Site-Specific Dissociation of DNA Bases by Slow Electrons at Early Stages of Irradiation

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At the very early time of irradiation, ballistic secondary electrons are produced as the most abundant of the radiolytic species directly within DNA or its environment. Here, we demonstrate the propensity of such low-energy (<3 eV) electrons to damage DNA bases via an effective loss of hydrogen located at the specific nitrogen positions. Since this site is directly implicated in the bonding of nucleobases within DNA and since dehydrogenation of the nucleic acid bases has been observed to be the predominant dissociative channel, the present findings foreshadow significant implications for the initial molecular processes leading to genotoxicity in living cells following unwanted or intended exposure to ionizing radiation (e.g., sunbathing, air travel, radiotherapy, etc.).

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The appearance of cancer cells induced by ionizing radiation (UV, x rays, etc.) results from genome mutagenesis, involving alteration and/or deletion in DNA [1,2], which carries the genetic information for cell replication and synthesis of many proteins. However, genomic lesions do not arise merely from the primary impact of the high-energy quanta, but rather from the reactions of the secondary particles, i.e., ions, radicals, and ballistic electrons, created along the ionization track [3,4]. These free electrons exist at the very early stages of irradiation (i.e., femtosecond to picosecond time scales), and are found to be the most abundant *secondary species*, created with an estimated quantity of $\approx 5 \times 10^4$ per MeV primary quantum deposited [5]. The larger majority possesses initial kinetic energies up to about 20 eV [6]. Inelastic collisions within the medium will quickly thermalize these electrons before being solvated, then as chemical rather inactive species. In addition to high-energy radiation, UV (*B* and *C*) radiation, which is known to be involved in skin cancer, can also directly ionize DNA thereby generating low-energy electrons within the nucleic acids [7].

The importance of reactions of *presolvated* electrons with biomolecules had been pointed out more than two decades ago by time resolved pulse radiolysis experiments [8,9]. The ability of *free* ballistic electrons (3–20 eV) to efficiently induce single and double strand breaks in supercoiled DNA had previously been shown by Sanche and co-workers [10]. This irreversible alteration of DNA is initiated by the formation of transient negative ion (TNI) states localized on the various building blocks [11–15]. These TNIs undergo dissociation, producing, for instance, dehydrogenated nucleobase anions [13–15]. Figure 1 indicates that dehydrogenation of the nucleobases can arise *a priori* from either N-H or C-H bond cleavage. Because of the implication of the N position of nucleobases within DNA (Fig. 1), and more crucial the fact that nucleobase dehydrogenation has been observed to be *predominant dissociative channel* [13,15,16], there is a need to unravel the action of low-energy (<3 eV) electrons on nucleic acid bases.

We have therefore performed electron/nucleobase collision experiments in a crossed-beam arrangement [17] consisting of an electron source, an oven, and a quadrupole mass analyzer (QMA). The components are housed in a UHV chamber at a base pressure of 10^{-8} mbar. A

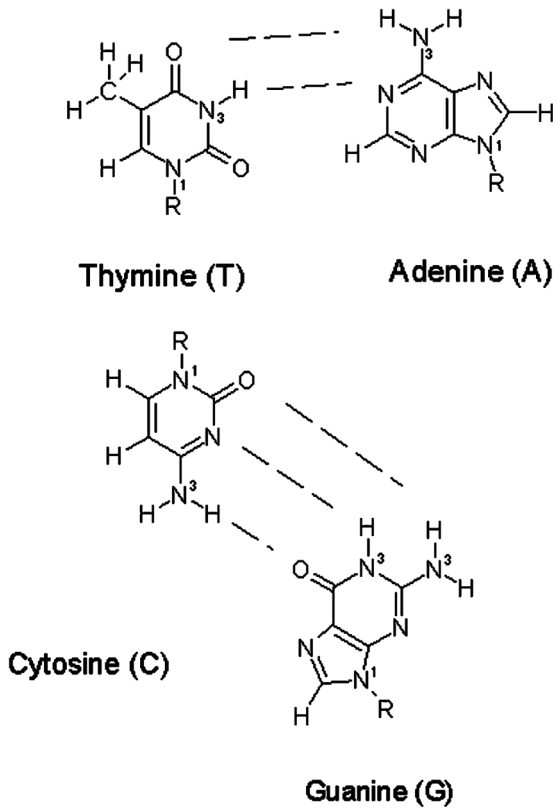

FIG. 1. Schematic representation of the adenine-thymine and guanine-cytosine base pairing within DNA. *R* represents the sugar unit of the backbone in DNA (nitrogen-carbon bond). In the isolated molecule, *R* corresponds to the H.

well-defined electron beam generated from a trochoidal electron monochromator (resolution ≈100 meV FWHM) [18] orthogonally intersects with an effusive molecular beam of T, A, C, or G. The nucleic acid bases emanate from a resistively heated oven [containing ≈50 mg of high purity powder (Aldrich Ltd.)] directly connected to the reaction chamber by a capillary. The temperature of the oven is measured by a platinum resistance to be approximately 450 K, which is considerably below the decomposition temperature of the nucleic acid bases (≈520 K). At these temperatures, the original structure of the investigated nucleic acid bases is not likely to be altered. Negative ions are extracted from the interaction area by a small draw-out field (<1 V cm^{-1}), analyzed by the QMA and detected by single pulse counting techniques. The electron energy scale is calibrated by an admixture of SF_6 gas flowing through the oven yielding the well-known SF_6^- resonance near 0 eV.

Figure 2 shows the incident electron energy dependence of the produced closed-shell dehydrogenated fragment anions $(M\text{-}H)^-$ from the four different gas phase DNA bases. The yield functions exhibit resonant structures reminiscent of resonant dissociative electron attachment (DEA), i.e., formation of a transient negative nucleobase

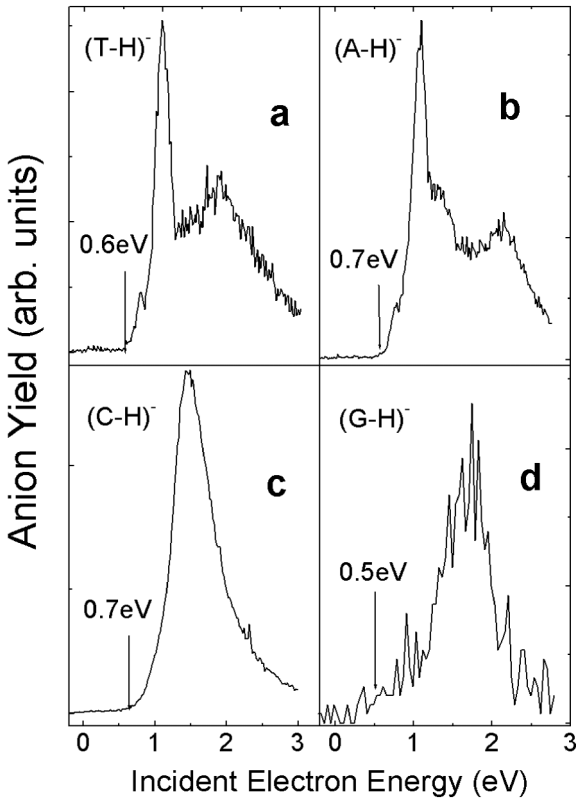

FIG. 2. Ion yield for $(M\text{-}H)^-$ formation (dehydrogenation) in (a) thymine (T), (b) adenine (A), (c) cytosine (C), and (d) guanine (G). With the exception of guanine, dehydrogenation is the dominant dissociative electron attachment channel (see the text).

ion, M^-, by accommodation of an extra electron into an unoccupied molecular orbital. M^- decomposes by ejecting a neutral hydrogen radical with the excess charge trapped by the positive electron affinity (EA) of the large fragment, viz.

$$e^- + M \rightarrow M^- \rightarrow (M\text{-}H)^- + H. \quad (1)$$

Electron capture leads to a variety of further negative fragments [13,15,16]; however, by far the dominant DEA channel is $(M\text{-}H)^-$ (with the exception of G, see below). From Fig. 2, it can be seen that the experimental appearance energy for dehydrogenation ranges between 0.5 and 0.7 eV. Electron attachment to the gas phase nucleobases has been recently studied by means of electron transmission spectroscopy (ETS) resulting in resonant features with some similarities to those observed in the present study. These resonances were ascribed to accommodation of the extra electron into empty antibonding π^* orbitals [19]. Bearing in mind that ET mirrors the energy of the transient negative ion state accessed by electron capture (the initial transition), DEA is a convolution of the capture process with the (energy dependent) decay probability into the particular channel (including autodetachment) [20]. The absolute cross section for $(T\text{-}H)^-$ formation can be estimated by taking the known cross section for thermal electron attachment to SF_6 as calibration standard (2.4×10^{-14} cm^2 [21]) and comparing with the present $(T\text{-}H)^-$ count rate. Assuming similar detection efficiencies for SF_6^- and $(NB\text{-}H)^-$ and by estimating the respective partial pressures [22], the cross section is evaluated to be $\approx 10^{-15}$ cm^2 (accuracy within 1 order of magnitude) at the peak of the dominant sharp resonance at 1.0 eV in agreement with the recently reported the value [15]. The cross sections for the other nucleobases are found to be within the same magnitude, indicating that the DEA cross section for hydrogen abstraction is on the order of the geometrical cross section of the respective molecule. Guanine is an exception insofar as (at a total DEA cross section of 10^{-15} cm^2) the dominant decay channel is formation of the pseudohalogen ion NCO^- while the intensity of the $(G\text{-}H)^-$ fragment is 1 order of magnitude lower.

Dehydrogenation of nucleobases can arise from either C-H or N-H bond cleavage. To clarify the situation, we have carried out experiments on partly deuterated thymine at the nitrogen positions, T_D. Figure 3 presents the yield function of $(T_D\text{-}H)^-$ (129 amu) a negative fragment. Both the energy dependence and the absolute intensity are virtually identical to those obtained from thymine $(T\text{-}H)^-$ (125 amu). More important, by switching the mass spectrometer to mass 128 amu [corresponding to $(T_D\text{-}D)^-$], the ion signal completely disappears. The present observation is a direct evidence that DEA generates the N-dehydrogenated anion $(T_D\text{-}H)_N^-$. The structures in the $(T_D\text{-}H)_N^-$ ion yield curve suggest that

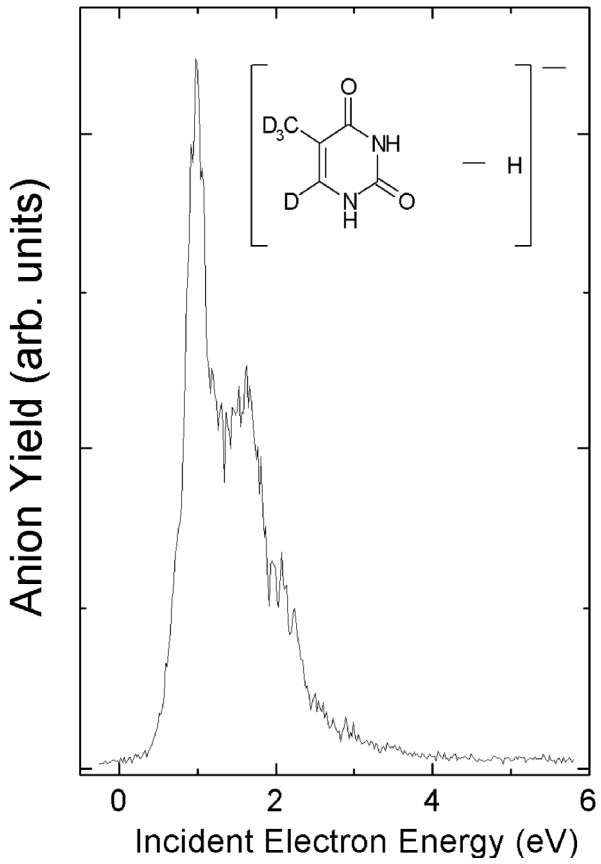

FIG. 3. $(M\text{-}H)^-$ formation from deuterated thymine (129 amu). The inset indicates the structure of the partly deuterated thymine. Formation of $(M\text{-}D)^-$ (128 amu) is not observable at energies up to 6 eV.

different electronic states of the precursor ion are involved. Any of these states, however, decay by hydrogen cleavage from the N sites, but not from the C positions.

The carcinogenic toxicity of a species can be related to its electron affinity [23], which is a measurement of the ability to trap an electron. From the appearance energies on the dehydrogenated anion yield function (Fig. 2), we can deduce the electron affinity of the corresponding radical, as we shall exemplify for the system thymine. The appearance potential (AP) of the dehydrogenated anion, $AP[(T\text{-}H)^-]$, can be expressed as [24]

$$A\,P(T\text{-}H) \approx D(T\text{-}H)_N - EA(T\text{-}H)_N, \qquad (2)$$

with $D(T\text{-}H)_N$ the N1-H or N3-H bond dissociation energy in T and $EA(T\text{-}H)_N$ the electron affinity of the dehydrogenated radical. While there are no explicit values available from experiment nor from theoretical prediction, high level *ab initio* calculations performed on uracil (U) [13] (which is structurally very close to thymine [25]) computes $D(U\text{-}H)_{N1} = 4.42$ eV and $D(U\text{-}H)_{N3} = 5.35$ eV, and the electron affinity of the corresponding radicals to be $EA(U\text{-}H)_{N1} = 3.6$ eV and $EA(U\text{-}H)_{N3} = 4.0$ eV. These calculations predict that dehydrogenation of uracil from the N1 site is the energetically most favorable pathway. Assuming $D(U\text{-}H)_{N1} \approx D(T\text{-}H)_{N1}$, we estimate $EA(T\text{-}H)_{N1}$ to be 3.8 ± 0.3 eV. This high value can be compared to the electron affinity of uracilyl radical (i.e., ≈ 3.2 eV) [13,26], produced from electron-induced reaction to the bromouracil radiosensitizer [27]. Although we did not carry out explicit experiments on deuterated A, C, and G, we anticipate from the similarities of the $(M\text{-}H)^-$ formation that, also in these nucleobases, dehydrogenation occurs from the N positions, and the electron affinities of the corresponding N-dehydrogenated radicals lie in the range 3.5–4 eV.

In conclusion, the present results demonstrate the propensity of free electrons at subexcitation energies (i.e., below the level for electronic excitation) to efficiently induce damage to nucleobases. This alteration arises from dehydrogenation of the nucleobases at the specific nitrogen positions. Since within DNA the nucleobase is bound to its respective pair via the N3-H site, the highly mobile hydrogen radical created can then induce further damage to nucleic acid.

More important is the fact that within DNA the nucleobases are bound to the sugar backbone via an N-C bond, as opposed to the N-H bond in the isolated molecule. Since an N-C bond tends to be appreciably weaker, cleavage of nucleobases from the backbone is expected to be even more favorable than dehydrogenation in isolated nucleobases. Such a nucleobase excision would result in a substantial loss of information for the genome.

Generally, reactions identified in the isolated bases will be modified in the condensed phase to some degree. It has been shown that coupling of a molecule to a dissipative environment can result in a decrease but also in an *increase* of its reactivity following electron attachment [28,29]. Cross-section enhancement for DEA arises from the medium-enhanced lifetime of the TNI with respect to electron loss. A further point concerns the molecular orbitals involved. If π^*-type resonances dominate, coupling to the N-H bond is not direct and may decrease the reactivity in the condensed phase. On the other hand, *ab initio* calculations on halouracils predict the existence of both π^* and σ^* resonances at low energy [30,31] which may also exist in a more realistic DNA subunit, e.g., a nucleobase bound to the sugar backbone. Evidence for such antibonding N-C character has in fact very recently been found in thymidine (thymine bound to a sugar unit). In that system, the cleavage of the N1-C bond was already observed at electron energies close to 0 eV [32].

The understanding of the details of such processes at the molecular level has significant potential to predict and possibly modify the long-term genotoxic effects of ionizing radiation with respect not only to the unwanted exposure (e.g., UV radiation, nuclear accidents, flight traveling, etc.) but also for the intended exposure in the course of radiotherapies.

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[1] M. Grundestrup and H. H. Storm, Lancet **354**, 2029 (1999).
[2] T. E. Wheldon, Phys. Med. Biol. **45**, R77 (2000).
[3] C. von Sonntag, *The Chemical Basis for Radiation Biology* (Taylor & Francis, London, 1987).
[4] *Radiation Damage in DNA: Structure/Function Relationships at Early Times*, edited by A. F. Fuciarelli and J. D. Zimbrick (Batelle, Columbus, OH, 1995).
[5] International Commission on Radiation Units and Measurements, ICRU Report 31 (ICRU, Washington, DC, 1979).
[6] S. M. Pimblott, J. A. La Verne, A. Mozumder, and N. J. B. Green, J. Phys. Chem. **94**, 488 (1990).
[7] Although the ionization energies of isolated DNA bases are found to be in the range 8–9 eV [S. D. Wetmore, R. J. Boyd, and L. A. Ericsson, Chem. Phys. Lett. **322**, 129 (2000)], those of dinocleotides (pGpA) were reported to be as low as 2.0 eV [N. S. Kim, Q. Zhu, and P. R. LeBreton, J. Am. Chem. Soc. **121**, 11 516 (2000)]; i.e., ionization is easily accessible by UV (*B* and *C*) radiations.
[8] A. D. Lenherr and M G. Omerod, Nature (London) **225**, 546 (1970).
[9] W. A. Bernhard, in *The Effect of Radiation in DNA*, edited by E. M. Fielden and P. O'Neil, NATO ARW Series (Springer, New York, 1991).
[10] B. Boudaïffa, P. Cloutier, D. Hunting, M. A. Huels, and L. Sanche, Science **287**, 1658 (2000).
[11] H. Abdoul-Carime, P. Cloutier, and L. Sanche, Radiat. Res. **155**, 625 (2001).
[12] D. Antic, L. Parenteau, M. Lepage, and L. Sanche, J. Phys. Chem. **103**, 6611 (1999).
[13] G. Hanel, B. Gstir, P. Scheier, M. Probst, B. Farizon, M. Farizon, E. Illenberger, and T. D. Märk, Phys. Rev. Lett. **90**, 188104 (2003).
[14] R. Abouaf, J. Pommier, and H. Dunet, Int. J. Mass Spectrom. Ion Processes (to be published).
[15] S. Denifl, S. Ptasinska, M. Cingel, S. Matejcik, P. Scheier, and T. D. Märk, Chem. Phys. Lett. **377**, 74 (2003).
[16] H. Abdoul-Carime, M. A. Huels, E. Illenberger, and L. Sanche (unpublished).
[17] S. Gohlke, A. Rosa, F. Brüning, M. A. Huels, and E. Illenberger, J. Chem. Phys. **116**, 10 164 (2002). In addition, during evaporation the pressure reading is in the range of 10^{-6} mbar at one of the flanges corresponding to about of 10^{-4} mbar directly in the collision region. The entire experimental chamber is maintained at elevated temperatures by two *in vacuo* halogen lamps during the experiments to prevent powder condensation on the surfaces (plates, chamber walls).
[18] A. Stamatovic and G. J. Schulz, Rev. Sci. Instrum. **39**, 1752 (1968).
[19] K. Aflatooni, P. D. Burrow, and G. A. Gallup, J. Phys. Chem. **102**, 6205 (1998).
[20] E. Illenberger and J. Momigny, *Gaseous Molecular Ions. An Introduction to Elementary Processes Induced by Ionization*, Topics in Physical Chemistry Vol. II (Steinkopff/Springer, Weinheim, New York, 1992).
[21] L. G. Christophorou and J. K. Olthoff, Int. J. Mass Spectrom. Ion Processes **205**, 27 (2001).
[22] H. Abdoul-Carime, M. A. Huels, F. Brüning, E. Illenberger, and L. Sanche, J. Chem. Phys. **113**, 2517 (2000).
[23] J. E. Lovelock, Nature (London) **196**, 540 (1962).
[24] The quantities on both sides of Eq. (2) are not exactly equal due to (a) thermal excitation of the molecule and (b) the appearance of reaction excess energy. However, both effects point into opposite directions with respect to AP and roughly cancel each other. The stated uncertainty on the present estimate of EA(T-H) is based on the uncertainties in determining the experimental appearance energy (± 0.1 eV), its possible shift with respect to the energy threshold (± 0.1 eV), and the accuracy in calculating the bond dissociation energies (± 0.1 eV).
[25] Replacing the CH_3 group in thymine by H results in uracil.
[26] H. Abdoul-Carime, M. A. Huels, E. Illenberger, and L. Sanche, J. Am. Chem. Soc. **123**, 5354 (2001).
[27] W. Szybalski and B. Djordjevic, Genetics **44**, 540 (1959).
[28] L. Sanche, A. D. Bass, P. Ayotte, and I. I. Fabrikant, Phys. Rev. Lett., **75**, 3568 (1995).
[29] Y. LeCoat, R. Azria, M. Tronc, O. Ingolfsson, and E. Illenberger, Chem. Phys. Lett. **296**, 208 (1998).
[30] X. Li, L. Sanche, and M. D. Sevilla, J. Phys. Chem. A **106**, 1124 (2002).
[31] T. Sommerfeld, Chem. Phys. Chem. **2**, 677 (2001).
[32] H. Abdoul-Carime, S. Gohlke, E. Fischbach, J. Scheike, and E. Illenberger, Chem. Phys. Lett. **387**, 267 (2004).