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Bond Breaks of Nucleotides by Dissociative Electron Transfer of Nonequilibrium Prehydrated Electrons: A New Molecular Mechanism for Reductive DNA Damage

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A much deeper understanding of fundamental mechanisms of cancer biology and therapies can lead to improved clinical outcomes.[1] Understanding the fundamental mechanisms (and new molecular pathways) that induce DNA damage and cell death (apoptosis) should lead to a clearer picture of the cause of cancers and benefit the development of improved strategies for cancer treatment.[1] Oxidative molecular pathways leading to DNA damage and apoptosis are relatively well-known in their relation to human cancers and cancer treatments.[2] However, little is known about the reductive molecular pathway.

Radiotherapy is still the main curative therapy for cancers. Exposure of living cells to ionizing radiation, such as hard X-rays and γ rays, leads to biological damage by both direct and indirect interactions with the cell components. In regard to direct damage to DNA, there have been intense studies of the effect of dissociative electron attachments (DEAs) of low-energy (0−20 eV) *free* electrons in damaging DNA components in the gas phase and *dry* DNA in vacuum.[3,4] DEA resonances lying energetically below their parents are called “Feshbach resonances”, while resonances lying above their parents are called “shape resonances”. A number of recent theoretical studies[5] have shown that an aqueous environment may have an inhibiting or enhancing effect on direct damage to DNA by DEAs of low-energy free electrons. The direct energy deposited in the DNA, however, accounts for only one-third of the energy deposited into the cell. The indirect action takes about two-thirds of the deposited energy, which is absorbed initially by water (the cell contains 70−80% water).[3,6] It is known that the biological damage induced by free radicals from radiolysis of water far exceeds that by direct energy deposited in the target (DNA).[6] Indeed, Ito et al.[7] have observed that the yields of single-strand breaks (SSBs) and double-strand breaks (DSBs) of DNA by γ-ray radiation in an aqueous solution are 3 orders of magnitude higher than those for dry DNA.

As has been known for over 40 years, $OH^{\bullet}$ and the hydrated electron (e^-_{hyd}) are the major radicals produced by radiolysis of water.[3,6] Despite its higher yield, e^-_{hyd} trapped in a deep potential well (at roughly −3.2 eV) is ineffective at inducing biological damage.[3,6] Thus, almost all of the indirect damage to DNA has been attributed to the attack by the *oxidizing* hydroxyl radical, $OH^{\bullet}$.[3,6] However, it has been pointed out that lesions produced in DNA by $OH^{\bullet}$ acting alone are unimportant and ineffective in cell killing, as they can be efficiently repaired.[8] In contrast, lesions from multiply damaged sites (MDSs) have important consequences for biological effects because they are difficult for the cell to repair. More remarkably, it has been observed that even very high concentrations of $OH^{\bullet}$ scavengers cannot completely quench the DNA damage, especially DSBs.[9] There is 30−65% “nonscavengable” DNA damage, which has been attributed to direct action of radiation in the DNA.[6,9] This seems inconsistent with the observed enhancement by orders of magnitude of ionizing-radiation-induced DNA damage in the presence of water solution.[7] Indeed, a long-standing mystery has been the exact role of water in DNA damage induced by ionizing radiation.[10]

The advent of femtosecond (1 fs = 10^{-15} s) time-resolved laser spectroscopy (fs-TRLS) has provided an unprecedented level of understanding of the radiolysis of water. It is now known that prior to the formation of e^-_{hyd}, an excess electron in water is rapidly located at precursor states with finite lifetimes of <1 ps (1 ps = 10^{-12} s); this is the so-called prehydrated electron (e^-_{pre}).[11] Although experimental and theoretical studies[11] once gave very diverse lifetimes and physical natures of e^-_{pre} states, we recently showed that they are electronically excited states and have lifetimes of ~200 and 500 fs after identification and removal of a coherent spike.[12] An e^-_{pre} is only weakly bound (−1.5 to −1.0 eV[12]) and has the highest quantum yield, which is nearly double that of its ending product (e^-_{hyd})[11d] or the $OH^{\bullet}$ radical. There is also evidence that e^-_{pre} can be attached to amino acids and nucleotides: Hunt and co-workers[13a] obtained indirect evidence by monitoring the initial yield of e^-_{hyd} at 30 ps in picosecond radiolysis, while Gauduel et al.[13b,c] observed ultrafast one-electron reduction of oxidized pyridine nucleotides and cystamine by e^-_{pre}. Using fs-TRLS, we have *directly observed the dissociation (bond breakage)* of halopyrimidines (XdU), hypoxic sensitizers for radiotherapy of cancer, caused by the *dissociative electron transfer (DET)* reaction e^-_{pre} + XdU → XdU*$^-$→ dU$^{\bullet}$ + X$^-$.[14] *However, little is known about reductive DNA damage induced by DET of* e^-_{pre} *with energies below 0 eV.*

In this study, we employed fs-TRLS to study the molecular mechanism of indirect damage to nucleotides (dXMPs), the DNA basic units, induced by e^-_{pre} under ionizing radiation. Our results demonstrate a novel DET mechanism for *reductive* DNA damage that may be related to various diseases such as cancer and stroke. Moreover, our finding challenges the conventional notion that damage to the genome by ionizing radiation is mainly induced by the $OH^{\bullet}$ radical and may therefore lead to a new understanding of many aspects of the biological actions of radiation and the development of improved strategies for radiotherapy of diseases such as cancer and for radioprotection of humans exposed to radiation.

The standard methodology for pump−probe femtosecond transient absorption measurements has been described previously.[14] A pump wavelength of 318 nm was used to generate excess electrons in water, and a probe wavelength at ~330 nm was used to probe the intermediate state (dXMP*$^-$) of the reaction of e^-_{pre} with a dXMP.[14] As shown in Figure 1, this is a key step in inducing DNA strand breaks. fs-TRLS allows us to observe the DET reaction in real time. The formation and decay of dXMP*$^-$ can be expressed in the following reaction:

$$e^-_{pre}(<0\ \mathrm{eV}) + \mathrm{dXMP} \rightarrow \mathrm{dXMP}^{*-} \rightarrow \text{dissociation or dXMP}^- \quad (1)$$

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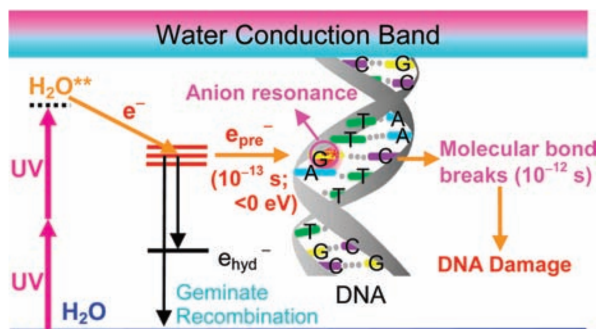

Figure 1. Two-UV-photon excitation of water leads to the formation of an electron localized in a p-like excited precursor state (e^-_{pre}), which then solvates to the equilibrated s-like hydrated state (e^-_{hyd}). When DNA is near the e^-_{pre}, dissociative electron transfer (DET) can occur, forming a transient molecular anion resonance that then leads to molecular bond breaks in DNA bases followed by strand breaks of the DNA.

where X denotes a DNA base (A, T, G, or C) and dXMP*− is a vibrationally excited intermediate anion state. It is well-known that the electronic absorption spectrum of dXMP arises solely from the excitation of the π-electron system of the DNA base X. According to the results for halodeoxyuridine anions (CldU*−, BrdU*−, and IdU*−),[14] the electronic absorption of dXMP*− or its vibrationally relaxed anion dXMP− is expected to have a UV absorption band at 300−350 nm, slightly red-shifted from that of the neutral counterpart. It is also known that the autodetachment of a molecular anion resonance occurs on time scales of 10^{-16} to 10^{-14} s and that the vibrational relaxation times of molecules range from 0.1 to 1.0 ps[15a] and are 0.5−1.0 ps for nucleotides.[15b,c] Here the detected real-time signal is the intensity of the electronic absorption, which is identical for both dissociative dXMP*− and nondissociative dXMP−, independent of the vibrational states.[14] Thus, the signal decay for $t \geq 1.0$ ps simply reflects the dissociation of the transient anion (dXMP*−); that is, a flat signal indicates no dissociation but rather formation of a stable anion (dXMP−) only.[14] Figure 2 shows transient absorption kinetic traces of dXMP*− species produced by DET reactions of four dXMPs with e^-_{pre}. First, it is interesting to observe that the formation (rising) of dXMP*− is complete within the lifetime (<1 ps) of e^-_{pre}. Second, it is found that purines (dGMP and dAMP) are more efficient in capturing e^-_{pre} than pyrimidines (dTMP and dCMP): the dGMP/dAMP/dTMP/dCMP initial electron capture efficiency ratio is 13:8:6:5. Third, the data also clearly indicate that e^-_{pre} can be attached to A and C to form only the stable anions dAMP− and dCMP−, respectively; that is, no decay (dissociation) of dAMP*− or dCMP*− occurs. In contrast, DET of e^-_{pre} to G is most efficient, forming a dGMP*− that dissociates rapidly within ∼1.8 ps: ∼60% of dGMP*− dissociates and ∼40% becomes a stable dGMP−. Similar DET of dTMP also occurs, but only ∼35% of dTMP*− dissociates while 65% becomes a stable dTMP−.

Furthermore, we also measured transient absorption kinetic traces of G*−, dG*−. and dGMP*− from the DET reactions with e^-_{pre}. The results in Figure 2c show the following: (1) A clear decay of G*− for the G base is observed, indicating that *the direct dissociation of G*− does occur*. (2) The lifetime of G*− decreases in the order G > dG > dGMP. This lifetime decrease is most likely due to the different environments around the G base in G, dG, and dGMP molecules. Indeed, the absorption intensities of G*−, dG*−, and dGMP*− decrease in the same order as those of their neutral counterparts (G > dG > dGMP), leading to a nearly identical peak intensity after the correction, as shown in Figure 2c. One might consider that intramolecular electron transfer (ET) from G*− to the sugar unit or the phosphate group would modify the electronic absorption signal of dG*− or dGMP*−. However, this consideration seems to be inconsistent with the fact that if effective

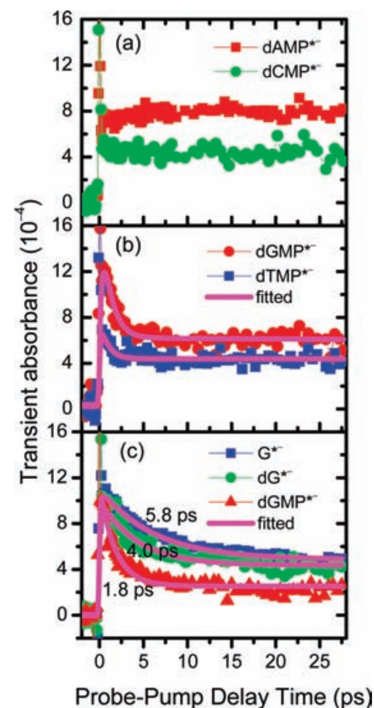

Figure 2. Femtosecond transient absorption kinetic traces of dXMP*−, dG*−, and G*− resulting from the DET reactions of e^-_{pre} with (a) 50 mM dAMP and dCMP in water, (b) 50 mM dGMP and dTMP in water, and (c) ∼45 mM G, dG, and dGMP in 90 mM NaOH, pumped at 318 nm and probed at 330 nm. Here, the sharp peak at time zero is the coherence "spike" of the pump and probe pulse.[12] All of the spectra were corrected by subtraction of the spectrum for the solvent, and the differences in absorption coefficients of dXMP*−, dG*−, and G*− were corrected using those of neutral counterparts, which are 15.3×10^3, 9.3×10^3, 10.2×10^3, 13.7×10^3, 17.1×10^3, and 30.4×10^3 M^{-1} cm^{-1} for dAMP, dCMP, dTMP, dGMP, dG, and G, respectively. Solid lines in (b) and (c) are best fits to the experimental data obtained using a least-squares fitting program.

ET from G*− to the sugar unit or the phosphate group occurs, then similar ET would also occur for other base anions, especially A*− in dAMP*−. Using an electron acceptor as a probe, we have observed that adenine is the most effective electron transporter among the four DNA bases after capture of an e^-_{pre},[16] while no ET from A*− to either the sugar unit or the phosphate group in dAMP*− was observed (Figure 2a). Thus, the intramolecular ET interpretation seems to be unsupported by our observed data, which indicate that direct dissociation at the G and T bases occurs after capture of an e^-_{pre}. Further studies to determine exactly which bond(s) (N−H, C−H, etc.) is (are) broken are being undertaken.

Strikingly, our above results show that only T and especially G are vulnerable to DETs of e^-_{pre} leading to bond breaks, while the electron can be stably trapped at C and especially A to form stable anions. These results provide a molecular mechanism for e^-_{pre}-induced DNA strand breakage. Our results are partially consistent with the theoretical results by Bera and Schaefer,[4d] who predicted the N−H bond dissociation in the G base, and with the experimental results of Ray et al.[4c] showing that the capturing probability of ∼1.0 eV free electrons by dry single-stranded DNA oligomers increases with the increasing number of G bases included. Our results are also partially consistent with the recent observation of the stable anionic states A−, C−, and T− (but not G−) by Bowen and co-workers.[4g,h] In comparison, our present results provide the *first real-time observation* of the DET-induced *dissociations* of G and T and the formation of all four stable anions (A−, G−, C−, and T−) in *aqueous solution*.

It is interesting to compare our results for indirect DNA damage induced by DETs of weakly bound e^-_{pre} in water with those for *direct*

DNA damage by DEAs of low-energy (0−20 eV) *free* electrons. In 2000, Boudaïffa et al.[4a] reported the first experimental result that DEAs of 3−20 eV free electrons cause SSBs and DSBs of dry DNA in vacuum. In 2002, Simons and co-workers[5a−c] reported the first theoretical studies of DEAs of low-energy free electrons to DNA in aqueous solution and proposed that SSBs can effectively occur via formation of a π^* anion shape resonance at the DNA base after attachment of an excess electron with an energy of ∼1 eV if the DNA is stabilized by water solvation. In 2004, Martin et al.[4b] showed a higher yield of DNA SSBs for ∼1 eV electrons but no yield of DSBs induced via DEA shape resonances of 0−4 eV free electrons. In 2005−2006, Illenberger and co-workers[4e,f] reported experimental results for DEAs of near 0 eV electrons to gaseous DNA bases and the phosphate group, while Schaefer and co-workers[5d,e] and Bao et al.[5f] reported theoretical calculations of DEAs of near 0 eV electrons to nucleotides in both the gas phase and aqueous solution. However, none of those experimental studies were done in aqueous solution, and none of the theoretical studies predicted our current observation that dissociation occurs only for $dGMP^{*-}$ and $dTMP^{*-}$ but not for $dAMP^{*-}$ and $dCMP^{*-}$.

The observed fact has indicated that water plays a dominant role in causing DNA SSBs and DSBs under ionizing radiation.[7] However, the water environment is unlikely to enhance DEAs of molecules at electron energies higher than 1.0 eV.[5a−c] In fact, Lu and Sanche[17] have observed that DEAs of many molecules to low-energy free electrons with energies above 1.0 eV, which are effective in the gas phase, are completely quenched when they are adsorbed on H_2O ice because of the polar environment. Thus, it is most likely that water has a protective effect on the DNA potentially damaged by DEAs at electron energies above 1.0 eV. In contrast, cross sections for DEA of molecules to ∼0 eV electrons were observed to be significantly enhanced by the presence of H_2O ice. The latter was due to the DET mechanism: the ∼0 eV electron is first trapped to become a presolvated e^-_{pre} in the polar medium, which is subsequently transferred to a molecule, leading to its dissociation.[17] As we have recently demonstrated for halopyrimidines and CCl_4,[14] DEA resonances observed for near 0 eV electrons in the gas phase shift to −1.0 to −1.5 eV in water because of the polarization effect, in Feshbach resonance with e^-_{pre} in energy, so effective resonant DETs of e^-_{pre} to these molecules were observed. DSBs can be induced by the DET process of G or T. The bond dissociation induced by DET can result in SSBs on one strand of the DNA, and the dissociation products can then react further to break the other strand of the DNA.[3] One e^-_{pre} can in this way produce multiply damaged sites, thus amplifying the complexity of DNA lesions from a single radiation track. Notably, the residence time of low-energy free electrons in water is very short, on time scales of a tenth to a few femtoseconds; they rapidly thermalize to become weakly bound e^-_{pre} with lifetimes of 200−500 fs.[11,12] Moreover, the autodetachment of the resultant Feshbach anion resonance cannot occur in water after transfer of an e^-_{pre} to a molecule.[14c] Hence, DET reactions of e^-_{pre} should play a dominant role in inducing DNA damage. Indeed, we have observed significant SSBs and DSBs induced by e^-_{pre} of aqueous DNA under ionizing radiation.[18]

In summary, we have presented the first *real-time* observation of DET reactions of e^-_{pre} with DNA nucleotides in aqueous solutions. The results not only challenge the conventional notion that damage to the genome by ionizing radiation is mainly induced by the oxidizing $OH^\bullet$ radical but also provide a deeper fundamental understanding of the molecular mechanism of the DNA damage caused by a *reductive* agent (e^-_{pre}). This finding may be applied to develop new strategies for more effective radiotherapies of diseases such as cancer. Furthermore, the direct observation of DNA-base-specific damage by DET of weakly bound electrons has a broad significance, as there are sources of weakly bound electrons in biological systems. The resultant DNA strand breaks, if not repaired quickly, could cause genetic mutation and even serious diseases such as cancer. The oxidative damage at the guanine (G) base and its relation to human cancers have been exploited.[2] The present findings of the most fragile point at the G base and a new molecular mechanism of *reductive* DNA damage could also play a vital role in various diseases such as cancer and stroke. This work may therefore have general significance for a deep understanding of DNA damage and repair processes in biological systems and for developing effective therapies for diseases such as cancer and stroke.

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References

(1) (a) Varmus, H. *Science* **2006**, *312*, 1162. (b) Alberts, B. *Science* **2008**, *320*, 19.
(2) Denissenko, M. F.; Pao, A.; Tang, M. S.; Pfeifer, G. P. *Science* **1996**, *274*, 430. David, S. S.; O'Shea, V. L.; Kundu, S. *Nature* **2007**, *447*, 941.
(3) Michael, B. D.; O'Neill, P. *Science* **2000**, *287*, 1603.
(4) (a) Boudaïffa, B.; Cloutier, P.; Hunting, D.; Huels, M. A.; Sanche, L. *Science* **2000**, *287*, 1658. (b) Martin, F.; Burrow, P. D.; Cai, Z.; Cloutier, P.; Hunting, D.; Sanche, L. *Phys. Rev. Lett.* **2004**, *93*, 068101. (c) Ray, S. G.; Daube, S. S.; Naaman, R. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 15. (d) Bera, P. P.; Schaefer, H. F., III. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 6698. (e) Abdoul-Carime, H.; Langer, J.; Huels, M. A.; Illenberger, E. *Eur. Phys. J.* **2005**, *D35*, 399. (f) König, C.; Kopyra, J.; Bald, I.; Illenberger, E. *Phys. Rev. Lett.* **2006**, *97*, 018105. (g) Haranczyk, M.; Gutowski, M.; Li, X.; Bowen, K. H. *Proc. Natl. Acad. Sci. U.S.A.* **2007**, *104*, 4804. (h) Stokes, S. T.; Li, X.; Grubisic, A.; Ko, Y. J.; Bowen, K. H. *J. Chem. Phys.* **2007**, *127*, 084321.
(5) (a) Barrios, R.; Shurski, P.; Simons, J. *J. Phys. Chem. B* **2002**, *106*, 7991. (b) Berdys, J.; Anusiewicz, I.; Skurski, P.; Simons, J. *J. Am. Chem. Soc.* **2004**, *126*, 6441. (c) Simons, J. *Acc. Chem. Res.* **2006**, *39*, 772. (d) Gu, J.; Xie, Y.; Schaefer, H. F., III. *J. Am. Chem. Soc.* **2006**, *128*, 1250. (e) Gu, J.; Xie, Y.; Schaefer, H. F., III. *Nucleic Acids Res.* **2007**, *35*, 5165. (f) Bao, X.; Wang, J.; Gu, J.; Leszczynski, J. *Proc. Natl. Acad. Sci. U.S.A.* **2006**, *103*, 5658. (g) Kumar, A.; Sevilla, M. D. *J. Phys. Chem. B* **2007**, *111*, 5464. (h) Schyman, P.; Laasksonen, A. *J. Am. Chem. Soc.* **2008**, *130*, 12254.
(6) Lehnert, S. *Biomolecular Action of Ionizing Radiation*; Taylor & Francis Group: New York, 2008; Chapter 6.
(7) Ito, T.; Baker, S. C.; Stickley, C. D.; Peak, J. G.; Peak, M. J. *Int. J. Radiat. Biol.* **1993**, *63*, 289.
(8) Ward, J. F. *Prog. Nucleic Acids Res.* **1988**, *35*, 95.
(9) deLara, C. M.; Jenner, T. J.; Townsend, K. M. S.; Marsden, S. J.; O'Neill, P. *Radiat. Res.* **1995**, *144*, 43.
(10) Chapman, J. D.; Gillespie, C. J. *Adv. Radiat. Biol.* **1981**, *9*, 143. Nikjoo, H.; O'Neill, P.; Goodhead, D. T.; Terrissol, M. *Int. J. Radiat. Biol.* **1997**, *71*, 467.
(11) (a) Migus, A.; Gauduel, Y.; Martin, J. L.; Antonetti, A. *Phys. Rev. Lett.* **1987**, *58*, 1559. (b) Rossky, P. J.; Schnitker, J. *J. Phys. Chem.* **1988**, *92*, 4277. (c) Long, F. H.; Lu, H.; Eisenthal, K. B. *Phys. Rev. Lett.* **1990**, *64*, 1469. (d) Laenen, R.; Roth, T.; Laubereau, A. *Phys. Rev. Lett.* **2000**, *85*, 50. (e) Borgis, D.; Rossky, P. J.; Turi, L. *J. Chem. Phys.* **2007**, *127*, 174508.
(12) Wang, C.-R.; Luo, T.; Lu, Q.-B. *Phys. Chem. Chem. Phys.* **2008**, *10*, 4463.
(13) (a) Aldrich, J. E.; Lam, K. Y.; Shragge, P. C.; Hunt, J. W. *Radiat. Res.* **1975**, *63*, 42. (b) Gauduel, Y.; Berrod, S.; Migus, A.; Yamada, N.; Antonetti, A. *Biochemistry* **1988**, *27*, 2509. (c) Gauduel, Y.; Gelabert, H.; Guilloud, F. *J. Am. Chem. Soc.* **2000**, *122*, 5082.
(14) (a) Wang, C.-R.; Lu, Q.-B. *Angew. Chem., Int. Ed.* **2007**, *46*, 6316. (b) Wang, C.-R.; Hu, A.; Lu, Q.-B. *J. Chem. Phys.* **2006**, *124*, 241102. (c) Wang, C.-R.; Drew, K.; Luo, T.; Lu, M.-J.; Lu, Q.-B. *J. Chem. Phys.* **2008**, *128*, 041102.
(15) (a) Foggi, P.; Bussotti, L.; Neuwahl, F. V. R. *Int. J. Photoenergy* **2001**, *3*, 103. (b) Hernanz, A.; Bratu, I.; Navarro, R. *J. Phys. Chem. B* **2004**, *108*, 2438. (c) Terpstra, P. A.; Otto, C.; Greve, J. *Biopolymers* **1997**, *41*, 751.
(16) Wang, C.-R.; Lu, Q.-B. In preparation.
(17) Lu, Q.-B.; Sanche, L. *Phys. Rev. B* **2001**, *63*, 153403. Lu, Q.-B.; Sanche, L. *J. Chem. Phys.* **2001**, *115*, 5711. Lu, Q.-B.; Sanche, L. *J. Chem. Phys.* **2004**, *120*, 2434.
(18) Nguyen, J.; Wang, C.-R.; Lu, Q.-B. In preparation.

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