Sensitizing DNA to Secondary Electron Damage: Resonant Formation of Oxidative Radicals from 5-Halouracils

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Substitution of thymine (T) with 5-halouracil (5-X-U) in the genetic sequence of cellular DNA leads to greater sensitivity to ionizing radiation,[1−3] without changing the normal gene expression in unirradiated cells. Although potential clinical applications of 5-X-Us as tumor-specific sensitizers promise cancer therapies at significantly reduced doses and hence less patient side-effects, the nascent mechanisms by which they enhance radiation damage to DNA are not understood. Here we show that the fundamental radio-sensitizing nature of 5-X-Us (X = F, Cl, Br, and I) relates to their substantial and unique propensity for *resonant* dissociation into various genotoxic radicals during 0−3 eV secondary electron (SE) attack to the molecule. Nonhydrated, low-energy SEs are an abundant initial species in irradiated cells (5×10^4/MeV deposited),[4] and their ability to induce substantial single- and double-strand breaks (SSB and DSB) in unsensitized DNA, prior to solvation (10^{-12} s) at energies above 4 eV, has only been demonstrated recently.[5]

The traditional model of radiosensitization involves the reduction of 5-X-U, substituted for thymine in DNA, by a radiation-induced hydrated electron. Subsequent dehalogenation yields a uracil-5-yl radical,[6,7] (U-yl)•, which is believed to be the sole reactive precursor responsible for the strong enhancements in genotoxic damage, such as unrepairable DNA strand breaks. However, the efficiency for cellular DNA radiosensitization[1,2] depends not only on the type of halouracil but also on its degree of incorporation[3] (mono- or bifilar) in DNA, neither of which can be reconciled with the traditional hydrated-electron model involving only (U-yl)• reactive precursors. Furthermore, SSBs and DSBs, induced in unsensitized DNA by free SE with ballistic energies above 4 eV,[5] are initiated locally within the DNA by the same type of *resonant* single and multibond ruptures that are observed in isolated DNA components, such as gas-phase cytosine or thymine.[8] Thus, the latter "isolated molecule" techniques are ideally suited to probe the nascent mechanisms behind the radiosensitivity of 5-X-Us to SE at the most fundamental molecular level.

The key questions are essentially whether the intrinsic sensitivity of 5-X-Us involves nonhydrated SE and also extends beyond thermal SE energies and most importantly *whether it implicates more than one dissociation pathway*. Furthermore, the biochemical activity of a molecule or radical relates to its electron affinity (EA),[9] which is proportional to its redox potential;[10] however, no values for either have been reported to date for (U-yl)•.

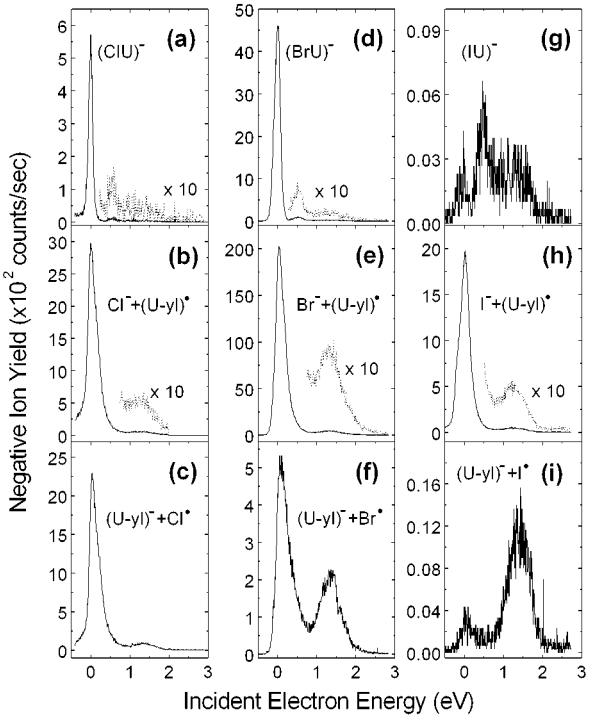

Figure 1. Anion yields and fragmentation pathways produced by electron impact to gas phase 5-X-Uracil, (X = Cl, Br, I) as functions of incident electron energy. The dotted curves have been multiplied by a factor of 10 for visibility. The experiments were carried out at the Berlin laboratory in a crossed beam apparatus described elsewhere.[8] An electron beam (∼10 nA, fwhm ≈ 0.12 eV) from a trochoïdial monochromator orthogonally intersects an effusive molecular beam emanating from a resistively heated oven (∼420 K) containing high-purity 5-halouracil powder (Aldrich Ltd.). Anions, formed via electron−molecule collisions, are extracted from the reaction volume by a small electric field toward a quadrupole mass analyzer and are detected by single-pulse counting techniques. The electron energy scale is calibrated via measurements of SF_6^- ion production, which exhibits a sharp peak at 0 eV (within the experimental uncertainty ±60 meV) of a known cross section.[11]

To address these questions, we have directly measured the sensitivity of isolated 5-X-Us to SE attack at biologically relevant SE energies between 0 and 3 eV. Our high-vacuum electron-microbeam techniques[8,11] allow us to determine the individual 5-X-U decomposition pathways and probabilities, but also the EA of (U-yl)•, by systematic substitution of different halogens at the carbon 5 position in 5-X-U.

The results in Figure 1 show that 0−3 eV resonant capture of a free electron by 5-X-Us induces not only the formation of parent anions $(5\text{-X-U})^-$ but also molecular decompositions leading to the formation of Cl^-, Br^-, or I^- (plus a (U-yl)• radical) *as well as* $(U\text{-yl})^-$ *plus a free halogen radical*; however, for 5-F-U no F^- or $(U\text{-yl})^-$ are produced for incident electron energies below 3 eV. Near 0 eV incident electron energy (±60 meV), the production of long-lived (up to 50 μs) parent anions may arise a priori from formation of either "dipole-bound" anions,[12] where

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(1) Zamenhof, S.; DeGiovanni, R.; Greer, S. *Nature* **1958**, *181*, 827.
(2) McLaughlin, P.; Mancini, W.; Stetson, P.; Greenberg, H.; Nguyen, N.; Seabury, H.; Heidorn, D.; Lawrence, T. S. *Int. J. Radiat. Oncol., Biol., Phys.* **1993**, *26*, 637.
(3) Lawrence, T. S.; Davis, M. A.; Maybaum, J.; Stetson, P. L.; Ensminger, W. D. *Radiat. Res.* **1990**, *123*, 192.
(4) ICRU Report 31, International Commission on Radiation Units and Measurements, Washington DC, 1979.
(5) Boudaiffa, B.; Cloutier, P.; Hunting, D.; Huels, M. A.; Sanche, L. *Science* **2000**, *287*, 1658.
(6) Bhatia, K.; Schuler, R. H. *J. Phys. Chem.* **1973**, *77*, 1888.
(7) E. Rivera, R. H. Schuler, *J. Phys. Chem.* **1983**, *87*, 3966.
(8) Huels, M. A.; Hahndorf, I.; Illenberger, E.; Sanche, L. *J. Chem. Phys.* **1998**, *108*, 1309, communications.
(9) Lovelock, J. E. *Nature* **1961**, *189*, 729. Lovelock, J. E. *Nature* **1962**, *198*, 540.
(10) Ruoff, R. S.; Kadish, K. M.; Boulas, P.; Chen, E. C. M. *J. Phys. Chem.* **1995**, *99*, 8843.
(11) Illenberger, E. In *Gaseous Molecular Ions, Topics in Physical Chemistry*; Baumgärtel, H., Frank, E. U., Grünbein, W., Eds.; Steinkopff, Springer: Darmstadt, New York, 1992; Vol. 2, part III and references therein.

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the excess electrons are weakly bound to the molecules via long-range multipolar forces, or by formation of covalent anions (i.e., resonances). The resonant mechanism for anion and radical fragment formation is interpreted within the framework of dissociative electron attachment theory,[13] and has been shown recently to be active even in unsensitized DNA.[5] The incident-electron is captured by the neutral target molecule to form a transient molecular anion, that is, a resonance, viz.

$$e^- + \text{5-X-U} \rightarrow (\text{5-X-U})^{*-} \qquad (1)$$

This resonance may stabilize, autodetach the electron, or undergo unimolecular dissociation into a negative ion and its neutral radical counterpart, that is

$$(\text{5-X-U})^{*-} \rightarrow X^- + (\text{U-yl})^\bullet, \text{ or } X^\bullet + (\text{U-yl})^- \qquad (2)$$

Other fragmentation pathways, such as 5-X-U^{*-} $\rightarrow$ OCN$^-$ + (H+XH$_2$C$_3$NO)$^\bullet$ (and others, not shown here[14]), are also observed at 1.5 eV and higher electron energies; this demonstrates even more complex *fragmentations involving aromatic ring cleavage* of the base substituent.

On the basis of our results in Figure 1, the known C$-$X mean bond dissociation energies, BDE(C$-$X),[15] and the large halogen electron affinities (EA(X) > 3.0 eV),[16] we can estimate the excess energy for X$^-$ formation via reaction sequence (1$-$2) by

$$\epsilon_{exc} = E(e^-) + \text{EA(X)} - \text{BDE(C}-\text{X)} + E_{th} \qquad (3)$$

where $E(e^-)$ corresponds to the incident electron energy and E_{th} < 0.15 eV is the total internal energy of the target molecule.[17] Thus, at 0 eV incident electron energy the formation of Cl$^-$, Br$^-$, and I$^-$ ions is *exothermic* by about 0.01, 0.24, and 0.42 eV, respectively, but *endothermic* by 0.9$-$1 eV for F$^-$ formation. A salient fact is that here we also observe stable (U-yl)$^-$ formation from 5-Cl-U, 5-Br-U, and 5-I-U; this indicates that the e^- + 5-X-U $\rightarrow$ 5-X-U^{*-} $\rightarrow$ (U-yl)$^-$ + X$^\bullet$ reaction *is also exothermic or at least thermoneutral* ($\epsilon_{exc}\sim$0), and immediately gives a lower limit for the EA[18] of (U-yl)$^\bullet$ of about 3.2 eV via eq 3. Similarly, since here (U-yl)$^-$ formation is not observed for 5-F-U, or elsewhere for thymine,[8] under identical experimental conditions, this indicates that the EA of (U-yl)$^\bullet$ is smaller than $\sim$3.5 eV (i.e., the thymine C$-$CH$_3$ BDE). Thus, we find that the EA of the (U-yl)$^\bullet$ radical is high, about 3.2 to 3.5 eV, which suggests that the (U-yl)$^\bullet$ redox potential is also substantial, possibly surpassing that of OH.[19]

Most importantly, however, the present yields of (U-yl)$^-$ from 5-X-Us are equal to those of *free halogen radicals*, X$^\bullet$, which have not been previously anticipated in the traditional model. The branching ratios, (U-yl)$^\bullet$/X$^\bullet$, for gas-phase production (at about 10^{-7} Torr in vacuo) of these highly reactive radical species from 5-Cl-U, 5-Br-U, and 5-I-U, are directly given by our measurements of X$^-$/(U-yl)$^-$, and are estimated to be 1.3, 40, and 490, respectively, near 0 eV. In other words, low-energy SE damage to 5-I-U produces almost exclusively (U-yl)$^\bullet$ (bound to the ribose moiety in DNA), which can lead to SSBs, for example, via 4′ hydrogen abstraction from the ribose, when the 5-I-U is incorporated within only a single strand of cellular DNA[3] (the closed shell halogen anions are believed to be inert); only upon bifilar incorporation of 5-I-U in such irradiated DNA are DSBs induced. However, in the case of 5-Br-U substitution, and even more so for 5-Cl-U (see Figure 1), the increased probabilities for formation of both (U-yl)$^-$ *and* free atomic halogen radicals may then enhance genotoxic damage, in agreement with observations reported elsewhere;[3] this may be due to subsequent reactions of the halogen radicals within their vicinity (e.g., via oxidation on the opposite strand leading to a SSB), even if the 5-Br-U is only incorporated in one DNA strand.[20] Compared to the redox potentials of genotoxic radiolytic species such as OH$^\bullet$ or O$_2$($^1\Delta_g$), that is, 2 and 0.65V, those of atomic halogens are similar, or higher, viz. I$^\bullet$(1.3 V) < Br$^\bullet$(2 V) < Cl$^\bullet$(2.6 V).[21] In addition to their redox potential, the pernicious nature of the halogen radicals is intensified by the redistribution of ϵ_{exc} in terms of kinetic energy, which favors lighter fragments; thus, the Cl, Br, and I radicals receive about 76, 58, and 47% of the ϵ_{exc}, respectively. This may enhance their mobility within a small volume of DNA but also their reactivity, for example, by $\sim$1 eV for SE energies near 1.5 eV. The overall radiosensitivity of 5-X-U-substituted DNA to SE attack will likely depend on the branching ratios but also on the cross sections for the formation of the various reactive radicals: for (U-yl)$^\bullet$ production by 0$-$3 eV electrons they are found to be larger for 5-Br-U than for either, 5-Cl-U or 5-I-U, whereas for *free halogen radical* formation they are largest for 5-Cl-U than for 5-Br-U or 5-I-U.

This propensity of 5-halouracils for fragmentation by abundant 0$-$3 eV SE, formed along radiation tracks in living tissue, is likely responsible for their radiosensitizing effect within cellular DNA. C*ontrary to the traditional model*, our results show that the formation of *at least* two highly reactive radicals, that is, (U-yl)$^\bullet$ *and* free halogen, and their different formation and reaction cross sections, relate to the sensitization dependence on halouracil type and degree of substitution (mono- or bifilar) observed in irradiated cells. Our results also suggest that 5-Cl-U may be a more effective radiosensitizer for production of lethal DSBs than the other halouracils. Since thermal electrons are belived to migrate along the DNA base π-stack,[22] our results imply that novel photodynamic therapies involving intercalating electron donors might be enhanced, or even controlled, by combination with a judicious choice of 5-halouracil incorporation in DNA.

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(12) Desfrançois, C.; Abdoul-Carime, H.; Schulz, C. P.; Schermann, J. P. *Science* **1995**, *269*, 1707.

(13) Christophourou, L. G. In *Electron−Molecule Interactions and their Applications*; Christphourou, L. G., Ed.; Academic Press: New York, 1984; Vol.1.

(14) Abdoul-Carime; Huels; Illenberger; Sanche, to be published.

(15) Lowry, T. H.; Richardson, K. S. *Mechanism and Theory in Organic Chemistry*, 3rd ed.; Harper Collins Publications: New York, 1987.

(16) Janousek, B. K.; Brauman, J. I. In *Gas-Phase Ion Chemistry*; Bowers, M. T., Ed.; Academic Press Inc: New York, 1979; Vol. 2, pp 53−86.

(17) SantaMaria, R.; Charro, E.; Zacarías, A.; Castro, M. *J. Comput. Chem.* **1999**, *20*, 511. Vibrational spectra calculated via Hartree−Fock ab initio method for nucleic acid bases exhibit only three active vibrational modes (VM) at energies below 280 cm^{-1} (35 meV, 420 K in the present experiments). If each of these VM absorbs 35 meV thermal energy, the internal energy of the neutral target molecule, E_{th}, is at most 0.15 eV, and thermal effects on the dissociation dynamics are believed to be small.

(18) For gas-phase molecules the EA is actually the vertical detachment energy (VDE) of the extra electron in the molecular anion at its most stable geometry; relative to the gas-phase adiabatic EA (AEA) the VDE ≥ AEA; e.g., for OH, AEA = VDE = 1.83 eV.

(19) The relative redox potential $E_r \propto$ EA $-$ $\Delta\Delta G_s$ (ref 10); $\Delta\Delta G_s$ is the solvation energy difference (gas phase to solution) between a neutral and its anion and depends strongly on degree of charge (or radical site) localization. For neutral (U-yl)$^\bullet$, this is likely to occur on C5. If the $\Delta\Delta G_s$(U-yl)$^\bullet$ ranges between values for aromatics (ref 10) and OH (Coe, J. V.; Earhart, A. D.; Cohen, M. H.; Hoffman, G. J.; Sarkas, H. W.; Bowen, K. H. *J. Chem. Phys.* **1997**, *107*, 6023), 1.5 V ≤ E_r(U-yl)$^\bullet$ ≤ 3.5 V. This will be explored and discussed in detail elsewhere (ref 14).

(20) The notion that atomic halogens may react over a distance similar to the DNA's diameter (2$-$3 nm) and induce a SSB on the opposite strand, is in part based on the observation that other reactive species, such as O$^-$ radicals, may react in simple hydrocarbon films on similar distances (e.g.: Bass, A. D.; Parenteau, L.; Huels, M. A.; Sanche, L. *J. Chem. Phys.* **1998**, *109*, 8635; observed reactions include hydrogen abstraction and reactive charge transfer). Simultaneously, in DNA the (U-yl)$^-$ might, e.g., abstract a proton from its ribose, leading to phosphate elimination and a second SSB, hence a DSB.

(21) Wardman, P.; *J. Phys. Chem. Ref. Data* **1989**, *18*, 1637. All values are relative to the standard hydrogen electrode and in aqueous solution.

(22) Kelley, S. O.; Barton, J. K. *Science* **1999**, *283*, 375 and references therein.