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Deprotonation dynamics of guanine radical cations

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ABSTRACT

This review is dedicated to guanine radical cations (G\(^{+}\))\(^*\) that are precursors to oxidatively generated damage to DNA. (G\(^{+}\))\(^*\) are unstable in neutral aqueous solution and tend to lose a proton. The deprotonation process has been studied by time-resolved absorption experiments in which (G\(^{+}\))\(^*\) radicals are produced either by an electron abstraction reaction, using an external oxidant, or by low-energy/low-intensity photoionization of DNA. Both the position of the released proton and the dynamics of the process depend on the secondary DNA structure. While deprotonation in duplex DNA leads to (G-H1)\(^*\) radicals, in guanine quadruplexes the (G-H2)\(^*\) analogues are observed. Deprotonation in monomeric guanosine proceeds with a time constant of ~60 ns, in genomic DNA is completed within 2 \(\mu s\) and spans from at least 30 ns to over 50 \(\mu s\) in guanine quadruplexes. Such a deprotonation dynamics in four-stranded structures, extended over more than three decades of times, is correlated with the anisotropic structure of DNA and the mobility of its hydration shell. In this case, commonly used second order reaction models are inappropriate for its description.
INTRODUCTION

Guanine radical cations (G\(^{+}\))\(^{\bullet}\) are precursors to DNA damage under oxidative stress (1). These species are unstable in neutral aqueous solutions and tend to lose a proton, giving rise to deprotonated guanine radicals (G-H)\(^{\bullet}\) (2). Alternatively, (G\(^{+}\))\(^{\bullet}\) may follow other reaction path; for example, undergo hydration, which subsequently leads to the well-known oxidation marker 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodG) (1). The relative yields of the final lesions resulting from various reactions in competition depend, among others, on the dynamics of the associated reactions. In other terms, the faster the reaction rate, the higher the probability to observe lesions resulting from this reaction (3).

Information about the deprotonation dynamics of (G\(^{+}\))\(^{\bullet}\) in room temperature solutions has been obtained by time-resolved techniques, pulsed radiolysis (4, 5) and flash photolysis (6-13), probing the absorption of guanine radicals on the UV-visible spectral domain. Depending on the way in which the (G\(^{+}\))\(^{\bullet}\) are generated, we can distinguish two main experimental approaches. On the one hand, (G\(^{+}\))\(^{\bullet}\) may be produced by electron abstraction from DNA through a reaction with an external oxidant; this approach is named hereafter “indirect”. On the other, (G\(^{+}\))\(^{\bullet}\) may result from photoionization of DNA, without mediation of other molecules, which we coin “direct” approach.

In 1992, Candeias and Steenken observed that radicals of monomeric guanosine derivatives generated by photoionization in neutral aqueous solutions are already deprotonated in 200 ns (14). The first determination of (G\(^{+}\))\(^{\bullet}\) deprotonation dynamics was achieved a decade later by Kobayashi and Tagawa (4). This study, as well as subsequent ones performed for different
types of DNA multimers, revealed that the \((\text{G}^+)\)\(^*\) deprotonation rate strongly depends on the secondary structure (4, 6-9, 11-13). Despite the qualitative agreement of the findings published by various groups, important discrepancies regarding the reported rate or time constants appeared, mostly in the case of guanine quadruplexes (G-Quadruplexes). These four-stranded DNA structures are formed by folding and/or association of guanine rich DNA/RNA strands in the presence of metal cations (Figure 1); the latter affect both the folding geometry and the stability of the resulting scaffold (15). G-Quadruplexes are extensively studied because of their important role in numerous biological functions (16, 17) and their potential applications in biosensors (18-20).

>Figure 1<

The objective of the present review is to (i) highlight the major outcomes on the dynamics of \((\text{G}^+)\)\(^*\) deprotonation in neutral aqueous solutions reported from different groups, (ii) compare the conclusions reached following different experimental methodologies and (iii) discuss the origins for the observed inconsistencies. As experiments providing information on reaction dynamics require the knowledge of the absorption spectra of the radicals, we start our review by presenting their features. In a next step, the basic principles underlying direct and indirect \((\text{G}^+)\)\(^*\) generation are outlined. Then, we present the main results acquired by both indirect and direct approaches. The latter were obtained in our group exploiting a recently discovered unexpected phenomenon: absorption directly by DNA of single photons with energies significantly lower compared to the vertical ionization potential of nucleobases (21) may provoke electron ejection (22-24, 13, 25). The photoionization process as well as the general methodology adopted for the quantification of the ejected electrons and guanine radicals have
been recently highlighted (13). Here, we insist on quantitative aspects related to the deprotonation dynamics, enriching the existing picture with a novel methodology for the determination of the \((G^+)^*\) survival probability in G-Quadruplexes. Finally, after comparing the various findings, we discuss the occurrence of non-constant reaction rates involving DNA.

**ABSORPTION SPECTRA: A PREREQUISITE FOR DYNAMICAL STUDIES**

The absorption spectra of both \((G^+)^*\) and the resulting deprotonated radicals were first determined by Candeias and Steenken (26). In the case of the nucleoside (dG) and nucleotide (dGMP), they found that the proton in position 1 of the guanine is lost upon deprotonation; but if the release of this proton is not possible, as happens in the case of 1-methyl guanine, the proton in position 2 of the amino group takes over (Figure 2, insets). The ensued deprotonated radicals, noted \((G-H1)^*\) and \((G-H2)^*\), respectively, exhibit quite different absorption spectra. As the relevant feature for the determination of the deprotonation dynamics is the spectral change occurring during this process, we present in Figure 2 the differential spectra corresponding to \((G^+)^* \rightarrow (G-H1)^*\) and \((G^+)^* \rightarrow (G-H2)^*\) deprotonations.

>Figure 2<

The spectral features of the various guanine radicals were recently rationalized by quantum chemistry calculations (27, 28). In addition, these studies showed, that their main characteristics in the visible spectral domain are maintained upon hydrogen-bonding and base-stacking. Computations also indicated subtle differences in duplex spectra depending on
whether the H1 proton is transferred either to the bulk water or to the cytosine (Figure 3a).

However, the sensitivity of time-resolved measurements does not allow a clear distinction between these two transfers.

Globally, the spectra of deprotonated radicals in DNA multimers match quite well those of monomeric guanosine in the visible spectral domain (9, 13). But moving towards the UV, the agreement may be less good, because of the contribution of the “initial” multimer absorption (see Figure 9 in reference (7)). As a matter of fact, due to the electronic coupling among nucleobases, the Franck-Condon excited states of DNA multimers differ from those of non-interacting monomers (29, 30, 28, 31) and this is reflected in their time-resolved absorption spectra.

>Figure 3<

The absorption spectra of \((G^+)\)• in certain G-Quadruplexes were determined experimentally for solutions with pH 3, where deprotonation is known to be hindered for monomeric guanosine derivatives (26). It was observed that the spectral intensity below 450 nm is significantly lower than that of the monomeric analogue (7, 9). This finding is supported by quantum chemistry computations (7, 28), which also showed that the electron hole may be delocalized over parallel stacked guanines (32-36, 28). Such a delocalization could influence the deprotonation rate. However, its occurrence has not been clearly established on the time-scales and temperatures discussed here.

GENERATION OF RADICAL CATIONS
All the studies that reported deprotonation dynamics following the indirect approach, used 
(SO$_4^-$)$^+$ as external oxidant to produce (G')$^+$ radicals.

\[(SO_4^-) + G \rightarrow SO_4^{2-} + (G')^+\]  \hspace{1cm} \text{(reaction I).}

The oxidant itself is formed from a peroxodisulfate salt via a two-step or one-step process. In pulsed radiolysis (4, 5), first, the interaction of an electron beam with water gives rise to hydrated electrons e$_{\text{hyd}}^-$ and HO$^+$ radicals. The latter are scavenged by tert-butyl alcohol, which is also added in the solution so that to avoid their reaction with DNA. Then, hydrated electrons react with peroxodisulfate anions:

\[e_{\text{hyd}}^- + S_2O_8^{2-} \rightarrow SO_4^{2-} + (SO_4^-)^+\]  \hspace{1cm} \text{(reaction II).}

If laser irradiation is used instead of an electron beam, the oxidant is generated by a single photochemical reaction (6, 10):

\[h\nu + S_2O_8^{2-} \rightarrow 2(SO_4^-)^+\]  \hspace{1cm} \text{(reaction III).}

In the low-energy photoionization studies, (G')$^+$ generation takes place at 266 nm (13). The key point of this approach is the use of laser intensities ($\leq 2 \times 10^6$ Wcm$^{-2}$) significantly lower compared to those used to study final lesions (37). Under these conditions, the e$_{\text{hyd}}^-$ originate only from DNA, without interference from solvent ionization. Consequently, they can be quantified and compared with the radical concentration through the corresponding molar absorption coefficients ($\varepsilon$) as explained in reference (13). For the experiments reported here, DNA was dissolved in the phosphate buffer, composed of an equimolar mixture of NaH$_2$PO$_4$/Na$_2$HPO$_4$ or KH$_2$PO$_4$/K$_2$HPO$_4$ in concentrations of 0.015 - 0.15 mol·L$^{-1}$ each. Under these conditions, e$_{\text{hyd}}^-$ decay in less than 2 µs, reacting with H$_2$PO$_4^{-2}$ (13).

We remark that, going from pulse radiolysis to photosensitized electron abstraction and further to photoionization, both the number of reaction steps necessary to generate (G')$^+$ and the number of chemicals present in the solution, decrease. The counterpart is that, in parallel, the
intensity of the transient absorption signals decreases, typically by two orders of magnitude (compare, for example, Figure 5 in reference (4) and Figure 4 in this manuscript). Therefore, low-energy photoionization studies require long measurement times. In addition, both DNA and the buffer ingredients need to be purified so that to avoid spurious signals from impurities and/or their interaction with radicals.

We stress that the initial electron holes generated by either the indirect or the direct method are not necessarily located on guanine sites. On the one hand, (SO$_4^-$)$^\cdot$ may oxidize other nucleobases (38, 39), and, on the other, low-energy photoionization takes place also in DNA multimers devoid of guanines (23, 24). But, as guanine has the lowest oxidation potential among nucleobases (40), the electron hole is rapidly trapped by guanines following a charge transport process (41-47) and only guanine radicals are observed in the experiments described here.

**MAIN FINDINGS: INDIRECT APPROACH**

In their seminal work, Kobayashi and Tagawa (4) performed pulse radiolysis and probed the transient absorption at 625 nm (see Figure 2a). They found that (G$^\cdot$)$^\ast$ $\rightarrow$ (G-H1)$^\ast$ deprotonation in dG proceeds with a rate constant of $1.8 \times 10^7$ s$^{-1}$ (56 ns). Recent flash photolysis measurements yielded a similar rate constant ($1.5 \times 10^7$ s$^{-1}$ / 67 ns) (10).

Kobayashi and Tagawa also studied the deprotonation dynamics of (G$^\cdot$)$^\ast$ in a series of model duplexes with 12-13 base-pairs containing G, GG and GGG steps at different positions (4). In this case, the fit of the transient signals required two-exponential functions, provided rate constants of $1.3 \times 10^7$ s$^{-1}$ (78 ns) and $3 \times 10^6$ s$^{-1}$ (330 ns). The fastest one was attributed to
guanines located close to the ends of the duplex, while the slowest was assigned to internal guanine sites.

The first study on G-Quadruplexes was performed by Su and co-workers, using photosensitized electron abstraction (6). They examined four different sequences forming monomolecular, bimolecular and tetramolecular structures (Figure 1). They came to two important conclusions. First, they showed that, in contrast to what was observed for duplexes, (G')\(^{\ast}\) deprotonation in these systems does not involve release of the H1 proton but of the H2 proton (Figure 1b). This conclusion was attested by the appearance in the transient absorption spectra of a band peaking in the red, which is typical of the (G-H2)\(^{\ast}\) radicals (26, 48). It was explained by the fact that H1 protons are engaged in the Hoogsteen hydrogen bonds connecting the guanines within the guanine tetrads (Figure 3b). Later, quantum chemistry calculations found that, indeed, (G-H2)\(^{\ast}\) radicals in G-quadruplexes are more stable than the (G-H1)\(^{\ast}\) analogues (9). The second conclusion was that (G')\(^{\ast}\) \(\rightarrow\) (G-H2)\(^{\ast}\) deprotonation in G-Quadruplexes is slower compared to (G')\(^{\ast}\) \(\rightarrow\) (G-H1)\(^{\ast}\) in duplexes. Rate constants ranging from \(1.4 \times 10^{6} \text{ s}^{-1}\) (0.7 µs) and \(2 \times 10^{5} \text{ s}^{-1}\) (5 µs) were reported.

A subsequent study on two G-quadruplexes, also performed by photosensitized electron abstraction reached a different conclusion (49). The transient absorption spectra recorded at 50 µs were attributed to (G-H1)\(^{\ast}\) deprotonated radicals but the discrepancy with previously reported studies (6, 7) was not discussed.

MAIN FINDINGS: DIRECT APPROACH
In photoionization studies, a full spectral characterization of the radicals is possible only after 2-3 µs. This is due, on the one hand, to the low intensity of the transient absorption signals, which are noisier on shorter times, and, on the other, to the presence of hydrated ejected electrons, whose broad and intense absorption is extended over the whole visible domain (13). Yet, some delicate measurements were performed on the sub-microsecond time-scale by adding in the solution efficient electron scavengers, N\textsubscript{2}O or NO\textsubscript{3}\textsuperscript{-} (50).

For all the DNA systems studied by this method, the guanine radical concentration at 2-3 µs was found to be equal to that of the hydrated ejected electrons \([e_\text{hyd}]_0\), determined by independent experiments on the sub-microsecond time-scale (13). Thus, it was possible to quantitatively follow the entire radical population during the time. We note that these “initial” concentrations correspond to electron and holes that escaped recombination (51) and may provoke DNA damage.

The differential absorption spectra recorded on the microsecond time-scale for model duplexes and purified calf thymus DNA (CT-DNA) (52, 8, 13) correspond to that of \((G-H1)^\cdot\) radicals, showing that \((G^\cdot)^\* \rightarrow (G-H1)^\cdot\) deprotonation takes place earlier. The deprotonation dynamics was determined in the case of CT-DNA by recording transient absorption signals at 700 nm. The latter exhibit a rise, which can be described by a mono-exponential function with a time constant of 320 ± 30 ns; after 2µs, the signal starts decreasing slowly (Figure 4) and subsequently it decays on the millisecond time-scale (13).

>Figure 4<
A more complex picture was found in the case of G-Quadruplexes. The study of 7 different systems showed that a significant population of the initially generated \((G^+)^*\) is still present at 3 \(\mu\)s (Table 1). Depending on the G-quadruplex type, the concentration of this “long-lived” \((G^+)^*\) population varies from 25% to 60% of \([e_{\text{hyd}}]_0\) while the remaining part corresponds to \((G-H2)^*\) radicals.

>Table 1<

The percentages presented in Table 1 were determined by reconstructing the transient absorption spectra obtained for the studied G-Quadruplexes by linear combinations of the spectra reported for monomeric guanosine radicals the \((G^+)^*\) and \((G-H2)^*\). This reconstruction takes into account not only the spectral shape but also the spectral intensities. To this end, the differential absorption \(\Delta A\), observed over an optical path-length of 1 cm, was divided by the concentration of hydrated ejected electrons, while the monomer spectra were considered with their \(\varepsilon\) (26). An example is given in Figure 5a where the transient absorption spectrum of OXY/Na\(^+\) at 3 \(\mu\)s is presented together with that corresponding of a linear combination of \((G^+)^*\) and \((G-H2)^*\) radicals at a ratio of 45/55 while the sum of their concentrations is equal to the detected \([e_{\text{hyd}}]_0\).

>Figure 5<

For some G-Quadruplexes, deprotonation toward \((G-H2)^*\) becomes much more obvious on later times. For example, the spectrum of \((T\text{G}_{4}T)_{4}/Na^+\) at 50 \(\mu\)s (Figure 5 in reference (9)) strongly resembles that of monomeric \((G-H2)^*\). At even longer times, \((G-H2)^* \rightarrow (G-H1)^*\)
tautomerization, which is in competition with other reaction paths, may be observed (13). But this aspect is out of the scope of the present review.

For a few cases, the early deprotonation dynamics could be resolved. This is presented in Figure 6 for TEL21/K\(^{+}\), OXY/Na\(^{+}\) and OXY/K\(^{+}\): the transient signals recorded at 620 nm exhibit a rise, corresponding to the appearance of (G-H2)\(^{\cdot}\) radicals (see Figure 2b). Although these signals are very noisy, it is clear that a fast deprotonation process takes place in less than 1 \(\mu\)s; the time constants, derived from fits with mono-exponential functions (150 ± 15 ns in Figure 6a and 200 ± 30 ns in Figure 6b), are smaller than that found for CT-DNA (320 ± 30 ns, Figure 4).

Coming to the slow deprotonation process, it could be visualized directly by transient absorption signals only in the case of tetramolecular G-quadruplexes (9, 11). For these systems, the (G-H2)\(^{\cdot}\) population at 50 \(\mu\)s is still equal to that of the total radical population at 3 \(\mu\)s. Thus, the decay of the differential absorbance at 500 nm and the rise at 600 nm reflect directly the deprotonation dynamics. This is shown in Figure 7a for (TG\(_{4}\)T)\(_{4}\)/Na\(^{+}\), where both the decay and the rise are approximated by single exponential functions, with the same time constant of 6 ± 1 \(\mu\)s (9). The deprotonation is practically completed at 20 \(\mu\)s. For other systems, the total radical population, determined by the transient absorption decays at 510-515 nm as explained in reference (13), starts decreasing before completion of the deprotonation processes. For this reason, a different method was adopted in order to determine the reaction dynamics of (G\(^{\dagger}\))\(^{\cdot}\). Several time-resolved spectra were recorded on the microsecond time-scale and, subsequently, reconstructed in the same way as that followed for the 3 \(\mu\)s spectra. As
example is presented in Figure 5b the reconstruction of the spectra recorded for TEL21/Na⁺ at 25 µs. At this time, the total radical concentration corresponds to 85% of the hydrated ejected electrons; the missing part was associated with formation of 8-oxodG which was detected by analytical methods (7). Finally, the \((G^+)\) survival probability, determined as a percentage of the initially generated electron holes that have escaped recombination, equal to \([e_{hyd}]_0\), was plotted. Figures 7b and 7c show the results derived from such an analysis performed for the OXY/Na⁺ and TEL21/Na⁺. At times longer than 50 µs for the former system and 40 µs for the latter, the errors related with the spectral analysis increase to provide reliable data. At those times, about ~10% of the initial population is still present in the G-Quadruplex.

>Figure 7<

**COMMON CONCLUSIONS AND DISCREPANCIES**

The above described studies carried out by different groups using different approaches lead to some common conclusions regarding the \((G^+)\) deprotonation dynamics. First, similar rate constants were found for deprotonation in dG via the indirect method using either pulse radiolysis (4) and flash photolysis (6). Second, the characteristic time determined by photoionization of CT-DNA \((320 \pm 30 \text{ ns}; \text{Figure } 4)\) (13) matches well the longest time constants \((330 \text{ ns})\) derived from the study of model duplexes by pulsed radiolysis (4). The shortest time constants reported in the latter study had been correlated with guanines located close to the ends of the duplexes and it is normal that they are not detected for genomic DNA which is very long macromolecule.
Third, the specificity of $(G^+)^\cdot$ deprotonation towards $(G-H2)^\cdot$ in G-Quadruplexes, initially deduced from photosensitized electron abstraction studies, carried out by Su and co-workers (6), was largely confirmed by our photoionization studies (11, 13, 12, 9, 8, 7). And it is important to note that oxidatively generated lesions to DNA resulting from $(G-H2)^\cdot$ radicals have not been documented so far. Yet, a study Merta et al. using, as the Su group, photosensitized electron abstraction (reaction I), failed to detect the presence of $(G-H2)^\cdot$ in telomeric G-Quadruplexes (49).

Another common conclusion drawn by the Su group and our work is that $(G^+)^\cdot$ radicals in G-Quadruplexes are still observed on the microsecond time-scale. But quantification of $(G^+)^\cdot$ population, possible only through photoionization studies, revealed that these “long-lived” species, whose decays were characterized in the 3 - 50 µs interval (Figure 7), represent only part of the initially generated electron holes (see Table 1). As shown in Figures 6a and 6b, an important part of $(G^+)^\cdot$ deprotonates on much shorter times, even faster compared to CT-DNA (Figure 4).

**REACTION RATES IN ANISOTROPIC STRUCTURES**

In order to understand the discrepancies regarding the rate constants determined for $(G^+)^\cdot$ deprotonation in G-Quadruplexes, a closer look at the reaction mechanism underlying their determination may be informative. Proton transfer from $(G^+)^\cdot$ to water is considered to be a second order reaction. Given that the bulk concentration of water molecules is much higher than that of $(G^+)^\cdot$, this second order reaction is approximated by a pseudo-first order one, as indicated by the fact that the rate constants are expressed in $s^{-1}$ (4, 6, 10). However, the validity of such an approximation requires certain conditions to be fulfilled. The reaction rate needs to
be controlled by diffusion of the reactants and the probability of their encounter should be uniform over the three-dimensional space over the time-window of the experimental measurements (53). This is the model of a well-stirred reactor.

The conditions corresponding to the simplified pseudo-first order reaction kinetics are far from valid in G-Quadruplexes. As a matter of fact, analysis of several crystal structures revealed the existence of well-defined water networks, whose properties depend on the topology of G-Quadruplex; the primary water molecules may be hydrogen bonded to phosphate and/or sugar groups and even to guanines at the edges of the G-quadruplexes (54).

Heterogeneities are encountered not only in the static arrangement of the DNA hydration shell but also on the lability of its water molecules which may be modulated by slow conformational motions of the nucleic acids (55). Interestingly, the hydration cell has been found to be thicker around G-quadruplexes than duplexes and more difficult to be disrupted by solute molecules (56). According to two-dimensional NMR experiments, the residence times of the water molecules belonging to the hydration shell of model duplexes do not exceed 1 ns at room temperature (57, 58). Consequently, these effects should be averaged on the time-scale on which \((G^+)\) deprotonation dynamics is detected for CT-DNA by transient absorption (Figure 4). In contrast, longer water rotation and residence times, extended on the microsecond time-scale, have been reported for OXY/Na\(^+\) G-Quadruplexes (59), explaining, at least partly, the observed slower \((G^+)\) deprotonation.

Second order kinetics in systems with spatial and dynamical heterogeneity may give rise to multiscale survival probabilities of the reactants (60). Such reaction patterns artificially appear to be mono-exponential if the observation and/or analysis of the time-resolved signals is restricted over a small time-window. In addition, different time-windows result to different
time constants. It is worth-noticing that multiscale dynamics characterizes also the excited state relaxation in DNA, where electronic coupling, affected by conformational motions among nucleobases (61), is operative (62).

Multiscale reaction rates may also concern \((G^+)\cdot\) generation in DNA multimers using \((SO_4^-)\cdot\). Reaction I takes place between negatively charged electron acceptors and spatially correlated electron donors located on a polyelectrolyte. It is possible that the rate constants determined for the oxidation process reflect only part of the reaction. In this case, a simple change in the concentration ratios between DNA and the oxidant or the duration of the laser pulse may modify the oxidation rates and, hence, those of deprotonation. This could provide an explanation why \((G-H2)^+\) radicals have gone undetected in one study (49) while they were readily detected in another (6).

Focusing on the deprotonation process determined by the photoionization method, the time constants derived from the fits with exponential functions (Figures 4, 6 and 7) do not necessarily correspond to reaction rate constants; they are simply useful for a phenomenological comparison of the transient absorption traces on a given time-window. In this respect, the first photoionization study of G-Quadruplexes reported that \((G^+)\cdot\) survive in TEL21/Na\(^+\) on the millisecond time-scale signals (7). This conclusion was based on time constants derived from exponential fits of the transient absorption signals which, following the above reasoning, may lead to erroneous results. The methodology presented here for the determination of the \((G^+)\cdot\) survival probability (Figure 5b), based on the transient absorption spectra, provided shorter lifetimes. Therefore, we conclude that deprotonation in G-Quadruplexes spans over three orders of magnitude of time, from at least 30 ns to over 50 µs, the limits being determined by the time resolution and the sensitivity of the experimental set-
up. This anisotropic deprotonation dynamics can be also viewed as the occurrence of
dynamically anisotropic pKa values.

As analytical formulas derived from simple reaction patterns cannot describe correctly the
\((G^+)^*\) deprotonation dynamics, specific theoretical developments are needed blatantly. A first
step toward this direction was made in a recent quantum chemical study: it was shown that a
cluster of four water molecules must be taken into account to correctly reproduce the
temperature dependence of the reaction rates \((G^+)^* \rightarrow (G-H1)^*\) in \(dG\) determined
experimentally as a function of temperature (10). Regarding DNA multimers, theoretical
studies should encompass, in addition, molecular dynamics simulations on tens of
microseconds, already used to describe interaction of DNA with biomolecules (63, 64). In this
way, it should be possible to establish a correlation between DNA topology and \((G^+)^*\)
dynamics.

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REFERENCES


Table 1. Concentrations of \((G^+)^*\) and \((G-H2)^*\) radicals present in G-Quadruplexes at 3 \(\mu\text{s after}\) photoionization, expressed as percentage of \([e_{\text{hyd}}]^0\); error: \(\pm 5\%\).

<table>
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<th>Type</th>
<th>Sequence</th>
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<th>((G-H2)^*)</th>
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<td>monomolecular</td>
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<td>55</td>
</tr>
<tr>
<td></td>
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<td>TEL21/Na$^+$† (7)</td>
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<td>50</td>
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<td></td>
<td></td>
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<td>40</td>
</tr>
<tr>
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<td>OXY/Na$^+$††</td>
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<td></td>
<td></td>
<td>OXY/K$^+$ (13)††</td>
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<td>(TG$_4$T)$_4$/K$^+$ (11)</td>
<td>40</td>
<td>60</td>
</tr>
</tbody>
</table>

†TEL: containing the human telomeric repeat TTAGGG; ††OXY: containing the oxytricha nova telomeric repeat TTTTGGGG.
FIGURE CAPTIONS

Figure 1. Schematic illustration of the G-Quadruplexes discussed in this review: (a) monomolecular, formed by folding of a single strand, (b) bimolecular, formed by folding and association of two single strands and (c) tetramolecular, formed by association of four single strands; red spheres represent metal cations (Na$^+$, K$^+$) located in the central cavity. Guanines are shown in violet and the backbone in grey.

Figure 2. Spectral differences corresponding to ($G^+$)$^* \rightarrow (G\text{-H}1)^*$ (a) and ($G^+$)$^* \rightarrow (G\text{-H}2)^*$ (b) deprotonation, obtained by subtracting the spectrum of the radical cation from those of deprotonated radicals determined for monomeric guanosine derivatives (2, 48). The position of H1 and H2 hydrogen atoms is indicated in the insets.
Figure 3. Location of the hydrogen atoms H1 and H2 in (a) a Watson-Crick guanine-cytosine pair; (b) a tetrad composed of four guanines interconnected via Hoogsteen hydrogen bonds.

Figure 4. Transient absorption signals (black) recorded for N₂O-saturated solutions of CT-DNA at 700 nm. The red line in (a) is derived from a fit with the model function A₁(1-exp(-t/τ₁))+A₀ with τ₁ = 320 ± 30 ns. Adapted from reference 9 with ACS permission.
Figure 5. Reconstruction of the transient absorption spectra determined for G-Quadruplexes (circles) with linear combinations of the \((G^+)\) and \((G-H2)^+\) spectra reported for monomeric guanosine derivatives (solid lines), represented with their molar absorption coefficient \((26, 48)\).

(a) OXY/Na\(^+\) at 3 \(\mu\)s; \([ (G^+) ] / [ (G-H2)^+ ] = 45/55, \[(G^+)\] + \[(G-H2)^+\] = \([e_{hyd}]_0\). (b) TEL21/Na\(^+\) at 25 \(\mu\)s; \([ (G^+) ] / [ (G-H2)^+ ] = 20/80, \[(G^+)\] + \[(G-H2)^+\] = 0.85\([e_{hyd}]_0\).

Figure 6. Deprotonation of in G-Quadruplexes on the sub-microsecond time scale. Transient absorption signals recorded for \(N_2O\)-saturated solutions of TEL21/K\(^+\) (red) \((12)\), OXY/Na\(^+\) (green) and OXY/K\(^+\) (pink) \((13)\). Yellow lines correspond to fits with mono-exponential functions, \(A_1(1-exp(-t/\tau))+A_0; \tau = 150 \pm 15\) ns \((a)\) and \(200 \pm 30\) ns \((b)\); adapted from reference \((13)\) with ACS permission).
Figure 7. Reaction dynamics of (G⁺)⁺ in G-Quadruplexes on the microsecond time-scale. (a) transient absorption signals recorded at 500 nm (green) and 600 nm (red) for (TG₄T)_₄/Na⁺; their intensity has been arbitrarily scaled; reproduced from reference 24 with ACS permission. Survival probability of the (G⁺)⁺ population (circles) in OXY/Na⁺ (b) and TEL21/Na⁺ (c), determined from reconstruction of the time-resolved spectra, as shown in Figure 5. Black lines correspond to fits with exponential functions A₁ (1-exp(t/τ₁))+A₀ (rise) and A₁ exp(-t/τ₂)+A₀ (decays) with time constants: 6 ± 1 µs (a); 22 ± 2 µs (b) and 7 ± 0.5 µs (c).