

Excess electron transfer in G-quadruplex

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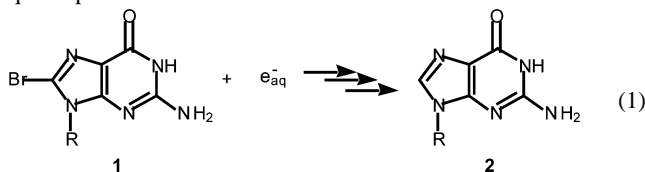
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The excess electron transfer in a G-quadruplex is successfully probed by using the reaction of hydrated electrons with quadruplex complex of pentamers and the 8-bromoguanine moieties as the detection system.

DNA is an efficient trap for electrons. The one-electron reduction of DNA gives a radical anion, which may easily be transferred and thus is often referred to as *excess* electron.¹ Photoinduced electron injection experiments have been the main approach. Carell and coworkers developed well-defined donor–DNA–acceptor model compounds to investigate the migration of excess electrons.² In particular, they studied the efficiency of T=T cyclobutane dimer repair *via* electron injection from a flavin nucleobase surrogate, and their findings indicated a thermally activated electron-hopping process. Lewis *et al.* have used synthetic hairpins with a stilbene electron donor “head” to study the picosecond dynamics of electron injection to different neighboring base pairs.³

Few years ago some of us reported on the reaction of hydrated electrons (e_{aq}^-) with 8-bromoguanosine.⁴ It was found that this bromide is prone to capture electrons with quantitative formation of guanosine (eqn. (1)). More recently the one-electron attachment reaction to double-stranded oligonucleotides that contain the 8-bromo-2'-deoxyguanosine moiety (G^{Br}) was also shown to afford the corresponding debrominated derivatives.⁵ The conversion of G^{Br} was found to be higher in Z-DNA than in B-DNA and the difference was explained by the specific conformation of G^{Br} in the B- and Z-DNA. Herein we wish to report on the reaction of e_{aq}^- with a variety of oligonucleotide (ODN) trimers that contain G^{Br} , which shows that G^{Br} may act as a sink for electrons in an appropriate sequence of oligodeoxynucleotides. This approach has been used to study the excess electron transport in a G-quadruplex.



De-aerated aqueous solutions containing 8-bromo-2'-deoxyguanosine (**1**) (*ca.* 1.5 mM) and *t*-BuOH (0.25 M) at pH \sim 7 were irradiated under stationary-state conditions[†] with a dose rate of *ca.* 20 Gy min^{-1} followed by RP-HPLC analysis. Compound **2** was the only detectable product and mass balances were close to 100%. Analysis of the data in terms of radiation chemical yield (G)[‡], gives $G(-\mathbf{1}) = 0.34$ and $G(\mathbf{2}) = 0.31 \mu\text{mol J}^{-1}$. Taking into account that $G(e_{\text{aq}}^-) + G(\text{H}^\cdot) = 0.33 \mu\text{mol J}^{-1}$ our results lead to the conclusion that solvated electrons and hydrogen atoms react with **1** to yield the observed product as in the *ribo* series.⁴

Next we investigated the reaction of e_{aq}^- with trimers ${}^5\text{TG}^{\text{Br}}\text{T}3'$, ${}^5\text{TG}^{\text{Br}}\text{G}3'$, ${}^5\text{CG}^{\text{Br}}\text{A}3'$, ${}^5\text{G}^{\text{Br}}\text{TT}3'$. De-aerated aqueous solutions buffered at pH 7 containing a trimer (*ca.* 1 mM) and *t*-BuOH (0.25 M) were irradiated at a dose rate of *ca.* 20 Gy min^{-1} . Fig. 1 shows

the irradiation dose profiles of disappearance of three starting trimers (solid circles) and the formation of their debrominated ODNs. The extrapolation to zero dose of the starting trimers gave an average value of $0.34 \mu\text{mol J}^{-1}$. On the other hand, the yield of the debrominated trimer $G(\text{product})$ values extrapolated at zero dose depended on the structure, in terms of the position along the chain of the brominated nucleotide and the flanking bases \S . All individual G extrapolated to zero dose are reported in Table 1.

Since the early work in radiation biology, it was suggested that protonation of anionic base radicals may have drastic effects on the chemical reactivity of the species involved. The initial reaction is certainly of statistical nature, since all the nucleic acid bases have similar reactivity towards the hydrated electron.^{4,6} The $G(\text{product}) = 0.08$ for ${}^5\text{CG}^{\text{Br}}\text{A}3'$ indicates, however, that only 30% of e_{aq}^- afforded the debromination product. We suggest that the latter results from the direct addition of hydrated electron to G^{Br} , whereas the electron adducts at C and A terminus are prone to protonation affording other products rather than transferring the excess electron to the G^{Br} moiety. In fact, it is known that electron adducts of adenine and cytosine nucleotides are strong bases and are rapidly protonated by water even in very strongly alkaline solutions.^{7,8} The relatively high $G(\text{product})$ values for ${}^5\text{TG}^{\text{Br}}\text{T}3'$ and ${}^5\text{TG}^{\text{Br}}\text{G}3'$ of 0.24 suggests that at least some of the initially formed electron-adducts of T and/or non-brominated G moieties have transferred their electrons to G^{Br} , affording the debrominated trimer at about

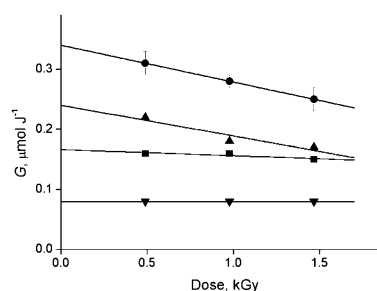


Fig. 1 Radiation chemical yield (G) as a function of irradiation dose for the consumption of trimers ${}^5\text{TG}^{\text{Br}}\text{G}3'$, ${}^5\text{CG}^{\text{Br}}\text{A}3'$, and ${}^5\text{G}^{\text{Br}}\text{TT}3'$, (●, average values) and the formation of ${}^5\text{TGG}3'$ (▲), ${}^5\text{GTT}3'$ (■), and ${}^5\text{CGA}3'$ (▼), from the continuous irradiation of vacuum degassed buffered solutions containing *ca.* 1 mM of a brominated trimer and 0.25 M *t*-BuOH at pH \sim 7. The lines are the linear fit to the data.

Table 1 Radiation chemical yield ($\mu\text{mol J}^{-1}$) of disappearance of starting trimers and formation of debrominated trimers from the γ -radiolysis^a

Starting trimer	$G(-\text{starting})$	$G(\text{product})$
${}^5\text{TG}^{\text{Br}}\text{T}3'$	0.31	0.24
${}^5\text{TG}^{\text{Br}}\text{G}3'$	0.33	0.24
${}^5\text{G}^{\text{Br}}\text{TT}3'$	0.33	0.17
${}^5\text{CG}^{\text{Br}}\text{A}3'$	0.37	0.08

^a Estimated to be accurate to $\pm 10\%$

90% yield (based on e_{aq}^-). The lack of neutralization of $T^{\cdot-}$ and $G^{\cdot-}$ prior to this electron transfer is in line with the fact that these radical anions are weak bases.^{8,9} The results with $5'G^{Br}TT3'$ indicate that 63% of e_{aq}^- afforded the debrominated trimer. We suggest that the electron-adduct of the T residue, which is located in the middle of sequence, is able to transfer the electron to G^{Br} , whereas the terminal T is probably protonated prior to electron transfer affording other products.

The pentamers $5'TGG^{Br}GT3'$ and $5'TG^{Br}GGT3'$ are known to form a parallel four stranded quadruplex complex.^{10,11} The radiolytic experiments have been carried out at an oligonucleotide concentration of 1.5 mM, *i.e.* two orders of magnitude higher than the concentration used for the CD analysis[¶]. Since the stability of the complex increases with the oligonucleotide concentration, it can be concluded that these experiments have been conducted on a parallel four stranded quadruplex structure, stabilized by a set of specific H-bonds arrays. De-aerated aqueous solutions buffered at pH 7 containing a pentamer (*ca.* 1 mM) and *t*-BuOH (0.25 M) were irradiated at a dose rate of *ca.* 20 Gy min^{-1} . The results for $5'TGG^{Br}GT3'$ are shown in Fig. 2. Extrapolation to zero dose of the starting pentamer gives $G(-5'TGG^{Br}GT3') = 0.32$, which is close to the already repeatedly mentioned sum of hydrated electron and hydrogen atom, *i.e.*, $G(e_{aq}^-) + G(H^{\cdot}) = 0.33 \mu mol J^{-1}$. On the other hand, the formation of the pentamer product occurs with only $G(5'TGGGT3') = 0.22$. This is close but still somewhat lower (82%) than $G(e_{aq}^-) = 0.27$ §. We therefore assume that $5'TGGGT3'$ results mainly from the e_{aq}^- reaction and do not explicitly consider any contribution by the hydrogen atom at this stage. Similar results were obtained for $5'TG^{Br}GGT3'$.

Since nucleic acid bases have similar reactivity towards hydrated electrons, it is reasonable to assume that e_{aq}^- adds to any of the 20 nucleotides of the quadruplex randomly. The facts that debromination is the only observed reaction and its radiation chemical yield is so high, suggest that almost any reacting electron is eventually transferred to G^{Br} . Fig. 3 shows the step-by-step debromination of quadruplex that is suggested to operate in order to obtain a high reaction yield. Our results demonstrate that adjacent guanines in G-quartets appear to be very effective in excess electron transfer and G^{Br} behaves as an ultimate sink for electrons due to the Br^- ejection.

In summary, the effectiveness of debromination of nucleotide trimers containing G^{Br} depended strictly on the flanking bases. It is envisaged that $T^{\cdot-}$ and $G^{\cdot-}$ moieties are transferring one electron to the neighboring G^{Br} , whereas $C^{\cdot-}$ and $A^{\cdot-}$ are rapidly protonated affording other products. The studies described on the $5'TGG^{Br}GT3'$ quadruplex clearly demonstrate that G^{Br} can serve as a useful probe for gaining deeper insight into the kinetic aspects of excess electron transfer through the G-quadruplex π -stack.

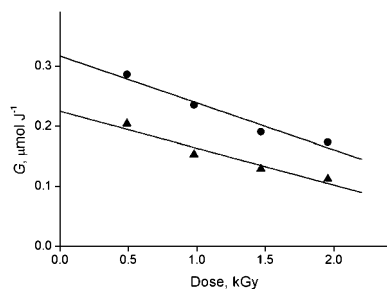


Fig. 2 Radiation chemical yield (G) as a function of irradiation dose for the consumption of $5'TGG^{Br}GT3'$ (●) and the formation of $5'TGGGT3'$ (▲) from the continuous irradiation of vacuum degassed buffered solutions containing *ca.* 1 mM $5'TGG^{Br}GT3'$ and 0.25 M *t*-BuOH at pH \sim 7. The lines are the linear fit to the data of $G(-5'TGG^{Br}GT3')$ and $G(5'TGGGT3')$.

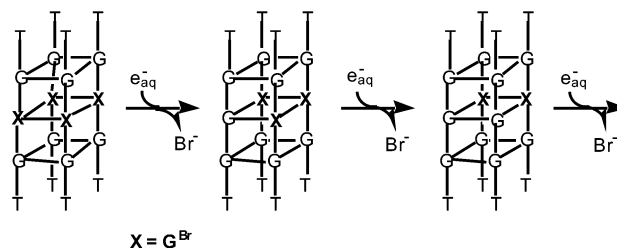


Fig. 3 Reaction mechanism for the reaction of hydrated electrons with $5'TGG^{Br}GT3'$, which is in the form of parallel four stranded quadruplex complex.

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Notes and references

† Radiolysis of neutral water leads to e_{aq}^- (0.27), HO^{\cdot} (0.28) and H^{\cdot} (0.062) where the values in parentheses represent the radiation chemical yields (G) in units of $\mu mol J^{-1}$.⁶ In the presence of *t*-BuOH, HO^{\cdot} is scavenged efficiently ($k = 6.0 \times 10^8 M^{-1} s^{-1}$), whereas H^{\cdot} reacts only slowly ($k = 1.7 \times 10^5 M^{-1} s^{-1}$).⁶

‡ The disappearance of the starting material or the appearance of the product ($mol kg^{-1}$) divided by the absorbed dose ($1 Gy = 1 J kg^{-1}$) gives the radiation chemical yield, *i.e.*, $G(-1)$ or $G(2)$, respectively.

§ The $G(e_{aq}^-)$ is the appropriate comparator because the H^{\cdot} is expected to react unselectively with both sugar and base moieties affording mainly other products.

¶ It has been found that their conformational behaviour, at 15 μM , in the buffer *t*-BuOH 0.25 M, 5 mM Na_2HPO_4 , 5 mM KH_2PO_4 , pH 7, is very similar to the one exhibited, at the same concentration, in the buffer 10 mM KH_2PO_4 , 1.0 M KCl and 0.1 mM EDTA, where G-rich sequences typically do form such quadruplex structures.¹⁰ Particularly in all the spectra a large positive band at 260 nm and a negative band at 240 nm were apparent, indicative of a parallel four stranded structure.¹²

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