Gas-Phase DNA: Oligothymidine Ion Conformers

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Interest in producing gas-phase nucleic acid ions has been stimulated by recent mass spectrometry (MS) based sequencing strategies for biomolecules. Recent mass spectra for plasmid and linear DNAs having molecular weights of 10^6 to 10^8 daltons have been recorded. In contrast to the wealth of structural data for nucleic acids in solution, there is no information about conformations of the gas-phase ions. The conformations of protein ions can influence fragment formation; thus, the issue of conformation is important for sequencing nucleic acids by MS. Here, we report the first study of the conformations of a deprotonated DNA oligomer in the gas phase: oligothymidine comprised of ten thymine nucleotides. Using electrospray ionization and ion mobility techniques, we have measured collision cross sections for the (T_{10}^-H)^{4+} - (T_{10}^-H)^{6+} states of oligothymidine with sodium ions attached (Na(T_{10}^- nH)^{n+}), where n = 5 - 7. A sharp structural transition is observed when four or more protons are removed; lower charge states favor compact globular conformers and higher ones favor elongated forms. Addition of a single sodium ion to deprotonated states stabilizes more compact conformers. Conformations that were derived using molecular modeling techniques provide complementary information that allows many important structural features to be delineated.

Our experimental apparatus and procedures have been described previously. Negatively-charged (deprotonated) oligothymidine ions were formed by electrospraying a solution of ammonium hydroxide. Sodium adducts are readily formed, although no sodium was added. Ions were extracted into a high-vacuum region and 30 microsecond pulses were injected into a drift tube containing ~3.0 Torr of helium in order to record ion mobility distributions. The drift times depend on the ions' energy, and thus, the issue of conformation is important for sequencing biomolecules.

Using electrospray ionization and ion mobility techniques, we have observed when four or more protons are removed; lower charge states favor compact globular conformers and higher ones favor elongated forms. Addition of a single sodium ion to deprotonated states stabilizes more compact conformers. Conformations that were derived using molecular modeling techniques provide complementary information that allows many important structural features to be delineated.


(7) Oligothymidine used was synthesized using an Applied Biosystem 391 DNA synthesizer utilizing the phosphoramidite method. The ions studied here are dephosphorylated at the 5' end.

Figure 1. Ion mobility distributions for deprotonated (T_{10}^-H)^{+} (solid lines) and (Na(T_{10}^-H)^{n+}) (dotted lines) ions formed by electrospray ionization. The distributions are shown on a modified time scale (t* z) which normalizes for differences in effective drift fields.
Figure 2. Experimental cross sections for the \(-2\) to \(-6\) states of \((\text{T}_{10}^{-} \cdot n\text{H})^{n-}\) (■) and \(-4\) to \(-6\) charge states of \((\text{NaT}_{10}^{-} \cdot n\text{H})^{(n-1)-}\) (●). The dotted line shows calculated cross sections for triam conformers with charges on adjacent phosphodiester linkages [averages of the data denoted with asterisks (*) in Table 1]. The dashed line shows the calculated cross sections that are obtained when the deprotonation sites are distributed more evenly along \(\text{T}_{10}\). Values plotted for evenly distributed sites correspond to averages of the data denoted with solid squares (■) in Table 1. Example model conformations are shown for the \(-2\) (1,5), \(-4\) (2,4,6,8), \(-5\) (1,2,3,4,5), and \(-5\) (1,2,3,5,7,9) states.

Cross sections are estimated by calculating the average projection when the coordinates are rotated through all possible orientations.

The calculations show that the gas-phase conformation depends strongly on the number and locations of charged sites. Deprotonation should occur along the nine phosphodiester linkages; the number of possible combinations of assigning charges to sites is \(9!/(9-n)!\).

A summary of calculated collision cross sections for several different site assignments is given in Table 1. The effect of varying the position of charges can be seen by considering the \(-5\) state. When charges are assigned at the 1,3,5,7,9 sites, the resulting structures have an average collision cross section of 608 ± 21 Å², near the experimental value (627 ± 6 Å²). Assignment of charges to the 1,2,3,4,5 adjacent sites results in conformers with a much smaller cross section (501 ± 19 Å²).

Although there is some question about the absolute accuracy of methods for calculating cross sections, relative changes in calculated values for different charge states allow us to derive probable charge site assignments. Experimental cross sections for the \(-4\), \(-5\), and \(-6\) states are factors of 1.20, 1.40, and 1.43 times larger than the compact \(-2\) and \(-3\) states. Calculated cross sections for the \(-4\), \(-5\), and \(-6\) states (with charges assigned to adjacent sites) are larger than the \(-2\) result by factors of only \(1.04, 1.08, \) and 1.17, respectively (far below the experimental increases). When charges are distributed more evenly (i.e., 1,3,5,7, 1,3,6,9, and 2,4,6,8 for the \(-4\) state; the 1,2,5,6,9 and 1,3,5,7,9 for the \(-5\) state; and 1,2,4,5,7,8,9 and 1,2,3,4,5,7,8,9 for the \(-6\) state), the
cross sections are distributed more evenly (i.e., 1,3,5,7, 1,3,6,9, and 2,4,6,8 for the \(-4\) state; the 1,2,5,6,9 and 1,3,5,7,9 for the \(-5\) state; and 1,2,4,5,7,8,9 and 1,2,3,4,5,7,8,9 for the \(-6\) state), the

Table 1. Experimental and Calculated Data for \((\text{T}_{10}^{-} \cdot n\text{H})^{n-}\) Ions

<table>
<thead>
<tr>
<th>charge state</th>
<th>charged site assignment</th>
<th>(\sigma_{\text{exp}}^{b}(\text{Å}^{2}))</th>
<th>(\sigma_{\text{calc}}(\text{Å}^{2}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>(-1)</td>
<td>(-2)</td>
<td>(2)</td>
<td>(4)</td>
</tr>
<tr>
<td>(-2)</td>
<td>(-5)</td>
<td>(4)</td>
<td>(6)</td>
</tr>
<tr>
<td>(-3)</td>
<td>(-6)</td>
<td>(2)</td>
<td>(4)</td>
</tr>
<tr>
<td>(-4)</td>
<td>(-7)</td>
<td>(1)</td>
<td>(3)</td>
</tr>
<tr>
<td>(-5)</td>
<td>(-8)</td>
<td>(1)</td>
<td>(3)</td>
</tr>
<tr>
<td>(-6)</td>
<td>(-9)</td>
<td>(1)</td>
<td>(3)</td>
</tr>
</tbody>
</table>

\(\sigma_{\text{exp}}^{b}\) Charge site assignment denotes the phosphate between the first two residues on the 5′ end as site 1. \(\sigma_{\text{calc}}\) Cross section derived from ion mobility data. Calculated cross sections for conformers generated by molecular modeling. Uncertainties are in parentheses.

The transition to more open conformers is driven by an increase in coulomb repulsion energy for high charge states; this disrupts hydrogen-bonding and van der Waals interactions that favor more compact structures. Examination of the model structures where charges are dispersed evenly or placed adjacent on \(\text{T}_{10}\) provides insight about the interactions that define the \(\text{in vacuo}\) conformations. When charges reside at adjacent sites, the conformer is essentially linear over the deprotonated region and globular over the remaining portion. An example is displayed for \(-5\) (1,2,3,4,5). The linear section results from large coulombic energies in the region of neighboring charges. Globular portions of these conformers typically contain several base stacking interactions and hydrogen bonds.

When charges are distributed more evenly throughout the oligomer, such as \(-5\) (1,3,5,7,9) also shown the conformation opens up with relative uniformity. The importance of electrostatic interactions is consistent with the differences in cross sections near the structural transition region observed for \((\text{T}_{10}^{-} \cdot 4\text{H})^{4-}\) and \((\text{NaT}_{10}^{-} \cdot 5\text{H})^{5-}\). Although \(\text{Na}^{+}\) is physically larger than a proton, the cross sections for \((\text{NaT}_{10}^{-} \cdot 5\text{H})^{5-}\), \((\text{NaT}_{10}^{-} \cdot 4\text{H})^{4-}\), and \((\text{NaT}_{10}^{-} \cdot 4\text{H})^{4-}\), are smaller than the corresponding charge states for deprotonated \(\text{T}_{10}\).

The localized positive charge on the \(\text{Na}^{+}\) leads to more compact conformations.

The conformations of nucleic acids in solution are governed by a combination of hydrophobic interactions and hydrogen bonds (attractive folding forces) and repulsive electrostatic forces. When denatured nucleic acids undergo a cooperative unfolding transition. In the gas phase, compact conformations are also held together by hydrogen bonds and van der Waals forces and are similarly disrupted by electrostatic interactions.

In the absence of solvent and counterions the multiple charges repel one another causing the gas-phase nucleic acid to unfold. The unfolding transition occurs when coulomb repulsion exceeds the hydrogen bonding and van der Waals interactions between residues. Here, this transition occurs when \(~\sim 50\%\) of the phosphodiester linkages are charged.

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