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International Journal of Mass Spectrometry 253 (2006) 238–248
International Journal of Mass Spectrometry
www.elsevier.com/locate/ijms

Stability of complementary and mismatched DNA duplexes: Comparison and contrast in gas versus solution phases

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Received 8 December 2005; received in revised form 28 March 2006; accepted 30 March 2006
Available online 11 May 2006

Abstract

We report herein a mass spectrometric study of a complete set of 9-mer DNA duplexes (5′-GGTTXTTGG-3′/3′-CCAAYAACC-5′, X/Y = G, C, A or T) with and without single internal mismatches. This work represents a first step toward establishing whether mass spectrometry can be used as a tool for examining both solution and gas phase stabilities of DNA duplexes, leading to an understanding of the intrinsic behavior of DNA.

First, we have found that the relative ion abundances of the mismatched and matched electrosprayed duplexes correlate to solution phase behavior. That is, duplexes that are more stable in solution have higher relative ion abundances in the gas phase. This is consistent with previous MS results on complementary duplexes, and is advantageous in that relative solution stabilities can be thus obtained much more quickly than by using traditional melting temperature methods. Second, the gas phase stabilities of all the XY duplexes have been characterized, using collision-induced dissociation (CID) as a method to assess stability. Of the 16 XY duplexes we studied, four duplexes (GG, AC, TC and CC) exhibit enhanced gas phase stabilities, two (TA and AT) are unstable in the gas phase relative to in solution, and the remaining 10 (GC, CG, GT, AG, TG, TT, GA, CT, AA and CA) show a linear correlation between the gas and solution phase stabilities. This direct comparison between the gas phase and solution phase stabilities allows us insight into how solvation and the ESI process may affect DNA stability. The effects of base stacking and hydrogen bonding in the gas phase versus in solution are discussed. Our data indicate that in the gas phase, as in solution, duplex stability reflects both hydrogen bonding and base stacking interactions. However, unlike in solution, hydrogen bonding forces dominate in the gas phase.

Keywords: DNA; Duplex; Energetics; Dissociation; Mismatches

1. Introduction

DNA is the carrier of genetic information in living organisms. The maintenance of higher order DNA structures and genomic integrity is essential in living cells. A high degree of precision in the replication of DNA is ensured by the well-known complementary Watson–Crick scheme, with G·C and A·T "matched" base pairing. Mistakes can occur during DNA replication, where non-Watson–Crick base pairs are formed between the normal bases (A, G, C and T) or when these bases are modified by chemical and physical agents [1,2].

Forces stabilizing DNA structures in the condensed phase are comprised of a variety of contributions: hydrophobic interactions driven by the solvent, hydrogen bonding and base stacking between nucleobases, cation binding with the sugar-phosphate backbone, and many non-classical interactions [3–6]. Furthermore, these factors are sensitive to the local medium and environment. The complexity of the condensed phase behavior emphasizes the significance of understanding intrinsic interactions without the influence of solvent or crystal packing. The study of DNA properties in the gas phase allows for discovery of inherent energetics and behavior and can serve as a starting point for extrapolation to other media.

Since the 1980s there has been a growing effort to study DNA [7–11] and its complexes [12–18] in the gas phase using mass spectrometry. Gas phase oligonucleotide ions can be produced by either electrospray ionization (ESI) [7,12,19,20] or matrix-assisted laser desorption ionization (MALDI) [8,21,22]. The gentle nature of electrospray has made possible the gas phase study of DNA complexes including oligonucleotide duplexes/triplexes/quadruplexes [13,14,18,19,23,24] and DNA–drug complexes [15,25]. Among non-covalent complexes, DNA duplexes are a particularly attractive model system for electrospray mass spectrometry (ESI-MS) examination, due

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doi:10.1016/j.ijms.2006.03.014

to their well-studied and established condensed phase behavior [17,26,27]. Since the observation of DNA duplexes using MS in the early 1990s [12,28], many mass spectrometric studies of DNA duplexes have been reported [11,19,20,24,29,30].

The gas phase stability of DNA duplexes was first quantitatively measured by Williams and co-workers using the blackbody infrared radiative dissociation (BIRD) technique. They reported the gas phase activation energies for the dissociation of 4-mer and 7-mer double-stranded DNA duplexes [19]. This early and seminal work compared fully complementary and fully non-complementary DNA duplexes to establish that Watson–Crick base pairing appears preserved for the complementary duplexes in the gas phase. More recently, Gabelica and De Pauw, and Gross have focused on the use of in-source and ion trap collision-induced dissociation (CID) methods, rather than BIRD, to assess relative oligodeoxynucleotide stability [16,20,23,24,30]. These studies have focused on the stability of complementary duplexes and have demonstrated that the relative DNA stability in the gas phase appears to be consistent with that in solution, with the conservation of Watson–Crick hydrogen bonding and base stacking interactions.

Although many solution phase and solid state studies have been conducted on non-complementary, or mismatched DNA duplexes (that is, double strands where one or more base pairs are not "matched" Watson–Crick G·C and A·T base pairs) [2,31–42], few gas phase studies have been carried out [19]. One recent study examined *RNA* duplexes, focusing on only GA mismatches. The main conclusion from this work is that hydrogen bonding interactions are more important than base stacking in their gas phase RNA duplexes. These results, however, are for a limited data set (only one mismatch), and are for RNA, which can have markedly different behavior than DNA [43].

We describe herein a systematic study of complementary and mismatched DNA duplexes. Gas phase stabilities are evaluated and compared to the corresponding solution stabilities. Specifically, a set of non-self-complementary nonadeoxyribonucleotide octaphosphates, 5′-d(GGTTXTTGG)-3′ and 5′-d(CCAAYAACC)-3′, where the central base is G, C, A and T, was examined. We refer to these duplexes as "XY" duplexes, where X represents the central base in the sequence 5′-d(GGTTXTTGG)-3′ and Y represents the central base in 5′-d(CCAAYAACC)-3′. These sequences were chosen for several reasons. First, the duplexes are long enough to form the familiar double helix, but still short enough such that changing one central base will have a measurable effect on stability [44,45]. Second, these particular duplexes have been extremely well-characterized in solution, which gives us a good comparison point [45]. Third, these duplexes, because of the terminal G and C bases that help maintain helical structure during dissociation, are particularly well-suited to the traditional two-state dissociation model that allows for accurate theoretical prediction of melting temperatures [26]. Fourth, nucleobase mutation can lead to cell death, genetic disease and carcinogenesis. Even a single mismatch can have deleterious effects, and nature relies on proofreading and repair enzymes to recognize and excise incorrect base pairs [34]. This comprehensive and systematic study of the stabilities of DNA duplexes with and without a single internal mismatch will lend insight into the intrinsic differences among matched and mismatched duplexes. Last, we are ultimately driven by a biological problem, which is the toxicity of the mutated guanine base, O^6-methylguanine (OmG). It has been shown in solution that these duplexes are good models for examining the effect of OmG, and these studies herein represent a starting point for future biological studies [45].

2. Experimental methods

2.1. Sample preparation

A set of non-self-complementary nonadeoxyribonucleoside octaphosphates, 5′-d(GGTTXTTGG)-3′ and 5′-d(CCAAYAACC)-3′, where X and Y are G, C, A and T was purchased from Sigma Genosys (The Woodlands, TX) and used without further purification. To anneal the duplexes, stock solutions containing 62.5 μM of each strand in 40 mM NH_4OAc aqueous solution at pH 7.0 were heated to 90 °C for 10 min then cooled down slowly to 0 °C. The final ESI solution consisted of 12.5 μM DNA duplex in 40 mM NH_4OAc mixed with 20% methanol. To simplify the nomenclature of the duplexes in this paper, we use only the variable central base of each strand to represent the whole duplex. For example, a duplex called "GC" refers to the duplex 5′-d(GGTT**G**TTGG)-3′/3′-d(CCAA**C**AACC)-5′, where X = G, Y = C. The various combinations of X and Y can form four complementary (GC, CG, TA and AT), and 12 mismatched duplexes.

2.2. ESI-quadrupole ion trap mass spectrometer

Negative ion ESI-MS spectra were obtained with the Finnigan LCQ mass spectrometer (San Jose, CA). The 0 °C solution was infused at 25 μL/min directly into the mass spectrometer. The spray voltage was −4.0 kV while the capillary temperature was 175 °C. Collision-induced dissociation was performed in the mass analyzer by varying the relative collision energy with a default activation time of 30 ms and a q value of 0.25. The applied collision energy is a normalized collision energy (in %) that corrects for the m/z dependence of the activation voltage required for ions of different m/z ratios [46]. The relative mass difference between the base pair with the highest m/z ratio (GG) and with the lowest m/z ratio (CC) is only 1.5%. Given the similar sequences and m/z ratio, we assume that the energies deposited into the ions are the same when the same normalized collision energies are applied. The gas phase stability of the duplexes is measured in a relative way by subjecting the duplex parent ions to increasing collision energies during the CID event in an ion trap. E_{50} is defined as the collision energy at which 50% of the duplexes are dissociated into single strands, and is used to characterize the gas phase stability [24]. A higher E_{50} corresponds to a more stable duplex in the gas phase. Although CID is a kinetic experiment, the dissociation is assumed to be endoergic enough such that the barrier and the endoergicity are similar (vis a vis the Hammond Postulate – no reverse activation barrier) [19,23,47–49]. The term coined by Gabelica and De Pauw for this sort of measurement is "kinetic stability" [23]. When

we refer to gas phase stability in this paper, we therefore mean kinetic stability. Using E_{50} to compare stabilities does have limitations. Most importantly, E_{50} is only useful when comparing duplexes of the same size, structure and fragmenting route [23]. These duplexes herein differ only by the central base and therefore should be of the same size and structure. Those studied by CID dissociate into single strands as the major pathway (>80%). Due to the similarities among the studied substrates, it is also fair to assume that the entropies of activation for all the duplexes are similar. The internal energy distribution of the parent ion is poorly defined due to the multiple collision events in the ion trap. We therefore do not intend to report absolute duplex dissociation energies, but rather relative gas phase stabilities as reflected by the E_{50}'s [16,23,24,30,43,46,50].

Experimental conditions were tuned by optimizing the −4 charged duplex ions from GC (*m*/*z* 1358); the conditions thus obtained were applied to all the duplexes. Duplex abundance is normalized by using the equation: % duplex = (2 × [all duplexes])/([all single strands] + 2 × [all duplexes]), where the values in brackets are absolute ion abundances [23]. The reported duplex abundance is an average of six full-scan measurements; the average standard deviation is 2.04%. Duplex dissociation profiles were fitted with sigmoid equations, and the corresponding E_{50} values were derived using Origin 6.0 software. In order to ensure consistency of our relative stability ranking, each CID experiment was performed under two different parent ion isolation widths: $w = 5$ and 4 Da. Since the collision energy is applied across the full isolation width, increasing the isolation width decreases the actual collision energy that a given parent ion experiences. Therefore, for a given parent ion, the E_{50} at a wider isolation width is always greater than that at a narrower isolation width. The reported E_{50} value for each XY duplex is an average of three measurements. The average standard deviations for the measurement of E_{50} for all the XY duplexes are 0.07% for $w = 5$ Da and 0.06% for $w = 4$ Da. The Student *t*-test (to the 90% confidence limit) was used to differentiate close values.

2.3. Prediction of the melting temperature in solution

The ESI solutions are 40 mM NH_4OAc, with a duplex concentration of 12.5 μM. Melting temperatures (T_m) are dependent on duplex concentration and solution composition [26,51]. We can estimate the T_m's for the XY duplexes under our ESI conditions using the program "MELTING" [52]. The settings are as follows: (1) hybridization type: dnadna (for a DNA duplex); (2) nearest neighbor parameters set: all97a.nn [26]; (3) salt concentration: 0.04 M; (4) nucleic acid concentration: 12.5 μM; (5) nucleic acid correction factor: 4 (for non-complementary duplex) [53] and (6) salt correction: san98a [54]. The error associated with the parameterized information used in MELTING is ±1.6 °C [54].

The solution phase stabilities of these XY duplexes have also been measured by Gaffney and Jones [45]. Their reported T_m's (experimental error, ±0.3 °C) differ from our calculated "MELTING" values since the solution conditions are not the same as our ESI solution conditions, and T_m is environment-dependent. Although absolute thermodynamic parameters for DNA duplex studies in different solution conditions are not comparable, relative parameters within a given study where conditions are constant *are* comparable. We therefore expect a correlation between our calculated "MELTING" $T_{m,calc}$ and the Gaffney–Jones' experimental $T_{m,expt}$ (Fig. 1). The linear correlation between our calculated "MELTING" $T_{m,calc}$ and Gaffney–Jones' experimental $T_{m,expt}$ is: $T_m = 1.1482 \times T_{m,expt} - 19.944$ ($R^2 = 0.9876$), where duplexes AC and CA are omitted.

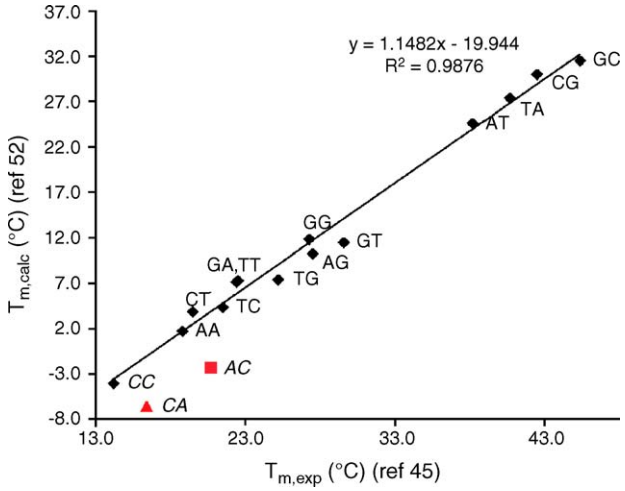

Fig. 1. Correlation of T_m of XY duplexes (5′-d(GGTTXTTGG)-3′/3′-d(CCAAYAACC)-5′) calculated using "MELTING" program with experimental T_m values.

Duplexes AC and CA, in contrast to the other duplexes, do not appear to correlate with Gaffney–Jones' experimental data (Fig. 1, in italics). Assuming that the error is in the calculated value, we can use the linear correlation established in Fig. 1 to estimate "calibrated" T_m's for AC and CA under our ESI conditions. The predicted, calibrated T_m's for AC and CA are 3.8 and −1.1 °C, respectively. We also used the linear correlation to "calibrate" all of our "MELTING" $T_{m,calc}$ data with the experimental data. The calibrated T_m's are used throughout the paper and are referred to simply as "T_m" [55].

3. Results and discussion

3.1. Solution phase stability of XY series duplexes

To simplify the nomenclature of each duplex in this paper, we use only the variable central base of each strand to represent the whole duplex. For example, a duplex called "GC" refers to the duplex 5′-d(GGTT**G**TTGG)-3′/3′-d(CCAA**C**AACC)-5′, where X = G, Y = C [45]. The various combinations of X and Y can form four complementary (GC, CG, TA and AT), and 12 mismatched duplexes, all of which we have studied.

When the temperature of a DNA duplex solution is slowly increased, the ordered double helical structures dissociate into single strands. The midpoint of this transition is called the "melting temperature" (T_m) and is used to characterize the stability of a double helix in solution. The higher the T_m, the more stable the duplex. The solution T_m's for our XY duplexes are shown in Table 1 (second column). In solution, the four complementary

Table 1
Calculated T_m and experimental E_{50} values for the XY duplexes

XY	T_m (°C)[a]	E_{50} (%) (w = 5 Da)	E_{50} (%) (w = 4 Da)
GC	32.2	9.96 ± 0.06	9.76 ± 0.09
CG	28.9	9.78 ± 0.01	9.52 ± 0.01
GG	11.4	9.69 ± 0.06	9.43 ± 0.09
AC	3.8	9.39 ± 0.11	9.12 ± 0.08
CC	−3.6	9.37 ± 0.13	9.07 ± 0.19
TC	4.7	9.36 ± 0.02	9.08 ± 0.05
GT	14.0	9.30 ± 0.07	9.00 ± 0.07
AG	11.6	9.27 ± 0.07	8.97 ± 0.05
TA	26.8	9.23 ± 0.13	8.98 ± 0.05
TG	9.0	9.23 ± 0.10	8.93 ± 0.07
AT	23.9	9.11 ± 0.09	8.89 ± 0.04
TT	5.9	9.10 ± 0.03	8.86 ± 0.01
GA	5.8	9.07 ± 0.06	8.76 ± 0.02
CT	2.4	8.94 ± 0.03	8.76 ± 0.00
CA	−1.1	8.86 ± 0.04	8.73 ± 0.04
AA	1.6	8.81 ± 0.06	8.58 ± 0.06

[a] Error associated with the data used to parameterize MELTING is ±1.6 °C (Refs. [52,53]). Error associated with experimental data used to calibrate these T_m values is ±0.3 °C (Ref. [45]). The T_m value reported herein is a calibrated, calculated value; see text.

duplexes (GC, CG, TA and AT, bold entries in Table 1) have much higher T_m's than do the mismatches. That is, in solution, the complementary duplexes are more stable than duplexes containing a mismatch. Although the structural disruption caused by a single mismatch is quite localized, exposure to solvent and counter ions increases, thus leading to the overall drop in stability as compared to fully complementary duplexes [56]. Overall, the solution phase stability ranking of the XY duplexes is: GC > CG > TA > AT > GT > AG ≈ GG > TG > TT ≈ GA > TC > AC > CT > AA > CA > CC; GC is the most stable matched duplex while AT is the least. For the mismatched duplexes, GT is the most stable while CC is the least stable.

3.2. Full-scan mass spectrometry of XY series duplexes

In neutral solution (pH 7), only the phosphate backbone of a DNA duplex is charged. This electrostatic force field accumulates an atmosphere of cations around the polyanionic backbone. In the ESI process, some but not all of the negatively charged phosphates are protonated, such that the oligonucleotides are vaporized as anions of varying charge states. The first question we sought to answer is whether the resultant mass spectrum is *quantitatively* a snapshot of solution. That is, do the relative ion abundances of the duplex ions correlate to their solution phase stabilities? It has been shown for other non-covalent complexes, such as enzyme-inhibitor complexes, that ion abundances do reflect the corresponding solution phase composition [19,28,57,58]. However, there has not been a systematic study to establish this correlation quantitatively for DNA duplexes and we wanted to examine this aspect for our XY series. Fig. 2 shows the full-scan mass spectra of GC, GT and CC; these particular duplexes are examples of a complementary, a heterogeneous mismatched, and a homogeneous mismatched XY duplex. GC is the most stable matched duplex in solution; GT is the most stable mismatch in solution. CC is chosen to show that even the least stable duplex in solution yields detectable duplex ions in the gas phase. In each spectrum, the duplex ions appear at two charge states, −4 and −3, with the former being more prevalent. The spectra indicate peaks corresponding to single strands as well. We believe these mainly reflect the solution composition; the T_m's are low enough, such that even for GC, some single strands are present in solution (Fig. 2).

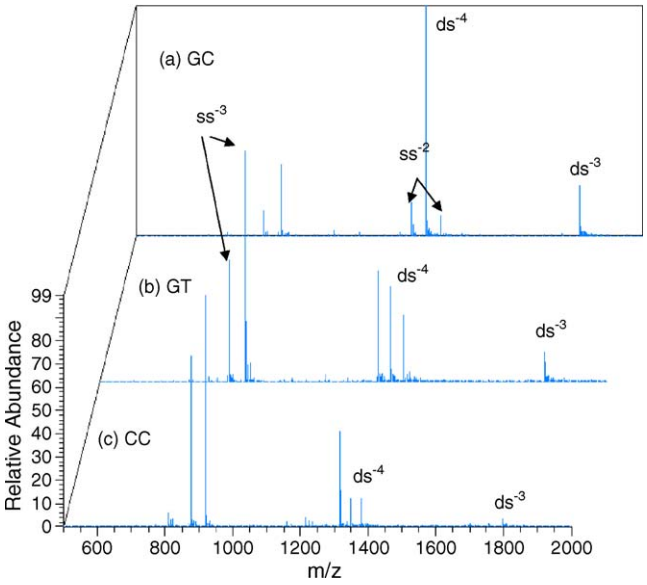

Fig. 2. Full-scan mass spectra for three XY duplexes 5′-d(GGTTXTTGG)-3′/3′-d(CCAAYAACC)-5′: (a) complementary GC duplex, (b) GT mismatched duplex and (c) CC mismatched duplex; "ds" indicates double strand and "ss" indicates single strand.

To assess the correlation between the ion abundance and solution phase composition, the duplex abundance in the gas phase is plotted versus the solution phase T_m values (Fig. 3). A good linear relationship is found, indicating that duplex ion abundances can be used to assess relative solution phase stabilities. That is, the electrosprayed duplexes appear to volatilize with relative integrity, such that the mass spectrum is a "snapshot" of what is observed in solution [10,11,19,23,28,29,57–61]. This is the first study establishing a correlation between solution phase stability and mass spectrometric signal abundance for *both* complementary and mismatched duplexes, and is extremely valuable. The ability to quickly assess, by mass spectrometry, the solution

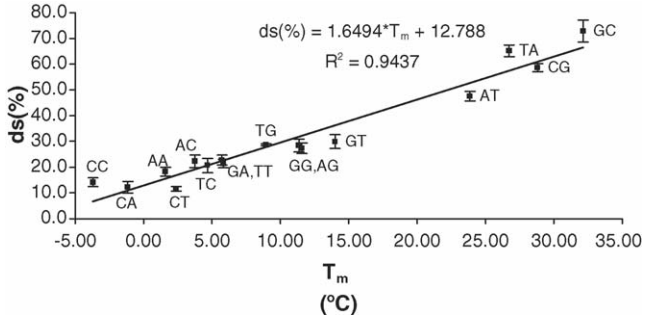

Fig. 3. Correlation of mass spectrometric duplex ion abundances with corresponding T_m values. Error bars indicate the standard deviation for each "ds%" value.

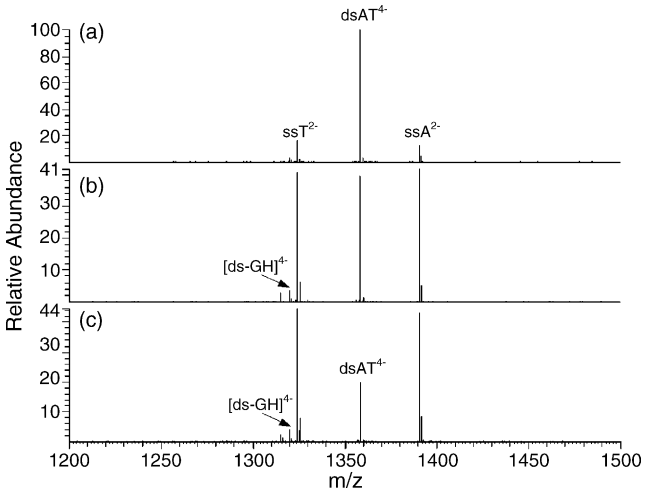

Fig. 4. CID spectra of the duplex $[AT]^{4-}$ ions at relative collision energies of (a) 8.2%, (b) 9.0% and (c) 9.8% (isolation width = w = 5 Da); "ds" indicates double strand; "ss" indicates single strand and "ds-GH" indicates neutral guanine loss from duplex.

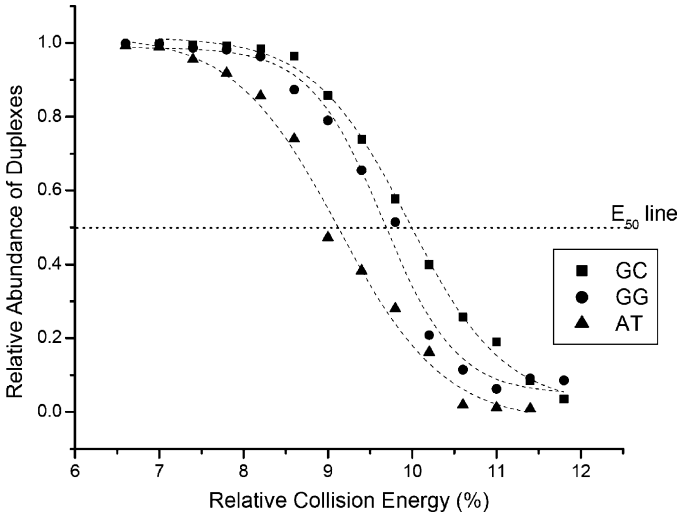

Fig. 5. Gas phase dissociation profiles of three XY duplex ions [5′-d(GGTTXTTGG)-3′/3′-d(CCAAYAACC)-5′]$^{4-}$, where X and Y are GC, GG and AT (isolation width = w = 5 Da).

phase non-covalent complex abundance has implication for the development of efficient screening assays for potential DNA binders [15,17,24,25,62,63].

3.3. Collision-induced dissociation of XY series duplexes

Under gentle CID conditions in the ion trap (<20% relative collision energy), we find that dissociation of the −4 duplex into its constitutive single strands is the major pathway (Fig. 4). The dissociation of the −3 charged duplexes preferentially yields extensive cleavage of covalent bonds, with little non-covalent dissociation. The effect of charge state on duplex stability has been discussed previously [24], and stability comparison among different ions is valid only if all the duplexes have the same structure, fragmentation route, and charge states [23]. Therefore, we report the CID experiments on the −4 charged duplexes only.

The dissociation of the parent duplex ion is monitored by the disappearance of the duplex signal and the appearance of the single strands. The dissociation profiles of three duplexes (GC, GG and AT) are displayed in Fig. 5, as examples of a stable complementary duplex, a stable mismatched duplex, and a less stable complementary duplex. To achieve the same degree of dissociation among all these duplexes (as indicated by the E_{50} value), the GC duplex (filled squares) requires the highest collision energy, followed by GG (filled circles), then AT (filled triangles). This would indicate that the gas phase stability order of these three duplexes is GC > GG > AT.

Table 1 lists the E_{50} values for the 16 XY duplexes at w = 5 and 4 Da isolation widths, together with the predicted melting temperatures, to allow for direct comparison between gas phase and solution phase stabilities. The XY duplexes are listed in decreasing order of their E_{50}'s (at w = 5 Da). The E_{50} difference between the most stable GC duplex and the least stable AA duplex is only 1.15% at w = 5 Da (and 1.18% at w = 4 Da). However, these differences are significant because the average standard deviations are only 0.07% for w = 5 and 0.06% for w = 4 Da. Overall, the gas phase stability ranking of the XY duplexes from two sets of E_{50}'s (w = 5 and 4 Da) are consistent, and follow the order GC > CG > GG > AC, CC, TC > GT, AG, TA, TG > AT, TT, GA, CT > CA > AA. As one might expect, the two complementary duplexes GC and CG are the two most stable in the gas phase. However, the other two complementary duplexes, TA and AT, are less stable than some of the mismatches, such as GG, AC, CC and TC. CA and AA are the two least stable duplexes in the gas phase.

3.4. Comparison of gas phase and solution phase stabilities of XY series duplexes

The maintenance of DNA duplex structure in solution is controlled by four major factors: hydrophobic interactions driven by the solvent, electrostatic interactions between the negatively charged phosphate groups and counter cations, intermolecular hydrogen bonding between base pairs, and base–base stacking [6]. In the gas phase, a duplex ion represents a balance between Coulombic repulsion of the polyanionic backbone and attraction from hydrogen bonding and base stacking [17,59]. Herein, we attempt to compare and contrast solution and gas phase behavior, which will aid in understanding how solvation affects intrinsic DNA duplex stability [59].

To compare gas phase and solution phase stabilities, we plot the E_{50} values versus the T_m values (Fig. 6). At first glance, the plots look scattered and there does not appear to be a linear correlation between gas phase and solution phase stabilities for these XY duplexes. However, one can imagine a line through the *majority* of the data (the solid diagonal lines shown in Fig. 6a and b), encompassing CA, AA, CT, GA, TT, TG, AG, GT, CG and GC. The gas phase stabilities of these 10 duplexes seem to track quite well with their solution stabilities. The outliers can be grouped as those "above the line" (CC, TC, AC and GG) and those "below the line" (AT and TA). The "above" outliers can be

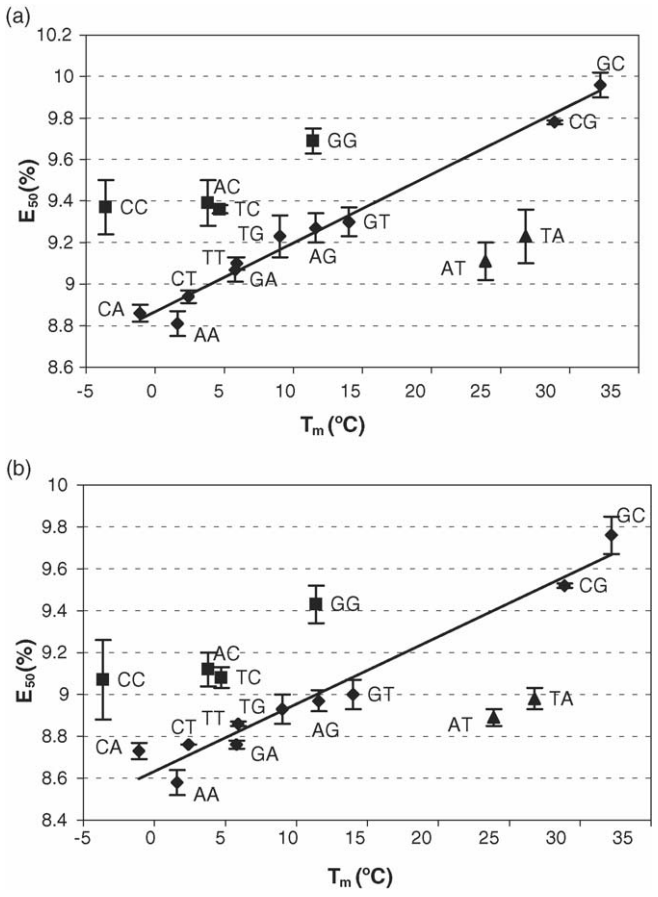

Fig. 6. Comparison of gas phase stability (E_{50}) and solution phase stability (T_m) of the 16 XY duplexes (5′-d(GGTTXTTGG)-3′/3′-d(CCAAYAACC)-5′) at isolation widths of (a) 5 Da and (b) 4 Da.

considered more stable in the gas phase than in solution, while the "below" group – TA and AT – is less stable in the gas phase than in solution.

These plots therefore indicate that the XY duplex stabilities in the gas phase do not always correlate to those in solution. These results are unexpected since to date, ESI-MS studies for complementary DNA duplexes indicate that gas phase and solution phase stabilities correlate; that is, trends in stabilities of complementary duplexes in solution are the same in the gas phase [16,20,23,24,30]. Specific questions that arise from our data are: (a) Why do some duplexes *not* show a gas-solution phase correlation? (b) Can the trends and observations for these XY duplexes be generalized for predictive power? In an effort to understand the observed gas phase DNA duplex stabilities, we analyzed the factors that play the largest role in dictating structure and stability: base stacking and hydrogen bonding.

3.4.1. Base stacking

Base stacking in DNA duplexes has long been studied in solution. The well-established nearest neighbor theory indicates that in solution, base composition is not the only factor that dictates DNA duplex stability [26,51,64]. For example, a duplex that contains a 5′-AC-3′/3′-TG-5′ sequence will not have the same stability as a duplex with a 5′-CA-3′/3′-GT-5′ sequence; that is, although both have the same composition, the sequence plays a large role in determining stability. The reason for this is that bases do not only hydrogen bond across a duplex; they also have stacking interactions. Essentially, a base's immediate "neighbor" on a given strand will stack with that base, so the next neighbor has a large role in dictating overall duplex stability; this feature is the foundation upon which solution phase melting temperatures are predicted [26,51,64–67].

Table 2
Gas phase ΔE_{50} values for selected isomeric XY duplexes

Isomeric pair	$\Delta E_{50,average}$[a] (%)
AC vs. CA	0.46
TC vs. CT	0.37
GC vs. CG	0.21
AG vs. GA	0.21
TA vs. AT	0.11
GT vs. TG	0.07

[a] The ΔE_{50} value is the averaged E_{50} (at $w = 5$ and 4 Da) for the more stable duplex minus the averaged E_{50} for the less stable isomeric duplex. For each isomeric pair, the more stable duplex is listed first. See text.

In the gas phase, by contrast, little work has been directed toward the analysis of base stacking in DNA duplexes. In a recent MS study of *RNA* duplexes, the authors concluded that base stacking interactions are "negligible" [43]. However, Gabelica and De Pauw found that two 16-mer complementary DNA duplexes with the same base composition but different sequences have different stabilities in the gas phase, which is attributable to differential base stacking [23].

In order to assess base stacking abilities, we compare duplexes of the same composition. Any differences in stability for duplexes of the same composition (and therefore the same number of hydrogen bonds) can be attributed to stacking [1,23,51]. There are six isomeric duplex pairs: AC versus CA, AG versus GA, AT versus TA, CG versus GC, CT versus TC and GT versus TG. The difference between the E_{50}'s (ΔE_{50}) for the two duplexes in a given pair should reflect the relative stacking stability since both isomers have the same number and kind of hydrogen bonding interactions [23]. Table 2 lists the average ΔE_{50}'s for six isomeric duplex pairs. The more stable duplex is listed first for each pair (for example, the first entry "AC versus CA" indicates that AC is the more stable of the two isomeric duplexes). The ΔE_{50} is equal to the averaged E_{50} (at $w = 5$ and 4 Da) for the more stable duplex minus the averaged E_{50} for the less stable isomeric duplex. The spread between the lowest and highest E_{50} is 1.17% (average of $w = 5$ and 4 Da); therefore, the largest ΔE_{50} (0.46%) is quite a large fraction of the total range. The rank ordering from the greatest to the smallest ΔE_{50} is (AC versus CA) > (TC versus CT) > (GC versus CG) ≈ (AG versus GA) > (TA versus AT) > (GT versus TG). The ΔE_{50} for GT versus TG is so small (0.07%, equivalent to the error associated with the measurement) that the two duplexes can be considered to have about the same gas phase stability. Therefore, in the gas phase, AC has a much greater stacking contribution than does CA; the same is true for TC, relative to CT. This becomes less true as one moves "down" Table 2, until one compares GT and

Table 3
Base stacking analysis for isomeric XY duplexes (5′-GGTTXTTGG-3′/5′-CCAAYAACC-3′) in the gas phase[a]

	Y = C	Y = G	Y = A	Y = T	Stacking ability of X
X = T	+ 0.37	0 0.07	+ 0.11	n/a	①
X = A	+ 0.46	+ 0.21	n/a	− 0.11	②
X = G	+ 0.21	n/a	− 0.21	0 0.07	③
X = C	n/a	− 0.21	− 0.46	− 0.37	④
Stacking ability of Y	(1)	(2)	(3)	(4)	

[a] A positive sign "+" indicates that an XY duplex has a higher E_{50} than its isomer YX duplex and a negative sign "−" denotes the opposite trend. A"0" is used for the cases in which two E_{50} values are the same within experimental error. The values of ΔE_{50} are also listed for each entry.

TG, for which the ΔE_{50} is so small that presumably stacking contributes comparably to both sequences.

These data can also be used to order the relative, intrinsic stacking ability of each central (X and Y) base in each sequence. Toward this end, the ΔE_{50} data from Table 2 are collated in a slightly different fashion in Table 3. Each entry in Table 3 is the ΔE_{50} between the corresponding XY duplex and its isomeric pair. It should be stressed that stacking ability is sequence-dependent; that is, since the sequence studied is 5′-GGTTXTTGG-3′/5′-CCAAYAACC-3′, we are assessing the stacking propensity of X when flanked by two T's, with a paired AYA strand. For the first entry, the value of "+0.37" reflects the E_{50} of the XY duplex (TC) minus the E_{50} of its isomer (CT) (this value can also be found in Table 2), with the positive sign indicating that this duplex (TC) is more stable than its isomer CT in the gas phase. Another example is AT/TA (second row, fourth column entry). The value of "−0.11" in this entry reflects the E_{50} of this XY duplex (AT) minus the E_{50} of its isomer (TA), with the negative sign indicating that the duplex AT is less stable than its isomer TA in the gas phase. In order to compare the relative base stacking abilities for the variable base X, each row in Table 3 must be compared to the other rows. The row having the most positive signs implies that that base X has the best stacking ability, while the row having the most negative signs implies the worst stacking ability. The first "X = T" row, which represents the stacking ability of T, has two positive signs and one zero; the second row, which represents the stacking ability of A, has two positive signs and one negative sign; the third row, which represents the stacking ability of G, has one positive sign, one zero, and one negative sign and the fourth row, which represents the stacking ability of C, has three negative signs. Therefore, the stacking ability of the X base in the sequence GGTTXTTGG follows the order T > A > G > C. For the stacking ability of Y, one can compare the columns. The first "Y = C" column has three positive signs; the second "Y = G" column has one positive sign, one zero and one negative sign; the third "Y = A" column has one positive sign, and two negative signs and the fourth "Y = T" column has three negative signs. Therefore, the relative stacking ability of Y in the sequence CCAAYAACC follows the order of C > G > A > T in the gas phase. Because next neighbor interactions are key, we would expect that any sequence with a central TXT/AYA motif would show the same base stacking trends that we have found.

3.4.2. Hydrogen bonding

Hydrogen bonding between base pairs is one of the most basic factors dictating DNA structure and recognition. There are eight possible base mismatches (A·A, A·C, A·G, C·C, C·T, G·G, G·T and T·T) that can compete with the Watson–Crick matched G·C and A·T pairs. In aqueous solution, hydrogen bonding and base stacking are both important in stabilizing the helical structure; recent experiments indicate that base stacking in particular may be the predominant factor in solution [68,69]. Gas phase ab initio calculations indicate that hydrogen bonding interactions are stronger than stacking interactions in vacuum for free base pairs [70,71]. Also in the gas phase, the experimental binding energies of free nucleic acid and mononucleotide base pairs have been reported by several groups [72–75]. These results, however, have an element of ambiguity since gas phase structure is difficult to ascertain experimentally [11,29,61,76].

Because obtaining structure is so challenging in the gas phase, calculations are often used to interpret structure [11,19,29,59]. Rueda et al. recently used molecular dynamics methods to demonstrate that complementary duplexes can exist in the gas phase; the structure is slightly distorted but retains the same general features that are found in aqueous solution [59]. Bowers and co-workers have also used molecular dynamics coupled with ion mobility experiments to show that under their experimental conditions, duplexes retain helical, solution structures [29]. We therefore assume that our 16 XY duplexes maintain B-like helical structures in the gas phase.

The structures of experimentally determined (X-ray diffraction and NMR) H-bonded base pairs that exist in B-DNA duplexes are shown in Fig. 7 [2,34,77]. The interaction energies of all possible base pairs in the gas phase have been calculated; the highest level values are listed in Table 4, left column [70,78–80]. The nominal structures of these calculated base pairs are analogous to those shown in Fig. 7, with three exceptions: A·A, C·C and A·C. (Note that the bullet indicates a free base pair. Therefore "A·T" indicates the free base pair formed from A and

Table 4
Comparison of calculated interaction energies of H-bonded base pairs and experimental gas phase E_{50} values for the corresponding XY duplexes[a]

ΔE, calculated for free base pairs (kcal mol^{-1})[b]	E_{50}, average for XY duplexes (%)
−24.5 (G·C) [GCWC]	9.86 (GC); 9.65 (CG)
−22.8 (G·G) [GG1]	9.56 (GG)
−14.1 (G·T) [GT2]	9.15 (GT); 9.08 (TG)
−14.1 (A_{anti}·G) [GA1]; −12.5 (A_{syn}·G) [GA3]	9.12 (AG); 8.92 (GA)
−12.9 (T·A) [TAWC]	9.11 (TA); 9.00 (AT)
−10.8 (C·T) [CT2]	8.85 (CT)
−10.5 (T·T) [TT1]	8.98 (TT)

[a] The structures of the H-bonded base pairs can be found in Fig. 7.
[b] Names in brackets refer to nomenclature from original reference; calculations are at MP2 using a modified aug-cc-pVDZ basis set (see Ref. [78]).

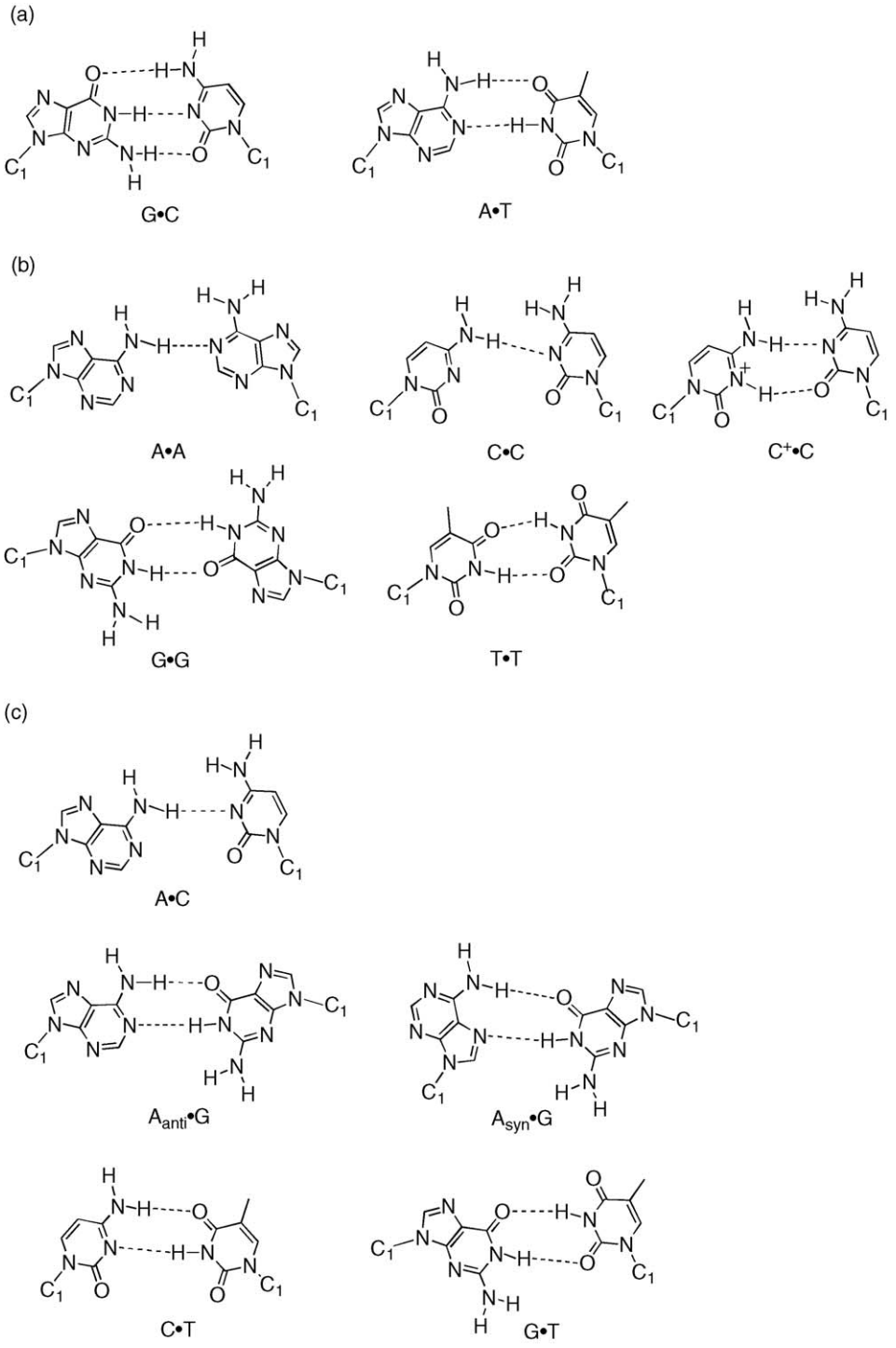

Fig. 7. Base pair structures: (a) Watson–Crick G·C and A·T base pairs, (b) homogeneous mismatched base pairs and (c) heterogeneous mismatched base pairs.

T, whereas "AT" indicates the corresponding XY duplex and "TA" indicates the isomeric XY duplex.) In the gas phase, these three *free* base pairs are calculated to assume, as their most stable structures, an arrangement which is not geometrically possible in the helical duplex (Fig. 8); therefore, these data are not listed in Table 4.

In order to interpret the effects of hydrogen bonding in the XY duplexes, we compare our E_{50} values with the calculated base pairing energy values (Table 4). Presumably if the gas phase hydrogen bonding interaction energies for the free base pairs correlate in trend to the gas phase stabilities of the XY duplexes, one can postulate that hydrogen bonding plays a major role in stabilizing the duplexes. However, such a direct comparison requires caution because base stacking also plays an important role in determining duplex stability and one cannot assume that only hydrogen bonding determines stability. That is, if sequence and base stacking play a significant role in stability, hydrogen bonding effects cannot be separated out. How can we account for base

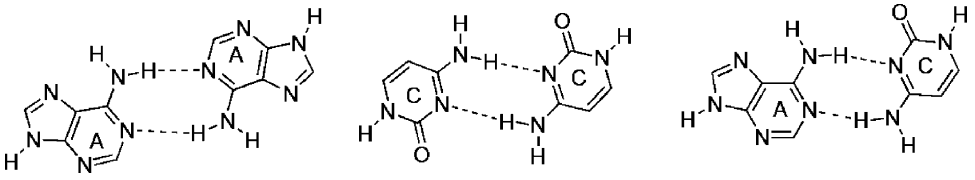

Fig. 8. Structures showing hydrogen bonding patterns for the most stable calculated configuration for free base pairs A·A, C·C and A·C (Refs. [70,78–80]).

stacking? As delineated in the previous section, the E_{50} difference for isomeric duplexes, which have an equivalent number of hydrogen bonds, can be used to assess the magnitude of base stacking effects. AC versus CA and TC versus CT have the largest ΔE_{50}'s (Table 2). This would imply that these sequences are sensitive to base stacking, and that AC and TC appear to have large stacking contributions; therefore, we omit these values from Table 4. We include CT; CA is excluded on the basis of the geometry of its calculated base pair (vide supra). Therefore, there is a total of 11 XY duplexes listed in Table 4, corresponding to seven H-bonded base pairs G·C, G·G, G·T, A·G, T·A, C·T and T·T. Based on our stacking analysis, we predict that the base stacking in the XY duplexes involving these six H-bonded base pairs is similar; GC versus CG, GT versus TG and AG versus GA all have smaller ΔE_{50}'s (Table 2). Although we cannot separate out the stacking contributions for GG and TT, from Table 3 we can see that TT is the best X stacker but the worst Y stacker. We assume that stacking is not a major influence for TT since it has a very good, but also a very bad contributor. A similar argument can be made for GG (X stacking ability ranked third; Y stacking ability ranked second, also "canceling" each other out). Given the assumption that stacking is minimal, the ranking of the duplexes' E_{50} values should directly reflect the role of hydrogen bonding in stabilizing the duplex in the gas phase. In Table 4, the calculated hydrogen bonding energies are listed in the left column from the most stable G·C pair to the least stable T·T pair. The experimental E_{50}'s of the XY duplexes with the corresponding H-bonded base pairs are listed in the right column. As can be seen, the most stable calculated hydrogen bonded base pair is G·C, which corresponds to the experimentally most stable XY duplexes in the gas phase: GC and CG have the highest E_{50} values. This correlation holds true for all the base pairs and duplexes except the "last" two (Table 4). The hydrogen bonded base pair C·T is calculated to be *very* slightly more stable than the base pair T·T (so slight that the two have essentially the same gas phase interaction energy). However, the corresponding duplexes track in the opposite direction; CT is slightly less stable than TT. This discrepancy is probably due to the fact that it is not possible to completely factor out and discount base stacking, and TT is a better stacking combination than CT (Table 3). We therefore have to bundle the last two as a group and note that C·T and T·T are the weakest hydrogen bonded pairs, and correspondingly, CT and TT are the weakest duplexes in the gas phase. Overall, the results in Table 4 imply that for these duplexes, hydrogen bonding is a dominant factor contributing to gas phase stability.

Now let us revisit the three pairs excluded from Table 4 (A·A, C·C and A·C). One would expect these three to be relatively unstable since each has only one hydrogen bond (Fig. 7). Consistent with this, AA is the least stable XY duplex. Likewise CA is the next most unstable XY duplex. However, CC and AC are quite stable in the gas phase, despite the single hydrogen bond. AC has been shown to be a good stacker (Tables 2 and 3), which would contribute to overall stability in spite of minimal hydrogen bonding. CC, however, is mysterious. As with GG and TT, we assume that the stacking contribution for this duplex is not overwhelming (vide supra). So why is CC so stable? It is known in solution that duplexes and triplexes containing CC(C) often undergo protonation of a cytosine at low pH [40,81–83]. It has also been shown that protonation of a cytosine significantly stabilizes these duplexes and triplexes. Perhaps in the ESI process, protonation of a cytosine occurs, leading to an unusually stable gas phase duplex; there is no evidence for this but it is a tantalizing proposal that we would like to test in the future.

What general conclusions about solvent effects can we now make? Clearly in the gas phase, hydrogen bonding and base stacking both play major roles in duplex stability. Although any analysis of hydrogen bonding in duplexes is complicated by stacking, it appears that the majority of the XY duplexes track with the gas phase hydrogen bonding energy calculations of the corresponding free base pairs (Table 4). This correlation implies that hydrogen bonding is probably the major force in determining gas phase duplex stability. This is in contrast to in solution, where base stacking is believed to dominate.

That being said, Fig. 6 indicates that despite changes in the factors contributing to stability, for most of our duplexes, gas phase stability correlates in a linear fashion with solution phase stability, indicating that solvation probably affects those particular duplexes in a similar relative way, such that gas phase trends track with solution phase trends. By contrast, the "outliers" clearly must be affected by solvation or possibly, the ESI process. The "above the line" outliers (TC, CC, AC and GG) are all more stable in the gas phase than in solution. These enhanced gas phase stabilities could arise from changes in hydrogen bonding and/or stacking and/or the effects of solvation. The high gas phase stability of the CC duplex leads us to propose protonation of C during ESI; however, CC could also be an outlier due to other structural and desolvation changes that we cannot quantitate. We will have to conduct further studies focusing on these mismatches in order to parse the factors contributing to the enhanced gas phase stabilities.

The "below" outliers should be more stable in solution than in the gas phase. AT and TA, however, are a real surprise. Both are Watson–Crick complementary matches and one would expect these duplexes to be stable in all media. The free A·T base pair is, however, surprisingly unstable (Table 4) in the gas phase, which

may explain the decreased gas phase stability of the corresponding AT and TA duplexes. Interestingly, it has also been proposed that in solution, a well-defined spine of water molecules is a major stabilizing force in AT-rich DNA duplexes [42,59]. The absence of this solvation in the gas phase would also contribute to the AT and TA duplexes being relatively unstable.

We should also make clear that Fig. 6 is overall surprising. Gas phase DNA studies to date have focused on complementary sequences; this is the first study of its kind and it is unexpected to see the majority of the duplexes form a clear line, with AT and TA *off* that line. This study represents a first step toward future work designed to probe the generality of the conclusions herein.

4. Conclusions

Understanding the features of electrosprayed oligonucleotides is important both for fundamental reasons (How does solvent affect DNA?) as well as applied (Does DNA behavior in the gas phase correlate to that in non-polar media such as enzyme active sites? How does DNA gas phase behavior impact MS sequencing and assay studies?).

In this work, a complete set of 9-mer DNA duplexes (5′-GGTTXTTGG-3′/3′-CCAAYAACC-5′, X/Y = G, C, A or T) with and without single internal mismatches were studied. To our knowledge, this is the first systematic study of a set of duplexes that differ in sequence by the central base only, allowing for the examination of complementary duplexes as well as duplexes containing one mismatch.

We have found that the relative ion abundances of the electrosprayed duplexes correlate to their relative solution phase stabilities. While this has been shown for complementary duplexes, this is the first establishment of such a correlation among a series of matched and mismatched duplexes. This correlation is valuable as it shows that mass spectrometry can be used as a method of quickly assessing solution phase duplex stability, which would be preferable to the more traditional (and tedious) method of obtaining the melting curve for each duplex of interest.

In addition to obtaining relative solution stabilities, we have also characterized the gas phase stabilities of all the XY duplexes, using collision-induced dissociation. By comparing isomeric duplexes, we are able to establish the relative stacking abilities of the central bases X and Y in our sequence. This is a first step toward providing predictive power for duplex gas phase stabilities by establishing what sequence combinations are most stable.

We have also shown that the gas phase stabilities of the majority of the duplexes track with the gas phase hydrogen bonding capabilities of the central XY base pair. That is, if a free base pair has a stronger hydrogen bond, the corresponding XY duplex is more stable in the gas phase. These trends are somewhat complicated since stacking can never be factored out, but the general trends imply a major role for hydrogen bonding in gas phase duplex stability, which is in contrast to in solution, where stacking is the major force. These results are commensurate with expectation, since in solution, hydrogen bonding between bases competes with hydrogen bonding to solvent, and stacking therefore prevails [1]. An additional point of interest is that the AT and TA duplexes, even though they are complementary, are more unstable than several mismatches in the gas phase; this result is consistent with the fact that A·T is not a particularly strong gas phase hydrogen bonded pair.

Last, we examined the relationship between gas phase and solution phase stability for each of the 16 XY duplexes. We find that four duplexes (GG, AC, TC and CC) exhibit enhanced gas phase stabilities, two (TA and AT) are unstable in the gas phase relative to in solution, and the remaining 10 (GC, CG, GT, AG, TG, TT, GA, CT, AA and CA) show a linear correlation between the gas phase and solution phase stabilities. These results are of interest since prior to this study, complementary DNA duplexes have been the main focus and the conclusion is that gas phase stability in general tracks with solution phase stability. We have shown this to be generally true, even for duplexes with just one mismatch, but that there are key outliers that do not follow the correlation, with AT and TA being particularly intriguing.

Future work will include studies to establish the generality of our conclusions toward gaining predictive power in terms of gas phase DNA duplex stability, and ascertaining why the relative gas phase stability of certain duplexes differs from that in solution.

Acknowledgements

We thank the National Science Foundation (CHE-0092215), the Alfred P. Sloan Foundation, and the Rutgers Busch Grant Program for support, and the National Center for Supercomputing Applications for computational resources. We are grateful to Dr. Valerie Gabelica and Dr. Jae C. Schwartz for helpful discussions.

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