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Fundamental Mechanisms of DNA Radiosensitization: Damage Induced by Low-Energy Electrons in Brominated Oligonucleotide Trimers

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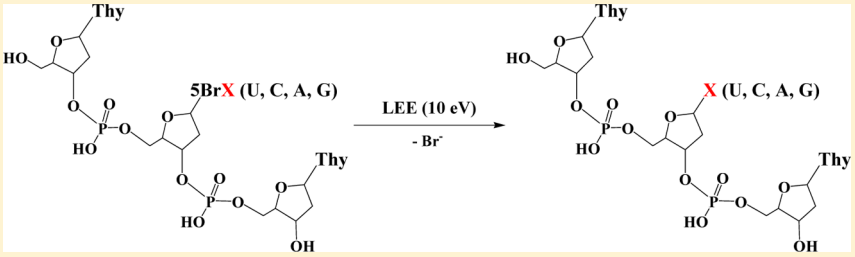
Supporting Information



ABSTRACT: The replacement of nucleobases with brominated analogs enhances DNA radiosensitivity. We examine the chemistry of low-energy electrons (LEEs) in this sensitization process by experiments with thin films of the oligonucleotide trimers TBrXT, where BrX = 5-BrU (5-bromouracil), 5-BrC (5-bromocytosine), 8-BrA (8-bromoadenine), or 8-BrG (8-bromoguanine). The products induced from irradiation of thin (~ 2.5 nm) oligonucleotide films, with 10 eV electrons, under ultrahigh vacuum (UHV) are analyzed by HPLC-UV. The number of damaged brominated trimers ranges from about 12 to 15 × 10^{-3} molecules per incident electron, whereas under the identical conditions, these numbers drop to 4−7 × 10^{-3} for the same, but nonbrominated oligonucleotides. The results of HPLC analysis show that the main degradation pathway of trinucleotides containing brominated bases involve debromination (i.e., loss of the bromine atom and its replacement with a hydrogen atom). The electron-induced sum of products upon bromination increases by factors of 2.1 for the pyrimidines and 3.2 for the purines. Thus, substitution of any native nucleobase with a brominated one in simple models of DNA increases LEE-induced damage to DNA and hence its radiosensitivity. Furthermore, besides the brominated pyrimidines that have already been tested in clinical trials, brominated purines not only appear to be promising sensitizers for radiotherapy, but could provide a higher degree of radiosensitization.

1. INTRODUCTION

Cellular DNA is the main target of anticancer radiotherapy. As opposed to proteins for which damage can be restored via appropriate gene expression, DNA damage, if not repaired, can lead to cell death or mutagenesis. When high energy radiation (e.g., electrons, protons, X-rays, or γ-rays) which is the basic tool in radiotherapy[1] interacts directly with DNA, excited molecules, radicals, ions, and secondary electrons are generated.[2,3] About 20% of the absorbed energy causes excitation, whereas the rest leads to ionization.[4] The latter process produces secondary electrons as the most abundant product of radiolysis.[5,6] Most secondary electrons have low energies with a distribution lying around 9 eV.[7] Similar interactions occur in water surrounding DNA in cells, to produce $H^{\bullet}$ and $HO^{\bullet}$ radicals and solvated electrons (e_{aq}^{-}),[8,9] which can also react with DNA (i.e., the indirect action of ionizing radiation).

The interaction of low energy electrons (LEEs) with DNA has been investigated with basic components of DNA (bases, sugar and phosphate groups),[10−16] oligonucleotides[10,17−19] and plasmids.[10,20−22] Together, these studies show that LEEs (1−30 eV) can generate single (SSBs), double (DSBs), and multiple strand breaks through a resonant process.[10,22] Electron attachment to DNA results in the formation of transient molecular anions, which dissociate, stabilize or decay via electron autodetachment.[23] The latter process can leave the target in an electronically excited state, which if dissociative leads to radical products. With the exception of stabilization which produces a local stable anion, these decay channels account for a large portion of strand breaks. Whereas SSB can be produced at any energy, DSBs formation occurs at electron energies higher than 5 eV. It has been suggested that formation of radicals from the decay of a transient anion into an

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electronically excited state followed by electron transfer to the other strand can cause a DSB.[24]

The dose required to damage DNA in the nucleus of cells can be reduced by substances that sensitize DNA to ionizing radiation. Such drugs decrease the radiation dose required to inactivate cancer cells and hence find application in anticancer radiotherapy.[25]

Halogenated nucleobases (Hal-NBs) can readily be incorporated into DNA, during cell replication, to render the molecule more sensitive to UV−light and γ radiation.[26] It seems, therefore, that owing to Hal-NBs properties (radio- and photosensitivity) these compounds could be used as efficient radiosensitizers in anticancer therapy.[27] Indeed, Djordjevic and Szybalski demonstrated an increase in the sensitivity of DNA to ultraviolet and X-ray radiation when 5-bromo-2′-deoxyuridine or 5-iodo-2′-deoxyuridine were introduced into mammalian cells.[28] Later on, it was shown that substitution of thymine with 5-bromouracil leads to an increased amount of DSB,[29] SSB,[30] and other forms of DNA damage as compared to the irradiated native biopolymer.

Several clinical trials with 5-bromo-2′-deoxyuridine (5-BrdU), 5-iodo-2′-deoxyuridine (5-IdU), and 5-fluoro-2′-deoxyuridine (5-FdU) have been reported.[31−34] Malignant brain tumors,[23] malignant glioma and anaplastic astrocytoma[32] were treated with 5-BrdU and radiation. Similarly, the influence of 5-IdU on the survival of anaplastic astrocytoma patient was examined by Urtasun et al.[33] Finally, 5-FdU was used in the management of localized pancreaticobiliary carcinoma.[34] In all of those cases, radiosensitization by halogenated pyrimidines was demonstrated and the degree of radiosensitivity was found to increase with increasing percentage of replacement of nucleic bases by their halogenated analogs.[35] It is worth noting, however, that so far Hal-NBs have not been introduced into clinical practice. Further understanding of the molecular mechanisms of Hal-NBs-induced DNA radiosensitivity may therefore contribute to improve their radiosensitizing action and their application in cancer therapy.

The sensitizing action of Hal-NBs may be illustrated by comparing the behavior of thymine (T) with that of 5-bromouracil (5-BrU) in the presence of solvated electrons.[36,37] Hydrated electrons (e_{aq}^-), produced during water radiolysis, attack 5-BrU and as a result of dissociative electron attachment (DEA) a bromide anion (Br^-) and highly reactive uracil-5-yl radical $(U\text{-}yl)^\bullet$ are formed. If 5-BrU is incorporated in DNA, this radical can abstract $H^\bullet$ from the adjacent 2-deoxyribose moiety in double stranded DNA and lead to the formation of uracil and a SSB.[38] In contrast, reaction of T with e_{aq}^- leads to the formation of the stable T^- anion that readily undergoes electron transfer to regenerate thymine. The production of SSBs and DSBs in DNA substituted with 5-BrU increases by 2 and 1.5 times, respectively, with regard to the unsubstituted biopolymer.[39]

The sensitivity toward radiation of other brominated nucleosides, i.e., 5-bromo-2′-deoxycytidine, 8-bromo-2′-deoxyadenosine, and 8-bromo-2′-deoxyguanosine, both isolated and incorporated into DNA, has been studied in the past. For instance, it was shown that 5-bromo-2′-deoxycytidine (5-BrdC) and 5-BrdU are incorporated to the same extent into a human cell line, producing approximately an equal degree of radiosensitization.[40] Moreover, 5-BrdC turned out to be an efficient radiosensitizer of rat glioma cells infected by an adenovirus.[41] Finally, Razskazovskii et al.[42] demonstrated, using low temperature EPR measurements, that 5-BrdC incorporated in DNA is a ca. 3 fold more efficient electron scavenger than 2′-deoxycytidine itself. Reactions between hydrated electrons and 8-bromo-2′-deoxyadenosine[43−45] or 8-bromo-2′-deoxyguanosine[46,47] were, in turn, addressed within radiolytic studies by the Chatgilialoglu group. They demonstrated that electron attachment to 8-bromo-2′-deoxyadenosine leads to a rapid bromine ion release followed by a fast hydrogen atom abstraction from the C5′ position of the 2-deoxyribose moiety. The latter species undergoes cyclization to afford the 5′,8-cyclopurine derivative. Moreover, the rate constant of formation of the 8-bromo-2′deoxyadenosine anion is increased by a factor of 2 in comparison to that of 2′-deoxyadenosine.[43] It was also shown that 8-bromo-2′-deoxyguanosine captured hydrated electrons with quantitative formation of 2′-deoxyguanosine in the presence of hydrogen donors. Therefore, this compound was employed as an effective detection system for excess-electron transfer in a variety of 8-BrdG-labeled single- or double-stranded oligonucleotides[48,49] and G-quadruplexes.[50]

Taking into account the above-mentioned increased radiosensitivity of the brominated nucleosides, the reaction of LEEs with trinucleotides substituted in the center position with brominated nucleobase (5-BrU, 5-BrC, 8-BrA, and 8-BrG) is examined in the present study. So far, the radiosensitizing effects of DNA have been studied mainly with halogenated pyrimidines. Here, we investigate the behavior of the brominated and nonbrominated purines and pyrimidines, so as to compare the degree of radiosensitization obtained by substituting bromine in any of the DNA basis. This requires a direct comparison of specific DNA damages between the brominated and unbrominated bases, under identical experimental conditions, to reduce relative errors to an acceptable limit. For this reason, previous measurements of damage to the unbrominated trimers[19,51] are repeated. The electron energy is chosen to be 10 eV. Brominated base-induced sensitization of DNA to 10 eV electrons is of particular interest for several reasons. First, secondary electrons emitted by high energy radiation have an energy distribution with a maximum lying around 9−10 eV in biological matter.[7] Furthermore, electrons around this range of energies have a high cross section to damage DNA[52] and have been shown to play an important role in radiosensitization.[53,54]

2. EXPERIMENTAL METHODS

2.1. Model Compounds. The sensitivity toward LEEs was measured for the nonmodified and brominated trinucleotides shown in Figure 1. The brominated trimers contained 5-bromouracil (5-BrU), 5-bromocytosine (5-BrC), 8-bromoade-

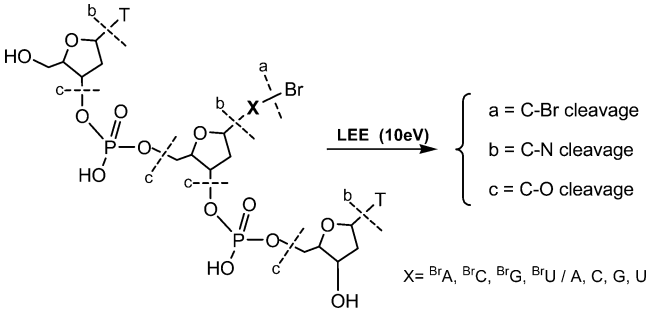

Figure 1. Possible fragmentation paths of a brominated (nonbrominated) oligonucleotide trimer via dissociative electron attachment (DEA).

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nine (8-BrA), or 8-bromoguanine (8-BrG) in the center and thymine moieties at the two terminal positions. The brominated trimers and TUT, TCT, TAT, TGT standards were purchased from Alpha DNA (Montreal, QC) and purified by high performance liquid chromatography (HPLC). Thymine (T) and monophosphates of nucleotides (pT and Tp) were purchased from Sigma-Aldrich (Milwaukee, MO). The purified solution was lyophilized to dryness and redissolved in sterile deionized (Millipore) water. The oligonucleotide solution was prepared without any added salt. The concentration of the stock solutions of trimers was estimated by optical density measurement at 260 nm using a Hitachi U-2000 spectrophotometer.

2.2. LEE Irradiation. The experimental details of the LEE irradiator and the procedure to irradiate samples have been reported elsewhere.[55] Briefly, it consists of two major steps, which include spin-coating to prepare samples and the UHV irradiation of samples with LEEs. The main advantage of this experimental setup is that it allows one to irradiate a relatively large area (26 cm^2) of a solid molecular film and hence to produce a sufficient amount of degraded molecules for chemical analyses. The samples of standard and brominated oligomers were prepared and irradiated under the same experimental conditions. Fourteen tantalum cylinders containing the standard (seven cylinders) and brominated oligomers (seven cylinders) films on the inside wall (3.2 cmx 2.5 cm diameter) were prepared by spin-coating at the same time and in the same system. The average thickness of each film was about 2.5 nm (approximately 5 monolayers (ML)), as estimated from the average density of single stranded DNA (1.7 g cm^{-3}).[56] Once spin-coated, the Ta cylinders were transferred to the UHV chamber, which was subsequently evacuated for ~24 h to reach a pressure of 10^{-9} Torr. The standard and brominated oligomer films were individually irradiated with a monoenergetic 10 eV electron beam; the number of electrons introduced into each sample was approximately 10^{16}. Three out of seven cylinders of the standard were irradiated, whereas four, nonirradiated, served as controls. The irradiation procedure was the same for the brominated oligomers. All procedures before and after LEE irradiation were carried out in a sealed glovebox under an atmosphere of dry nitrogen.

2.3. HPLC Analyses. The irradiated and nonirradiated samples were recovered by dissolution in 3 mL of pure water; afterward, they were frozen and lyophilized to dryness. Then, irradiated and nonirradiated samples were dissolved in 150 and 200 μL, respectively, of HPLC pure grade water and analyzed by HPLC-UV. These analyses were performed with a Waters Alliance HT system equipped with a thermostatted autosampler, a 2795 separations module and 2487 dual wavelength absorbance detector. The separation of products was achieved using an analytical YMC-Pack Pro C18 column (250 × 6 mm), maintained at 30 °C, using a linear gradient from 0% to 15% acetonitrile in buffer containing 20 mM ammonium acetate. The products were detected over an interval of 90 min, at a flow rate of 1.0 mL/min, by a UV detector operated at 210 and 260 nm. As in the past,[17] the identity of the specific compound corresponding to such peak in the chromatograms was obtained by comparison with the retention times of stable reference compounds. This identification procedure was limited by the availability of reference compounds. In the present study, in order to minimize inaccuracies related to the desorption of molecules from the film surface during irradiation and the absorption of products and substrates to the tantalum surface, we estimated total damage from the sum of UV-absorbing products in HPLC analyses The values are therefore smaller than those of the total damages reported in the previous work,[57] where the amount of total damage was deduced from the decrease of the main peak in the chromatograms after irradiation (i.e., the total loss of substrate was calculated as the ratio of the integrated HPLC peaks of substrate after and before irradiation).

3. RESULTS

The chromatograms shown in Figure 2, and in the Supporting Information (Figures S1 to S3), enable comparison between

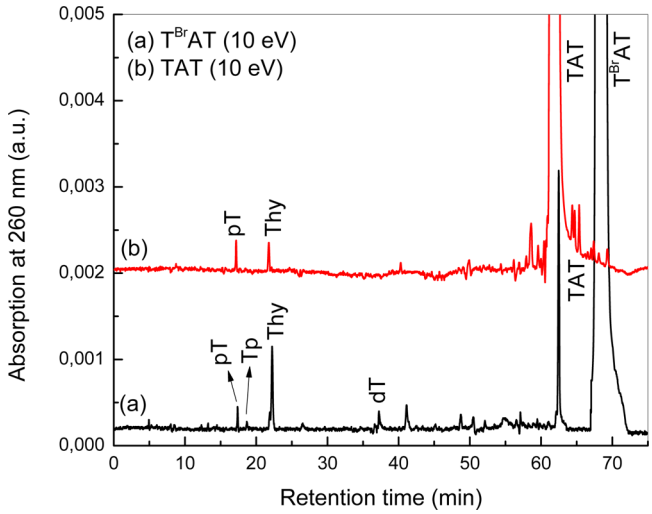

Figure 2. Chromatograms of TBrAT and TAT trinucleotides irradiated with 10 eV electrons. Chromatograms (a) and (b) refer to the brominated trimer TBrAT and nonbrominated trimer TAT.

the brominated (a) and nonbrominated (b) trimers: TAT, TGT, TCT, and TUT, respectively. In all figures the magnification of the signal is the same in (a) and (b), but the peak amplitudes are not corrected for differences in their extinction coefficients at 260 nm, whereas the values in Table 1 were corrected for UV absorption differences of the monomer, dimer, or trimer. In all figures in (a), the debrominated trimers are the dominant products of $T^{Br}XT$ irradiation. The most intense peak around 68 min in Figure 2(a) corresponds to the parent peak, $T^{Br}AT$, and the next larger one around 62 min, to the debrominated peak, TAT. Similarly, peaks at 65 and 57 min in Figure S1(a) correspond to $T^{Br}GT$ and TGT, peaks at 69 and 59 min in Figure S2(a) correspond to $T^{Br}CT$ and TCT, and finally peaks at 75 and 65 min in Figure S3(a) correspond to $T^{Br}UT$ and TUT, respectively. In Figure S1(a), two unidentified products appear near 35 and 50 min with appreciable intensity. Similarly for $T^{Br}CT$ (Figure S2) unidentified products between 65 and 68 min appear with considerable magnitude. Without suitable reference compounds, the identity of such peaks can be assessed by detail LC/MS-MS analysis.[58] So far, such analysis of LEE-induced products has only been performed with TTT, for which the series of peaks near the parent one in the chromatograms could be assigned to modification of the trimer to XpTpT, TpXpT and TpTpX, where X = 5,6-dihydrothymine.[58] According to comparison of the chromatogram in Figure S2(a) with that of TTT,[58] the unidentified peaks between 65 and 68 min could very well correspond to the formation of 5-bromo-5,6-

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Table 1. Yield of Products from LEE Induced DNA Damage ((Number of Product Molecules Per Incident Electron) × 10^3)[a]

	debromination		base release	strand break	
sample TXT	TXT	%[c]	T	fragments	sum of products by HPLC[b]
X = 8BrA	5.1 ± 0.8	42%	4.9 ± 3.0	1.9 ± 1.3	12.2 ± 3.5
X = 5BrC	4.8 ± 1.7	34%	4.4 ± 3.3	0.9 ± 0.9	14.1 ± 3.8
X = 8BrG	3.8 ± 1.3	26%	6.5 ± 1.0	1.3 ± 0.8	14.8 ± 2.0
X = 5BrU	6.1 ± 0.7	60%	2.8 ± 2.4	1.3 ± 0.7	10.2 ± 2.5
X = A	n.d.	n.d.	1.2 ± 0.5	1.6 ± 2.5	3.8 ± 2.8
X = C	n.d.	n.d.	4.3 ± 1.9	0.7 ± 0.7	6.8 ± 2.4
X = G	n.d	n.d.	2.3 ± 0.2	0.9 ± 0.7	4.6 ± 0.8
X = U	n.d.	n.d.	1.2 ± 0.5	1.4 ± 1.9	4.8 ± 2.1

[a]Each value shows the average and standard deviation of three to six independent experiments (n.d.: not detected). [b]Sum of products by HPLC was estimated by adding the areas under the 260 nm absorbing peaks in the chromatogram of irradiated samples minus those in nonirradiated samples. [c]Percentage of sum of products.

dihydrocytosine, which may elute immediately before the brominated trinucleotide. Similar peaks appear, but with less intensity, in the chromatograms of TAT, TGT, and TUT.

The identified products in all chromatograms correspond to bases related to the *N*-glycosidic bond cleavage and the products of the C−O bond cleavages related strand breaks. With the exception of base release, their yields were much smaller than those of debromination. A similar conclusion had previously been drawn from the LEE-induced degradation of $T^{Br}UT$ trimers.[57] In the case of nonbrominated trimers in Figures 2(b) and S1−S3(b), the dominant process is base (T) release from the end position of the trimer. Small amounts of pT, Tp, pXT, TXp, and dT are also observed (see panel (b) in Figures 2 and S1−S3). All these products from LEE-bombardment of the nonbrominated trimers TXT (X = U, C, A, or G) films have been previously reported.[51] They were measured in the present experiment to obtain an accurate comparison with the data from the brominated trimers.

The sum of products estimated by HPLC and the individual product yields are expressed as the number of product molecules per incident electron (see Table 1). The yields were estimated as the ratio of products to parent compound on the basis of integrated HPLC peaks corrected for differences in their extinction coefficients at 260 nm.[59] The average values in Table 1 and their standard deviations refer to three to six independent experiments. The most dominant product, corresponding to Br release from the brominated trimers has a magnitude 1.1 to 4.4 times higher than that of nonbrominated ones. It accounts for 42%, 34%, 26%, and 60% of the sum of products, respectively (see Table 1). These results indicate that 10 eV electrons mainly lead to the cleavage of the Br−C bond. In general, base release at the terminal position is the second most dominant process in the brominated trimers and the yields of T account for 40%, 31%, 44%, and 28% of the sum of products in $T^{Br}XT$ (^{Br}X = ^{Br}A, ^{Br}C, ^{Br}G, and ^{Br}U), respectively (see Table 1). We have also observed small fragments corresponding to stand breaks such as pT, Tp, dT, pXT, and TXp but their yields are much smaller than those observed for the two main products; i.e., ∼6 to 16% of sum of products (Table 1). The HPLC signals corresponding to pXT and TXp were below the limit of quantification.

4. DISCUSSION

Wetmore et al.[60] calculated at the B3LYP level the gas phase and solution electron affinities (EA) as well as energy barrier for the dissociation of the resulting 5-XU anions for the series of 5-halouracils (5-XU; X = F, Cl, and Br). The EA were predicted to decrease in the following order: 5-BrU > 5-ClU > 5-FU. On the other hand, energy barriers for the dissociation of 5-XU^- to X^- and uracil centered radical $(U\text{-yl})^\bullet$ were found to decrease along the series 5-FU^- > 5-ClU^- > 5-BrU^-,[60] which was also confirmed by the B3LYP calculations of Li et al.[61] Since EA is larger, while kinetic barrier for the release of X^- anion is substantially smaller for 5-bromouracil than for the remaining 5-halouracils,[60] we have decided to use trinucleotides containing bromonucleobases within our LEE-bombardment studies.

Basically, electron attachment to the studied trimers may trigger the DEA processes leading to the fragmentations depicted in Figure 1. The pT, Tp, pXT, and TXp fragments (see Figures 2 and S1−S3) are evidence of direct strand breaks. Indeed, the sugar−phosphate backbone must be broken in order to produce those fragments. They are mostly formed via electron transfer from the flanking thymines or middle bases to the respective phosphodiester bonds (see Figure 1).[62]

On the basis of DEA measurements from the gas phase 5-BrU in the electron energy range 0−16 eV, Abdoul-Carime et al.[63] proposed a mechanism of electron-induced degradation of 5-bromouracil. Their experiments indicate that unstable transient anions of 5-BrU undergo dissociation into a negative ion and a neutral atom or radical: Br^- + $(U\text{-yl})^\bullet$ or $Br^\bullet$ + $(U\text{-yl})^-$; the branching ratio $(U\text{-yl})^\bullet/Br^\bullet$ was estimated to be 40. The theoretical studies of Wetmore et al.[60] and Sevilla et al.[61] on the electron-induced degradation of halouracils predict that formation of the 5-BrU anion both in the gas phase and aqueous solution is exergonic and leads to the uracil centered radical and the bromide anion with a small kinetic barrier of 0.26[60,61] and 3.20[60] kcal/mol, respectively. Furthermore, very recently Chomicz et al.[64]described, at the B3LYP/6-31++G** level, debromination (release of the bromide anion) of the four methylated nucleobases anions, i.e., the C8-Br or C5-Br bond dissociation in the 8-bromo-9-methyladenine, 8-bromo-9-methylguanosine, 5-bromo-1-methylcytidine, and 5-bromo-1-methyluracil valence anions in the gas phase and aqueous solution. They found out that the presence of the electronegative halogen atom (bromine) in nucleobase increases the stability of the corresponding nucleobases transient anions (generated after electron attachment to brominated nucleobase) with regard to the unsubstituted nucleobase. The stability of those anions significantly increases in solvent as compared to the gas phase. The reaction profiles for the electron-induced decomposition of bromonucleobases demonstrate that the overall thermodynamic stimulus for the process proceeding in water is rather high (ca. −60 kcal/mol) and very similar for the four potential radiosensitizers, indicating thus comparable radiosensitizing properties of the considered bromoderivatives. Similarly, the previous[57] and current results obtained for LEE irradiation of TBrUT show that the main DEA product is TUT, which could be formed in the reaction between $(U\text{-yl})^\bullet$ and a H-atom donor.[65,66] Thus, the first of two mechanistic possibilities proposed by Abdoul-Carime et al.[63] seem to be operative.

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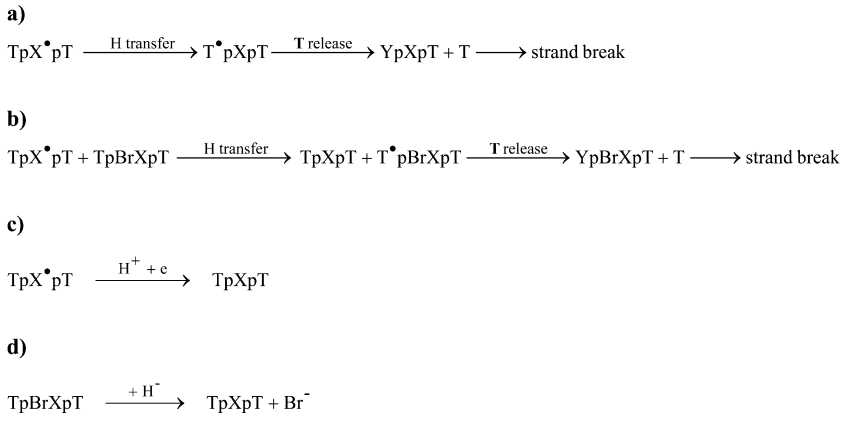

Figure 3. Secondary processes leading to thymine release (a and b), formation of debrominated products (b–d), and secondary strand breaks (a and b). X•, T•, and Y stands for the 5-yl or 8-yl radical, the radical at T site, and an abasic site, respectively.

The 5-yl or 8-yl radicals formed in DEA process involving the brominated bases may lead to secondary strand breaks that could occur as a result of hydrogen atom abstraction by those radicals. In our recent ESD work,[67] it was demonstrated the bromide anion desorption to be the second strongest resonance induced by bombardment with 3–20 eV electrons of the same brominated trimers. The bromide anions (ESD signal) must originate from the brominated bases and the reactive nucleobase radicals (5-yl or 8-yl) should be the second product of the CX-Br (X = 5 or 8) bond dissociation. These radicals may abstract a hydrogen atom from the adjacent sugar in the same trimer (intramolecular hydrogen atom transfer; see Figure 3a and ref 57) or from the sugar residue of another trimeric molecule (intermolecular hydrogen atom transfer; see Figure 3b). Typically, H atom abstraction does not lead to a direct strand break but to an abasic site (coupled to the formation of, e.g., 2′-deoxyribonolactone with half-life >20 h at 37 °C) formation of which involves a release of a nucleobase from the adjacent nucleoside,[68,69] i.e., thymine in the studied cases (see Figure 3a,b; under cellular conditions this type of damage may be converted to a strand break). Interestingly, the smallest ESD signal related to Br^- release, observed for TBrCT,[67] was significantly smaller than that in the remaining cases. This results agrees well with the fact that increase in thymine release with regard to the nonbrominated system is the smallest for the trimer containing BrdC (see Table 1). The unidentified peaks observed in all chromatograms (see Results and Figures 2 and S1–S3) might be related to the products with an abasic terminal site. The above reasoning may explain why, for the brominated systems, we observe an increase in thymine release compared to the nonsubstituted trimers (see Table 1). Moreover, the lack of products arising from the *N*-glycosidic bond dissociation in the brominated nucleosides is a strong argument indicating that while the electron attaches to the middle nucleoside, it triggers an immediate dissociation of the CX-Br bond leading to the 5-yl or 8-yl reactive radicals, which in secondary processes can abstract a sugar hydrogen atom (see Figure 3a,b).

On the other hand, the debrominated products (TXT; X = U, C, A, or G) may be produced via several secondary processes: intermolecular H atom abstraction by the 5-yl or 8-yl radicals from another trimer (Figure 3b), reduction of the 5-yl or 8-yl radicals and addition of a proton (Figure 3c and ref 57), and attachment of the hydride anions to the C5 or C8 position of brominated bases followed by elimination of the bromide anion (see Figure 3 and ref 57). It should be noted that in our recent ESD studies on the same brominated trimers,[67] the most intensive ESD signal corresponded to that of the resonance in the H^- yield.

The results presented here enable one to juxtapose the sensitivity to 10 eV electrons of brominated nucleobases incorporated into the same DNA context. It turns out that the sensitivity of brominated trimers is quite similar (cf. the sum of products in Table 1). The debromination accounts for a quarter to almost two-thirds of the products and the yields of base release and strand breaks also increase in comparison to those of nonbrominated ones (see Table 1). The yield of degradation of $T^{Br}UT$ indicates that the presence of the bromine atom on uracil doubles the damage induced by LEE irradiation compared to the unsubstituted TUT trimer (see Table 1).

According to the values presented in Table 1, one can conclude that in all studied cases bromination significantly increases the sensitivity of a trimer to the LEE-induced damage. The damage to brominated trimers is increased by a factor of 3.2, 2.1, 3.2, and 2.1 for TBrAT, TBrCT, TBrGT, and TBrUT, respectively (see Table 1). Thus, according to these values, bromination of the trimers produces an overall larger sensitization to LEE in the purine than in the pyrimidine bases (i.e., TBrAT = TBrGT > TBrCT = TBrUT). Interestingly, debromination is not directly related to the increase in the amount of degradation products found in the films. It is, however, related to the desorption of Br^-. In fact, Polska et al.[67] found that desorption of Br^- induced by 3–20 eV electrons impact on thin films containing the studied trimers, increased in the order of TBrUT > TBrAT > TBrGT > TBrCT. Thus, total debromination and debromination by Br^- desorption are highest for TBrUT followed by TBrAT.

Finally, one should note that the trimers labeled with BrdC, BrdA, and BrdG behave in a manner similar to that observed for the trimer labeled with 5BrdU whose radiosensitizing properties in cells are well established.[29,30] Since all studied trimers display similar electron degradation patterns under the UHV conditions, one can draw the conclusion that BrdC, BrdA, and BrdG should have radiosensitizing properties comparable to those of 5BrdU at the cellular level.

If the radiosensitivity of the cellular DNA would be increased by a similar value (ca. 2–3 fold) upon labeling it with bromonucleobases, cellular radiosensitivity could be considerably enhanced. However, cellular DNA is a much more complex entity than a short DNA strand. The process of

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electron attachment is known to be influenced by environmental changes. In cellular DNA, the presence of hydrogen bonds between complementary bases[70] as well as π-stacking interactions[71−74] could modify the electron-base interaction. Therefore, appropriate model systems comprising all the interactions present in double stranded DNA should be investigated before reaching a final conclusion about the radiosensitization properties of brominated bases in cellular DNA. Moreover, to be applied as a radiosensitizer a given bromoderivative has to be incorporated into DNA in vivo and the promising characteristics of the LEE-induced damage will depend on how well the modifications are incorporated into cancer cells.

5. SUMMARY

In the present work, damage induced by 10 eV electrons in trimeric oligonucleotides, TBrXT (X = dA, dC, dG, or dU), has been studied. The main degradation pathway is related to debromination of brominated bases (a quarter to almost two-thirds of the sum of products). The other identified products generated are T, pT, and Tp, in small amounts, and base release. The damage to brominated trimers is ca. 2.1 to 3.2 times higher than that observed in nonbrominated ones. This comparison confirms that substitution of natural DNA bases with their brominated derivatives makes DNA more sensitive to LEEs. These studies suggest that all brominated bases are potential radiosensitizers for anticancer therapy.

■ ASSOCIATED CONTENT

Supporting Information

Chromatograms of TBrGT/TGT, TBrCT/TCT, and TBrUT/TUT trinucleotides irradiated with 10 eV electrons; Complete ref 32. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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