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An ESR and DFT study of Hydration of the 2'-Deoxyuridine-5-yl Radical: Possible Hydroxyl Radical Intermediate

Lidia Chomicz^b, Alex Petrovici^a, Ian Archbold^a, Amitava Adhikary^a, Anil Kumar^a, Michael D. Sevilla^a, and Janusz Rak^b

Michael D. Sevilla: sevilla@oakland.edu; Janusz Rak: janusz.rak@ug.edu.pl

^aDepartment of Chemistry, Oakland University, Rochester, Michigan 48309, USA ^bDepartment of Chemistry, University of Gdansk, 80-308 Gdansk, Poland

Abstract

The mechanism of radiation-induced frank strand break formation in irradiated 5-bromo-2'-deoxyuridine (BrdU)-labelled DNA is still unclear, despite the proven radiosensitizing property of BrdU. Combination of ESR spectroscopy and quantum chemical modelling points to a simple reaction between the uridine-5-yl radical and water molecule that produces the genotoxic hydroxyl radical.

Hypoxia is common for solid tumors and makes them 2.5 to 3 times more radioresistant compared to normally oxygenated tissues.¹ As a result, efficient radiotherapy (RT) protocols often employ chemical agents that mimic oxygen to sensitize cells to ionizing radiation (IR). *In vitro* experiments, carried out in early 1960s, demonstrated that 5-bromo-2'-deoxyuridine (BrdU) is capable of sensitizing hypoxic cancer cells to IR.² Since then, BrdU has been extensively studied as a potential radiosensitizer in model DNA fragments,³ on cell cultures⁴ and even *in vivo* during clinical trials.⁵ Although, BrdU has not been routinely clinically employed, halogen derivatives of uracil, are still considered to be potentially useful in RT as indicated by recently published results on the clinical trial of 5-iodo-pyrimidine-2'-deoxyribose on cancer patients.⁶

BrdU can be classified to the group of radiosensitizers - whose activity is related to their incorporation into cellular DNA.⁷ The radiosensitivities of cells with BrdU-labeled DNA are reported to be significantly higher than the control.⁸ The incorporation of BrdU into cellular DNA results in an increase in the IR-induced formation of strand breaks (single (SSBs) and double (DSBs)); primarily, the amount of DSBs correlates with cell killing.^{8,9}

Correspondence to: Michael D. Sevilla, sevilla@oakland.edu; Janusz Rak, janusz.rak@ug.edu.pl.

[†]Electronic Supplementary Information (ESI) available: ESR experimental and DFT calculation details, ESR spectra in D₂O, Kinetic Isotope Effect discussion, optimized structures of substrates and transition states for the uridine radical hydration, optimized structures of various stereoisomers of 5-UHOH• and 6-UHOH• and their hyperfine couplings, discussion of the computational results concerning stereoisomers and effects of the rotation of the OH group in the P2 radical, xyz coordinates of the structures presented in Figures 2 and S2, additional references. See DOI: 10.1039/c000000x/

Solvated electrons are one of the main products of water radiolysis and especially in the absence of oxygen, add to BrdU.¹⁰ BrdU is susceptible to efficient dissociative electron attachment (DEA) which leads to the formation of uracil-5-yl radical (5-U-yl•) and bromide anion.¹¹ As proposed in the literature, (5-U-yl•) then undergoes a H-atom abstraction from an adjacent deoxyribose group resulting in a strand break.⁹ We note here that, in double stranded (ds) B-DNA, which is a relatively stiff molecule, the 5-U-yl• could only abstract either the C2'-H atom or the C1'-H atom of the adjacent 2'-deoxyribose as only these two H-atoms are close enough to the radical site of 5-U-yl•.¹² Since the C1'• and C2'• do not yield frank strand breaks in dsDNA, the increased number of strand breaks observed in IR-irradiated cell cultures under anoxic conditions⁴ cannot be fully accounted for after the formation of 5-U-yl• in B-form of dsDNA.

In the present communication, we propose a new mechanism of BrdU degradation by the excess electron that could explain the occurrence of strand breaks without the involvement of oxygen. First, ESR (Electron Spin Resonance) spectra of the γ -irradiated frozen (77 K) aqueous glasses (7.5 M LiCl in H₂O or in D₂O) containing BrdU show the formation of 5-U-yl• and its subsequent hydration producing the hydrate radical (5-UHOH•). Second, density functional theory (DFT) calculations show the lowest barrier mechanism for the reaction of the 5-U-yl• with water molecule involves a simple mechanism of a H-atom transfer from water to the C5 of uracil moiety in 5-U-yl•. This reaction produces the hydroxyl radical (OH•) and uracil; subsequent electrophilic addition of OH• to the C5 of uracil produces 5-UHOH• which is observed experimentally by ESR. For BrdU-incorporated DNA, OH• formed via the reaction of 5-U-yl• with surrounding water could attack the C3', C4', or C5' sites of the sugar moiety adjacent to 5-U-yl• or its own sugar which in the subsequent steps may give rise to strand breaks. Thus, OH• produced in the simple reaction of 5-U-yl• with water, could lead to strand break formation in BrdU-labelled dsDNA.

Figure 1 depicts the initial ESR spectrum recorded at 77 K (Figure 1A) and the spectra resulted from annealing (Figure 1B–D) in H₂O. The details of ESR experiments as well as the corresponding spectra for the matched BrdU sample in D₂O are shown in supplementary information (SI). Spectra recorded in D₂O for lower temperatures of annealing were found to be similar to the A–C spectra in H₂O except that little reaction occurs (Figure S1). The spectrum 1A shows electron attachment to BrdU at 77 K resulting in formation of the π -anion radical of the uracil base. Weak low field lines due to Cl₂•[–] have been subtracted out. Cl₂•[–] is scavenged by [Fe(CN)₆]^{–4} on annealing.

After 15 min annealing at ca. 145 K the 5-U-yl• is formed by Br[–] loss (see the broadened central component in Figures 1B and 1C). This species can form a weak complex with the leaving Br[–] that can result in substantial hyperfine coupling constant (HFCC) values of the bromine atom; however, these are not apparent in Figures 1B and C. The 5-bromouracil (BrU) π -anion radical and 5-U-yl• with its weak interaction with Br[–] as a $\sigma\sigma^*$ 5-U••Br•[–] radical complex have been previously reported for BrU in LiCl glasses.^{13,14} Voit et al. also found the weak 5-U-yl••Br•[–] $\sigma\sigma^*$ complex is formed in irradiated BrdU substituted DNA at low temperatures.¹⁵

For BrU, the 5-U-yl• was previously shown to react with water to form the “hydrate” radical, 5-UHOH•¹³. This radical is now proposed to be produced via •OH addition to the C5=C6 bond in dU. Previous pulse radiolysis and product analyses studies have established the predominant (ca. 80%) addition of •OH at C5 of the uracil base forming 5-UHOH•.¹⁶ •OH addition to dU forms R and S stereoisomers of 5-UHOH• each in two conformers H-axial or equatorial. The HFCC values are sensitive to these conformations (see supplement for the DFT calculations). It is therefore expected that for the nucleoside (BrdU), that 5-UHOH• formation should occur after electron attachment. Indeed, we find that after 15 min annealing at 160 K can be clearly noted by the formation of four line components in ESR spectrum (see the red simulated spectrum based on the interaction of ca. 70% of 5-UHOH• (H-axial, see supplement) due to one an isotropic α proton (HFCC= 9, 21, 31 G) along with one isotropic β proton having HFCC = 41 G (quartet). Further we find ca. 30% of a 20G doublet from 5-UHOH• (H-equatorial, see supplement). Note that DFT calculations show that 6-UHOH• would also produce a doublet (ca. 20 G, see supplement) and can also account for a portion of the doublet spectrum found. Owing to the low experimental temperatures, spectrum 1D is the additive result of individual conformers described above not their thermal averaging. We find the exchange of solvent (from H₂O to D₂O) significantly slows the reaction. Indeed, data obtained in D₂O, show the formation of the π anion radical at 77 K, 5-U-yl• forms at ca. 150 K and then undergoes a small amount of reaction with clear observation of deuteron couplings expected for 5-UDOD• (D-axial) (see Figure S1). The π -anion radical also undergoes deuteration at C6 to form the 6-D-5BrU• (Figure S1D, SI). The 6-D-5BrU• species, shows large anisotropic HFCC (114, -32, -20) G from bromine and a 32 G isotropic doublet arising from the one β -H at C6. These couplings closely agree with the earlier work for this radical in single crystals of BrdU.¹⁷ Since no significant formation of the corresponding C-6 protonated π anion radical (6-H-5BrU•) was observed in LiCl glasses in H₂O (compare Figure 1 with Figure S1), the formation of 6-D-5BrU• in D₂O is quite unexpected. Clearly, our results in H₂O and D₂O glasses show that the reaction of 5-U-yl• observed in H₂O has a significant solvent kinetic isotope effect (KIE) which retards reaction of 5-U-yl• with water. The protonation reaction of the π anion radical, therefore, must have a lower KIE than the competitive hydration of 5-U-yl• and thus becomes favored in D₂O systems. The KIE is discussed in more detail in the supplement.

This experimental picture can be elucidated with the help of quantum-mechanically (QM) derived energetics and kinetics concerning, in particular, the water reaction step. The formation of the π -anion radical of BrdU/BrU and $\sigma\sigma^*$ 5-U-yl••Br•⁻ complex has been previously studied^{11,18} at various DFT levels and very recently with the MD *ab initio* approach.¹⁹ All those theoretical endeavors demonstrate that BrU is an excellent electron acceptor and its π -anion radical decomposes, with a tiny kinetic barrier, to a weakly bonded σ complex that is by ca. 9 kcal/mol more stable than π -anion radical (hence, there is a significant thermodynamic stimulus that drives the dissociation of the BrU anion radical).¹¹ However, to the best of our knowledge, the hydration of the 5-U-yl•, as shown here by ESR experiments (Figures 1 and S1), has not been studied with QM methods so far. We modeled the energetics and kinetics of the studied reactions using the M06-2x density functional theory²⁰ with the 6-31++G(d, p) basis set²¹ and the PCM model for water.²²

In Figure 2 two possible products of 5-UHOH• (radicals P1 and P2), resulting from reaction of 5-U-yl• with water are presented. We note that P1 and P2 are tautomers. The water molecule can react with 5-U-yl• in two ways at least: approaching the radical with its oxygen (path A in Figure 2) or hydrogen atom (path B in Figure 2). Path A results in the formation of P1; path B leads to the H-atom abstraction by 5-U-yl• leading to OH• production. Path B, leading to OH• formation, is more competitive due to its relatively low activation barrier ($G^* = 12.1$ kcal/mol; compare with $G^* = 27.8$ kcal/mol for the P1 product, see Figure 2) and close to zero thermodynamic stimulus ($G = 0.14$ kcal/mol). It is worth noticing that even at ambient temperature path B will be greatly favored based on the thermodynamic free energies. In the subsequent step (path B; see Figure 2B) the complex of the neutral uracil and hydroxyl radical (U+OH•) can be transformed into experimentally observed product P2 (see Figure 1D) with a very low ($G^* = 2.6$ kcal/mol, see Figure 2B) activation barrier. Since, P1 species is simply a tautomeric form of P2 and since the P1 to P2 reaction has a highly favorable free energy change, $G = -7.0$ kcal/mol, we conclude that P2 is the only likely product from either pathway. We note similar OH• addition species to P2 are products of hydroxyl radical attack on pyrimidines.¹⁰

The hydroxyl radical may, in principle, react also with the C6 site of dU leading to the formation of 6-UHOH•. However, both the ESR spectrum shown in Figure 1D and previous reports¹⁶ demonstrate that this reaction pathway is disfavored (see the discussion above). In order to explain the observed preference we compared the M06-2x kinetic barriers for the formation of both C5 and C6 •OH adducts. Our calculations demonstrate that the barrier which leads to the C6 analogue of that depicted in Figure 2 amounts to 2.72 kcal/mol. Thus, the difference of 0.56 kcal/mol accounts for the observed preference for 5-UHOH• formation, especially at low temperatures employed during the ESR experiment (the kinetic preference for the •OH attack at the C5 site is discussed in more detail in the supplement).

Conclusions

In summary, our calculations strongly suggest that 5-U-yl• produces OH• while interacting with water even at ca. 155 K. It was demonstrated recently that in low temperature argon matrices containing the phenyl radical and H₂O, the hydroxyl radical can be produced.²² It should be noted, however, that this reaction is not spontaneous and the OH• are produced only after irradiation of the experimental system with visible light. It seems therefore, that the hydration of the 5-U-yl•, described in the current communication, is a rare example of spontaneous H-atom transfer from water to a carbon centered radical. Owing to the unusual nature of our prediction we have checked the energetics of the reaction with the thermodynamically reliable G3B3 level²³ of calculations in which each of the reacting species in the hydrogen abstraction reaction [1-methyluracil-5-yl• + H₂O → OH• + 1-methyluracil] were treated separately and found $G = -0.2$ kcal/mol in the gas phase and $G = 1.3$ kcal/mol in water (PCM) for the overall reaction free energy change. This shows that the C5-H bond energy is comparable to the OH bond in water and strongly supports our DFT results.

Moreover, our findings may explain the radiosensitizing properties of those uracil derivatives which reacting with solvated electrons produce the 5-U-yl•. It is commonly

assumed that 5-U-yl• incorporated in DNA can easily abstract hydrogen from the adjacent sugar which leads to SSB because a good radiation sensitizer should increase the number of DNA strand breaks.⁹ However, in ds DNA only 5'-neighbour 2'-deoxyribose C2'-H/C1'-H hydrogens are close enough to be abstracted producing the C2' or C1' radical centers.¹² Yet, none of these two radical centers can directly lead to a strand break. Moreover, a hydrogen shift between C3'/C5' and C2' that could produce the C3'/C5' radical vulnerable to direct strand break is not possible due to very unfavorable kinetic barrier of that shift.²⁵ The situation becomes completely different if one assumes that OH• is released during reaction between 5-U-yl• and H₂O. This is because the OH• in a close proximity to DNA can easily produce radical centers on the C3', C4', or C5' sites of the sugar moiety which then transform into strand breaks. Indeed, it is known that the genotoxicity of OH• produced during radiotherapy is related to DNA strand break formation that result due to the abstraction of sugar hydrogens.¹⁰

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1. ESR spectra of BrdU in 7.5 M LiCl in H₂O with K₄[Fe(CN)₆] as hole scavenger (A) immediately after γ -irradiation; (B)– (D) obtained after annealing the sample for 15 min; all recorded at 77 K. Structure of the radical assigned to each spectrum is shown. The simulated (red) spectrum (1D) employed is a combination of ca. 70% quartet from 5-UHOH• (H-axial to the radical site *p*-orbital, see supplement) and of ca. 30% doublet from 5-UHOH• (H-equatorial to the radical site *p*-orbital, see supplement) as well as from 6-UHOH• and is superimposed on the experimental (black) spectrum for comparison (for simulation parameters, see text). The three triangular field markers are individually separated by 13.09 G with the central marker at $g=2.0056$.

Figure 2.

Possible reaction paths calculated at MO6-2x/6-31++G(d, p) level for 2'-deoxy-5-U-yl• and water molecule system. G and G^* stand for reaction free energy and activation free energy, respectively. All values are expressed in kcal/mol. For 2D models R refers to 2'-deoxyribose. $P1$ and $P2$ are tautomers with $P2$ being most stable.