Gas-Phase Conformations and Folding Energetics of Oligonucleotides: dTG^- and dGT^-

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The use of mass spectrometry to study biomolecules in the gas phase has rapidly progressed the past few years due to the continuing development of MALDI[1] and ESI[2] ionization sources. Unfortunately, while MALDI-MS and ESI-MS have provided an enormous amount of information on peptides and proteins, they have been less successful in their applications to DNA. The problem is that the polynucleotides tend to fragment, especially the longer chains, thus limiting the size of polynucleotide that can be studied by MS and hindering structural assignments if small changes such as methylation or mutations are to be examined.

The fragmentation of polynucleotides is believed to involve the protonation of a base, which is then eliminated, followed by cleavages of the backbone.[3−6] To verify this mechanism, Hillenkamp and Gross examined the fragmentation of a group of oligonucleotides, including dGTTT, dTGTT, dTTGT, and dTTTG, by metastable decay and H/D exchange.[7,8] They chose this particular group because guanine (G) has the highest proton affinity of the DNA bases, and thymine (T), the lowest.[9] Therefore, G should be the base that is most likely to be eliminated according to the proposed mechanism. While the positive ions did, indeed, show loss of G, the mass spectra of deprotonated $dTGTT^-$ and $dTTGT^-$ showed loss of T instead. It was proposed that $dTGTT^-$ and $dTTGT^-$ formed salt-bridge structures, with two deprotonated phosphates and a protonated guanine, which stabilized the oligonucleotide and prevented the loss of G from occurring. Because so little is known about the structures and energetics of nucleotides in solvent-free environments,[10] these proposed fragmentation mechanisms must be viewed as suggestive only at this stage.

When investigating the fragmentation of biomolecules (or macromolecules in general), it is not only important to consider the composition and sequence of the molecule, but also its low-energy conformations and the energy barriers that separate them. Accordingly, we initiated a series of studies on the gas-phase conformations of a group of oligonucleotides. Although data has been obtained on several di-, tri-, and tetranucleotides, only the dinucleotides dTG^- and dGT^- will be discussed here.

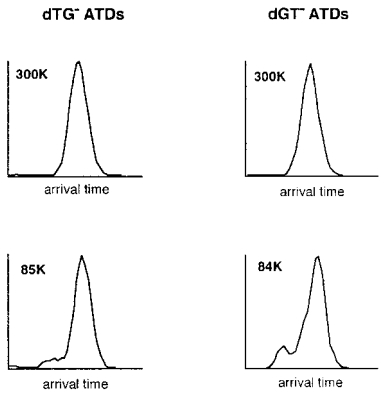

Figure 1. Arrival time distributions for dGT^- and dTG^- at 300 and 80 K. The smaller, shorter time peak is the folded form, and the larger, longer time peak is the open form.

Information about the conformations and folding energetics of these two dinucleotides was obtained from ion mobility[11,12] experiments and molecular modeling calculations. In the experiments, dTG^- or dGT^- ions (formed by MALDI) are injected at low energy into a drift cell[13] containing ~3 Torr of He. The ions are rapidly thermalized by collisions with the gas and drift through the cell under the influence of a weak electric field (5−25 V/cm). Ions exiting the cell are collected as a function of time, yielding an arrival time distribution, or ATD. The ions' drift times are directly proportional to their collision cross-sections.[14] Compact ions with small cross-sections drift faster than more extended ions with larger cross-sections. Thus, different conformers will appear as different peaks in the ATDs if their cross-sections are significantly different (>2%)[15] and they do not rapidly interconvert while they drift.[16]

A series of annealings and energy minimizations,[16] using AMBER 4.0 programs[17] with updated nucleotide parameters,[18] were used to generate 150 low-energy structures for each dinucleotide. The angle-averaged collision cross-section of each structure was then calculated using previously developed Monte Carlo algorithms.[15] Scatter plots of cross-section versus energy were then used to help identify the ions observed in the ATDs.

ATDs for dTG^- and dGT^- are shown in Figure 1. At 300 K, only one peak appears in the ATDs, but the 80 K ATDs clearly show two peaks, indicating the presence of two conformers. The drift times of the two peaks correspond to a difference in cross-section of 20 ± 2 Å^2.

Theory also predicts two families of structures with cross-sections differing by 23 ± 5 Å^2. Examples of each family are shown in Figure 2. The "folded" family, with the smaller cross-section, is characterized by the interaction between the two bases: a "π-stacked" arrangement for dTG^- and a quasi-perpendicular arrangement for dGT^-. In both cases, there is a hydrogen-bond interaction between the NH_2 group on guanine

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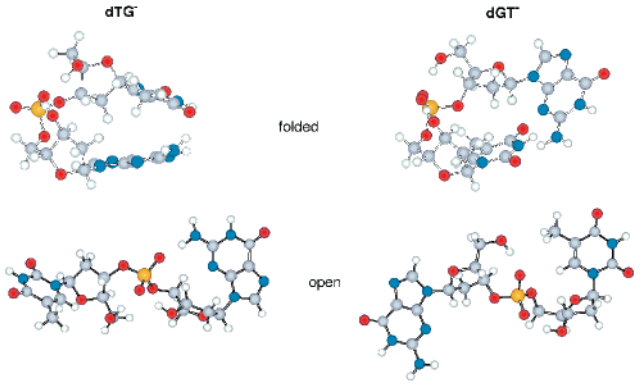

Figure 2. Low-energy structures of the folded and open forms of dTG^- and dGT^-. Carbon atoms are gray, oxygens are red, nitrogens are blue, hydrogens are white, and the phosphorus atom is orange.

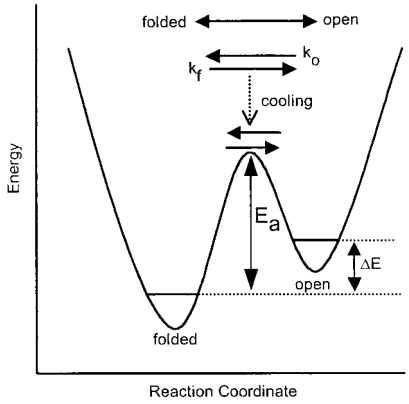

Figure 3. Reaction coordinate diagram for the isomerization of the folded and open forms of dTG^- and dGT^-.

and a carbonyl oxygen on thymine. The "open" family, on the other hand, is primarily stabilized by hydrogen bonds between the phosphate oxygens and the terminal OH groups. A third H-bond between a phosphate oxygen and the NH_2 group on guanine is also theoretically predicted for dTG^- (but not dGT^-).

The fact that two peaks are observed in the 80 K ATDs but only one in the 300 K ATDs indicates that the two forms rapidly isomerize at the higher temperatures. A reaction coordinate diagram qualitatively describing the isomerization is given in Figure 3.

At 300 K, the average energy in the system is well above the isomerization barrier, and the two forms can rapidly interconvert (leading to a single peak in the ATD at a weighted average drift time). As the temperature is lowered, the average energy in the system decreases and eventually becomes comparable to the barrier height, thus slowing the isomerization. Once below the barrier, the isomerization stops, and the two conformers are essentially frozen out.

The barrier height (E_a) can be determined by fitting the ATDs with a theoretical model based on kinetic theory.[19] In the fits, the only variables are the isomerization rate constants, k_f and k_o. Measurements were made for ATDs between 130 and 250 K and Arrhenius plots of ln k_f versus $1/T$ are given in Figure 4. The slopes of the lines are proportional to E_a, yielding barrier heights of 3.9 kcal/mol for dTG^- and 2.8 kcal/mol for dGT^-. These

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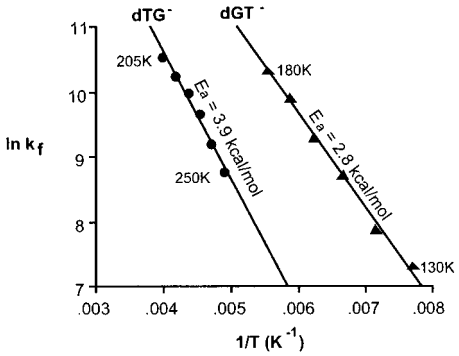

Figure 4. Arrhenius plot of ln k_f versus $1/T$ determined from the ATD fits.

barriers are clearly different and indicate that the sequence of the bases has a definitive influence on the energetics and dynamics of the folding of oligonucleotides.

The relative stabilities of the two forms (ΔE) can also be determined by fitting the relative peak intensities in the 80 K ATDs. At this temperature, it is assumed that the isomerization has stopped and that the relative peak intensities are accurate representations of the actual abundances of the two forms. The densities of states of the two forms were calculated at various values of ΔE until their ratio matched the relative intensities in the ATDs. This yielded a ΔE value of 0.3 kcal/mol for dGT^-. The molecular mechanics (AMBER) calculations gave a ΔE value of ~3−4 kcal/mol for both dTG^- and dGT^-, and subsequent density functional theory[20] (B3LYP/6-31G) optimizations of dGT^- gave a ΔE of 2.4 kcal/mol. The calculated relative energy of the open form of dGT^- is somewhat higher than the experimental value, although this could be due in part to the difficulty in calculating accurate densities of states for systems of this size.

The low-energy conformations given in Figure 2 indicate that base sequence strongly affects conformation, even for simple dinucleotides. To explore the factors that determine conformation, we decided to examine all 16 possible dinucleotides. While the details will be reported later, preliminary results indicate several open conformations and at least three different folded conformations exist: the two folded forms shown in Figure 2 and another folded form with the bases side-by-side (i.e., in the same plane). The isomerization barrier heights between different conformations also vary over a wide range from approximately 0 to 5 kcal/mol. It appears that the degree of hydrogen bonding in a given conformation is the primary determinant in energy.

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