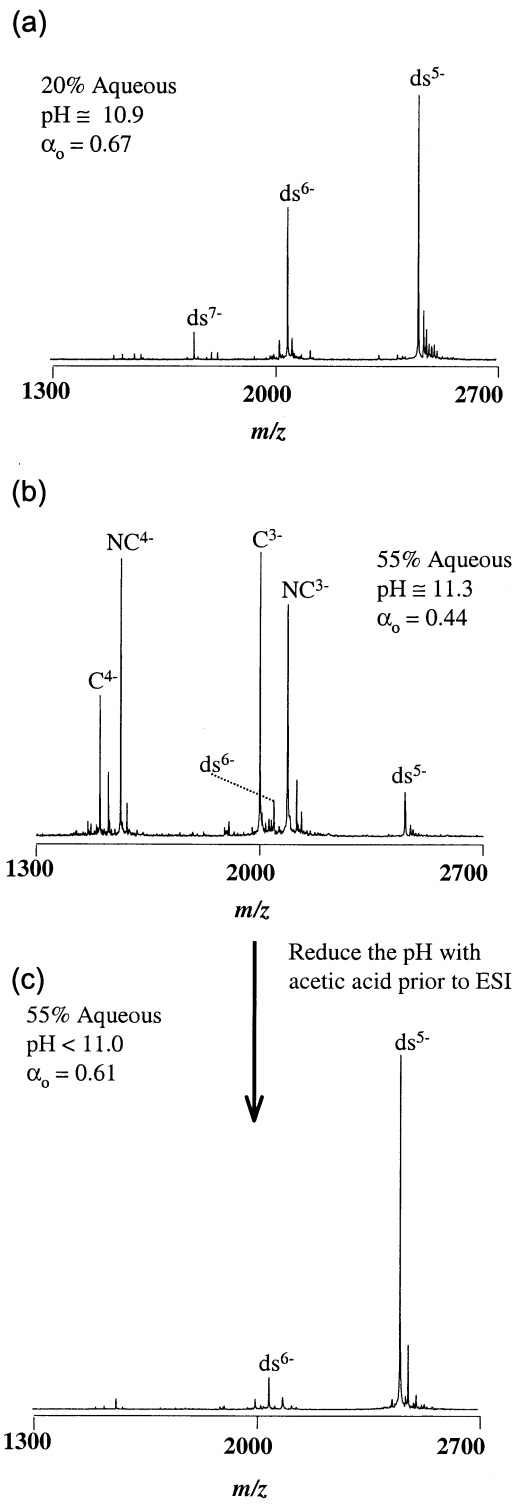
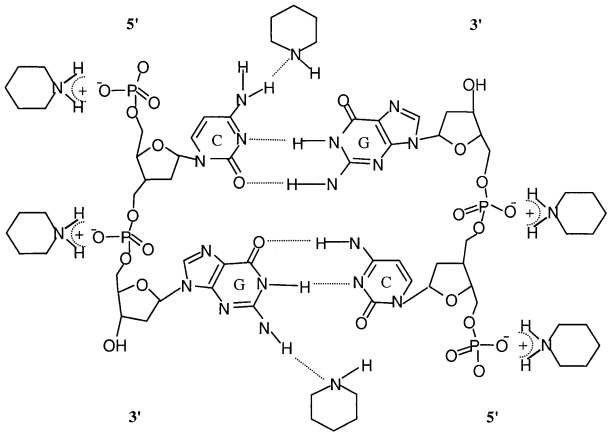


Figure 6. ESI-FTICR mass spectra of the 20-mer oligonucleotide duplex at ambient temperature showing that the stability of the DNA duplex is affected by fraction of protonated piperidine in the ESI solution. (**a**) With a 20% aqueous solution, apparent pH of 10.9, the duplex structure is retained. (**b**) At 55% aqueous content, apparent pH of 11.3, destabilization of the helical conformation is promoted. (**c**) In the same 55% aqueous solution with the apparent pH adjusted to slightly less than 11.0 by the addition of acetic acid, the double-stranded conformation is retained (the pH is below the pKa of piperidine).



Scheme 1

tion is 55% aqueous inducing the production of single-stranded moieties. Figure 6c shows the mass spectrum using a 55% aqueous ESI solution where apparent pH has been slightly lowered below 11.0 with the addition of a small amount of acetic acid. While all single-stranded species are present in the ESI solution at a pH greater than the pKa of piperidine, the duplex state of the 20-mer is retained when the apparent pH of the same ESI solution is reduced below the pKa of piperidine. The *average* charge-state for the duplexes shown in Figure 6a with 6c are 5.6 and 5.1, respectively. This decrease in the *average* charge-state is due to the approximately 6% increase in protonated piperidine which reduces charge-states [43].

Scheme **1** illustrates the proposed mechanism for piperidine interaction based upon the experimental data and the α-plot for piperidine. At pH values below the pKa of piperidine, the excess of protonated piperidine acts as a cation and electrostatically binds to the phosphate backbone neutralizing the repulsive negative charges and thus creating an effect similar to peptide nucleic acid (PNA) duplex stability where Coulombic repulsions have been eliminated [47]. This effect is analogous to the Na^+ cation binding to the negatively charged phosphate backbone stabilizing the duplex by reducing the electrostatic repulsion [48, 49]. At pH values above the suggested pKa of piperidine, the excess of neutral piperidine can hydrogen bond with the nucleobases destabilizing the double helix analogous to the denaturant formamide [50].

Further evidence in support of this mechanism was accomplished by studying the melting temperatures of the 20-mer duplex (T_m = 71.6 °C) in two ESI solutions with slightly different apparent pH values. Figure 7 is a plot of relative ion intensity of the single-stranded 20-mer versus the temperature of the resistively heated transfer line for two 30% aqueous ESI solutions at pH 10.9 and 10.7; the latter pH was decreased by the addition of acetic acid. When electrosprayed from an ESI solution of pH 10.9 at room temperature (28 °C), the mass spectrum shows duplex DNA. Heating the trans-

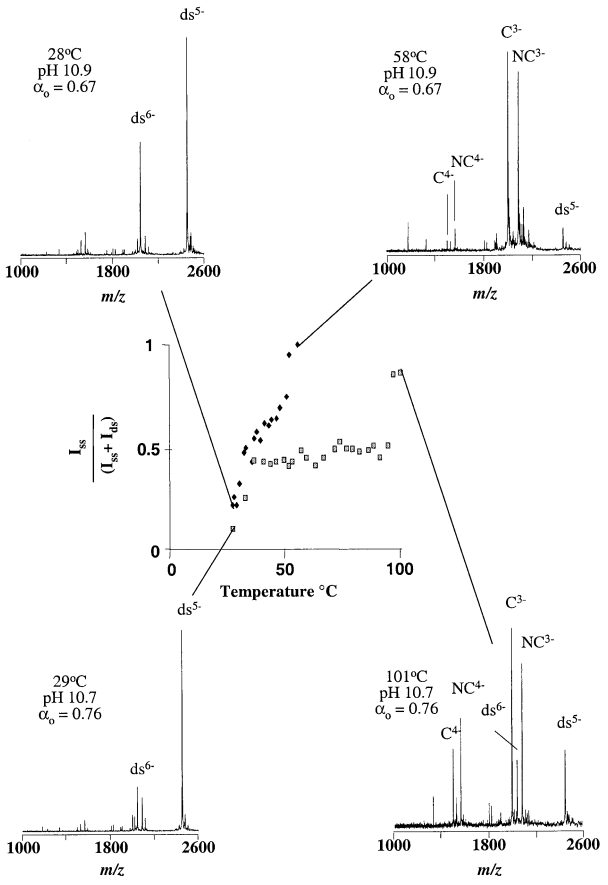


Figure 7. ESI-FTICR mass spectra of the 20-mer oligonucleotide duplex in 30% aqueous ESI solution at pH 10.9 (filled diamond) and pH 10.7 (filled square). A plot of relative ion intensity of the single-stranded species versus temperature of the transfer line is shown.

fer line to 58 °C results in almost **complete denaturation** of the 20-mer, as shown in the mass spectrum, with only minimal duplex structure remaining. However, the effect of lowering the pH with acetic acid (i.e., producing more protonated piperidine, pH = 10.7), clearly increases the stability of the duplex structure as well as increasing the T_m of the 20-mer oligonucleotide. Temperatures reaching 100 °C were necessary to achieve dissociation of the duplex structure. Analogous to PNA–PNA duplex stability [47], the oligonucleotide electrosprayed from a solution of lower pH (i.e., more protonated piperidine) stabilizes the anionic phosphate backbone of the double helix and melts at a temperature that is roughly twice that of the duplex electrosprayed from a higher pH solution. The decrease in the pH from 10.9 to 10.7 is also reflected in the charge-states of the duplex structures at ambient temperature; when the concentration of protonated piperidine increases by 9%, the *average* charge-state decreases.

In addition to the role piperidine plays on duplex stability, the degree of duplex hydration also contributes to the destabilization effects on the duplex structure. Swelling of the DNA duplex occurs as the double helix becomes completely hydrated with approximately 15–20 water molecules per nucleobase [51]. This swelling would allow the neutral piperidine easier accessibility to the nucleobases and promote the disruption of the hydrogen bonding as described by the proposed mechanism.

Due to the relatively complex ESI solution compositions and the numerous factors that play a role in DNA duplex stability (e.g., ionic strength which is pH dependent, aqueous content, temperature), more investigations are warranted to fully test the proposed mechanism. Currently, we are carrying out a titration experiment of piperidine concentration relative to aqueous content and accurately determining ionic strength (using doubly-dialyzed solutions to reduce alkali metal concentrations to a level in which they will no longer play a role) by carefully measuring the conductivity of each ESI solution. These experiments should enable us to develop stronger mechanistic arguments in the near future.

Conclusions

We have demonstrated the ability to routinely generate single-stranded amplicons prior to entrance into the mass spectrometer. Stability of the DNA duplex has been shown in this research to be affected by many parameters including temperature, ionic strength, and aqueous content of the ESI solution, all of which play a combined role in stabilizing or destabilizing the helical structure of DNA. Single-stranded moieties can be routinely generated from a double-stranded PCR product. The resistively heated metal transfer line, used to thermally dissociate the duplex structures, allowed control of which physical state of the DNA was observed. The state of piperidine as controlled by pH in the aforementioned ESI solution was found to affect duplex stability by disrupting the hydrogen bonds between the nucleobases or by stabilizing the phosphate backbone as described by the proposed mechanism. Higher aqueous contents, resulting in DNA swelling, of the ESI solutions enable neutral piperidine to interact and disrupt the hydrogen bonds found within the groove of the helical DNA structure. With the ability to continually and rapidly generate single-stranded amplicons, the potential role of automated high throughput genotying of complex STRs by ESI-FTICR-MS becomes feasible.

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