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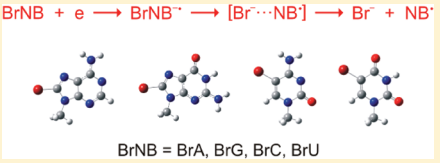
Electron-Induced Elimination of the Bromide Anion from Brominated Nucleobases. A Computational Study

Lidia Chomicz, Janusz Rak, and Piotr Storoniak*

Department of Chemistry, University of Gdańsk, Sobieskiego 18, 80-952 Gdańsk, Poland

ABSTRACT: The enhancement of radiodamage to DNA labeled with halonucleobases is attributed to the reactive radical produced from a halonucleobase by the attachment of an electron. We examined at the B3LYP/6-31++G** level electron capture by four brominated nucleobases (BrNBs): 8-bromo-9-methyladenine, 8-bromo-9-methylguanine, 5-bromo-1-methylcytosine, and 5-bromo-1-methyluracil followed by the release of the bromide anion and a nucleobase radical. We demonstrate that neutral BrNBs in both gas and aqueous phases are better electron acceptors than unsubstituted NBs and that resulting anion radicals, $BrNBs^{\bullet-}$, can easily transform into the product complex of the bromide anion and the nucleobase radical ($[Br^{-}\cdots NB^{\bullet}]$). The overall thermodynamic stimulus for the process starting with the neutral BrNB and ending with the isolated bromide anion and the $NB^{\bullet}$ radical is similar in the case of all four BrNBs studied, which suggests their comparable radiosensitizing capabilities.



I. INTRODUCTION

The high-energy radiation used in radiotherapy triggers a direct and indirect DNA damage. It is believed that about one-third of the damage is direct and results from energy deposited in DNA itself, while two-thirds is indirect as is induced by reactive species released by ionizing radiation from water and other molecules located in the vicinity of DNA.[1] Radiolysis of neutral water leads to the formation of solvated electrons e_{hyd}^{-} and free radicals ($HO^{\bullet}$ and $H^{\bullet}$).[2] These products are capable of interacting with DNA and other molecules in the cell, causing so-called indirect damage.

The susceptibility of bare DNA to damage by low-energy electrons was demonstrated quite recently[3] and attracted considerable attention. As a consequence of the exposure of dry films of DNA under ultrahigh vacuum to low-energy electrons (LEEs), single and double strand breaks were detected.[3−12] Under such conditions, LEEs have also been shown to induce base fragmentation[13−16] or base release.[7,10−12] Interaction of DNA with weakly bound prehydrated electrons (e_{pre}^{-}), which are precursors of hydrated electrons (e_{hyd}^{-}) formed by ionizing radiation in aqueous media, can also result in single or multiple strand breaks.[17]

An important issue in radiotherapy is to restrict the damaging action of X- or γ-rays to the tumor area as well as to minimize the radiation dose. Thus, sensitization of cancer cell DNA to radiation by the incorporation of radiosensitive nucleosides could help to reduce the therapeutic dose and, therefore, the side effects of radiotherapy. Halogenated pyrimidines, 5-halouracil[18−25] and 5-halocytosine,[20,21,26−28] have long been recognized as radiosensitizing agents with potential clinical applications and still are the subjects of in vitro and in vivo experiments on cancer cells[29−39] as well as on model DNA.[40−43] The most widely investigated halogenated base is 5-bromouracil (5-BrU) which, when incorporated into DNA, increases in particular the number of single and double strand breaks in the genetic material of cells exposed to ionizing radiation.[30,34,44−46] To our knowledge, there are no similar reports on the application of halogenated purine analogues in anticancer therapies.

The radiosensitizing properties of halogenated pyrimidines are attributed to their enhanced electron affinity in comparison with other DNA components.[47,48] Their pronounced tendency to trap electrons is coupled with the subsequent efficient decomposition of their respective anions to genotoxic intermediates.

The fast reaction of 5-halouracils with electrons generated in aqueous solution has been studied by nanosecond pulse radiolysis[49−53] and more recently by time-resolved femtosecond laser spectroscopy.[54] Electron adducts (radical anions) of halouracils were observed to undergo halide elimination, yielding nearly quantitatively the highly reactive uracil-yl radicals and bromide ions. The uracil-yl radical can, in turn, abstract $H^{\bullet}$ from nearby molecules, thereby causing further damage. Hydrogens available to the uracil-yl radical may come from the adjacent deoxyribose, and it has been established that the loss of any H atom by the sugar moiety may lead to a direct DNA strand break.[55]

In the context of radiation-induced DNA damage, the most important halogenated purines are 8-bromo analogues. The affinities of 8-bromopurines for hydrated electrons are high, significantly exceeding the electron affinities of the non-halogenated parents.[2,56] Radiolytic studies of the interaction of e_{hyd}^{-} with 8-bromo-2′-deoxyadenosine (8-BrdA) have shown that 8-BrdA captures electrons and rapidly loses the bromide ion to yield the corresponding, highly reactive, C8 radical.[56,57] Similarly, e_{hyd}^{-} captured by the brominated guanine derivatives 8-bromoguanosine and 8-bromo-2′-deoxyguanosine results in the quantitative release of Br^{-}.[58−60] Except for a certain amount of information available from radiolytic experiments on halogenated nucleobases in aqueous solution, reports on their

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interactions with electrons in the gas phase are available only for 5-halouracils.[48,61−65] In contrast to the extensive studies on the radiosensitizing properties of 5-halouracils, there are far fewer reports in this respect on the other halogenated DNA nucleobases. To partially fill this gap, the current work presents the results of a systematic DFT study in the gas phase and aqueous solution comparing the sensitivity of four brominated nucleobases (BrNBs)—8-bromo-9-methyladenine (BrA), 8-bromo-9-methylguanine (BrG), 5-bromo-1-methylcytosine (BrC), and 5-bromo-1-methyluracil (BrU)—to electron attachment. To mimic the sugar-binding sites present in nucleosides, the bases are methylated at sites 1 and 9 in pyrimidines and purines, respectively. We restricted our study to the formation of the respective anion radical and its subsequent decomposition into the Br^- anion and nucleobase radical, which is the first and probably crucial stage in the mechanism of halonucleobase-labeled DNA degradation induced by excess electrons.

II. COMPUTATIONAL METHODS

We applied the density functional theory method with Becke's three-parameter hybrid functional (B3LYP)[66] and the 6-31++G** basis set[67] to the gas-phase calculations and additionally the Polarizable Continuum Model (PCM)[68] to the aqueous solution. Although nucleobases present in double-stranded DNA (dsDNA) are not fully hydrated due to π-stack interactions and hydrogen bonds between complementary bases, the computational characteristics obtained for the aqueous environment reflect the limiting effects in solutions of DNA. Additionally, in the single-stranded DNA fragments occurring in dsDNA which in vivo exist under a number of physiological conditions like DNA replication, transcription, homologous recombination, in telomeres, and DNA bulges and mismatches,[41] nucleobases are fully hydrated, and hence the PCM results should be directly related to such a system.

All the geometries except that of $BrA^{\bullet-}$ were fully optimized without any geometrical constraints, and analysis of harmonic frequencies demonstrated that all of them were geometrically stable (all force constants were positive) or first-order saddle points (all but one force constant positive). Since the planar geometries of anionic nucleobases support a pure π^* state of somewhat higher energy than that of the nonplanar minimum,[48] the unconstrained geometry optimizations assured the studied anions converged to the nonplanar, mixed π^*/σ^* lowest-energy minima.

Optimization of $BrA^{\bullet-}$ leads to the $[Br^-\cdots A^\bullet]$ complex without a kinetic barrier. To obtain an estimate of its adiabatic and vertical stability and the energetics associated with the conversion of the anion radical into the radical complex, the geometry of $BrA^{\bullet-}$ was optimized with the C−Br distance frozen at the value equal to the equilibrium C−Br distance for the anionic BrG in aqueous solution (i.e., at 1.898 Å; see Table 1).

The energies of particular reactions (ΔEs) were calculated as the differences between the electronic energies of substrates and products, while the Gibbs free energies of these reactions (ΔGs) were ΔEs corrected for zero-point vibration terms, thermal contributions to energy, the pV term, and the entropy term. These terms were calculated in the rigid rotor-harmonic oscillator approximation for T = 298 K and p = 1 atm. To calculate ΔG for the solvated systems, the same correction terms were applied to the solute as were used for calculating the gas-phase free energies.[69]

The adiabatic electron affinity, AEA_E, is defined as the difference in the electronic energies (no zero-point energy correction) of the neutral and the respective anion radical at their corresponding fully relaxed structures. Moreover, the adiabatic electron affinity, calculated using Gibbs free energies at 298 K of the fully optimized neutral and the corresponding anion, i.e., the AEA_E value corrected for zero-point vibration (ZPV) terms, thermal contributions to energy, the pV term, and the entropy term, is denoted by AEA_G.

Electron vertical detachment energies (VDEs) were calculated as the difference between the electronic energies of the neutral and the anion radical at the geometry of the fully relaxed anion radical, while the vertical electron affinities (VEAs) are the difference between the electronic energies of the neutral and anion radical at the geometry of the fully relaxed neutral radical.

All quantum chemical calculations were carried out with the GAUSSIAN09[70] code on dual Intel Itanium 2 nodes at the Academic Computer Centre in Gdańsk (TASK), and the images of the molecules were plotted with the GaussView package.[71]

Table 1. Equilibrium C−Br Distance (Å) in the Neutral Bromo-Substituted Nucleobases (BrNB), Their Anions ($BrNB^{\bullet-}$), and Product Complexes ($[Br^-\cdots NB^\bullet]$) in the Gas Phase and Aqueous Solution

NB	BrNB	$BrNB^{\bullet-}$	$[Br^-\cdots NB^\bullet]$
		gas phase	
A	1.876	-	2.559
G	1.878	1.895	2.630
C	1.898	1.930	2.696
U	1.877	1.907	2.618
		aqueous solution	
A	1.875	-	2.685
G	1.876	1.898	2.679
C	1.895	1.916	2.793
U	1.882	1.903	2.705

III. RESULTS AND DISCUSSION

The mechanism of C−Br bond breakage following electron attachment in brominated nucleobases can be divided into three steps: (1) electron binding to the neutral BrNB, which results in the formation of the adiabatically bound $BrNB^{\bullet-}$ radical anion; (2) conversion, with a small kinetic barrier, of the electron adduct, $BrNB^{\bullet-}$, into the radical anion complex, $[Br^-\cdots NB^\bullet]$; (3) complete separation of the Br^- anion and neutral radical $NB^\bullet$ (d(C−Br) ≈ ∞):

$$BrNB + e \rightarrow BrNB^{\bullet-} \quad (1)$$

$$BrNB^{\bullet-} \rightarrow [Br^-\cdots NB^\bullet] \quad (2)$$

$$[Br^-\cdots NB^\bullet] \rightarrow Br^- + NB^\bullet \quad (3)$$

It is worth noticing that reaction 2 proceeds through a transition state that will be discussed in details in the section concerning the reaction profiles. We obtained stationary geometries for all the species involved in steps 1−3 except for $BrA^{\bullet-}$, in which the C−Br bond is elongated to 2.56 Å, yielding the $[Br^-\cdots A^\bullet]$ complex directly during the geometry optimization of the respective electron adduct. Thus, the $BrA^{\bullet-}$ anion radical appeared to be unstable both in the gas phase and in aqueous solution, which is in line with the fast release of Br^- from 8-bromoadenine observed in a previous experimental−computational study.[57] The gas-phase geometries of the radical anions with their corresponding singly occupied molecular orbitals (SOMOs) are presented in Figure 1.

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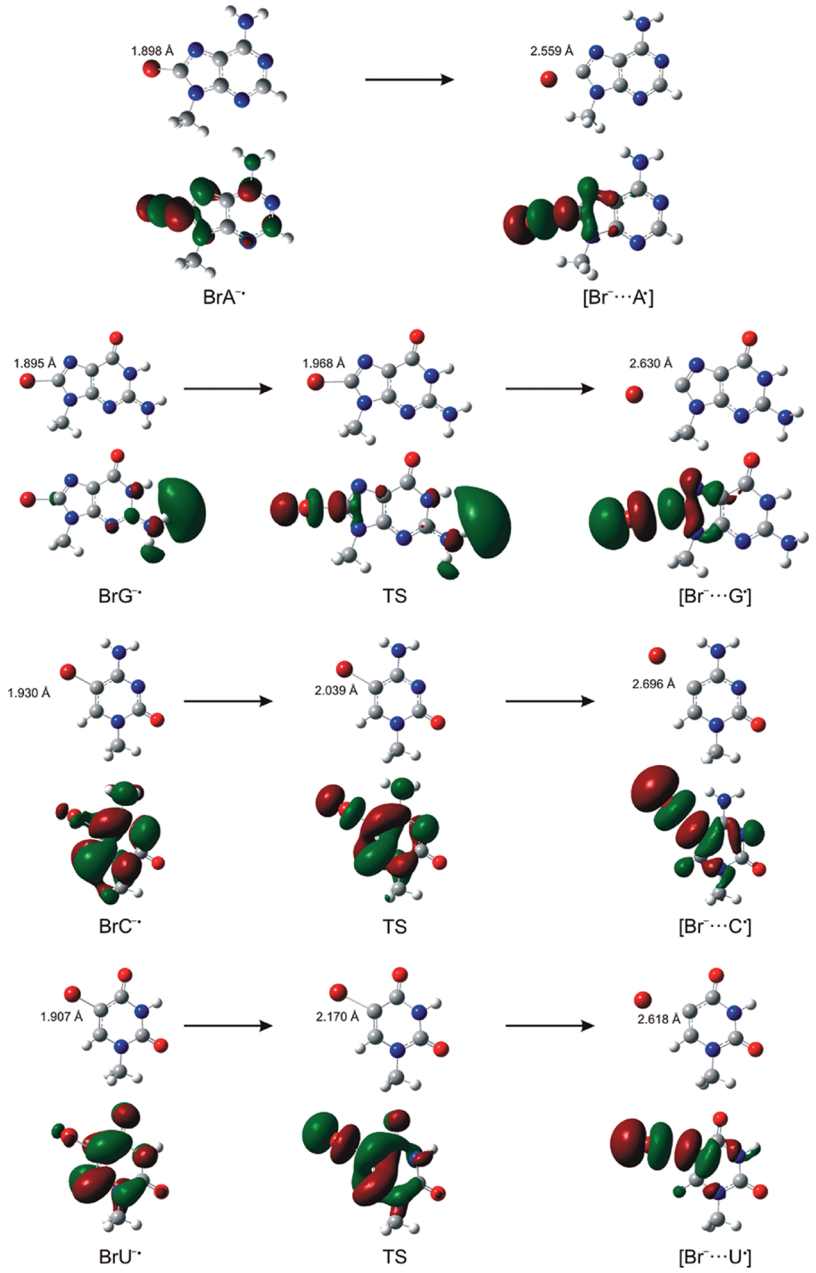

Figure 1. Gas-phase stationary geometries and singly occupied molecular orbitals (SOMOs) of anion radicals ($BrNB^{\bullet-}$), transition states (TS), and product complexes ($[Br^{-}\cdots NB^{\bullet}]$) calculated at the B3LYP/6-31++G** level. The geometry of $BrA^{\bullet-}$ results from constrained optimization with the C−Br distance frozen at 1.898 Å. Orbitals plotted with a contour value of 0.05 $(Bohr)^{-3/2}$.

Electron attachment to neutral BrNBs slightly elongates the C−Br bond (by 0.02−0.03 and 0.02 Å for the gas-phase and aqueous solution, respectively) in the resulting anions. This distance is longer still in the $[Br^{-}\cdots N^{\bullet}]$ complexes by 0.7−0.8 Å in the gas phase and by 0.8−0.9 Å in aqueous solution (see Table 1). The influence of solvent on the C−Br distance is most visible in the $[Br^{-}\cdots N^{\bullet}]$ complexes: it is longer by 0.05 Å ($[Br^{-}\cdots G^{\bullet}]$), 0.09 Å ($[Br^{-}\cdots U^{\bullet}]$), 0.1 Å ($[Br^{-}\cdots C^{\bullet}]$), and 0.13 Å ($[Br^{-}\cdots A^{\bullet}]$) than the gas-phase lengths (see Table 1). Generally, the geometries of these anions and the shapes of their singly occupied molecular orbitals do not change significantly on transfer to water. The only exception is the radical anion of BrG (see Figure 1). Owing to its substantial dipole moment (7.62 D compared with 4.78, 4.93, and 1.33 D for BrU, BrC, and BrA, respectively), $BrG^{\bullet-}$ supports a dipole bound state rather than a valence π^* anion in the gas phase. However, in aqueous solution a typical valence anion of BrG (as for the other brominated nucleobases) is developed, as such diffuse states are strongly destabilized in a condensed phase.[72] The $BrG^{\bullet-}$ and $[Br^{-}\cdots G^{\bullet}]$ structures, along with their SOMOs, resulting from the PCM calculations are shown in Figure 2.

Electron Affinities and Vertical Detachment Energies. The VEA values gathered in Table 2 demonstrate that BrA (−0.61 eV), BrG (−0.02 eV), and BrC (−0.09 eV) cannot bind an excess electron without geometrical relaxation in the gas phase. Only BrU is characterized by the positive VEA equal to 0.16 eV. This figure agrees well with the gas-phase VEA of 0.11 eV reported in the earlier DFT studies for the nonmethylated BrU.[48] On the other hand, in the aqueous solution the VEA values of all BrNBs are positive and span the range of

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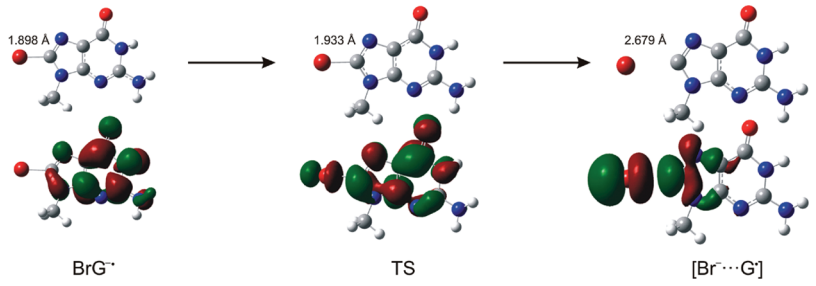

Figure 2. Stationary geometries and singly occupied molecular orbitals (SOMOs) of the radical $BrG^{\bullet-}$, transition state, and the radical $[Br^{-}\cdots G^{\bullet}]$ anionic complex in aqueous solution (PCM model). Orbitals plotted with a contour value of 0.05 $(Bohr)^{-3/2}$.

Table 2. Adiabatic Electron Affinity (AEA), Vertical Electron Affinity (VEA), and Vertical Detachment Energy (VDE) of Bromo-Substituted Nucleobases, BrNBs, and Corresponding Anion Radicals[a]

	AEA			
	AEA_E	AEA_G[b]	VEA	VDE
		gas phase		
BrA	0.01[c]	-	−0.61	0.99[c]
BrG	(0.02)[d]	(0.11)[d]	(−0.02)[d]	(0.06)[d]
BrC	0.27	0.45	−0.09	0.76
BrU	0.49	0.69	0.16	1.02
	0.51[e]		0.11[e]	1.21[e]
		aqueous solution		
BrA	1.87[c]	-	1.31	2.91[c]
BrG	1.36	1.55	1.00	2.04
BrC	2.07	2.26	1.65	2.51
BrU	2.27	2.48	1.90	2.74
	2.44[e]			

[a]All values in eV. [b]Calculated at 298 K. [c]Constrained geometry optimization with the C−Br distance frozen at 1.898 Å. [d]The dipole bound state characteristics are less accurate than the remaining ones due to the methodology/basis set not perfectly suited for the description of DB anionic states. [e]Values calculated at B3LYP/6-31+G(d) from ref 48.

1.00−1.90 eV (see Table 2). Here again BrU is the most prone to attach the excess electron, and the electrophilic character of the studied compounds increases in the order BrG < BrA < BrC < BrU.

The positive VDE and AEA values confirm the adiabatic and vertical stability of the BrNB anion radicals, in both the gas phase and aqueous solution (see Table 2). The calculated AEA_Gs for BrC and BrU are 0.45 and 0.69 eV in the gas phase.

In general, the presence of the highly electronegative bromine atom in the BrNB molecules makes their anions adiabatically stable. Indeed, it was demonstrated computationally that the AEA_E of valence anions of the unsubstituted pyrimidines were close to zero,[73−77] while those of adenine and guanine were highly negative.[73] The aqueous environment strongly stabilizes the valence anions of BrNBs, which is expressed by their aqueous solution AEA_G ranging from 1.55 to 2.48 eV (see Table 2). The enhanced adiabatic stability of the anions in water compared with the gas phase can be roughly described by the Born term, which for a typical DNA base anion is ca. 2 eV.[72]

As indicated by the calculated AEA values, bromopyrimidines are better electron trappers than bromopurines. The ease of attaching an electron, both in the gas phase and in solution, increases in the following order: BrG ≤ BrA < BrC < BrU (AEA_E, Table 2). As can be anticipated from the VDE values obtained (0.06−1.02 and 2.04−2.91 eV in the gas phase and in solution, respectively; Table 2), the vertical stabilities of the resulting radical anions, $BrNBs^{\bullet-}$, almost follow the trend of their adiabatic electron affinities. The only difference concerns the vertical stability of BrA, which is the second most stable in the gas phase and the most stable in aqueous solution (see Table 2). One should, however, realize that our estimates of $BrA^{\bullet-}$ anion stability are quite approximate since to obtain them we had to carry out frozen C−Br distance optimizations (no kinetic barrier for bromide anion release from $BrA^{\bullet-}$ (see Computational Methods)).

The above-mentioned series of adiabatic stabilities predicted for BrNB radical anions corresponds qualitatively to the results of the ESR experiments performed by Sevilla et al. for brominated DNA in ice-cooled aqueous solutions, which demonstrates that 5-bromocytosine and especially 8-bromoguanine do not compete for the electron as efficiently as the 5-bromoderivative of thymine (5-bromo-6-hydroxy-5,6-dihydrothymine).[78] Here, it is also worth emphasizing that both the AEA_E (0.49 and 2.27 eV) calculated in the gas phase and in solution and the gas-phase VDE (1.02 eV) for BrU (see Table 2) remain in good agreement with those obtained at the B3LYP/6-31+G(d) level by Li et al., who estimated these values to be 0.51, 2.44, and 1.21 eV, respectively.[48] The small discrepancy between our values and the ones in the literature may be due to differences in the BrU structures compared: our calculations refer to 5-bromo-1-methyluracil, while Li et al. reported data for unsubstituted BrU. Furthermore, we employed additional polarization and diffuse functions in our basis set.

Reaction Profiles for the Electron-Induced Dissociation of BrNBs. Following thermodynamically favorable electron attachment to a brominated nucleobase (see the previous section), the ensuing π^* radical anion (the dipole bound anion in the case of BrG) converts in the gas phase to the σ^* complex of the bromide anion and nucleobase neutral radical, $[Br^{-}\cdots NB^{\bullet}]$, where both species are separated by ∼2.6 Å (step (2); Figure 1). Gathered in Table 3, the energy and free energy changes for this reaction indicate that the formation of the $[Br^{-}\cdots N^{\bullet}]$ complex from $BrNB^{\bullet-}$ is thermodynamically feasible. For gaseous BrG, BrA, BrC, and BrU, the respective energies of step 2 are calculated to be ca. −12, −13, −13, and −3 kcal/mol (see Table 3). The presence of solvent makes this dissociation step much more advantageous, as demonstrated by the respective reaction energies, i.e., −31, −17, −15, and −9 kcal/mol, for BrG, BrA, BrC, and BrU (see Table 3).

With the exception of BrA, for which the conversion to the complex is barrier-free, dissociation of the valence anions of BrNBs is associated with a small kinetic barrier (see Table 3). The largest activation free energy (2.38 kcal/mol) was

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Table 3. Thermodynamic (ΔE and ΔG) and Kinetic (ΔE^* and ΔG^*) Characteristics of Steps 2 and 3 (See Text) As Well As the Global (ΔE_g and ΔG_g) Driving Force for the Electron-Induced Release of the Bromide Anion from BrNBs[a]

	$BrN^{\bullet-} \rightarrow [Br^- \cdots N^{\bullet}]$				$[Br^- \cdots N^{\bullet}] \rightarrow Br^- + N^{\bullet}$		$BrN + e \rightarrow Br^- + N^{\bullet}$	
	ΔE^{*b}	$\Delta G^{*b,e}$	ΔE	ΔG^e	ΔE	ΔG^e	$\Delta E_g{}^c$	$\Delta G_g{}^{c,e}$
gas phase								
BrA	barrier-free		−13.46[d]	-	23.85	16.65	10.29	0.24
BrG	0.55	0.44	−12.35	−13.01	23.62	14.98	10.81	−0.62
BrC	0.38	0.55	−12.90	−12.07	28.06	19.02	8.96	−3.51
BrU	2.72	2.38	−2.52	−2.97	23.71	17.43	9.78	−1.52
aqueous solution								
BrA	barrier-free		−17.33[d]	-	7.35	0.63	−53.09	−63.69
BrG	1.19	0.41	−30.73	−29.45	9.24	1.20	−52.84	−63.94
BrC	2.24	2.15	−15.08	−14.44	8.25	0.15	−54.48	−66.40
BrU	2.42	2.54	−8.99	−8.00	7.91	0.32	−53.36	−64.96

[a]All values in kcal/mol. [b]Barrier heights. [c]ΔE_g and ΔG_g are the differences between the electronic energies/the Gibbs free energies of optimized isolated products (Br^- and $N^{\bullet}$) and the optimized neutral substrates BrN. [d]Constrained geometry optimization with the C−Br distance frozen at 1.898 Å. [e]Calculated at 298 K.

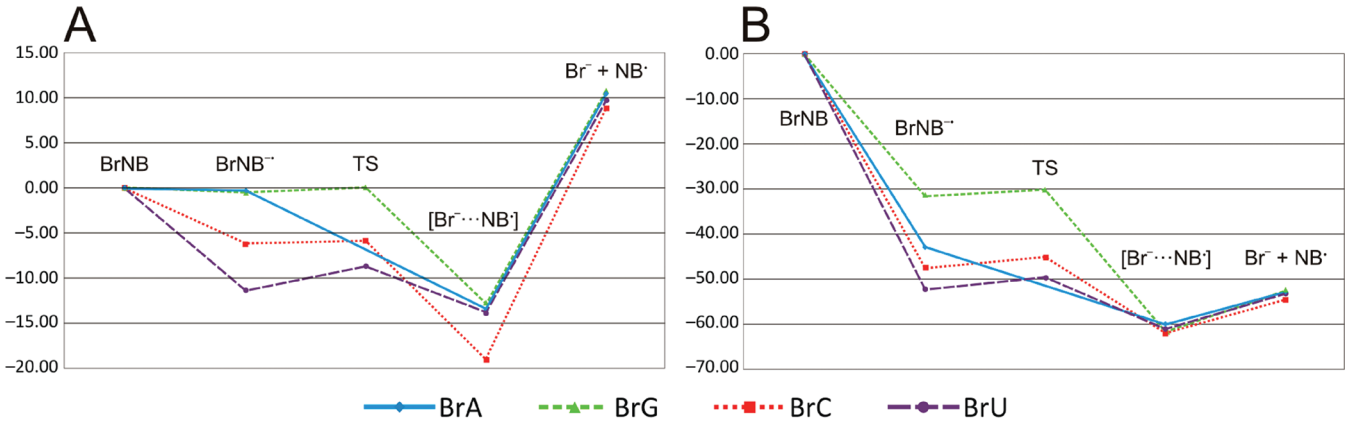

Figure 3. Reaction profiles (on the relative electronic energy scale, in kcal/mol) for electron-induced dissociation of BrNBs into the separate bromide anion and nucleobase-centered radical. Panel A, gas phase; panel B, aqueous solution.

calculated for $BrU^{\bullet-}$ and the smallest one (0.44 kcal/mol) for $BrG^{\bullet-}$ (see Table 3). The aqueous environment influences these barriers only insignificantly: as a result they were well below 3 kcal/mol in both media (see Table 3).

The last step of the process, i.e., the dissociation of $[Br^- \cdots N^{\bullet}]$ separating the bromide anion and neutral radical of the base (step 3), is endergonic both in the gas phase and in solution. In contrast to the findings of ref 47, where an exergonic effect is reported for $[Br^- \cdots U^{\bullet}]$ dissociation in aqueous solution, we predicted here that this reaction would be endothermic. Besides the differences in basis sets used in our work and in ref 47, we consistently optimized the geometries in solution, whereas gas-phase structures were used to consider the solvent effect in ref 47, which may account for this discrepancy.

Owing to solvation, the free energy of complex dissociation in solution amounts to only 0.15−1.2 kcal/mol, whereas in the gas phase (lack of solvent stabilization) the respective values range between 15 and 19 kcal/mol (see Table 3).

The reaction profiles on the electronic energy scale for the gas phase (panel A) and solution (panel B) are presented in Figure 3. The most striking dissimilarities between the processes occurring in these two media relate to step 3. In the gas phase, the complex dissociation is highly endothermic. At first glance, this suggests that thermodynamics prevents complex separation into the bromide anion and the corresponding radical. However, gas-phase studies on dissociative electron attachment to 5-bromouracil have demonstrated that 0 eV electrons efficiently decompose brominated uracil and that the cross-section for bromide ion formation is ca. 10-fold larger than that for the formation of the 5-BrU anion itself.[65] These experimental observations show that product complex separation is quite effective in the gas phase. The ostensible discrepancy between our computational findings relating to the gas phase and the experimental results of Abdoul-Carime et al.[65] can be explained by invoking a complete change of the Gibbs free energy relating to electron attachment, shown in the last column of Table 3, instead of just the energy change of step 3. Indeed, while ΔE and ΔG of step 3 for BrU, respectively, are 23.7 and 17.4 kcal/mol, ΔG for the overall process, starting from the neutral BrU and ending with the Br^- anion and $U^{\bullet}$ radical in separation, is −1.5 kcal/mol (see ΔG_g in the last column of Table 3). As BrU is a relatively small molecule, it has rather limited possibilities of accommodating the energy released in the exergonic steps 1 and 2 other than in the C−Br stretch vibrational degrees of freedom (weak vibrational coupling). Moreover, in the gas phase, under an experimental pressure of 3×10^{-8} Torr,[65] the dissipation of excess energy in molecular collisions is also rather improbable. Therefore, under such conditions, it is the total ΔG of the process under investigation rather than the thermodynamic characteristics of step 3 that should govern the yields of reaction products. Hence, the large cross-section of bromide anion release from BrU induced in the gas phase by 0 eV electron attachment is explained by the

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small negative value of the total ΔG. If such a reaction profile were valid for the native DNA environment, the attachment of an excess electron to a bromonucleobase would impede DNA breakage since the thermodynamics of step 3 would then prevent separation of the product complex. Indeed, because of its size, DNA possesses a large number of coupled vibrational degrees of freedom. Moreover, the number of collisions in solvent effectively dissipates the energy released in steps 1 and 2. Therefore, the product complex should be formed as a thermally equilibrated species, and then the thermodynamics of step 3 determines the complex dissociation. Here, it is worth noting that further reactions, necessary for strand breakage to occur, involve the nucleobase radical itself and would be sterically hindered by the presence of the bromide anion in the nearest neighborhood of that radical if the product complex were the most stable species. Nevertheless, as indicated in Figure 3, the energy profiles for BrNB + e → Br^- + $NB^\bullet$ are quite different in aqueous solution and in the gas phase. Solvation effects make the energy of the last step (complex dissociation) only slightly positive, and the free energy of this reaction amounts to ca. 1 kcal/mol (see Table 3). Thus, [Br^-···$NB^\bullet$] complex separation in water requires less than the thermal energy at 298 K and should, therefore, readily proceed at ambient temperature. As a consequence in solution, electron attachment to DNA labeled with bromonucleobases should end up with the separate bromide anion and the radical (being a part of the DNA strand). Without the steric barrier that might occur due to the small distance between the bromide anion and $NB^\bullet$ in the [Br^-···$NB^\bullet$] product complex, the $NB^\bullet$ radical could, in turn, abstract a hydrogen atom from its own or a neighboring sugar moiety and thus develop a DNA strand break. This analysis highlights the crucial role of the medium in the formation of strand breaks in DNA labeled with bromonucleosides. The presence of water facilitates the product complex dissociation and, therefore, allows for strand break occurrence.

Finally, one could ask which of the studied derivatives is the best potential radiosensitizer. As demonstrated above, these compounds differ in the stabilities of their valence anions. They also have slightly different barriers to bromide anion release. The most important thing, however, seems to be that the total thermodynamic stimulus associated with the electron-attachment-induced conversion of the neutral into the separate radical and bromide anion is rather high (especially in aqueous solution) and similar for all derivatives (see Figure 3B and Table 3). Indeed, pulse radiolysis studies of 5-bromouracil, 8-bromo-2′-deoxyadenosine, and 8-bromo-2′-deoxyguanosine have demonstrated that at pH = 7 the second-order rate constants for the attack of solvated electrons on the halonucleobase are $(1.4 \pm 0.2) \times 10^{10}$, 1.5×10^{10}, and 1.1×10^{10} M^{-1} s^{-1}, respectively.[51,56,58] The similarity of these rate constants suggests a similar sensitivity of brominated nucleobases toward electron attachment, which is in good agreement with the computational finding that the global thermodynamic stimulus for the dissociation processes under discussion here is almost identical for all the systems investigated.

4. CONCLUSIONS

In this study, we compare, at the DFT level, the susceptibility of four brominated nucleobases to decomposition into a bromide anion and a nucleobase radical after electron addition, which is the first step in the mechanism of enhancement of electron-induced DNA damage observed in biopolymers labeled with bromonucleobases.

The positive VDE and AEA values calculated at the B3LYP/6-31++G(d,p) level confirm the vertical and adiabatic stability of the bromonucleobase anion radicals in both the gas phase and aqueous solution and indicate that the presence of the electronegative bromine atom in nucleobase molecules increases the stability of the corresponding nucleobase anions. As demonstrated by the shape of SOMO, BrG supports a dipole-bound anion in the gas phase, which correlates with the large dipole moment of this molecule. On the other hand, in aqueous solution, all the bromonucleobases form valence anions in which the excess electron is localized on a π^* orbital. Moreover, the stability of those anions increases significantly in solvent as compared to the gas phase, which suggests that the aqueous environment facilitates electron attachment.

All the anions but BrA decompose with a small kinetic barrier, forming a product complex of the bromide anion and neutral nucleobase radical. Attachment of an electron to BrA does not produce a stable π^* anion, and consequently the product complex is formed without a kinetic barrier both in the gas phase and in solution.

The complete separation of the Br^- anion and $NB^\bullet$ radical would be suppressed in water if the thermodynamic barrier to the elemental [Br^-···$NB^\bullet$] → Br^- + $NB^\bullet$ reaction were comparable to that found for this step in the gas phase. In the aqueous environment, this barrier is, however, considerably lower, which enables the product complex dissociation to reach completion, even at ambient temperature.

The reaction profiles of the electron-induced decomposition of bromonucleobases demonstrate that the overall stimulus for the process proceeding in water is rather high (ca. −60 kcal/mol) and very similar for the four potential radiosensitizers. Moreover, the formation of noninteracting products, i.e., the neutral radical and bromide anion, which opens a route for strand break formation if bromonucleobases are incorporated in DNA, is accompanied by only a small kinetic barrier, which should be easily overcome during the course of dissociation.

In summary, our calculations indicate that if incorporated into DNA BrC, BrA, and BrG should sensitize the biopolymer to electron-induced damage to much the same extent as BrU does, which calls for experimental studies determining the actual radiosensitivity of DNA labeled with 5-bromocytosine and 8-bromopurines. Such experiments are in progress in our laboratory.

AUTHOR INFORMATION

Corresponding Author

*E-mail: pondros@chem.univ.gda.pl.

Notes

The authors declare no competing financial interest.

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