Detection of T-T Mismatches Using Mass Spectrometry: Specific Interactions of Hg(II) with Oligonucleotides Rich in Thymine (T)

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Electrospray ionization tandem mass spectrometry (ESI-MS/MS) was employed in a detailed study of the interactions of mercury dications with selected oligodeoxynucleotides rich and poor in thymine (T): d(5′-TT-3′), d(5′-TTT-3′), d(5′-TTTT-3′), d(5′-GG-3′), d(5′-CC-3′), d(5′-AA-3′), d(5′-GCTTGC-3′), d(5′-GTGCTC-3′), d(5′-GCATGC-3′), and d(5′-GCGCGC-3′). Specific interactions are observed for Hg^{2+} with pure and mixed thymine sequences in which simultaneous bonding between two thymine units is indicated, and this is consistent with a model proposed in the literature in which Hg^{2+} covalently coordinates to two thymines by replacing two N3 imino protons of the bases. The ESI-MS/MS measurements, combined with data on the thermal stability of mixed sequence hexadeoxynucleotides, indicate that mercury prefers thymines over the other binding sites in oligonucleotides both in solution and in the gas phase. These results point toward the effective use of Hg^{2+} in the fast detection of mismatch base pairs incorporated in oligonucleotide duplexes and longer mixed DNA sequences using ESI-MS/MS.

Introduction

The nature of the bonding of metal ions to biological molecules is undoubtedly fundamental to the biological effects that they exhibit.[1,2] Detailed knowledge of the modes of bonding as well as the kinetics and thermodynamics of the interactions of metal ions with biomolecules, including DNA, are crucial for a definitive understanding of the mechanisms of the physiological functions of metal ions. In vitro interactions of model oligonucleotides with heavy metals, such as Hg(II), can potentially provide a molecular basis for the genotoxicity and environmental impacts of mercury compounds.[3] Since the early and increasing use of mercury in human applications has resulted in an increase in environmental contamination, studies directed to an understanding of the fundamentals of the interactions of mercury ions with DNA and its model molecules are of growing significance.[4,5] Several molecular pathways through which mercury ions can exhibit their genotoxicity have been suggested in the literature to date:

(i) Hg^{2+} can promote formation of reactive oxygen species (ROS) and so contribute to the oxidative stress, peroxide oxidation of lipids (POL), that alters the structure of membranes and interferes with the mitochondrial function.[6]

(ii) Mercury may influence DNA repair mechanisms by displacing native Zn^{2+} cofactor from cysteine-rich sites of DNA repair enzymes and so inhibit their function.[7]

(iii) Mercury specifically binds to the main proteins constituting microtubules, tubulin and kinesin, and so blocks the biochemical cascade responsible for cellular functioning.[8]

(iv) Hg ions may interact directly with DNA molecules.[9] Hg^{2+} was demonstrated to covalently coordinate with the endo- and exocyclic nitrogen sites of nucleobases.[10] It was hypothesized that every three or four pairs of the nucleobases in a DNA helix are capable of binding one compound of organic (methylmercury, ethylmercury, phenylmercury) or inorganic (Hg^{2+}) mercury species. Hg^{2+} ions are believed to have the highest affinity for thymine base pairs that belong to two separate strands.[9,11] Over the years, there were numerous experimental studies supporting this model;[11] however, the overall structure of a DNA duplex adduct with Hg^{2+} remains unknown.

Electrospray ionization tandem mass spectrometry (ESI-MS/MS) has recently emerged as a powerful tool for structural elucidations of DNA and oligonucleotides as well as their complexes with metal ions, drugs, and small organic ligands.[12−15] Here we report the results of ESI-MS/MS studies with thymine-rich oligonucleotides and mercury dications along with measurements of the solution stability of the corresponding bare and mercuriated duplexes. They point toward a new methodology for the effective use of Hg^{2+} in the fast detection of mismatch base pairs incorporated in oligonucleotide duplexes and longer mixed DNA sequences using ESI-MS/MS.

Experimental Section

Electrospray ionization data were acquired in both positive and negative ion modes using an API 2000 (MDS-SCIEX, Concord, ON, Canada) triple quadrupole ($Q_1q_2Q_3$) mass spectrometer equipped with a "Turbo Ion Spray" ion source. Experiments were performed at an ion spray voltage of −5500 V, a ring-electrode potential of −300 V (used for ion beam confinement), and a range of potential differences between the orifice and the skimmer. N_2 was used as a curtain gas at a setting of 10 psi and air was used as a nebulizer at a flow rate of 8 L min^{-1}. Samples were directly infused into the electrospray source at a flow rate of 3 μL min^{-1}.

Tandem mass spectrometric (MS/MS) measurements were performed in the product ion and multiple reaction monitoring (MRM) modes with N_2 as collision gas at a pressure estimated to be about 3 mTorr (viz. multicollision conditions). The collision offset voltage (the potential difference between the

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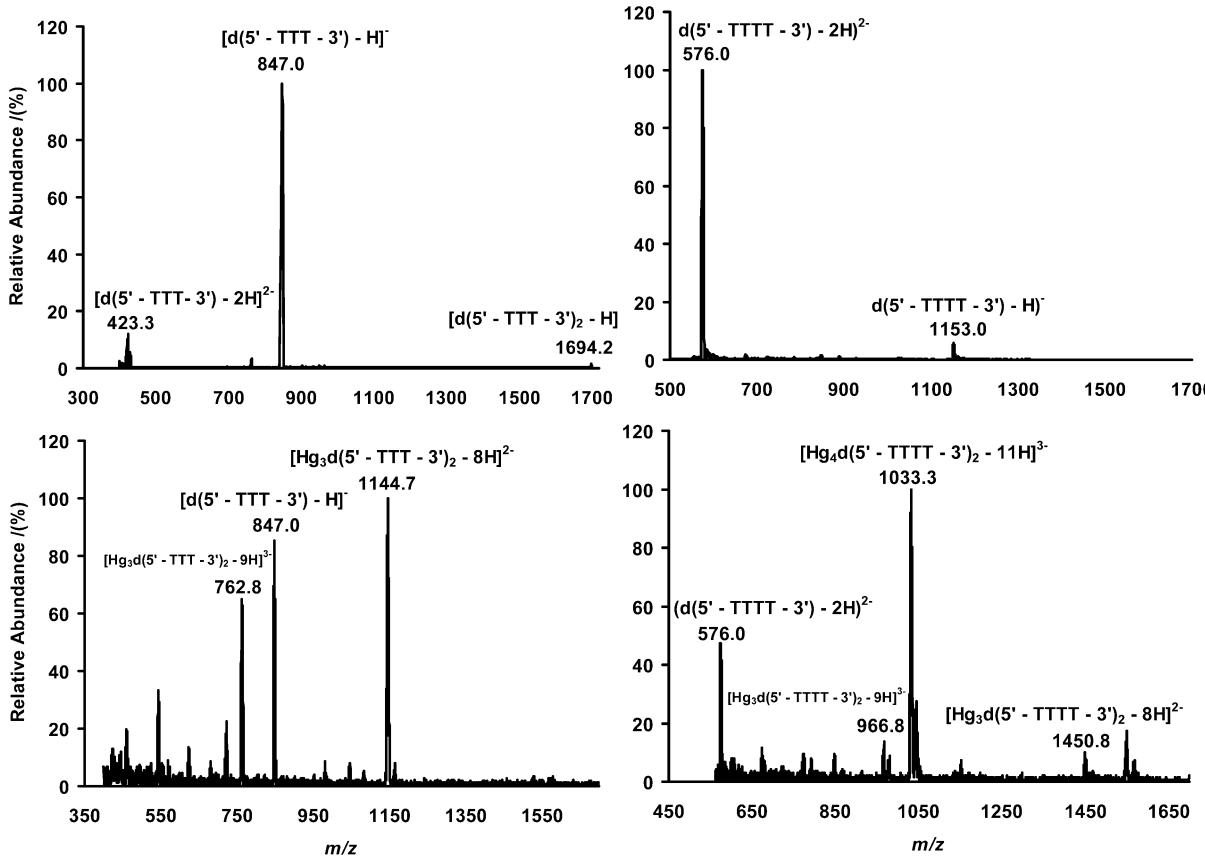


Figure 1. ESI mass spectra of 20 μM solutions of d(5′-TTT-3′) (left) and d(5′-TTTT-3′) (right) without (top) and with (bottom) added 0.5 equiv of Hg^{2+}. All the solutions were prepared in 20:80 water:methanol mixtures.

collision cell quadrupole (q_2) and the quadrupole entrance lens (q_0)), which nominally gives the laboratory frame collision voltage, was adjusted in 1 V intervals between −1 and −130 V or +1 and +130 V, depending on the ion mode. Space charge and contact potentials, field penetration, and field distortion all can influence the actual collision voltage offset but were not taken into account. Product ion spectra were obtained by scanning Q_3 over the range $m/z = 10-1800$. The interquadrupole lens potentials and the float potential of the resolving quadrupole Q_3 were linked to the q_2 potential to maintain proper transmission through Q_3.

The relative gas-phase stability of a particular ion of interest was evaluated utilizing so-called tangent voltage (TV) values determined by extrapolating the steepest slope of the breakdown curve of an ion of interest to the collision offset voltage axis.[16] The precision of the TVs is taken to be one standard deviation from the mean TV value obtained in several (four or more) repeated experiments. The TVs were not measured as a function of pressure.

Hg^{2+} was generated from its chloride salt (Aldrich, pa ≥99.99%). HPLC grade methanol and Millipore (18.2 mΩ) water were used to prepare the solvent mixture. All oligodeoxynucleotides (ODNs) were purchased from ACGT Corp. (Toronto, Canada), desalted, and cartridge purified. To form double-stranded species, the ODNs were annealed in 100 mM solution of ammonium acetate buffer by heating to 90 °C for 15 min and slow cooling down to room temperature over a 3 h period. Stock solutions were diluted in 20:80 methanol:water mixture to yield a 20 μM solution. The 100 mM solution of ammonium acetate also was prepared in a 20:80 methanol:water mixture so that the ratio of the solvents did not change in the final electrosprayed solutions in which the concentration of ammonium acetate was about 50 mM. The pH of the solutions was maintained with ammonium acetate buffer at around 6.8.

UV melting curves for the ODNs were obtained with a Varian Cary 100 UV−vis spectrophotometer at a heating rate of 0.2 °C min^{-1}. The actual values of the melting points were determined using "the first derivative" method incorporated into the thermal application of Cary WinUV software package. Solution concentrations of the ODNs utilized in the spectroscopic measurements were set to1 μM with $[Hg^{2+}] = 0.5$ μM.

Results and Discussion

1. Formation of Mercury−ODN Complexes. Figures 1 and 2 provide the negative ion ESI mass spectra that were recorded for solutions of the selected (annealed) ODNs, both in the absence and in the presence of 0.5 equiv of Hg(II) chloride. Enhanced resolution was employed to confirm the binding stoichiometry and charge state of the anion adducts of Hg(II) and deprotonated ODNs that were observed. Protons are lost in the mercuriated species both from the −NH groups on thymines to neutralize the charges on Hg(II), two for each Hg(II), and from the phosphate groups. For example, in the doubly mercuriated duplex trianion $[Hg_2(d(5'\text{-}GTCGTG\text{-}3')_2 - 7H]^{3-}$ the losses of protons can be indicated explicitly by the formula $[Hg_2(d(5'\text{-}G(T\text{-}\underline{H})CG(T\text{-}\underline{H})C\text{-}3')_2 - 3\underline{H}]^{3-}$, but this has not been done throughout to avoid complexity.

Two aspects of the observations in Figure 1 are worth noting. No adducts with Hg(II) are observed for the pure thymine *single-stranded* ODNs. On the other hand, multiply mercuriated duplexes of the general formula $[Hg_n(d5'\text{-}T_n\text{-}3')_2 - mH]^{l-}$ with $l = m - 2n$, $n = 2-4$ always appear in the presence of mercury. So it may be inferred that two strands can be cross bonded by the bridging of two thymines with Hg(II) with up to four Hg(II)

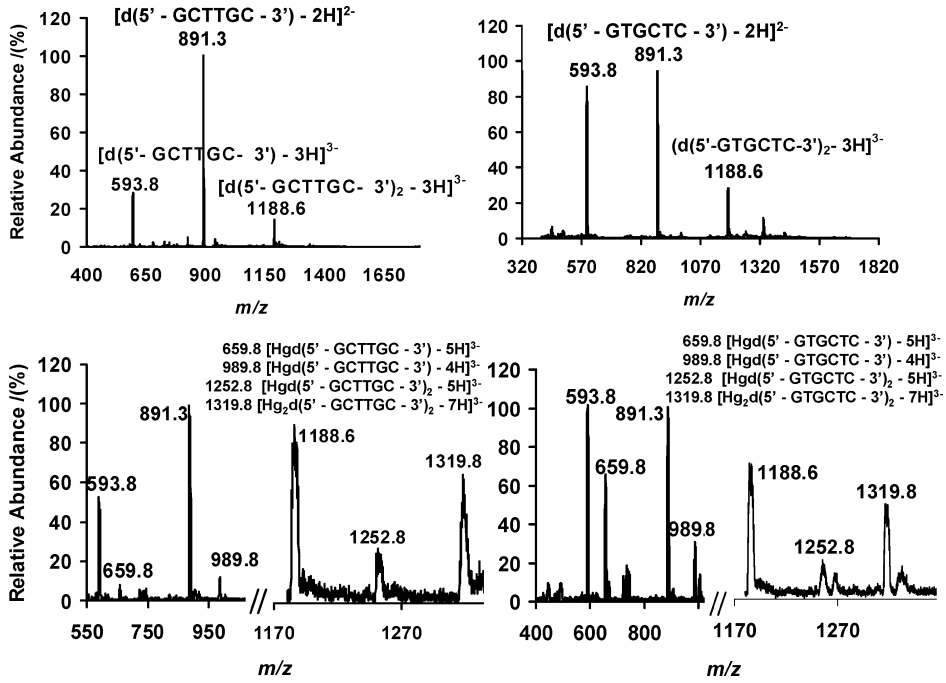
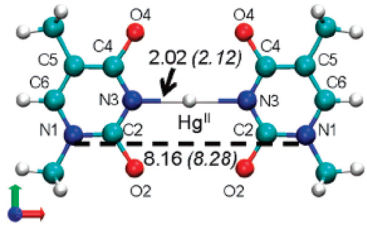


Figure 2. ESI mass spectra of 20 μM solutions of d(5′-GCTTGC-3′) (left) and d(5′-GTGCTC-3′) (right) without (top) and with (bottom) added 0.5 equiv of Hg^{2+}. All the solutions were prepared in 20:80 water:methanol mixtures.

SCHEME 1: Optimized Structure of T-Hg(II)-T[a]



[a] Aromatic rings of nucleobases are in a plane. Selected bond lengths at two levels of theory are shown in Ångstroms. Adopted from ref 17 with permission.

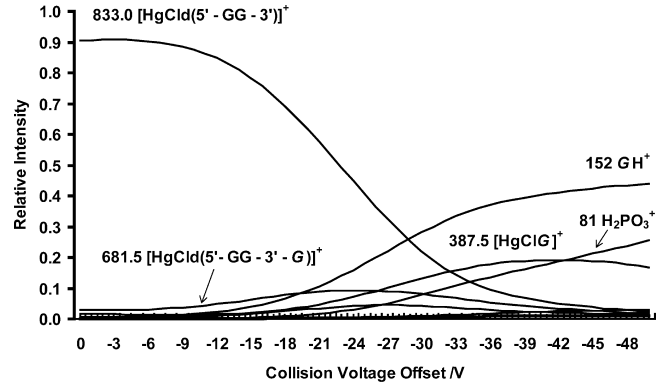


Figure 3. CID profiles for the dissociation of [HgCld(5′-GG-3′]$^+$. The concentrations in solution are as follows: [Hg^{2+}] = 10 mM, and [d(5′-GG-3′)] = 20 mM. *G* (in italics) designates the guanine nucleobase.

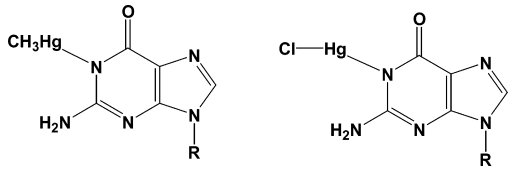
bridges forming between two strands of four thymines each. Such bonding has been described previously by Katz with a model in which Hg(II) coordinates to two thymines with the formation of T−Hg−T accompanied by the displacement of two N3 protons by one mercury dication (see Scheme 1).[11,17] This model has been recently subjected to theoretical modeling which confirmed the suggested Hg(II) coordination.[17]

The pertinent literature data indicate that Hg(II) is capable of interacting with the other three nucleobases.[9] According to the model, AA and CC should not be cross-bonded by Hg^{2+} since no NH protons are available whereas GG might be since one NH proton is present. So, we also investigated the interactions of the mercury dication with the homogeneous dideoxynucleotide sequences d(5′-AA-3′), d(5′-CC-3′), and d(5′-GG-3′) at a concentration ratio identical to that employed in the experiments with the d(5′-TT-3′) dideoxynucleotide. No detectable complexation was observed with d(5′-AA-3′) and with d(5′-CC-3′) in either the negative or positive ion mode. This does not preclude the possibility of Hg(II) complexation with adenine and cytosine-rich sequences at a higher concentration of Hg(II) since earlier studies performed with just adenine nucleobase showed that adenine interacts with methyl mercury at pH 9 and a methyl mercury:adenine ratio of 1:1.[18]

Only one type of mercuriated d(5′-GG-3′) was detected in the positive ion mode under the chosen concentration conditions: [HgCld(5′-GG-3′) − H)]$^+$. Our results for collision-induced dissociation measurements with [HgCld(5′-GG-3′) − H)]$^+$ species indicate that the monocation fragments primarily via the loss of a neutral guanine nucleobase (Figure 3). Surprisingly, no losses of HCl were observed in the applied range of the laboratory collision voltage. Losses of neutral inorganic acids from ion pairs were demonstrated previously.[19] Failure to observe HCl loss suggests the occurrence of direct binding of Cl^- to the mercury dication whose affinity for Cl^- is fairly high. Subsequent dissociation by loss of phosphodisaccaride to form *m*/*z* 387.5 (HgCl*G*)$^-$ implies that Hg^{2+} coordinates to a guanine nucleobase directly.

The literature data that refer to methylmercury interactions with nucleobases show that, depending on the ratio of CH_3Hg: guanine, different coordination compounds can be formed.[9] Under the experimental conditions of the study, the structure would be as indicated in the left side of Scheme 2.[9] This is the structure that was proposed to exist under pH 7−8, which is similar to the conditions in the experiments reported here. By analogy, one can visualize a hypothetical structure for [HgCld(5′-GG-3′) − H)]$^+$ as presented in the right-hand side of

SCHEME 2: Proposed Binding of Methyl Mercury and Inorganic Hg(II) with Guanine Nucleobase



Scheme 2. We have to highlight that the hypothesized structure is supported by the observed dissociation pathways of [Hg-Cld(5′-GG-3′) − H)]$^+$. The initial loss of a neutral guanine is followed by the loss of a neutral deoxyribose with the formation of [HgCl*G*]$^+$. The CID profiles clearly illustrate a higher onset for the formation of this ion. The observation that Hg(II) remains attached to the guanine and Cl^- supports the proposed binding mode.

We can also report that no Hg(II) adducts were observed with d(5′-GCGCGC − 3′) under similar conditions of concentration, with either the single or the double strands. So the cross bonding appears to be specific to TT under our experimental conditions.

For further insight to show that Hg^{2+} interacts with T independent of other, different adjacent nucleobases in the same strand, we investigated the mixed sequences d(5′-GCATGC-3′), d(5′-GCTTGC-3′), and d(5′-GTGCTC-3′) that contain some T.

With GCATGC strands that contain only one T, adducts with one Hg(II) were observed with negligible intensity for both single and double strands. No specific adducts are expected with the double strand since the alignment of the duplex is not mismatched (Watson−Crick type). In the presence of two Ts in two different single strands positioned in such a way so that they face each other in the duplex (a mismatch), as in d(5′-GCTTGC-3′) and d(5′-GTGCTC-3′) that were chosen for study, we observed, as before, only specific duplexes with two Hg(II) (70% conversion), whether the two Ts were adjacent or separated by GC. Only tiny amounts of Hg(II) adducts were observed with single strands (10% conversion).

Both the single- and double-stranded deprotonated hexadeoxynucleotides with d(5′-GCTTGC-3′) and d(5′-GTGCTC-3′) sequences were found to complex with Hg^{2+}. Single-stranded species were found to add at least one mercury dication under our experimental conditions. The ESI spectra of the double-stranded hexadeoxynucleotides (inserts in the bottom spectra) demonstrate the formation of three mercury-containing complexes: $[Hg_n(ODN - (2n+3)H)_2]^{3-}$ with $n = 1-3$ and ODN = d(5′-GCTTGC-3′) and d(5′-GTGCTC-3′). The distribution of these complexes in the ESI mass spectra suggests *specific binding of two mercury dications to two strands*. If Hg^{2+} interacted with d(5′-GCTTGC-3′)$_2$ and d(5′-GTGCTC-3′)$_2$ non-specifically, one could expect $[Hg(ODN - 5H)_2]^{3-}$ to dominate the spectrum, since in all the experiments the concentrations of the ODNs exceeded that of Hg^{2+} by a factor of 2.[20] However, we observed $[Hg_2(ODN - 7H)_2]^{3-}$ to be the most abundant species in the 1200−1700 *m*/*z* ranges of the spectra. We also studied complexation of d(5′-GCATGC-3′)$_2$ and d(5′-GCGCGC-3′)$_2$ with Hg^{2+} under similar concentration conditions (data is not shown) and observed addition of only one mercury dication to the deprotonated d(5′-GCATGC-3′)$_2$ and no addition to d(5′-GCGCGC-3′)$_2$. The latter observation supports sequence specificity of Hg^{2+} interactions with double-stranded oligonucleotides in that Hg^{2+} interacts only with thymine-containing duplexes. Our mass spectrometric measurements provide important information regarding the origin of thymine nucleobases in the T-Hg-T assembly: the data show that Hg^{2+} coordinates with thymines from two different strands rather than two adjacent nucleobases of the same strand. d(5′-GCATGC-3′)$_2$ and d(5′-GCGCGC-3′)$_2$ are Watson−Crick type ODNs while d(5′-GCTTGC-3′)$_2$ and d(5′-GTGCTC-3′)$_2$ contain two TT mismatches. Taking into account the proximity of thymines within d(5′-GCTTGC-3′)$_2$, one cannot exclude the possibility of an intrastrand coordination of Hg^{2+} to the adjacent nucleobase of the same strand. However, in d(5′-GTGCTC-3′)$_2$ thymines are spaced by GC and formation of the link between two nucleobases of the same strand would require significant structural rearrangement which is unlikely to occur. d(5′-GCTTGC-3′)$_2$ and d(5′-GTGCTC-3′)$_2$ were found to produce similar distributions of mercury complexes in their ESI spectra; the dominant species in the 1200−1700 *m*/*z* range of the spectra is $[Hg_2(ODN - 7H)_2]^{3-}$. Therefore, one can expect a similar binding mode (interstrand) for both mismatch sequences (Scheme 1). It is essential to highlight that we incubated mercury chloride with water:methanol solutions of d(5′-GCTTGC-3′) and d(5′-GTGCTC-3′) (data are not shown) without annealing the oligonucleotides to induce the formation of the duplex. No specific adducts of the duplexes with two Hg(II) were observed! This observation suggests sequential insertion of Hg(II) cations into the duplex rather than their interactions with single strands to form bridged structures.

2. Coordination with Hg^{2+}. The preference of the mercury dication to simultaneously coordinate two thymines may be related to its electronic configuration ($[Xe]5d^{10}6s^0$) and the propensity of the transition metal ions with closed d-shell to simultaneously coordinate two rather than just one ligand, as was previously reported in a number of studies.[21,22] The reason for this preference lies in the mechanism of binding that involves s-dσ hybridization.[21,22] s-dσ hybridization can be visualized as the following sequence of steps:

- Once the first ligand is brought to the proximity of the binding metal ion, the occupied dσ orbital of the latter hybridizes with the available empty valence s-orbital. The s-dσ hybridized orbital that was formed is situated along the axis of binding and interacts with the ligand accepting its electron density. On the other hand, the electron pair that originated from the dσ orbital is now localized on the s-dσ orbital situated perpendicular to the axis of binding. Overall, hybridization results in a decrease of the electron density of the metal ion along the axis of binding and as a consequence the repulsion between metal ion and ligand.
- The second ligand can also donate its electron density to the unoccupied s-dσ hybridized orbital and so, in a manner similar to that for the first ligand, experience a reduction in the metal ion−ligand repulsion. *The energetic cost of hybridization therefore is shared by two ligands*.[21,22]

Very recent DFT calculations[17] have demonstrated significant stabilization of the T-T mismatch base pair upon the insertion of a mercury dication between two facing thymines (viz. T−Hg−T). The stabilization is attributed to the interactions of an unoccupied 6p orbital of the metal ion with the 2p orbitals of thymine N3 atoms in the same manner as in a π-system of the stacked nucleobases in DNA. The DFT calculations predicted the T−Hg−T structure to be even more stable than the Watson−Crick A-T hydrogen bonding structure.[17] The computed energy minimum in the T−Hg−T interaction was estimated to be located at around 3.65 Å, which is similar to the distance between A-T nucleobases in natural B-DNA.

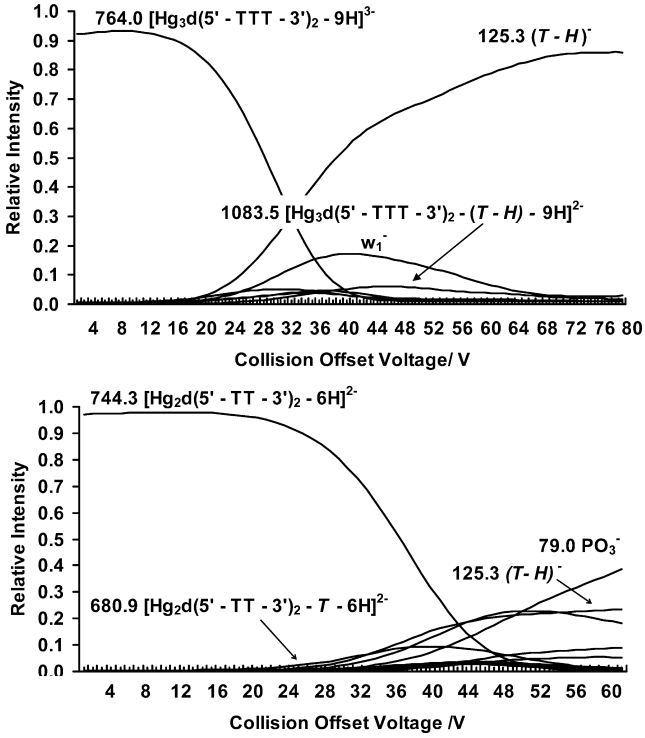


Figure 4. CID profiles for the dissociation of $[Hg_2d(5'\text{-TT-}3')_2 - 6H]^{2-}$ (top) and $[Hg_3d(5'\text{-TTT-}3')_2 - 9H]^{3-}$ (bottom). T designates the thymine nucleobase. w_1^- is one of the fragments resulting from the cleavage of the phosphodiester backbone of the oligonucleotide.[23] Sequence ions nomenclature is presented in Scheme S1 of the Supporting Information.

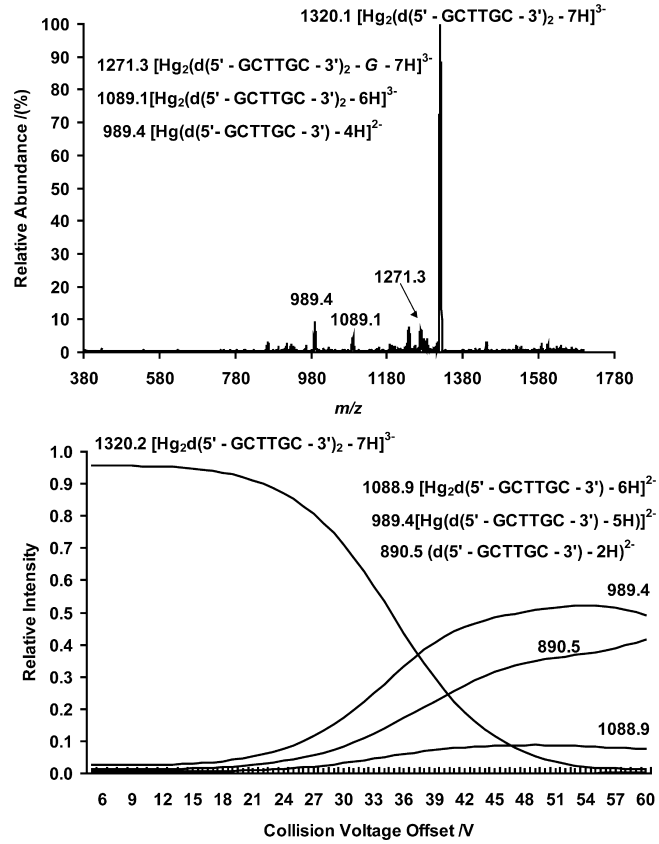


Figure 5. CID spectrum of $[Hg_2d(5'\text{-GCTTGC-}3')_2 - 7H]^{3-}$ at the collision offset voltage averaged for the region 5−20 V (top) and the corresponding CID profiles (bottom). *G* stands for guanine nucleobase. The profiles of the species whose intensities were not larger than 0.05 are not shown.

Therefore, multiple stacking of T−Hg−T structures within a DNA duplex should not significantly modify its structure.[17] These computations significantly strengthen our use of the proposed binding model to rationalize our experimental measurements, especially for the heterogeneous duplexes, since Hg(II) favors linear coordination with two N3 from two different strands. The ionic radius of Hg(II) is optimal among other dications for fitting into the duplex almost without disturbing its helical structure.[17]

3. Tandem Mass Spectrometric Measurements of Mercury(II) Complexes with Homogeneous Thymine Duplexes. Figure 1 demonstrates the abundant formation of mercury-bridged duplexes of pure thymine sequences (d(5′-TTT-3′) and d(5′-TTTT-3′)). Similar types of bridged species were observed also for the homogeneous thymine dideoxynucleotide, $[Hg_2(d(5'\text{-TT-}3')_2 - 6H)]^{2-}$ (data is not shown). Collision-induced dissociation of this ion indicates primarily loss of a neutral thymine nucleobase at a relatively low value of the laboratory collision offset voltage (Figure 4). This dissociation pathway is quite different from the pathway observed for the deprotonated duplex $(d(5'\text{-TT-}3')_2 - H)^-$ (data not shown) and the duplex−metal ion complexes $[M(d(5'\text{-TT-}3')_2 - 3H]^-$ ($M = Mg^{2+}, Pb^{2+}$) in that it involves the loss of a neutral molecule rather than strand separation as was observed for the other thymine dideoxynucleotide-containing species.

As expected, the formation of what presumably are covalent bonds between N3 of the four thymines of the two strands and two mercury dications changes the dissociation pathway of this small duplex from noncovalent strand separation to the loss of a neutral nucleobase.

Similarly, the lowest-energy primary dissociation pathway for the mercuriated duplex of the thymine *tri*deoxynucleotide was found to involve the loss of a thymine nucleobase. However, in the case of this triply charged species ($[Hg_3d(5'\text{-TTT-}3')_2 - 9H]^{3-}$) thymine was lost in its deprotonated form as the result of a charge separation process according to the following equation:

$$[Hg_3d(5'\text{-TTT-}3')_2 - 9H]^{3-} \rightarrow (T - H)^- + [Hg_3d(5'\text{-TTT-}3')_2 - (T - H) - 9H]^{2-}$$

Apparently the strong Coulomb repulsion experienced by the three permanent charges on the anion thermodynamically favors the charge separation over the loss of a neutral thymine. This observation is consistent with literature reports regarding the CID pathways of synthetic single-stranded oligonucleotides in different charge states.[23] The covalent cleavage observed for $[Hg_3d(5'\text{-TTT-}3')_2 - 9H]^{3-}$ also contributes to our suggestion that Hg(II) dications bridge the two strands via covalent bonds with the three thymines. Supporting this suggestion is the dissociation pathway observed for the self-assembling duplex of the d(5′-TTT-3′) at *m*/*z* 1694.2 (data not shown). Similarly to the studied hydrogen-bonded ODNs,[13] the fragmentation of the latter was found to include strand separation at a very low value of the laboratory collision voltage.

4. Tandem Mass Spectrometric Measurements of Hg(II) Complexes with d(5′-GCTTGC-3′) Duplexes. Figure 5 includes the CID spectrum of the doubly mercuriated d(5′-GCTTGC-3′) duplex averaged for the laboratory collision offset voltage between 5 and 20 V (top) along with the corresponding CID profiles (bottom). For a better understanding of the

TABLE 1: Tangent Voltages (V) (and Their Standard Deviation) for Doubly-Metalated Duplexes of and Melting Points of the Metal Ion−ODN Solutions[a]

M^{2+}	$[M_2d(5'\text{-GCTTGC-}3')_2 - 7H]^{3-}$	$T_{m,exp}$, °C (±0.3 °C)
Mn^{2+}	44.0 ± 0.4 (0.99)	N/a
Fe^{2+}	41.6 ± 0.7 (0.94)	N/a
Co^{2+}	34.7 ± 0.5 (0.78)	18.5
Ni^{2+}	36.1 ± 0.4 (0.81)	N/a
Cu^{2+}	34.1 ± 0.4 (0.77)	18.4
Zn^{2+}	33.8 ± 0.4 (0.76)	N/a
Hg^{2+}	*49.1 ± 0.9 (1.03)*	*22.5*
no M^{2+}	**30.8 ± 0.4 (0.72)**	**18.8**

[a] The corresponding apparent centre of mass (CM) energies (eV) are given in parentheses. The apparent center-of-mass energies were calculated according to $E^{ap}_{CM} = z \times TV \times m_{N2} \times (m_{N2} + m_{ion})^{-1}$, where z represents the charge of the corresponding parent ion.

structural specificity of Hg(II) interactions with TT mismatches in this mixed sequence, we compared the dissociation pathways and relative gas-phase stabilities of the doubly metalated complexes of this duplex and the first-row transition metal dications with the pathways and stability of the doubly mercuriated species. We observed two main dissociation pathways for doubly metalated complexes of the first-row transition metal dications with d(5′-GCTTGC-3′) duplex that are summarized in the equation below (a representative example of the CID profiles of metal dications other than Hg(II) is presented in the Supporting Information, Figure S1):

$$[M_2\text{ds} - 7H]^{3-} \rightarrow \begin{cases} [M\text{ss} - 3H]^- + [M\text{ss} - 4H]^{2-} \\ [M_2\text{ss} - 5H]^- + [\text{ss} - 2H]^{2-} \end{cases}$$

where ds = d(5′-GCTTGC-3′)$_2$ and ss = d(5′-GCTTGC-3′).

$[Hg_2(d(5'\text{-GCTTGC-}3')_2 - 7H]^{3-}$, however, was found to exhibit two additional fragmentation channels (see Figure 5), viz.:

$$[Hg_2\text{ds} - 7H]^{3-} \rightarrow \begin{cases} [Hg\text{ds} - G - 7H]^{3-} + G \\ [Hg_2\text{ss} - 6H]^{2-} + [\text{ss} - H]^- \end{cases}$$

The loss of a neutral guanine nucleobase in one of these channels was followed by the loss of a furfuryl alcohol molecule of the 5′-end of the ODN, indicating that upon dissociation of the doubly mercuriated duplex, covalent cleavage becomes as thermodynamically favored as the noncovalent strands separation. The presence of these two extra pathways can be understood in terms of structural differences between the doubly mercuriated duplex and the doubly metalated complexes of the first-row transition metal dications with d(5′-GCTTGC-3′)$_2$. Assuming that the interactions of the two mercury dications occur as described in Katz's model, we can expect strengthening of the binding between the two strands as the result of covalent cross-linking.[11] Similar dissociation pathways were observed for the $[Hg_2(d(5'\text{-GTGCTC-}3')_2 - 7H]^{3-}$ species.

We conclude that the major fragmentation of doubly mercuriated mismatch duplexes proceeds via strand separation, in spite of the presence of an extra dissociation channel associated with a covalent cleavage and elimination of a neutral guanine. Table 1 provides a comparison of the gas-phase stability of the mercury complex with the gas-phase stabilities of the doubly metalated duplexes of d(5′-GCTTGC-3′) of the first row transition metal dications that were investigated. The experimental results clearly indicate that complexation of the duplex with two mercury ions results in the most significant enhancement (compared to the case of the bare duplex) in the gas-phase stability among all the metal ions. Indirectly, this observation also suggests significant structural differences between the doubly mercuriated duplex and those of the first row transition metal ions.

5. Melting Points of the Metal Ion−ODN Solutions. To compare the solution stabilities of the mercuriated complexes of mixed sequences, we obtained the UV melting curves of the solutions containing the annealed hexadeoxynucleotides d(5′-GCATGC-3′), d(5′-GCGCGC-3′), and d(5′-GCTTGC-3′) (see Table 1). The melting curves were obtained by increasing the temperature continuously from 2 to 30 °C by increments of 0.3 °C. When the temperature of a DNA duplex slowly increases, the latter slowly dissociates into two single strands. This dissociation is accompanied by an increase in UV absorption of the solution at 260 nm, a phenomenon known as hyperchromicity. The midpoint of this transition reflects the melting point of a duplex DNA. To avoid variations in the melting temperatures due to differences in concentrations, care was taken to set the concentrations of all the solutions that were investigated to be the same. The melting points obtained for the solutions of d(5′-GCATGC-3′) and d(5′-GCGCGC-3′) in the absence of Hg(II) were found to be 18.0 and 22.3 °C respectively. There was no change in the melting point of these duplexes upon addition of mercury chloride (17.9 and 22.7 °C, respectively).

Addition of 0.5 equiv of Hg(II) salt to the d(5′-GCTTGC-3′)$_2$ resulted in a significant (almost 4 °C) increase in the melting point of this duplex, indicating a stabilizing effect of mercury dication on the T-T mispair. This result is consistent with the 1D ^{1}H NMR measurements by Miyake et al. in which the dissociation of imino protons of thymine residues in the d(5′-CGCGTTGCCC-3′)/d(5′-GGACTTCGCG-3′) duplex was monitored upon addition of 0.8 and 2 equiv of the divalent mercury salt.[24,25] A significant increase in the melting points of several thymine mismatching duplexes also was demonstrated.[26] Interestingly, our observation of an increase in the melting point of the duplex of d(5′-GCTTGC-3′) upon binding with Hg(II) did not extend to the other metal dications that we studied. No significant influence of Cu(II) or Co(II) upon the noncovalent dissociation of the duplex was observed in the solution phase (see Table 1). In the gas phase, however, we observed stabilization of the duplex upon its complexation with two metal dications. The most significant stabilization was achieved upon complexation with Hg(II). The latter observation further contributes to the hypothesis of specific interaction of mercury dications within the duplexes rich in thymine residues.

The measurements of the melting points in solution provide a means of evaluating the overall strength of hydrogen bonding between the nucleobases from two different strands. On the other hand, the gas-phase measurements of the dissociation of the metalated duplexes provide a means of the influence of metallocomplexation on the binding between two strands.

With Hg(II) we observed an increase in the melting point of the duplex in solution but no change with the other metals (Co(II), Cu(II)) that were studied (see Table 1). This result is consistent with a different mode of interaction of Hg(II) with DNA as suggested by Katz's model. A similar result is obtained for the measured dissociation threshold of the corresponding unsolvated gas-phase species (see Table 1). On the basis of these results, we hypothesize that metal ions other than Hg(II) interact with the phosphate groups from different strands and so increase the stability of the duplex against CID (reflected in the elevated

TV values). The much higher TV value for the mercuriated duplex supports the hypothesis of Hg(II) bridging two thymines from the different strands.

Conclusions

The results of the research presented here provide direct support for Katz's chain slippage model of the binding of Hg(II) to short homogeneous thymine oligonucleotide duplexes.[11] The stoichiometry of the complexes successfully transferred into the gas phase was found to be 1:2 (Hg(II):T) with thymine residues coming from two different strands. Significant differences in the gas-phase dissociation pathways of the bare and metalated thymine duplexes (with metal ions other than mercury) and Hg(II) containing double-stranded species provide further evidence for the specific interactions of thymine-rich duplexes and mercury dications. ESI and ESI-MS/MS measurements with the selected mixed sequences demonstrated preservation of preferential interactions of Hg^{2+} with thymine nucleobases rather than with other binding sites on the ODNs. Thermal denaturation curves of the bare and metalated duplexes offered insights into the relative solution stabilities of the species that were investigated and indicated a significant increase in the thermal stability of the T-T mismatch duplex bound to Hg^{2+}. The solution-phase stability and the gas-phase kinetic stability of the doubly mercuriated d(5′-GCTTGC-3′) duplex were demonstrated to be enhanced in comparison with both the bare and doubly metalated species (other than that involving mercury). Together, these results suggest preservation of the Hg^{2+} preference for thymine residues over other binding sites in mixed sequence oligonucleotides.

ESI-MS/MS has been shown elsewhere to provide invaluable information on secondary structure of DNA and DNA adducts with small molecules and drugs.[26−28] Here we demonstrate the capabilities of ESI-MS/MS for structural elucidation of complexes of DNA with mercury ions. Our results point toward a new methodology for the detection of mismatch base pairs using ESI tandem mass spectrometry. They may also provide an important step toward the advancement in single nucleotide polymorphism (SNP) analysis. SNP is a variation in a DNA sequence that results when a single nucleobase A, T, G, or G in the genome differs between the members of a species. This type of variation in the human genome affects how individuals develop diseases and respond to chemicals, medication, pathogens, and other agents. SNP analysis is believed to become one of the corner stones of "personalized medicine".[29]

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Supporting Information Available: Nomenclature for the fragmentation of nucleic acids. CID profiles. This material is available free of charge via the Internet at http://pubs.acs.org.

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