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Irreversible electron attachment - a key to DNA damage by solvated electrons in aqueous solution‡

K. Westphal, [a†], J. Wiczk, [a†], J. Miloch, [a] G. Kciuk,[b] K. Bobrowski[b] and J. Rak *[a]

[a]Faculty of Chemistry, University of Gdańsk, Wita Stwosza 63, 80-308 Gdańsk, Poland.
e-mail: janusz.rak@ug.edu.pl
[b]Centre of Radiation Research and Technology, Institute of Nuclear Chemistry and Technology, Dorodna 16, 03-195 Warsaw, Poland
[†]These authors contributed equally.
‡Electronic Supplementary Information (ESI) available: scheme showing digestion of a TXT trimer by micrococcal (MC) or P1 nuclease (P1), retention times of dimers and monomers obtained by enzymatic digestion, stable fragments formed due to γ-irradiations, molar absorption coefficients, molar contribution (in %) of individual products generated by irradiation of 3 x 10^{-5} M TBrAT solution in different Tris concentration, MS/MS spectra of gamma irradiated aqueous solution of TBrAT, TBrCT and TBrGT.

Abstract

The TYT and TXT trimeric oligonucleotides, where X stands for a native nucleobase, T (thymine), C (cytosine), A (adenine), or G (guanine), and Y indicates a brominated analogue of the former, were irradiated with ionizing radiation generated by a ^{60}Co source in aqueous solutions containing Tris as a hydroxyl radical scavenger. In the past, these oligomers were bombarded with low energy electrons under an ultra-high vacuum and significant damage to TXT trimers was observed. However, in aqueous solution, hydrated electrons do not produce serious damage to TXT trimers although the employed radiation dose exceeded many times the doses used in radiotherapy. Thus, our studies demonstrate unequivocally that hydrated electrons, which are the major form of electrons generated during radiotherapy, are a negligible factor in damage to native DNA. It was also demonstrated that all the studied brominated nucleobases have a potential to sensitize DNA under hypoxic conditions. Strand breaks, abasic sites and the products of hydroxyl radical attachment to nucleobases have been identified by HPLC and LC-MS methods. Although all the bromonucleobases lead to DNA damage under the experimental conditions of the present work, bromopyrimidines seem to be the radiosensitizers of choice since they lead to more strand breaks than bromopurines.

Introduction

Since the seminal paper by Sanche and colleagues was published,[1] the damaging potential of electrons toward DNA has continued to attract interest. The Canadian group mentioned above demonstrated that plasmid DNA adsorbed on a tantalum support developed single and double-stranded breaks when bombarded with low energy electrons (LEEs) in an ultra-high vacuum (UHV) with yields dependent on the electron energies.[1] This spectacular finding initiated a flood of experimental and theoretical papers that speculated on the mechanism of electron-induced strand break (SB) formation.[2-4]

Since hydrated electrons (e_{aq}^-) are one of the main products of water radiolysis in a low concentration of oxygen[5] (beside hydroxyl radicals (•OH)) and DNA is the most important target of radiotherapy,[6] the biological connotation of Sanche's discovery was obvious from the very beginning. However, one has to realize that both the original experiment by Sanche[1] and a number of subsequent studies[7] were carried out under ultra-high vacuum (UHV). To this end, it has to be emphasized that LEEs are unstable kinetic species, while prehydrated electrons (e_{pre}^-) or e_{aq}^- are chemical species trapped in potential energy wells.[8]

Hydrated electrons generated by low-LET (Linear Energy Transfer) ionizing radiation (*ir*) from a ^{60}Co γ-source are able to attach to nucleobases (NBs) as indicated by a transient absorption observed in pulse radiolysis studies of thymine aqueous solutions[9] and ascribed to the thymine anion. However, in a Biochemistry paper published in 2005[10], as well as in other publications from the same group, Sanche et al. showed that no SBs are formed in an aqueous solution containing a relatively short DNA duplex and irradiated with as much as 700 Gy of IR. To this end, one should realize that the usual ir dose employed in cancer patients does not exceed 40 Gy for the whole treatment and is always divided into single exposures with doses usually smaller than 1.5-2.0 Gy.[11] Recently, the Sanche group has studied the cisplatin enhancement of IR-induced DNA damage and demonstrated that in a dilute, deoxygenated,

aqueous solution containing plasmid DNA, the yield of SSBs generated by γ-radiation from a ^{60}Co source is doubled when the solution is saturated with N_2O, which converts all the e_{aq}^- into •OH radicals.[12] Their findings prove indirectly that hydrated electron attachment is inconsequential for DNA in an aqueous solution. All the above mentioned facts demonstrate that, in conventional radiotherapy, e_{aq}^- do not form SSBs in DNA.

It is worth noting that the radiation chemical yield of e_{aq}^- depends on the oxygen concentration in the irradiated water.[5] One should also remember that solid tumors suffer frequently from hypoxia due to insufficient angiogenesis and high metabolism.[13] For fully oxygenated cells, the main product of water radiolysis is the •OH radical[5] due to a very fast reaction between e_{aq}^- and oxygen (with $k = 2 \times 10^{10}$ $M^{-1}s^{-1}$) leading to the formation of superoxide $O_2^{\bullet-}$ radical anion. However, with increasing hypoxia e_{aq}^- becomes more and more abundant and in deoxygenated water the reaction mentioned above does not occur. As a consequence, the radiation chemical yield of e_{aq}^- generated by ionizing radiation becomes equal to the radiation chemical yield of •OH radicals.[5] Thus, the *enfant terrible* of radiotherapy – an oxygen effect,[13] i.e. 2.5-3.0-fold higher radioresistance of hypoxic cells compared to the well-oxygenated ones, can be explained by the fact that the •OH radicals induced damage is not "fixed" due to lack of oxygen[13] while e_{aq}^- do not produce serious damage to native DNA.[14]

The discussion above clearly demonstrates the necessity of the employment of radiosensitizers for an efficient *ir* treatment. One of the very promising radiosensitizing agents, conformed by clinical studies,[12] seems to be cisplatin as its adduct to DNA dramatically enhances the yield of strand break formation due to interactions with electrons generated by sparsely ionizing radiation.[12,15] 5-bromo-2'-deoxyuridine (BrdU) is another radiosensitizer that belongs to the class of thymidine analogues and utilizes hydrated electrons for SB formation.[16] The compound is recognized by human kinases[17] and polymerases,[18]

which enables newly synthesized DNA to be labeled efficiently during its replication or repair. Attachment of hydrated electron to such modified DNA triggers the dissociation of the C5-Br bond in BrU (5-bromouracil), which in turn produces the uracil-5-yl radical in DNA and releases a bromide anion (Br^-).[19-24] The latter process is an irreversible, low-barrier reaction that keeps the overall reaction moving to products.[9] The resulting reactive uracil-5-yl radical may abstract a hydrogen atom from the adjacent sugar residue forming a radical on sugar residue, which is involved in a sequence of subsequent reactions leading to a strand break.[25] As it has been recently demonstrated, the uracil-5-yl radical may also react with a water molecule to produce a genotoxic hydroxyl radical – a well-known DNA strand breaker.[26]

It was demonstrated in *in vitro* studies that BrU sensitizes cancer cells to γ-radiation. Indeed, tumor cells grown in media containing BrdU are 2-3 fold more sensitive to *ir*.[27] It is worth noting that not only the increased electron affinity of BrU, compared to that of T or U, is responsible for the observed effect. The irreversibility of e_{aq}^- induced degradation of the BrU labeled DNA is an even a more important factor. Indeed, a comparison of the radiolysis of BrU and T in aqueous solution revealed that an attachment of e_{aq}^- only to the former compound led to a measurable effect, i.e. to the efficient production of U.[9] In solutions containing T, the yield of degradation was negligible. Thus, the BrU anion, unlike the T one, is very unstable and undergoes C5-Br bond dissociation before transfer of the excess electron to water. Similar properties are displayed not only by BrdU but also by other brominated derivatives of nucleosides. Indeed, it was demonstrated, using low temperature Electron Spin Resonance (ESR), that 5-bromo-2'-deoxycytidine incorporated into DNA makes the modified biopolymer ca. 3-fold more sensitive to the e_{pre}^- attachment in comparison to native DNA.[28] On the other hand, in radiolytic studies at ambient temperature, Chatgilialoglu's group demonstrated that hydrated electron attachment to 8-bromo-2'-deoxyadenosine (8BrdA)[29] or

8-bromo-2'-deoxyguanosine (8BrdG)[30] leads to an efficient release of bromide anion and the formation of the respective radical localized on the nucleobase. Similarly, e_{aq}^- induced debromination of 8BrdA and 8BrdG was observed in DNA duplexes containing beside brominated nucleobases a light-dependent flavin electron injector.[31] The sensitizing properties of bromonucleosides were very recently confirmed by one of us in studies on the interaction between low energy electrons (LEEs) and brominated nucleobases incorporated into short (trimers) oligonucleotides.[32,33] Namely, the electron stimulated desorption (ESD) of small anions induced by LEE bombardment of the studied trimers adsorbed on a gold substrate (for the structures of these trimers, see Fig. 1) under ultra-high vacuum (UHV) was recorded.[32] In addition, the trimers degraded by LEEs were removed from the metal substrate and subjected to HPLC analyses. This approach enabled quantitative measurements of damage to oligonucleotides.[33] The ESD curves showed that the release of Br^- was one of the main reaction channels induced by LEEs in the studied trimers.[32] It was also demonstrated that brominated TYT oligonucleotides were 2-3 fold more reactive than their native counterparts.[33]

In the experiments described below, hydrated electrons were generated in a manner similar to that employed in radiotherapy, i.e. by γ-irradiation of deoxygenated water. The irradiated aqueous solutions contained the same short oligonucleotides (see Fig. 1) for which the ESD/HPLC studies mentioned above were performed in the past.[32,33] The obtained radiolytes were subjected to HPLC and LC-MS (LC-MS/MS) analyses that demonstrated degradation only in the brominated trimers. Since the irradiations were carried out in the presence of a •OH radical scavenger, this result shows unequivocally that only the brominated trimers are sensitive to the γ-radiation generated hydrated electrons. As the same trimers were subjected to LEE-induced damage under UHV, where both the labeled and native oligonucleotides turned out to be sensitive to LEEs, the current studies can be treated as a

direct proof that hydrated electrons do not lead to a serious DNA damage in aqueous solution. Therefore, some type of DNA sensitization is indispensable for electrons to play an active role in radiotherapy.

Materials and methods

General

All the trinucleotides of HPLC purity, synthesized using the phosphoroamidite method, as well as native and brominated nucleoside phosphoramidites were purchased from Metabion (Munich, Germany; brominated trimers) or Genomed (Warsaw, Poland; native trimers). The irradiated aqueous solutions (ultrapure water obtained using a Mili-Q system from Hydrolab, Poland HLP) containing ca. 3 x 10^{-5} M of a trinucleotide were freshly prepared before radiolysis. Tris(hydroxymethyl)aminomethane (Tris) and Triethylammonium acetate (TEAA) were purchased from Sigma-Aldrich (Poland), while HPLC grade acetonitrile (ACN) was from POCh (Gliwice, Poland).

γ-Irradiations

The γ-irradiations were carried out in a degassed solution that contained 30 mM Tris as a scavenger of the •OH radicals. Each sample was irradiated for one hour. The irradiation was carried out with the use of ^{60}Co γ-source (Issliedowatiel; 1.17 and 1.33 MeV). The radiation dose amounted to 140 Gy.

Chromatography

The HPLC separations were performed on a Dionex UltiMate 3000 System with a Diode Array Detector, which was set at 260 nm for monitoring the effluents. A Waters® XBridge™ OST C18 column (4.6 mm × 50 mm; 2.5 μm in particle size) and a linear gradient of 0-20% B

over 20 min was used (phase A: 50 mM TEAA + 1% ACN, phase B: 80 % ACN). The flow rate was set at 0.5 ml/min.

Preparation of chromatographic standards

Formation of strand breaks due to exposure of the labeled oligonucleotides to ionizing radiation results in the respective monomeric (pT, Tp) and dimeric (pXT, TXp) fragments originating from the irradiated TYT trimers (X, Y and p stand for native, and brominated nucleobase, and the phosphate group, respectively). Dimeric and monomeric standards were obtained by enzymatic digestion of 2 μg of a TXT trimer (Genomed, Poland). The fragments containing the 3' terminal phosphate group were obtained by the digestion of the native trimers with micrococcal nuclease (Sigma-Aldrich, Poland) (0.02U/μl) while the fragments containing the 5' terminal phosphate were obtained by the digestion with P1 nuclease (Sigma-Aldrich, Poland) (0.2U/μl) – see Scheme S1 (ESI). The retention times for the particular fragments are given in Table S1 (ESI).

Mass Spectrometry

Electrospray ionization-mass spectrometry (ESI-MS), MS and tandem MS (MS/MS) experiments were conducted on an HCTultra ion-trap mass spectrometer (Bruker Daltonics). The applied spray voltage was ±4.0 kV, the drying gas (N_2) pressure was 50 psi, the flow rate was adjusted 11 l/min and the temperature to 365 ^{o}C. The spectra were registered in the negative-ion mode. Each spectrum was obtained by averaging 3 scans, the time of each scan being 0.1 s.

LC-MS and LC-MS/MS conditions. An Agilent 1200 Technologies HPLC System was employed for the LC-MS/MS experiments. The same column, mobile phases and gradient were used as those described in the *Chromatography* section. The effluent was coupled to the

HCTultra ion-trap mass spectrometer, which was operated in the positive or negative ion mode in the LC-MS analyses while only in the negative ion mode in the LC/MS/MS assays.

Results and discussion

The aqueous solutions containing TYT trimers at the concentration of 3×10^{-5} M were irradiated with a dose of 140 Gy. The solution additionally contained 30 mM Tris as an •OH radical scavenger. Prior to irradiation, the samples were deoxygenated by purging with argon for 3 min. The applied dose was chosen on the basis of a series of separate irradiations (not shown) where the dose was varied over the range 35 to 600 Gy. Decomposition of the substrates was sufficiently high for 140 Gy to carry out reliable quantitative analyses while the dose itself was small enough to prevent a substantial degradation of the primary products of radiolysis. Exemplary HPLC runs for all the studied trimers are depicted in Fig. 2. Only the HPLC peaks that give rise to the appropriate MS signals are marked since only LC-MS analysis allows for product identification unless chromatographic standards are available. The employed dose causes significant damage to the labeled oligonucleotides as demonstrated by these chromatograms. Interestingly, practically no damage (note that 140 Gy greatly exceeds the doses routinely used in radiotherapy[11]) is produced by the same dose in the corresponding native trimers (see Fig. 2). Indeed, the only products (with molar contributions between 1-3% of the initial amount of the trimer) triggered by hydrated electrons in the solutions of the native TXT trimers, are those with the MS signals marked with TXT+2H (Figure 2). These products are probably native trimers containing the 5,6-dihydrothymine moiety. Hydrated electrons are stabilized by the solvation energy of only 1.6 eV,[34] so they can easily add to all nucleobases. Indeed, it is known that hydrated electrons react with nucleobases with a diffusion controlled rate.[35] Reacting with the native DNA hydrated electrons mainly add to

pyrimidines since they possess higher electron affinities than purines. As indicated by pulse radiolysis studies the pyrimidine radical anion is rapidly and reversibly protonated at O4.[36] The protonation at O4 competes with slower and irreversible protonation at C6.[36] The latter radical may subsequently be reduced giving a stable 5,6-dihydropyrimidine derivative.[37] The picture described above explains the formation of TXT+2H observed in our studies. Additionally, our finding remains in full accordance with the results obtained by Swart et al.[37] where the influence of the increase in hydration of DNA on its reaction with hydrated electrons was studied. These authors found that in a fully hydrated DNA (that resembles the situation of trimers in our studies) a small amount of 5,6-dihydropyrimidines and molecular hydrogen are formed due to the reaction with hydrated electrons. On the other hand, significant damage of the native trimers was observed in UHV experiments.[32,33] This is an essential finding of the current study that directly addresses a query about the biological role of hydrated electrons - an issue that has been posed a number of times over the last 15 years. Thus, the results shown here indicate unequivocally that, as far as damage to native DNA is concerned, the involvement of hydrated electrons generated by low LET ionizing radiation is negligible. One may contest the above-mentioned reasoning, claiming that the reactions of hydroxyl radicals with Tris generate a large number of radicals and products that in turn can react with hydrated electrons when the concentration of DNA targets is low. As a consequence, one might observe comparable amounts of damage to brominated and normal trimers at a higher concentration of trimers. If it was the case, the lack of reactivity of the native trimers would be concentration dependent. In order to exclude this possibility, we carried out additional experiments in which the concentration of TBrGT and TGT was 10 fold larger, i.e. 3×10^{-4} M. These experiments led to identical results to those obtained in diluted solutions. Namely, we did not observe products from TGT while the qualities of products assayed in both solutions containing TBrGT were the same.

Damage to each labeled trimer, in terms of the type of particular fragments, is qualitatively summarized in Table S2 (ESI). The particular chromatographic peaks (shown in Fig. 2), were identified using the LC-MS method and tandem mass spectrometry. The structures of the stable products formed during the course of γ-irradiation are shown in Fig. 2 and marked by nos. 1-13 while their abundances are collected in Table 1. The identification of particular species has been carried out by two different approaches, i.e. either by employing the retention times of the TXT and TYT trimers (both type of oligonucleotides were purchased, *(see the Materials and methods section)*, as well as of the $_{P}T_{OH}$, $_{HO}T_{P}$, $_{P}XT_{OH}$, $_{HO}T_{OH}$ fragments, which were generated by enzymatic digestion *(see the Materials and methods section)* or, when the chromatographic standards were unavailable, on the basis of distinctive fragmentation mechanisms revealed by the MS/MS analysis (e.g 1 and 6 in Fig. 2). Let us analyze, as an example, the data gathered in Fig. 3 for TBrUT. This system undergoes the most diverse damage (see Fig. 3). The chromatographic peaks numbered 2, 3, 4, 5, 7, 12 and 13 (see Fig. 2) correspond to $_{P}T_{OH}$, $_{HO}T_{P}$, $_{OH}T_{OH}$, $_{P}UT_{OH}$ $_{OH}TU_{OH}$/$_{OH}UT_{OH}$, TUT and TBrUT standards, respectively. Another confirmation of the identity of these species is the fragmentation pattern observed in the MS/MS spectra. Indeed, the fragmentation pathways for TBrUT (Fig. 3, product number 13) and TUT (Fig. 3, product number 12) are similar and proceed according to the well-known fragmentation behavior of oligonucleotides.[38] Indeed, the major fragmentation of electrospray-generated oligodeoxynucleotide anions involves cleavage of the 3' C–O bond of the sugar from which the nucleobase is lost to give [a-B] ions or their complementary w ions.[38] In the first step of fragmentation, the neutral 5' thymine is eliminated (m/z =709.2 and 789.3 for TUT and TBrUT, respectively (see 12 and 13 in Fig. 3)) and then the sugar residue is lost, which gives rise to the w_2 anion (m/z = 611.2 (5'-pUT) and 691.1 (5'-pBrUT), for TUT and TBrUT, respectively; cf. 12 and 13 in Fig. 3). A similar fragmentation occurs with respect to the loss of U, 5'-phosphate and sugar residue or BrU, 5'-

phosphate and sugar residue from the anionic 5'-pUT or 5'-pBrUT fragments, respectively. Thus, the further fragmentation of these 5'-pUT or 5'-pBrUT fragments leads to the same w_1 anion (pT, m/z = 321.0; see 12 and 13 in Fig. 3). Shorter fragments such as $_PT_{OH}$ (Fig. 3, product number 2), $_{HO}T_P$, (Fig. 3, product number 3) and $_{HO}T_{OH}$ (Fig. 3, product number 4) originate from radiation induced cleavage of the 5'or 3' C-O and 3' O-P bond, respectively. The fragmentation of $_PT_{OH}$ and $_{HO}T_P$ results in the loss of thymine as a consequence of the backside nucleophilic S_N2 attack on C1' by the negatively charged terminal phosphate[38], while the fragmentation of $_{HO}T_{OH}$ mainly leads to the negatively charged thymine.

Products 1, 6, 8 and 11 (Fig. 3) were exclusively identified using the MS/MS method since the appropriate chemical standards were unavailable. Nevertheless, the fragmentation patterns observed in the mass spectrometry experiments confirm the assumed structures. The fragment with the abasic site (Fig. 3, product number 11) is formed due to γ-irradiation via N-glycosidic bond cleavage, which results in the release of BrU. The fragmentation of this product (m/z = 725.0 (M-H); see 11 in Fig. 3) comprises the loss of 5'-thymidine (cleavage of the 3' C-O bond[38]) followed by the detachment of the 5'-phosphate group along with the sugar residue (499 → 321; see 11 in Fig. 3). $T_{HO}UT$ (Fig. 3, product number 8), in turn, loses thymine, which is coupled to the cleavage of the 3' C-O bond and release of the sugar residue along with the 5'-phosphate group. In the next step, hydroxyuracil, together with the second sugar residue, is detached to result in the formation of the w_1 anion (m/z = 321, see 8 in Fig. 3). The formation of products containing a carbonyl group (Fig. 3, product number 1 and 6) may occur when the radiation induced uracil-5-yl radical is transferred directly, or via the hydroxyl radical released from the water molecule[26], to the C3'/C5' carbon atom, which ultimately results in the O-P bond break.[39] The fragmentation pathway for dT=O comprises the loss of a sugar residue (Fig. 3, product 1), while for $O{=}UT_{OH}$, the sugar moiety and uracil

are lost since the fragmentation is coupled with the nucleophilic attack of the negatively charged 5' phosphate on C1'.

The radiation-induced products observed for the other modified trinucleotides (TBrAT, TBrCT, TBrGT) were identified in a similar way (see Figs. S1, S2 and S3 ESI).

The relative yields of particular degradation products were calculated regarding the amount of the trimer in the non-irradiated sample. The integrated HPLC peaks of a particular species, and that of the labeled trimer corrected for the difference in the molar absorption coefficients of the compared compounds, were used for this purpose (for molar absorption coefficients, see Table S3 ESI). The relative molar contributions (as percentages) of particular compounds in the analyzed radiolytes are collected in Table 1.

One may wonder if the employed Tris concentration of 30 mM is sufficient to scavenge all the hydroxyl radicals generated by the 140 Gy applied in our radiolytic experiments. Indeed, this dose produces 37.8 to 40 μM of •OH radicals (0.27-0.28 μM of OH per 1 Gy[5]). Taking into account that the rate constant of the •OH radical scavenging by Tris amounts to 1.1×10^9 $M^{-1}s^{-1}$, reported by Hicks and Gebicki,[40] it seems that 30 mM Tris used in this work was sufficient to completely scavenge the radiation-induced •OH radicals and, as a consequence, quench all chemical reactions induced by them (the scavenging efficiency also depends on the intensity of the ionizing photons flux, which in our case was relatively low, i.e. 2.3 Gy/min). In order to confirm the scavenging capabilities in our system, additional irradiations of the solutions containing TBrAT and three other concentrations of Tris: 0, 60 and 200 mM were carried out (see Table S4 ESI). The data gathered in Table S4 (ESI) confirm that 30 mM of Tris is sufficient to quench most of the chemical processes triggered by the •OH radicals. The amount of particular products is 3-5 fold higher for 0 mM of Tris than that for 30 mM Tris. However, the product yields change by only 5-10% (see Table 1 and Table S4 ESI) when the Tris concentration is increased to 60 mM and then to 200 mM (when the •OH scavenging

capacity is equal to the •OH radical diffusion distance[12]). This simple analysis, and the fact that the $T_{OXO}XT$ product forms in TBrAT without the addition of Tris, suggests that this product results from the TBrUT and TBrCT solutions containing 30 mM Tris as a result of the reaction between the uracilyl radical and water[26], rather than due to the involvement of radiolysis-generated •OH radicals.

The most abundant product for all the studied trimers results from the debromination of TYT, i.e. a respective TXT trimer (type 12, see Fig. 2 and Table 1). As far as the biological function of DNA is concerned, this is an irrelevant type of damage since a bromonucleobase is substituted with a native nucleobase. Such a lesion results from hydrogen atom abstraction by a radical localized on the nucleobase (the latter comes from DEA to BrX) from an external H-donor. This reaction may lead to DNA damage only if the radical originating from a hydrogen donor is sufficiently genotoxic like, for instance, the •OH radical that would be formed in the reaction between the uracil-5-yl radical and water.[26] Such an •OH radical could be produced in very close vicinity to the DNA helix, provided that BrX is incorporated in the double-helix, and be responsible for a strand break after hydrogen abstraction from a proximate sugar residue.[41] Moreover, since in the cell DNA interacts permanently with proteins, the process discussed above (abstraction of a hydrogen atom from a protein by a nucleobase radical) could also lead to a DNA-protein cross-link.

A strand break is the second most abundant damage in trimers labeled with the brominated pyrimidines. The $pXT_{OH}/_{HO}XT_{OH}$ (see 5/7 in Fig. 2) and $_{HO}TX_{OH}$ (see 7 in Fig. 2) fragments are the signatures of this process. The former fragments indicate breakage at the 5'-end while the latter fragment indicates a breakage at the 3'-end. The results collected in Table 1 suggest a preference toward the 5'-end break. Thus, the main reaction channel, as far as a SB is concerned, seems to be related to hydrogen atom abstraction from the sugar residue. In the subsequent step, the sugar radical may undergo either heterolytic dissociation[5] at the CX'-O

(X = 3 or 5) bond or homolytic dissociation at the O-P bond.[42,43] The former process produces the X'-OH on the deoxyribose (see 7 in Fig. 2), while the latter produces an aldehyde group on C5' (see 6 in Fig. 2). Both types of products are detected by MS/MS analysis (see Fig. 2 and Table 1).

The strand breaks are also visible in trimers labeled with bromopurines although this effect is significantly smaller than in trimers labeled with bromopyrimidines. Especially, the bromoguanine labeled oligonucleotide seems to be resistant to the formation of strand breaks. Other types of damage, observed only for the bromopyrimidine labeled trimers, are the substitution of the bromine atom with a hydroxyl group and formation of an abasic site. The former reaction suggests involvement of the $^{\bullet}$OH radical, while the abasic site is an indication of hydrolysis of the N-glycosidic bond.

Conclusions

In summary, all the studied bromonucleobases sensitize the trimeric oligonucleotides, and probably duplex DNA, to hydrated electrons generated by water radiolysis. Interestingly, in the analogous native trimers that comprise ordinary nucleobases, rather than their brominated analogues, hydrated electrons do not lead to serious damage. This finding directly addresses the question about the biological role of hydrated electrons produced during radiotherapy. Our results indicate that e_{aq}^- generated in water by low-LET ionizing radiation are unable to damage native DNA, whereas DNA labeled with bromoderivatives are vulnerable to damage. Moreover, under hypoxia, characteristic for solid tumor cells, the cytotoxic properties of •OH are highly limited.[13] Therefore, radiosensitizers that make hydrated electrons reactive to DNA are particularly valuable. Hence, modified nucleobases incorporated into DNA during its biosynthesis and undergoing an irreversible electron-induced dissociation are valuable radiosensitizers. The brominated nucleobases studied in this work belong to such a type of

sensitizer. Bromopyrimidines seem to be much better sensitizers than bromopurines since the yield of strand breaks induced by DEA is significantly higher for DNA labeled with the former. It is worth of noting that either damage to nucleobases or the formation of an abasic site is easily repaired by the enzymatic machinery of a cell. However, the same does not hold for strand breaks. Indeed, double strand breaks (DSBs) especially pose a real threat to cell survival[31] and the single strand breaks (SSBs), if formed in sufficiently large amounts, may lead to DSBs.

In order to sensitize a cell, the studied compounds must be incorporated into genomic DNA during cell division or repair. Labeling experiments employing tumor cells and the four studied bromonucleosides are underway in our laboratory.

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References

1 B. Boudaiffa, P. Cloutier, D. Hunting, M. A. Huels and L. Sanche, *Science,* 2000, **287**, 1658–1660.

2 J. Simons, *Acc. Chem. Res.*, 2006, **39**, 772–779.

3 J. Rak, K. Mazurkiewicz, M. Kobyłecka, P. Storoniak, M. Haranczyk, I. Dąbkowska, R. A. Bachorz, M. Gutowski, D. Radisic, S. T. Stokes, S. N. Eustis, D. Wang, L. Xiang, Y. J. Ko and K. H. J. Bowen, Stable Valence Anions of nucleic acid bases and DNA strand

breaks induced by low energy electrons, in *Radiation Induced Molecular Phenomena in Nucleic Acids,* eds.: M. Shukla and J. Leszczynski, Springer, Netherlands, 2008, p. 619-667.

4 J. Gu, J. Leszczynski, and H. F. Schaefer III, Chem. Rev., 2012, **112**, 5603-5640.

5 C. von Sonntag, *Free-radical-induced DNA Damage and its Repair. A Chemical Perspective*, Springer, Berlin, Heidelberg, Germany, 2006.

6 *DNA Repair in Cancer Therapy. Molecular Targets and Clinical Applications*, ed.: M. R. Kelly, Elsevier Inc, 2012.

7 L. Sanche, *Low-Energy Electron Interaction with DNA: Bond Dissociation and Formation of Transient Anions, Radicals, and Radical Anions* In *Radical and Radical Ion Reactivity in Nucleic Acid Chemistry*, ed.: M. M. Greenberg, John Wiley & Sons, Inc., Hoboken, New Jersy, USA, 2010, pp. 239-293.

8 B. Abel, U. Buck, A. Sobolewski and L. Domcke, *Phys. Chem. Chem. Phys.*, 2012, **14**, 22-34.

9 J. D. Zimbrick, J. F. Ward and L. S. Myers Jr, *Int. J. Radiat. Biol.*, 1969, **16**, 505-523.

10 S. Cecchini, S. Girouard, M. S. Huels, L. Sanche and D. J. Hunting, *Biochemistry,* 2005, **44**, 1932-1940.

11 *Basic clinical radiobiology*, eds.: M. Joiner and A. van der Kogel, Hodder Arnold, London, Great Britain, 2009.

12 M. Rezaee, L. Sanche and D. J. Hunting, *Rad. Res.* 2013, **179**, 323–331.

13 B. T. Oronsky, S. J. Knox and J. Scicinski, *J. Trans. Oncol.*, 2011, **4**, 189–198.

14 F. J. Nabben, J .P. Karman, H. Loman, *Int. J. Radiat. Biol.*, 1982, **42**, 23-30.

15 B. Behmand, J. R. Wagner , L. Sanche and D. J. Hunting, *J. Phys. Chem. B*, 2014, **118**, 4803–4808.

16 P. Wardman, *Clin. Oncol.*, 2007, **19**, 397-417.

17 L. S. Lee and Y. Cheng, *Biochemistry*, 1976, **15**, 3686-3690.

18 B. Goz, *Pharmacol. Rev.*, 1977, **29**, 249-272.

19 S. D. Wetmore, R. J. Boyd and L. A. Eriksson, *Chem. Phys. Lett.*, 2001, **343**, 151−158.

20 X. Li, L. Sanche and M. D. Sevilla, *J. Phys. Chem. A*, 2002, **106**, 11248−11253.

21 L. Chomicz, J. Rak and P. Storoniak, *J. Phys. Chem. B*, 2012, **116**, 5612-5619.

22 M. E. Dextraze, J. R. Wagner and D. J. Hunting, *Biochemistry*, 2007, **46**, 9089–9097.

23 T. G. Gantchev and D. J. Hunting, *J. Mol. Model.* 2008, **14**, 451–464.

24. M. E. Dextraze, S. Cecchini, F. Bergeron, S. Girouard, K. Turcotte, J. R. Wagner and D. J. Hunting, *Biochemistry*, 2009, **48**, 2005–2011.

25 W. Knapp-Pogozelski and T. D. Tullius, *Chem. Rev.*, 1998, **98**, 1089−1107.

26 L. Chomicz, A. Petrovici, I. Archbold, A. Adhikary, A. Kumar, M. D. Sevilla and J. Rak, *Chem. Commun.*, 2014, **50**, 14605–14608.

27 H. A. Curry and W. J. Curran Jr., *Chemoradiation Strategies for Patients with Malignant Gliomas* In *Chemoradiation in Cancer Therapy*, ed.: H. Choy, Humana Press Inc., New Jersey, USA, 2010, p. 140.

28 Y. Razskazovskii, S. G. Swarts, J. M. Falcone, C. Tayler and M. D. Sevilla, *J. Phys. Chem. B*, 1997, **101**, 1460−1467.

29 R. Flyunt, R. Bazzanini, C. Chatgilialoglu and Q. G. Mulazzani, *J. Am. Chem. Soc.*, 2000, **122**, 4225-4226.

30 M. Ioele, R. Bazzanini, C Chatgilialoglu and Q. G. Mulazzani, *J. Am. Chem. Soc.*, 2000, **122**, 1900-1907.

31 A. Manetto, S. Breeger, C. Chatgilialoglu,T. Carell, *Angew. Chem. Int. Ed.*, 2006, **45**, 318 –321.

32 K. Polska, J. Rak, A. D. Bass, P. Cloutier and L. Sanche, *J. Chem. Phys.*, 2012, **136**, 075101.

33 Y. Park, K. Polska, J. Rak, J. R. Wagner and L. Sanche, *J. Phys. Chem. B*, 2012, **116**, 9676-9682.

34 A. Kumar, J. A. Walker, D. M. Bartels, M. D. Sevilla, *J. Phys. Chem. A*, 2015, DOI: 10.1021/acs.jpca.5b04721

35 G. Scholes, *Effects of Ionizing Radiation on DNA. Physical, Chemical and Biological Aspects*, eds.: J. Huttermann, W. Kohnlein, R. Teoule and A. J. Bertinchamps, Springer-Verlag, Berlin, Heidelberg, New York, 1978, p . 153.

36 C. von Sonntag, H.-P. Schuchmann, *Int. J. Radiat. Biol.*, 1986, **49**, 1-34.

37 J. M. Falconea, D. Becker, M. D. Sevilla, S. G. Swarts, Radiat. Phys. Chem., 2005, **72**, 257–264.

38 K. X. Wan, J. Gross, F. Hillenkamp and M. L. Gross, *J Am Soc Mass*, 2001, **12**, 193-205.

39 J. Rak, L. Chomicz, J. Wiczk, K. Westphal, M. Zdrowowicz, P. Wityk, M. Żyndul, S. Makurat and Ł. Golon, *J. Phys. Chem. B* 2015, **119**, 8227-8238.

40 M. Hicks and J. M. Gebicki, *FEBS Lett.,* 1986, **199**, 1, 92-94.

41 W. Knapp-Pogozelski and T. D. Tullius, *Chem. Rev.*, 1998, **98**, 1089−1107.

42 L. Chomicz, L. Golon and J. Rak, *Phys. Chem. Chem. Phys.*, 2014, **16**, 19424-19428.

43 Ł. Golon, L. Chomicz and J. Rak, *Chem. Phys. Lett.*, 2014, **612**, 289-294.

Table 1 Molar contribution (in %) of individual products generated by irradiation of $3x10^{-5}$ M TYT solution also containing 30 mM Tris with 140 Gy (for individual molar absorption coefficients see Table S3 in ESI and for product symbols see Fig. 2)

Product	TBrUT	TBrCT	TBrAT	TBrGT
dT=O	0.71	0.89	-	-
$_{P}$T$_{OH}$	0.22	0.12	0.27	0.20
$_{HO}$T$_{OH}$/$_{HO}$T$_{P}$	1.92	0.14	0.53	0.50
O=XT$_{OH}$	1.79	1.17	-	-
$_{HO}$TX$_{OH}$/ $_{HO}$XT$_{OH}$	1.82	0.52	2.55	0.54
$_{P}$XT$_{OH}$	4.19	5.75	1.72	0.41
T$_{HO}$XT	1.78	0.98	-	-
T$_{OXO}$XT	-	-	-	3.77
abasic site	2.63	4.80	-	-
TXT	14.37	12.99	11.68	20.72

Figure Captions

Fig. 1 Structures of modified trimers.

Fig. 2 HPLC analysis of γ-irradiated aqueous solution containing TBrUT, TBrCT, TBrAT, TBrGT, TUT, TCT, TAT, TGT or TTT. Peak numbers displayed in particular chromatograms correspond to the structures presented in the bottom part of the figure. "TXT+2H" stands for the product trimer containing 5,6-dihydrothymidine.

Fig. 3 MS/MS (in the negative ionization mode) spectra of γ-irradiated aqueous solution of TBrUT (the arrows indicate the mass of pseudomolecular anions; for species symbols see Fig. 2).

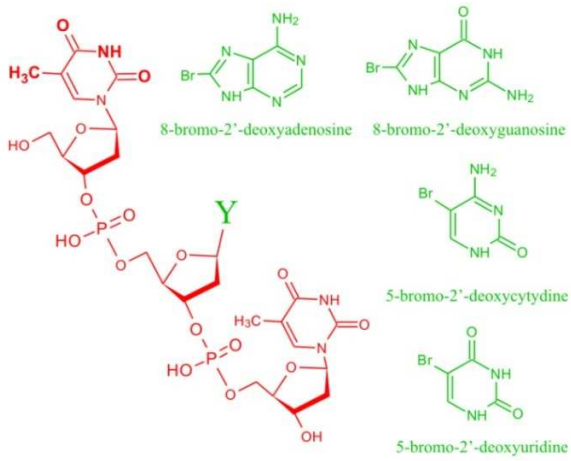

Fig. 1

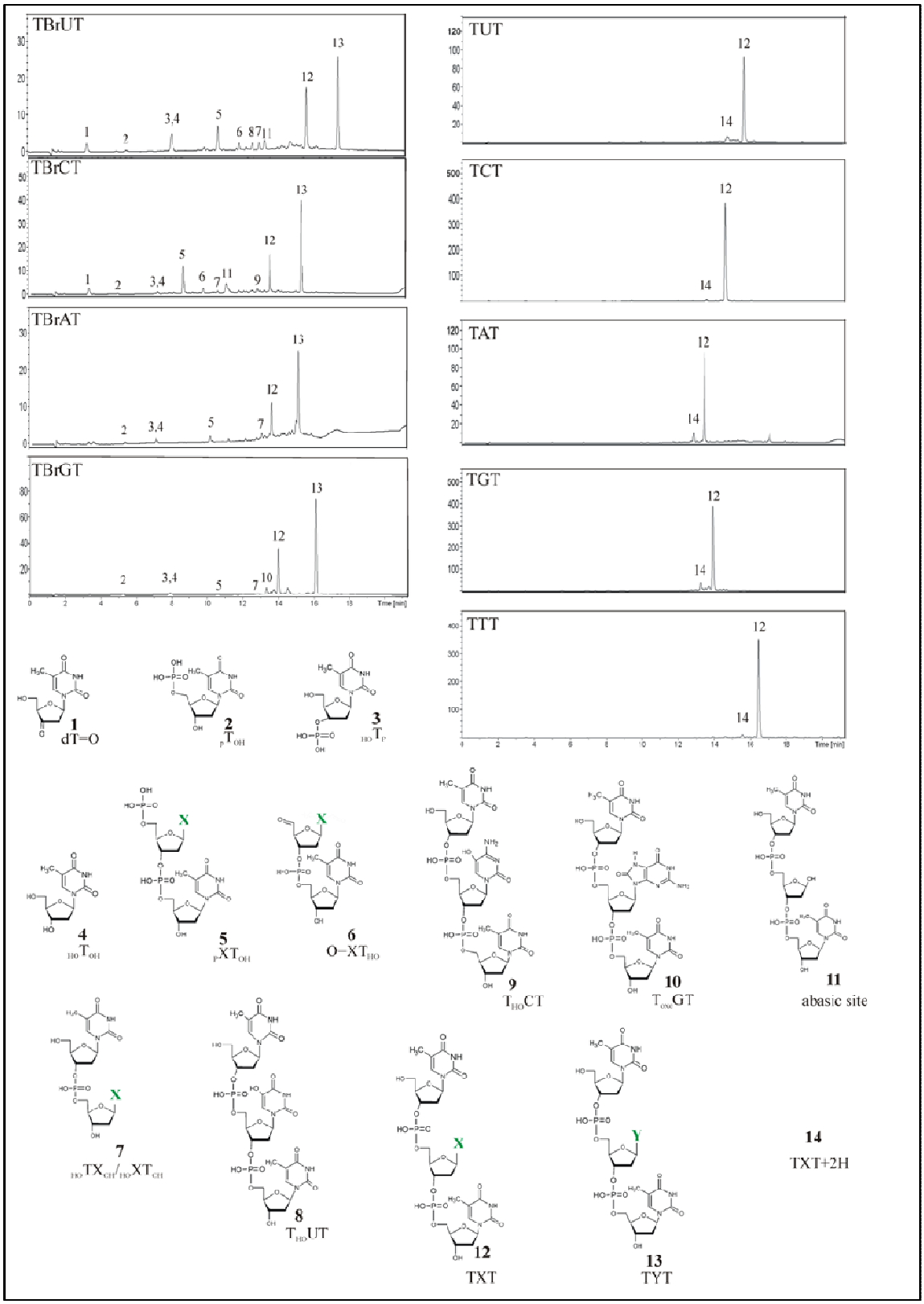

Fig. 2

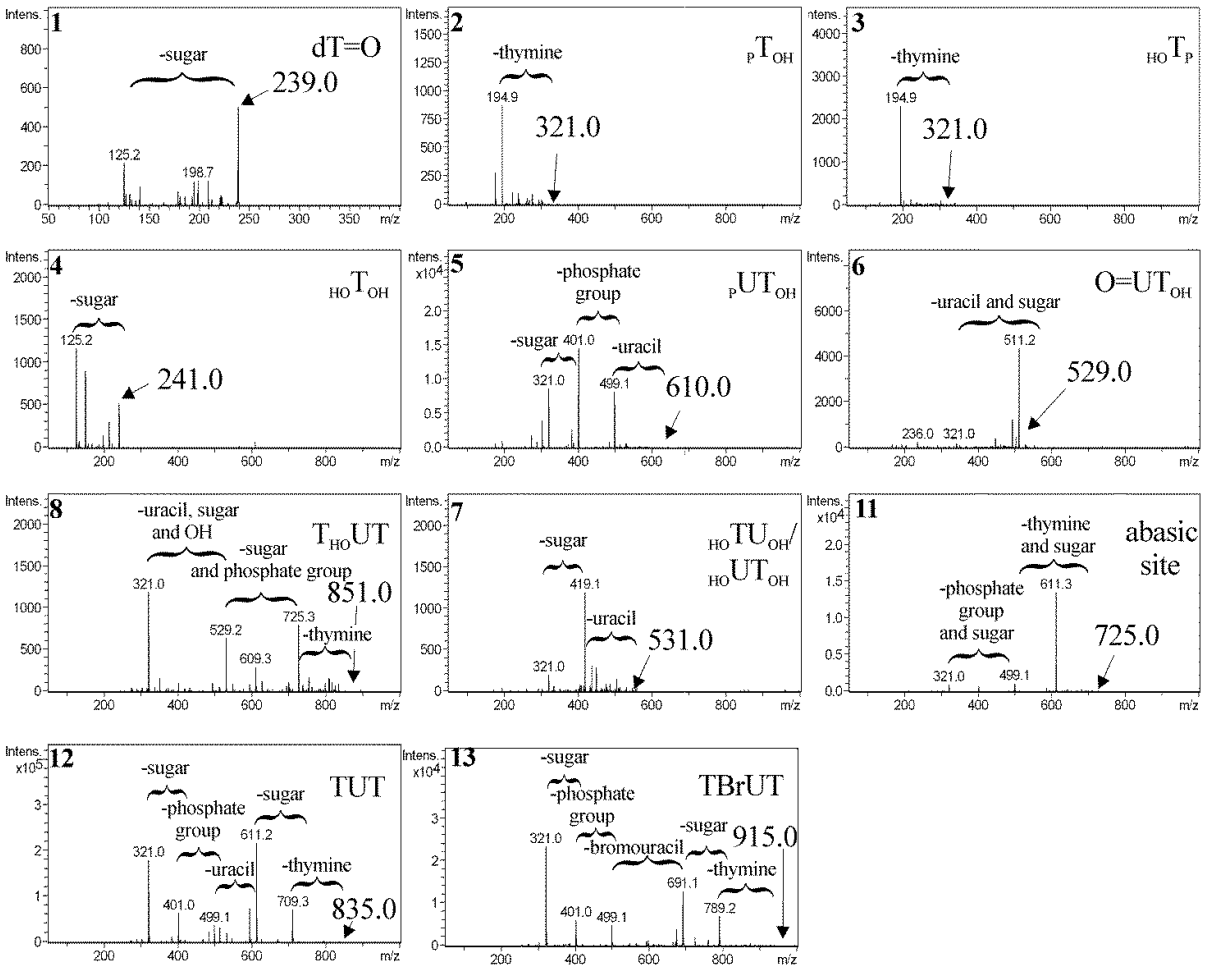

Fig. 3