Fast Gas Phase H/D Exchange Observed for a G-Quadruplex DNA

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Abstract

The gas phase H/D exchange kinetics of DNA G-quadruplexes has been investigated in an FTICRMS. The quadruplex [$(TGGGGT)_4 \bullet 3NH_4^+$] undergoes very fast H/D exchange, both in the positive and in the negative ion mode, compared to DNA duplexes and other quadruplexes tested, and compared to the corresponding single strand TGGGGT. Substitution of NH_4^+ for K^+ did not alter this fast H/D exchange, indicating that the hydrogens of the ammonium ions are not those exchanged. However, stripping of the inside cations of the quadruplex by source-CID in the positive ion mode showed that the presence of the inner cations are essential for the fast exchange to be possible. Molecular dynamics simulations show that the G-quadruplex is very rigid in the gas phase with NH_4^+ ions inside the tetrads. We suggest that the fast H/D exchange is favored by this rigid quadruplex conformation. This example illustrates that the concept that compact DNA structures exchange H for D slower than unfolded ones is a misconception.

Introduction

In 1993, the first reports on the observation of a DNA duplex by electrospray ionization were published[1;2]. Since then, ESI-MS has demonstrated its capabilities to detect various intact noncovalent DNA complexes[3;4], among which are DNA duplexes, quadruplexes[5;6] and triplexes[6]. Of particular interest is the use of ESI-MS for screening drug targeting these different structures[3;4;7-14]. Several reports have shown an excellent agreement between solution-phase binding affinities and the relative peak intensities in the ESI-MS spectra[14;15]. It is important to note that the conservation of the structure of the complexes in the gas phase is not required for the ion abundances in the ESI-MS spectra to reflect the composition of the solution, as long as the partners remain together in some way during their travel from the source to the mass analyzer. Consequently, the good correlations between solution data and ESI-MS observations can not be considered as a proof that the structures in the gas phase are the same as the structures in solution.

The question of the relationships between the solution and the gas phase structures is currently a central question in the mass spectrometry community. If the gas phase structures were the same as the solution phase ones, then mass spectrometric techniques could be used to get structural information in addition to the detection or quantification of the complexes. Several mass spectrometric techniques are available to probe directly or indirectly the gas phase structures of the complexes. The most readily available method is MS/MS. Collision-induced dissociation and BIRD experiments on different DNA duplexes suggested that the A-T and G-C hydrogen bonds and the base stacking interactions were conserved in the gas phase[16-18]. However MS/MS provides only very indirect information on the structures, as it depends on the dissociation rate of the complex, rather than on its stable conformation.

Several techniques exist to probe the gas-phase equilibrium conformations of biomolecules. In this paragraph we will shortly overview the different studies on the gas-

phase structures of DNA. Ion mobility spectrometry[19;20] allows probing a molecule's cross section, and therefore its relative folding/unfolding. Bowers et al. applied ion mobility spectrometry to the study of the conformations of di- and tri-nucleotides[21;22], and more recently to larger DNA duplexes and quadruplexes[23]. They showed that the duplexes are formed from (CG)n single strands where n=2 to 9. Globular structures dominate for n=2,3 and 4 while helices appear at n=4 (weak) and dominate for n= 5, 7 and 9. Parks et al. developed instrumentation to measure fluorescence resonant energy transfer (FRET) in an ion trap mass spectrometer[24]. FRET allows probing the distance between a donor and an acceptor group covalently attached to DNA. They applied this technique to the study of DNA duplexes, and their unzipping in particular[25]. Finally, some studies using gas-phase H/D exchange have been published. Hofstadler and Griffey[4] reported some measurements on DNA duplexes and the corresponding single strands, and showed that H/D exchange was slower for the duplex than for the single strands. Vairamani and Gross[26] recently reported H/D exchange on the thrombin binding aptamer GGTTGGTGTGGTTGG, which is known to form a quadruplex in solution with the inclusion of a cation. During gas phase H/D exchange in a quadrupole ion trap, they showed that less hydrogens were exchanged for deuterium for the monocationic adducts of the aptamer, and concluded that the K^+- or Sr^{2+}-bound aptamer had more compact structures (possibly quadruplex structures) than the aptamer with no cation.

In our study, we were initially interested in probing the conformation of quadruplexes in the gas phase, and in particular in probing the structure of the telomeric intramolecular quadruplex. In the course of the study which included other DNA duplexes and quadruplexes, a dramatically fast H/D exchange kinetics for the quadruplex [$(TGGGGT)_4 \bullet 3NH_4^+$] was measured. This prompted us to investigate this phenomenon in more detail, and the results are reported in the present paper.

Experimental section

Materials

All single stranded oligodeoxyribonucleotides were purchased from Eurogentec (Angleur, Belgium). Duplex and quadruplex solutions were prepared in 150 mM NH_4OAc (pH = 7.0). The oligonucleotides were used as received, without further desalting. Solutions were heated to 80 °C and cooled overnight to form the duplexes and quadruplexes. All DNA forms were injected in the mass spectrometer at a concentration [duplex] or [quadruplex] = 20 μM. For the quadruplex $(TGGGGT)_4$, we observed that annealing is not necessary, and that the tetramer forms in a few minutes at room temperature in 150 mM ammonium acetate. The DNA G-quadruplexes $[(TGGGGT)_4 \bullet (3-n)NH_4^+ \bullet nK^+]$ were generated simultaneously in a solution containing 20 μM quadruplex (i.e. 80 μM single strands), 150 mM NH_4^+ and 70 μM K^+, incubated at room temperature.

Mass Spectrometry

Experiments were performed with a modified APEX III Fourier transform mass spectrometer (Bruker Daltonics, Billerica, MA, USA) equipped with a 7 Tesla actively shielded superconducting magnet (Bruker Biospin, Wissembourg, France). A quadrupole mass filter and a collision cell containing a linear hexapole ion trap was used between ion source and the ion transfer optics and the ICR cell. For the electrospray ion generation a combined ion source[27] was used in the electrospray operation mode. Electrospray-generated ions were accumulated in a linear hexapole trap. Ions were accumulated for 5 seconds for each scan in the hexapole of the ion source; 8 scans have been added for each mass spectrum. The ion source parameters have been set to soft ionization conditions for best intensities of the complex. The DNA concentration (quadruplex and duplex) was 20 μM infused at 2 μL/min

with a syringe pump. Liquid CD_3OD with a purity of 99.8% was purchased from SIGMA-Aldrich (Steinheim, Germany). Te reactant CD_3OD was degassed employing multiple freeze-pump-thaw cycles. Gaseous CD_3OD was leaked into the analyzer using a leak valve. The experiments have been performed with a partial pressure of CD_3OD of 8.0 +/- 1.0 x 10^{-9} mbar. For kinetic experiments ions are injected into the ICR cell and after a certain reaction time with CD_3OD up to several minutes the ions have been detected.

Molecular dynamics

Molecular dynamics simulations were performed on the parallel quadruplex $d(TGGGGT)_4$ with 3 ammonium cations coordinated between the tetrads. The starting structure for the quadruplex was generated from the X-ray structure of $[(TGGGGT)_4 \bullet 3Na^+]$ (PDB code: 1DJ4). The three sodium cations between the tetrads were replaced by ammoniums (with a net charge of +1 per NH_4). In conformity to our electrospray experiments, a net charge of 5- was assigned to the complex $[(TGGGGT)_4 \bullet 3NH_4^+]$ (5- is the predominant charge state). The charge of the quadruplex was adapted using Orozco's protocol[28]: as there is no information on the localization of the charges in the quadruplex, the total charge of the phosphates was equally distributed along all phosphates. As each ammonium has a net charge of +1, the 20 phosphate groups have to share 8 negative charges so that the whole complex has 5 negative charges. The phosphate charge scaling factor was therefore equal to 8/20.

Calculations were carried out using the AMBER7[29] software suite, using the AMBER99[30;31] force field. The starting structure generated by replacing the sodium ions by the ammonium ions was first optimized to a RMS gradient of 0.5 kcal/Å mol) before starting the unrestrained molecular dynamics simulation itself. The MD simulation was performed *in vacuo* at 298 K for 10 ns, with no constrains, and a time step of 0.1 fs for the first 800 ps and 0.3 fs time step from 800 to 10 000 ps.

Results and Discussion

Among the different DNA duplexes and quadruplexes we investigated by gas-phase H/D exchange in the FTICR cell, one particular quadruplex, $[(TGGGGT)_4 \bullet 3NH_4^+]$ (Figure 1) shows a very different behavior from the other structures. Figure 2 shows the spectra of the duplex $[(CGCGAATTCGCG)_2]^{5-}$ and of the quadruplex $[(TGGGGT)_4 \bullet 3NH_4^+]^{5-}$ recorded after different times spent in the ICR cell with a partial pressure of CD_3OD of $(8.0 \pm 1.0) \times 10^{-9}$ mbar. The isotopic distribution of the duplex is slowly spreading as the reaction time increases, similarly to what has been reported in other studies[4]. The exchange kinetics of the single stranded GGGGTTTTGGGG, of the quadruplex $(GGGGTTTTGGGG)_2$, and of the telomeric DNA sequence GGGTTAGGGTTAGGGTTAGGG (which is susceptible of forming a quadruplex) were all similar to the exchange kinetics shown for the duplex in Figure 2a. Only the quadruplex $[(TGGGGT)_4 \bullet 3NH_4^+]$ showed a particularly fast exchange kinetics (Figure 2b), with very little spreading of the isotopic distribution.

To investigate the exchange kinetics in more details for this quadruplex, we plotted the mean number of deuterons incorporated as a function of the reaction time, and fitted the results with an equation corresponding to an exponential rise to maximum. The plots are shown in Figure 3, and the results of the fittings are given in Table 1. For the single stranded $TGGGGT^{2-}$, a single component exponential was sufficient to fit the data satisfactorily. For the two charge states 4- and 5- of the quadruplex, however, a 2-component fitting was obviously better than a single component fitting. The most surprising result is that for the charge states 4- and 5- of the quadruplex, respectively 16.8 ± 0.8 and 25.3 ± 0.5 hydrogens are exchanged for deuterons with a time constant around 3 s, which is extremely fast compared to the other duplexes or quadruplexes of the same size (having time constants in the range 25-80 s), and given the very low partial pressure of CD_3OD achieved in the ICR cell.

The second component is slower. It is noteworthy that the kinetics of the slow component for the charge state 5- is very similar to that of the single stranded $TGGGGT^{2-}$.

We suspected that the ammonium ions present inside the structures of the quadruplex were responsible for the unusually fast H/D exchange component. A good test would have been to compare with a different quadruplex having ammonium ions inside. Unfortunately our attempts to produce the quadruplex $(GGGGTTTTGGGG)_2$ with ammonium ions still inside were unsuccessful, presumably due to the lability of the ammonium ions in that structure[6], and to dissociation during the long storage times needed in the transfer hexapole. Another test is the comparison with the same quadruplex $(TGGGGT)_4$, but without the ammonium cations inside. Unfortunately again, for that quadruplex, in the negative ion mode the ammonium ions are so tightly bound that it is impossible to obtain source-CID or MS/MS conditions that allow stripping the ammonium ions off the tetramer without destroying the tetramer itself.

In the positive ion mode, however, stripping of the ammonium ions by source-CID is feasible. H/D exchange spectra have been recorded in conditions of partial source-CID allowing the simultaneous observation of the quadruplex with its 3 ammonium ions, and without ammonium ions. The results are presented in Figure 4 for the charge state 5+. Similarly to the negative ion mode, the quadruplex $[(TGGGGT)_4 \bullet 3NH_4^+]$ with charge states 4+ and 5+ also exchanges H/D very extensively and very fast. The results of the fittings for the ammonium-bound quadruplex in the positive ion mode are also included in Table 1. Comparatively, the exchange of the tetramer without the ammonium ions inside is dramatically slowed down, and is more similar to a "normal" behavior. We can therefore conclude that the presence of the ammonium cations inside the quadruplex structure is required to observe a fast H/D exchange.

A question that arises at this point is whether the hydrogen atoms of the ammonium (NH_4^+) are exchanged themselves or if the exchanged hydrogens are coming from the DNA

strands. We therefore tested the replacement of ammonium ions by potassium ions. The quadruplex $(TGGGGT)_4$ was prepared at concentration 20 μM in 150 mM ammonium acetate and 70 μM KCl. As the preference for K^+ over NH_4^+ is about 500-fold, this allows the formation of the quadruplex forms $[(TGGGGT)_4 \bullet (3-n)NH_4^+ \bullet nK^+]$, with n = 0-2. The H/D exchange kinetics for the charge state 5- is shown in Figure 5. In this case the spacing between the isotopic distributions is constant, indicating that the rate of H/D exchange is independent of the nature of the cation present inside the quadruplex.

The different H that can be exchanged in the quadruplex are situated on the phosphates (5 per strand), on the sugars at the extremities of the strands (2 per strand), on the guanines (3 per guanines, 12 per strand), on the thymines (1 per thymine, 2 per strand), and on the ammonium ions (4 × 3 = 12). The replacement of NH_4^+ by K^+ excludes the possibility of ammonium hydrogen exchange. Also, in the quadruplex structure, the exchangeable hydrogens on the guanines are not all available: 2 per guanine are involved in hydrogen bonding within the tetrads (Fig. 1a); only one H per guanine is accessible in the grooves of the quadruplex. The localization of the H/D exchange sites based solely on the deuterium incorporation count can be hazardous, but it is interesting to note that in the positive ion mode, for both charge states there are 28 fast H/D exchange sites. This could correspond to 7 exchanges per strand (the 5 phosphate O–H + the 2 sugar O–H). It must be underlined that, in the positive ion mode, the protonation sites are not known. If we attribute formally 3 positive charges to the ammonium ions, 1 or 2 protons remain to be attached, possibly on the bases themselves. However all phosphate groups are neutralized, giving a total of 28 exchangeable hydrogens on the backbone of the strands. This simple counting suggests that the fast H/D exchange sites are located on the phosphates and the sugars. In the negative ion mode, the number of fast H/D exchange sites is < 28, which is compatible with the fact that part of the phosphates are deprotonated. Additional refinements on the exchange sites location based on

simple site counts would be too speculative. In particular, the reason for the very different behavior of the charge states 4- and 5- is not elucidated yet.

The present study underlines that the interpretation of gas-phase H/D exchange kinetics is not as simple as the interpretation of H/D exchange in solution. In solution, the solvent is always "in excess" and the exchange rate depends primarily on the accessibility of the basic sites to the bulk solvent. In the gas-phase, a basic site encounters only one deuterated solvent molecule at a time, and the exchange kinetics depends not only on the site accessibility, but also on the availability of a neighboring site that can act as a relay in the H/D transfer mechanism[32;33]. The case of the quadruplex DNA we studied here represents an extreme case where a very compact structure promotes an unusually fast H/D exchange.

The rigidity of the cation-bound quadruplex structure has been investigated by molecular dynamics. By comparing cation-bound and cation-free quadruplexes, Spackova et al.[34] showed that the central cations K^+ and Na^+ are essential to maintain the stable and rigid quadruplex structure (the case of ammonium ions was not investigated). The rigidity of the cation-bound quadruplexes is exceptional compared to most other nucleic acid forms. For example, molecular dynamics on duplex DNA in the gas phase[28] have shown that the double helix progressively unwinds (elongates), although the Watson-Crick hydrogen bonds are maintained. To complete the description of the quadruplex [$(TGGGGT)_4$•$3NH_4^+$] in the gas phase, we performed unrestrained molecular dynamics for 10 ns in the gas phase, with 5 negative charges. The simulation procedure was the same as used by Rueda et al.[28] for their duplexes. The structure of the quadruplex obtained after 10 ns simulation is shown in Figure 1(c), and more details on the evolution of particularly interesting bond distances are given in Figure 6. During the simulation, the Hoogsteen type hydrogen bonds N_1–H_1···O_6 are retained. All four tetrads are still intact after 10 ns. The average hydrogen bond distances (1.87 Å in the first tetrad and 1.93 Å in the second) are similar to the distances in the starting X-ray crystal

structure of the sodiated quadruplex (1.91 Å and 1.90 Å respectively). The distances between the ammonium cations and the O_6 of the neighboring guanines has also been monitored (Fig. 6 d-g), and the results show that the ammonium cations stay remarkably in place between the tetrads.

In conclusion, although very compact and rigid, the DNA quadruplex [$(TGGGGT)_4 \bullet 3NH_4^+$] shows very fast H/D exchange in the gas phase. This example illustrates that the picture according to which compact DNA structures exchange H for D slower than unfolded ones is too simplistic. In the case of the quadruplexes, we believe that the inner cations do lock the exchange sites and the relay sites in a conformation that is particularly favorable to H/D exchange.

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Table

Table 1. Fitting (number of H exchanged for D and time constants) of the gas phase H/D exchange kinetics (Figure 3) of the quadruplex $[(TGGGGT)_4 \bullet 3NH_4^+]$ (charge states 4-, 5-, 4+ and 5+) and of the corresponding single strand $TGGGGT^{2-}$. Fittings were done with an equation representing an exponential rise to maximum: $y = \sum_{i=1}^{1or2} a_i*(1-\exp(-b_i x))$. a_i gives the number of H/D exchanges, and $1/b_i$ gives the time constants. The components have been classified in fast ($1/b_i$ < 10 s), medium (10 s < $1/b_i$ < 100 s), and slow ($1/b_i$ > 100 s) components to facilitate comparisons.

		$[(TGGGGT)_4 \bullet 3NH_4^+]^{4-}$	**$[(TGGGGT)_4 \bullet 3NH_4^+]^{5-}$**	**$TGGGGT^{2-}$**	**$[(TGGGGT)_4 \bullet 3NH_4^+]^{4+}$**	**$[(TGGGGT)_4 \bullet 3NH_4^+]^{5+}$**
Type of fit		2-component	2-component	1-component	2-component	2-component
Fast component	**# H/D**	16.8 ± 0.8	25.3 ± 0.5	-	28.3 ± 0.6	28.2 ± 0.6
	Time constant	2.9 ± 0.5 s	3.01 ± 0.14 s	-	0.90 ± 0.03 s	0.79 ± 0.03 s
Medium component	**# H/D**	23.2 ± 0.5	-	-	13.5 ± 0.5	7.4 ± 0.5
	Time constant	83 ± 14 s	-	-	12.5 ± 1.8 s	11.7 ± 3.0 s
Slow component	**# H/D**	-	6.2 ± 1.2	7.7 ± 1.6	-	-
	Time constant	-	450 ± 250 s	500 ± 250 s	-	-

Figures

Figure 1.

(a) Structure of a guanine tetrad, and (b) schematic structure of the parallel quadruplex $[(TGGGGT)_4 \bullet 3NH_4^+]$, and (c) structure of the $[(TGGGGT)_4 \bullet 3NH_4]^{5-}$ obtained after 10 ns of unrestrained molecular dynamics. G-tetrads are shown in dark grey sticks and the ammonium cations in light grey sticks. The DNA backbone and the thymine bases are left in wire frame.

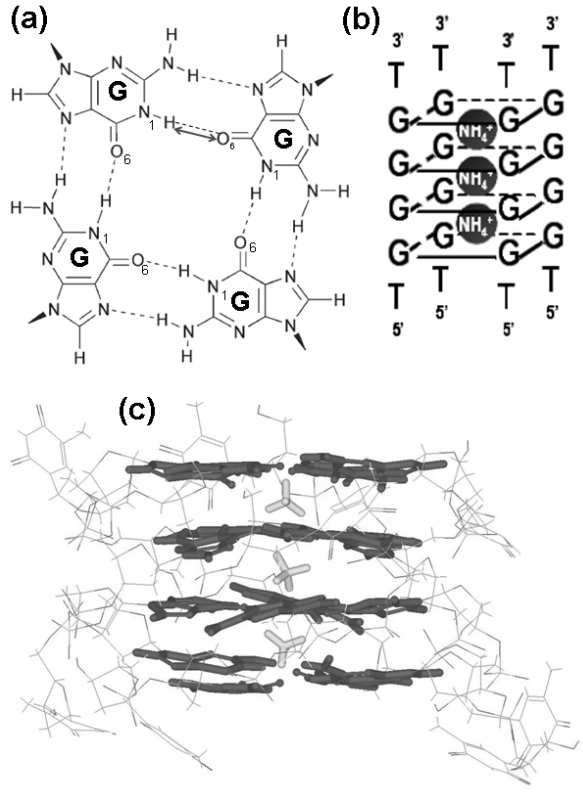


Figure 2.

Gas phase H/D exchange (a) on the DNA duplex $[(CGCGAATTCGCG)_2]^{5-}$ and (b) on the DNA G-quadruplex $[(TGGGGT)_4 \bullet 3NH_4^+]^{5-}$.

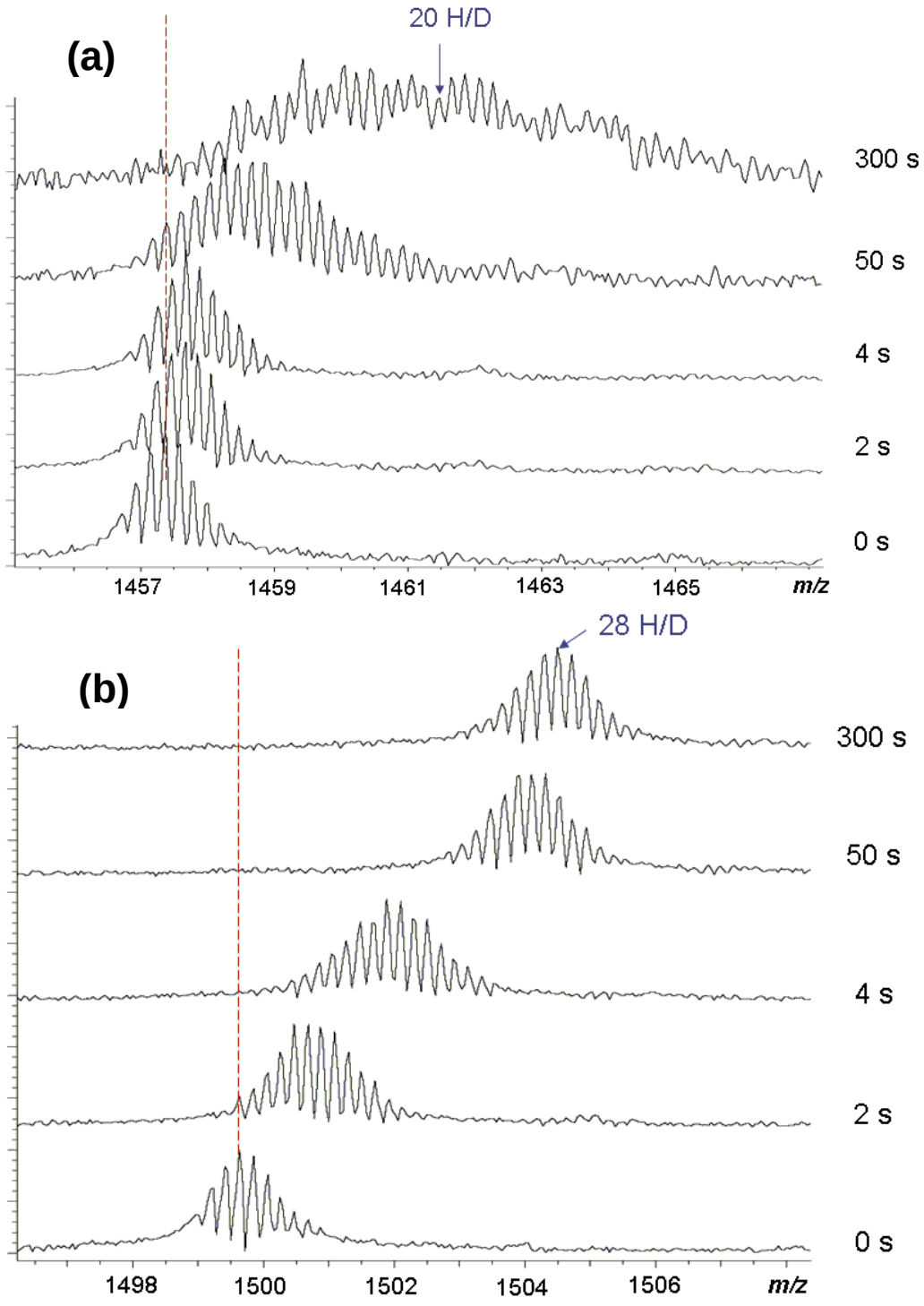


Figure 3.

Kinetics of gas phase H/D exchange of the quadruplex [$(TGGGGT)_4 \bullet 3NH_4^+$] (charge states 5- and 4-) and of the corresponding single strand $TGGGGT^{2-}$. The fitting parameters are given in Table 1.

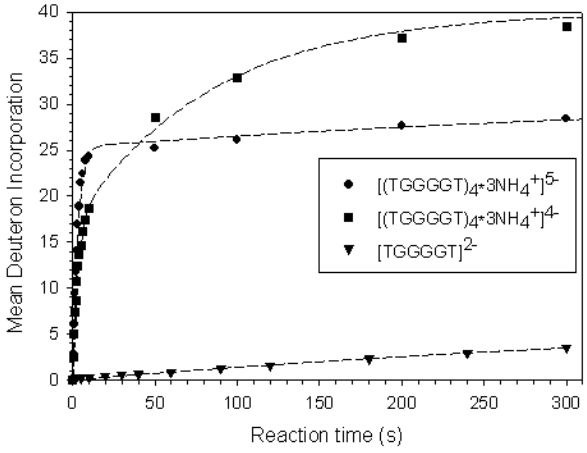


Figure 4.

Gas phase H/D exchange on the DNA G-quadruplexes $[(TGGGGT)_4 \bullet 3NH_4^+]^{5+}$, and of $[(TGGGGT)_4]^{5+}$ generated simultaneously by partial source-CID of the former.

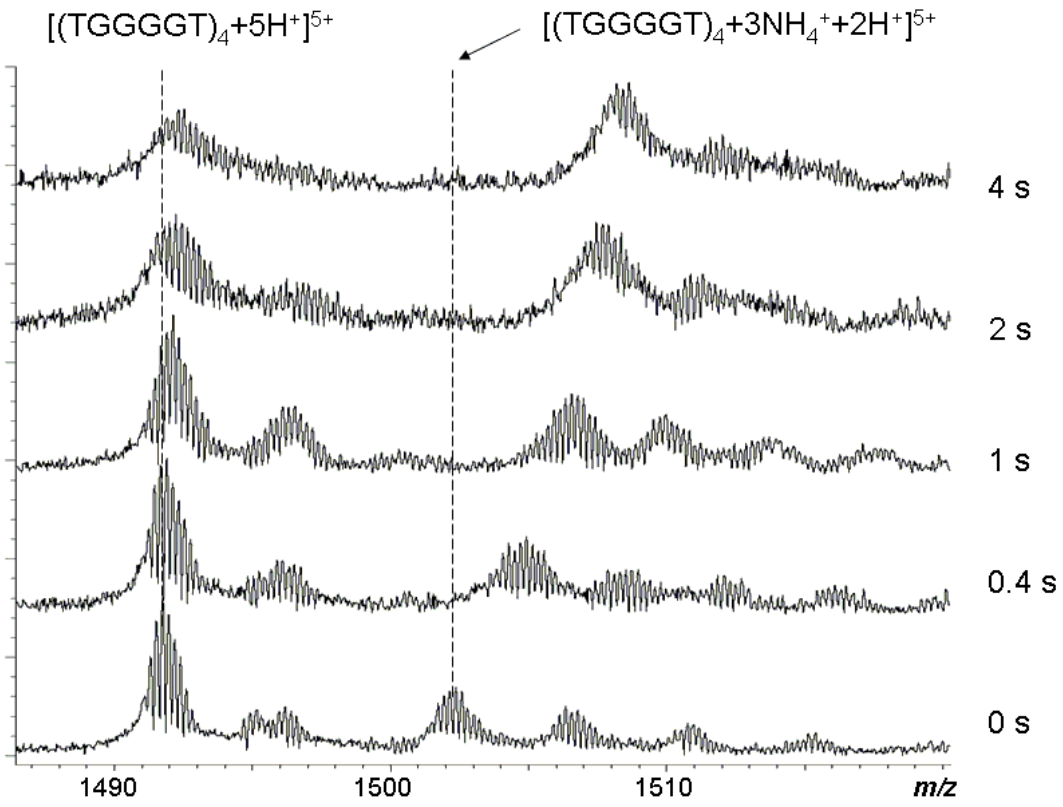


Figure 5.

Gas phase H/D exchange on the DNA G-quadruplexes $[(TGGGGT)_4 \bullet 3NH_4^+]^{5-}$, $[(TGGGGT)_4 \bullet 2NH_4^+ \bullet 1K^+]^{5-}$, and $[(TGGGGT)_4 \bullet 1NH_4^+ \bullet 2K^+]^{5-}$, generated simultaneously in a solution containing 20 μM quadruplex, 150 mM NH_4^+ and 70 μM K^+. All arrows on the picture have the same length, showing that the spacing between the isotopic distributions is constant.

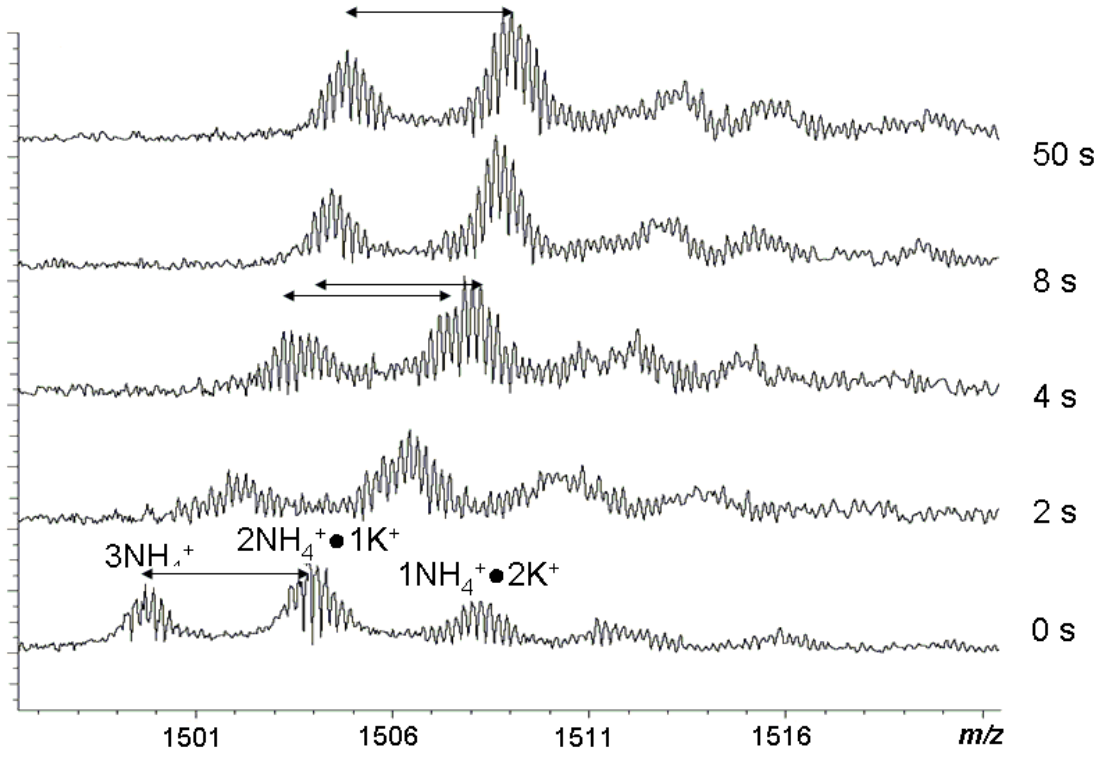
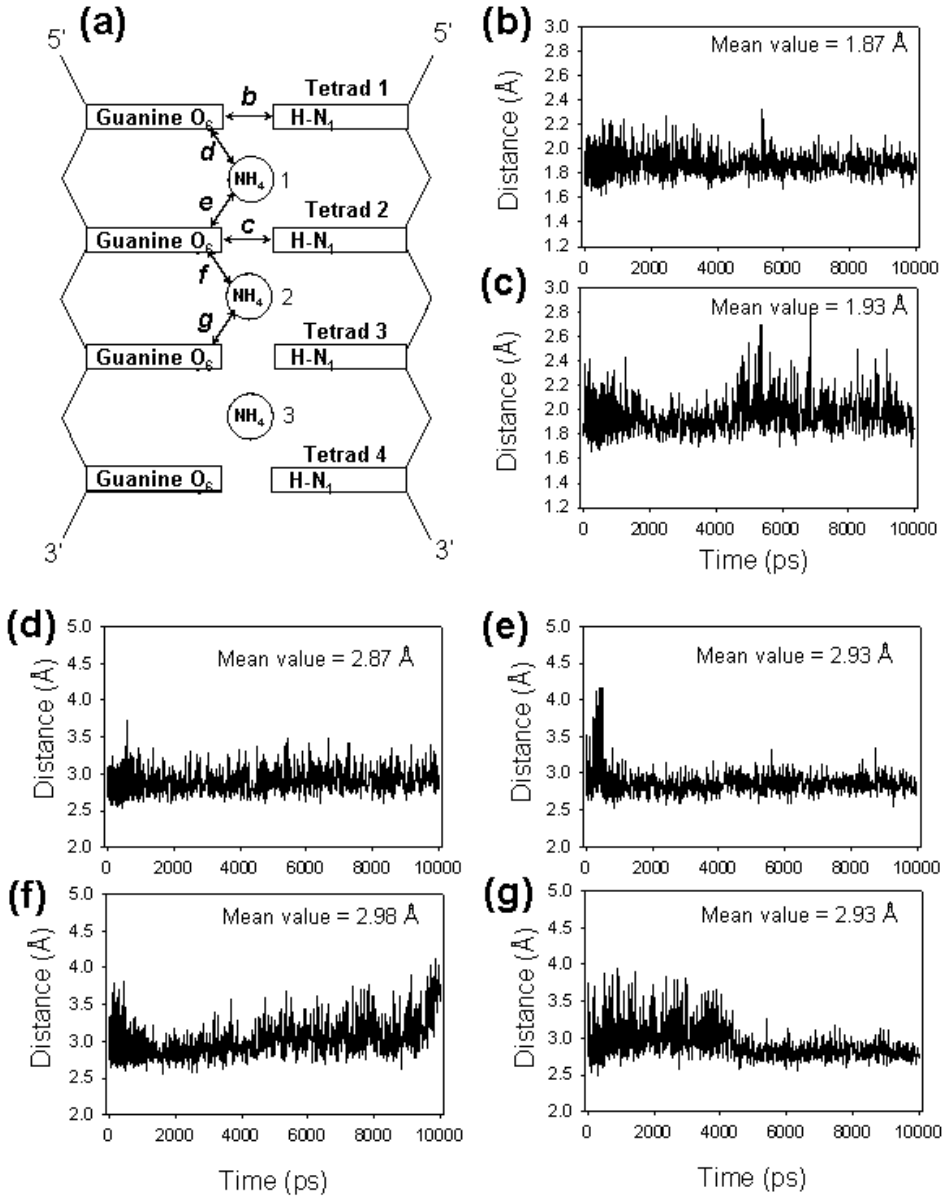


Figure 6.

Unrestrained molecular dynamics simulation on the quadruplex $[(TGGGGT)_4 \bullet 3NH_4]^{5-}$. **(a)** Scheme of the quadruplex, with italic letters indicating the bond distances that are shown in panels b-g. **(b-c)** Evolution of the distance of one of the four $O_6 \cdots H–N_1$ hydrogen bond (see also Fig. 1a) in the upper (b) and in the second (c) tetrad. **(d-g)** Evolution of the distances between the nitrogen of an ammonium cation and the O_6 of one of the neighboring guanines.



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