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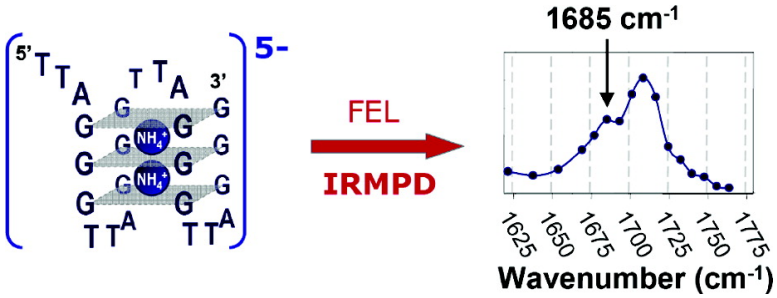

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Infrared Signature of DNA G-Quadruplexes in the Gas Phase

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Nucleic acid secondary structures are determined by hydrogen bonding interactions between nucleic bases. Besides the well-known Watson−Crick base pairing motif, there is a variety of other hydrogen bonding configurations possible. For instance, guanine-rich sequences can fold into G-quadruplex structures owing to Hoogsteen hydrogen bonding between four guanines, forming G-quartets.[1] As evidence is accumulating on in vivo G-quadruplex formation in telomeric DNA and in the promoters of some oncogenes, these structures have become key targets for anticancer strategies.[2]

Electrospray mass spectrometry (ESI-MS) can be used to transfer large biomolecular complexes from the solution to the gas phase.[3,4] However, a longstanding question is whether the gas-phase multiply charged ions produced by ESI-MS keep a folded conformation in the absence of solvent. Infrared (IR) spectroscopy is an ideal approach for studying nucleic acid conformation in solution.[5] In the gas phase, infrared multiphoton dissociation (IRMPD) spectroscopy has been demonstrated for large proteins,[6,7] but the interpretation cannot be reasonably done through comparison with high-level calculations of all possible low-energy conformers.[8] A different investigational approach is needed, based on a thoughtful design of control experiments, as proposed in this study on DNA ions. We studied here the IR signature of two quadruplex-forming sequences: dTG_4T, forming a tetrameric quadruplex $[(dTG_4T)_4{\cdot}(NH_4^+)_3]$ that is highly stable in solution[9] and in the gas phase,[10] and the human telomeric sequence dTTAGGGTTAGGGTTAGGGTTAGGG (noted T_4), forming an intramolecular antiparallel quadruplex in NH_4OAc solution. The IR spectra of DNA negative ions were studied using an electrospray quadrupole ion trap mass spectrometer (Esquire 3000, Bruker Daltonics, Germany) modified to inject an IR beam in the trap through the ring electrode. All experiments were carried out at the CLIO free electron laser (FEL) center (Orsay, France), which provides an intense and continuously tunable source from 5 to 25 μm with a resolution $d\lambda/\lambda \leq 1\%$. IRMPD spectra are recorded by monitoring the relative fragmentation efficiency of mass-selected parent ions as a function of the excitation wavenumber, in the range of 1000−2000 cm^{-1}. Additional experimental details and typical IRMPD mass spectra are provided as Supporting Information.

In order to determine a spectral signature of structural changes, the IR spectrum of the $(dTG_4T)_4$ quadruplex has been recorded and compared to that of the single strand (Figure 1). Given the strand stoichiometry and the selective incorporation of three ammonium cations, there is little doubt about the quadruplex structure of $[(dTG_4T)_4{\cdot}(NH_4^+)_3]^{5-}$. The IR spectrum of the quadruplex exhibits three distinct bands that can be assigned by

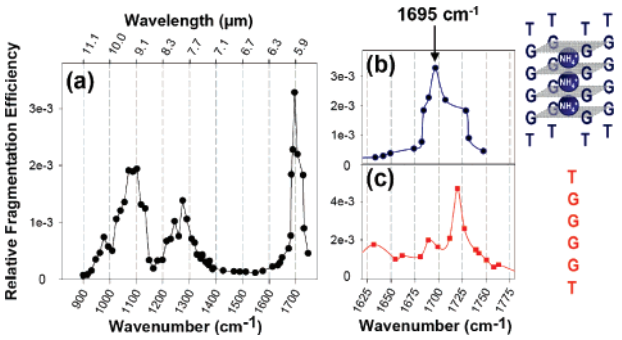

Figure 1. IRMPD spectra of TG_4T. (a) Full spectrum and (b) zoom on the carbonyl stretch region of the quadruplex $[(TG_4T)_4{\cdot}(NH_4^+)_3]^{5-}$. (c) Zoom on the carbonyl stretch region of the single strand $[TG_4T]^{2-}$.

referring to nucleic acid IR bands in solution.[5] The bands centered at 1080 and 1260 cm^{-1} correspond to the symmetric and antisymmetric PO_2^- vibrations, respectively. This explains the high efficiency of IRMPD fragmentation of DNA with CO_2 lasers.[11] The band centered around 1700 cm^{-1} corresponds to C═O stretching modes of the nucleic bases.

The gas-phase IR spectra of the single strand and the quadruplex show no significant differences in the region of the PO_2^- vibrations. In contrast, the C═O stretching mode region shows significant differences between the quadruplex $[(dTG_4T)_4{\cdot}(NH_4^+)_3]^{5-}$ (Figure 1b) and the single strand $[dTG_4T]^{2-}$ (Figure 1c). A band observed at 1720 cm^{-1} for the single strand is shifted to 1695 cm^{-1} in the quadruplex. The band broadening observed in the quadruplex is likely due to a splitting between the unshifted thymine and the shifted guanine contributions. As the base composition of the species compared in the two IRMPD spectra is exactly the same, the red shift can be attributed to the engagement of C═O bonds in hydrogen bonding with other bases and with the ammonium cations. Bending modes of ammonium cations expected at 1460 cm^{-1} with a lower absorption cross section compared to the carbonyl stretches remain undetected because of the low number of active modes compared to the size of the system.

We then turned to the human telomeric sequence T_4 (7575.0 Da) to detect a signature of intramolecular hydrogen bond formation. The full scan mass spectrum of T_4 depends on the acceleration voltages set in the electrospray source (see Supporting Information). Under soft source conditions (low voltages), the major peak is at $m/z = 1520.8$, which corresponds to $[T_4{\cdot}(NH_4^+)_2]^{5-}$ containing the T_4 sequence with two embedded ammonium cations. Under harsher source conditions (higher voltages), the most abundant peak of T_4 appears at $m/z = 1514.0$, without ammonium cations. The more energetic collisions with the residual gas in harsher source conditions result in the loss of the two inner ammonium cations, thereby breaking the native interactions between the guanine quartet and

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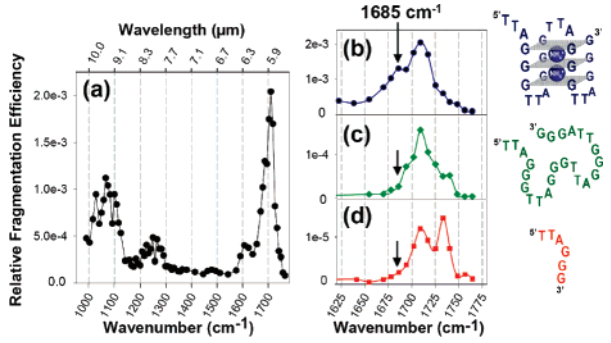

Figure 2. IRMPD spectra of telomeric DNA sequences. (a) Full spectrum of $[T_4{\cdot}(NH_4^+)_2]^{5-}$. (b−d) Zooms on the carbonyl stretch regions of (b) $[T_4{\cdot}(NH_4^+)_2]^{5-}$, (c) $[T_4]^{5-}$, and (d) $[T_1]^{2-}$.

the inner cations. The comparison between the IR spectra of $[T_4{\cdot}(NH_4^+)_2]^{5-}$ and that of $[T_4]^{5-}$ might therefore reveal marker bands of guanine−cation interactions. Furthermore, the hydrogen bonding network between guanine tetrads can also be destabilized because of the absence of cations.[12] To evaluate the hydrogen bonding status of T_4, we used the short single strand dTTAGGG (noted T_1, 1893.7 Da) as a reference. T_1 was added to the solution containing T_4 in 150 mM ammonium acetate. The IRMPD spectra (Figure 2) of $[T_4{\cdot}(NH_4^+)_2]^{5-}$, $[T_4]^{5-}$, and $[T_1]^{2-}$ were recorded simultaneously from the same sample solution, by changing source conditions and the *m*/*z* selected at each wavenumber. Full IR multiphoton dissociation spectra are provided as Supporting Information.

Of the vibrations considered here, the C═O stretching region is again the most sensitive to the secondary structure. For the single strand T_1 (Figure 2d), two bands are observed at 1710 and 1735 cm^{-1}. Vibration frequencies of isolated thymine and of 1−9 ketoguanine have been calculated at the DFT B3LYP(6-311++G**) level and corrected by the scaling factor of 0.9793.[13] The thymine $C_2{=}O_2$ and $C_4{=}O_4$ carbonyl stretches lie at 1761 and 1715 cm^{-1}, respectively, and the guanine $C_6{=}O_6$ mode is calculated at 1753 cm^{-1}. The two bands experimentally observed in the single strand T_1 can thus be assigned, respectively, to the thymine $C_4{=}O_4$ mode and a combination of overlapping guanine $C_6{=}O_6$ and thymine $C_2{=}O_2$ modes that cannot be separated with our resolution. For T_4 (Figure 2c), the large increase in intensity of the 1710 cm^{-1} band compared to the 1740 cm^{-1} transition might reflect the formation of intramolecular hydrogen bonding between guanine bases. Because thymines are not involved in the hydrogen bonding network, their carbonyl stretching vibrations should be barely affected by the formation of intramolecular G-tetrads. Therefore, in $[T_4]^{5-}$, the red-shifted guanine $C_6{=}O_6$ vibration overlaps with the thymine $C_4{=}O_4$ mode around 1710 cm^{-1}. In $[T_4{\cdot}(NH_4^+)_2]^{5-}$ (Figure 2b), the further red shift of the carbonyl band now lying at 1685 cm^{-1} is likely due to the influence of the ammonium cations on the guanine carbonyl stretching modes.

In ion mobility spectrometry,[14] $[T_4]^{5-}$ and $[T_4{\cdot}(NH_4^+)_2]^{5-}$ have a similar collision cross section (789 $Å^2$) that is compatible with a preserved G-quadruplex fold, but the actual status of hydrogen bonding is unknown. Our IR data show significant red shift of the carbonyl band in $[T_4]^{5-}$ as compared to that in T_1 as expected if hydrogen bonds are formed. However, the further decrease of the band at 1735 cm^{-1} in $[T_4{\cdot}(NH_4^+)_2]^{5-}$ compared to that in $[T_4]^{5-}$ suggests that the hydrogen bonding network between the guanines is not fully formed in $[T_4]^{5-}$. This is in line with previous experiments showing that ammonium cations play a role in the gas-phase stability of the G-quadruplex structures.[12] The further red shift of the guanine carbonyl frequency in $[T_4{\cdot}(NH_4^+)_2]^{5-}$ supports the idea that ammonium cations insert between the guanine tetrads and provides strong evidence of G-quadruplex formation in telomeric DNA in the gas phase.

Comparison with FTIR spectroscopy also supports the above assignment. In solution[5] for single-stranded DNA, thymine $C_2{=}O_2$ and $C_4{=}O_4$ carbonyl stretches are observed in D_2O at 1698−1691 and 1655−1671 cm^{-1}, with medium and strong intensity, respectively. The guanine $C_6{=}O_6$ stretch is observed at 1660−1673 cm^{-1} with strong intensity. These bands are significantly red-shifted compared to the gas phase due to hydrogen bonding with the solvent. For G-quadruplexes, a recent study[15] has shown that guanine $C_6{=}O_6$ carbonyl stretching is sensitive to the conformation. The band is consistently found at 1693.3 ± 2.1 cm^{-1} for parallel quadruplexes and at 1681.3 ± 2.3 cm^{-1} for antiparallel quadruplexes. These values are strikingly close to the gas-phase values (1695 and 1685 cm^{-1}, respectively). Gas-phase values being so close to solution-phase values can be explained by the fact that, in both cases, water does not interfere with the $C_6{=}O_6$ hydrogen bonding. Although this hypothesis needs further testing with other DNA hydrogen bonding motifs, such as duplex or triplex structures, the present study paves the way toward gas-phase conformational analysis of DNA ions using their infrared signature.

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Supporting Information Available: Methods and IRMPD tandem mass spectra are available. This material is available free of charge via the Internet at http://pubs.acs.org.

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