Gas-Phase Reactions

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Bond- and Site-Selective Loss of H Atoms from Nucleobases by Very-Low-Energy Electrons (< 3 eV)**

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Excess charge deposited on gas-phase thymine (T) and uracil (U) by resonant attachment of low-energy (0–3 eV) electrons induces the loss of hydrogen, which exclusively takes place from the N positions. This bond selectivity can be made site selective by properly adjusting the electron energy. While electrons at 1 eV result in loss of hydrogen from N1, the reaction can be switched to loss of hydrogen from N3 by tuning the electron energy to 1.8 eV. We find that any energy (and charge) transfer is completely blocked when the N–H bond is replaced by N–CH_3. The present results have significant consequences for the exploration of the initial molecular processes leading to DNA damage, specifically in relation to recent observations of strand breaks in plasmid DNA induced by very low energy (0–4 eV) electrons.[1]

Recent gas-phase experiments on the isolated nucleobases (NBs) thymine (T), cytosine (C), adenine (A), guanine (G), and uracil (U) have demonstrated that they all effectively capture low-energy electrons in the range below 3 eV .[2–6] The generated transient negative ion (TNI) subsequently decomposes by the loss of a neutral hydrogen atom. The overall dissociative electron attachment (DEA) reaction can be expressed as Equation (1), in which $NB^{-\#}$ is the TNI of the

$$e^- \ (< 3\,eV) + NB \rightarrow NB^{-\#} \rightarrow (NB{-}H)^- + H \qquad (1)$$

corresponding nucleobase and $(NB-H)^-$ is the closed-shell anion formed by the ejection of a neutral hydrogen radical whereby the excess charge remains on the nucleobase. The reaction is effective already at energies below the threshold for electronic excitation (at subexcitation energies) and driven by the appreciable electron affinity of the (NB–H) radicals, which is in the range between 3 and 4 eV, dependent on the site from which the hydrogen atom is ejected[2,6] (see below). Experiments with partly deuterated thymine[5] demonstrated that hydrogen abstraction occurs exclusively from the N sites, although H loss from the C sites is energetically accessible within that energy range. In the present contribution we demonstrate by means of methylated thymine and uracil that by properly adjusting the electron energy, the loss of hydrogen can be made even site selective with respect to the N1 and N3 positions. In light of strong efforts to induce cleavage of particular bonds by coherent laser control using tailored ultrafast pulses[7] the present result is very remarkable.

In addition to these basic aspects, our findings have direct implications for the molecular description of radiation damage in biological systems, more specifically, for DNA in living cells. It is well accepted that the main biological effect is usually not produced by the primary interaction of the high-energy quanta with the complex molecular network within a living cell, but rather by the action of the secondary species generated along the ionization track.[8] The interaction of these secondary species (ions, electrons, radicals) with DNA and its surrounding can cause mutagenic, genotoxic, and other potentially lethal DNA lesions such as single-strand breaks (SSBs) and double-strand breaks (DSBs).

Electrons are by far the most abundant of these secondary species and have an initial energy distribution extending to about 20 eV.[9,10] For the understanding of the effects of radiation in cells, it is therefore essential to investigate the action induced by these electrons on the vital cellular components such as water and DNA. In experiments directly exposing plasmid DNA on a surface to an electron beam of variable and well-defined energy, Boudaiffa et al.[11] showed that electrons below the ionization threshold can produce SSBs and DSBs. The same research group showed very recently that in plasmid DNA SSBs are produced at energies as low as the nominal zero-energy threshold of the electron beam and that the yield as a function of the energy exhibits a sharp peak at 0.8 ± 0.3 eV and a broader feature centered at 2.2 eV.[1]

On the other hand, the explicit molecular mechanism how low energy electrons damage DNA is yet unknown. Here we study in detail the processes that are initiated in isolated thymine (T) and uracil (U) by additional experiments on the compounds methylated at one of the N positions. These results have direct implications for the initial steps towards strand breaks in DNA.

The present investigations were performed in a crossed electron/molecule beams device previously described in detail.[12] The electron beam is formed in a hemispherical electron monochromator, operated at an energy resolution between 60 and 110 meV and an electron current of 5–8 nA. The molecular beam emanates from a source consisting of a temperature-regulated oven and a capillary. Evaporation at 385–400 K results in an effusive beam of intact molecules. Negative ions formed in the collision zone are extracted by a weak electric field toward the entrance of the quadrupole mass spectrometer and are detected by a single-pulse counting technique. The intensity of a particular mass-selected negative ion is then recorded as a function of the electron

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energy. The electron energy scale is calibrated using the well-known Cl^-/CCl_4 signal near 0 eV. Methylated thymine and uracil were purchased from Sigma–Aldrich at a stated purity of 98%.

Figure 1 shows the loss of a neutral hydrogen atom from thymine methylated at the N1 position (m1T) compared to

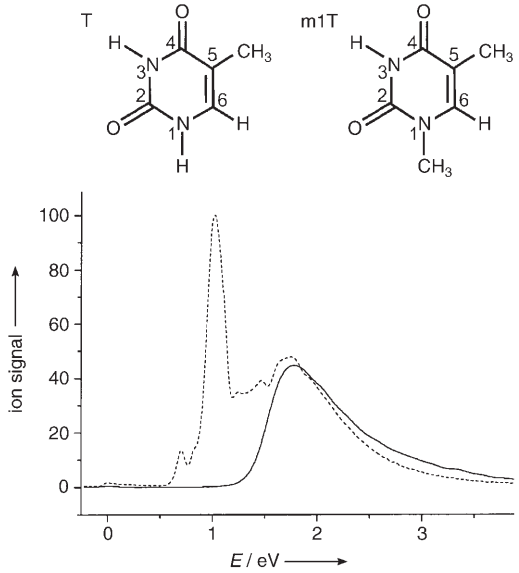

Figure 1. Plot of the ion yield of the fragment ion $(M-H)^-$ (loss of a hydrogen atom) from thymine methylated at the N1 position (m1T, ——) with that of thymine (T, -----). E = electron energy.

the yield from (non-methylated) thymine (T), in both cases by measuring the yields of the corresponding anions $(M-H)^-$ [Eq. (1)]. For a detailed interpretation of these results we have to recall a previous study on thymine deuterated at the C positions[5] which demonstrated that hydrogen loss at sub-excitation energies occurs exclusively at the N positions. Figure 1 directly shows that in m1T hydrogen loss is completely suppressed below 1.4 eV, and we consequently assign the smooth feature peaking at 1.8 eV to H loss from the N3 position. H loss is hence not only exclusively restricted to the N–H bonds, it can further be made site selective by tuning the electron energy.

Accordingly, uracil methylated at the N3 position (m3U) shows a nearly complementary behavior (Figure 2); in other words, hydrogen loss primarily occurs within the sharp feature at 1.0 eV, which we consequently assign to H loss from the N1 site. It should be noted that the relative shape of the ion yields due to H loss in T and U are virtually identical (as is also apparent from the spectra for T and U in Figure 1 and Figure 2, respectively). While in m3U some intensity on the $(M-H)^-$ signal (N1–H loss) remains above 1.4 eV, that in m1T (N3–H loss) below 1.4 eV is completely suppressed. This complete site selectivity for N1–H loss is a result of the corresponding energy threshold, which we shall briefly consider.

The threshold E_0 for the DEA reaction [Eq. (1)] is given by Equation (2), in which D is the corresponding bond

$$E_0 = D - EA(M-H) \qquad (2)$$

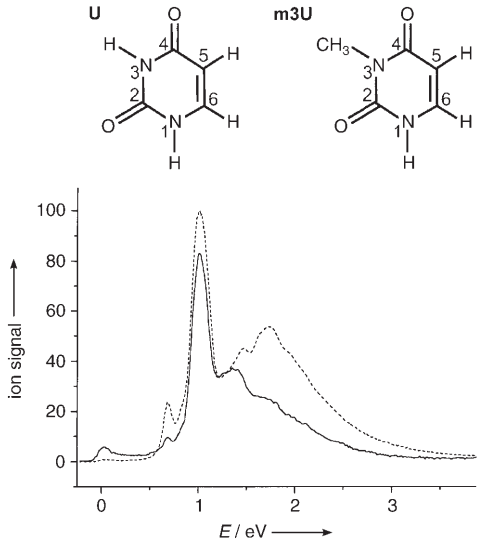

Figure 2. Plot of the ion yield of the fragment ion $(M-H)^-$ (loss of a hydrogen atom) from uracil methylated at the N3 position (m3U, ——) with that of uracil (U, -----). E = electron energy.

dissociation energy ((N-H)/(C-H)) and $EA(M-H)$ is the electron affinity of the radical formed by the loss of H from the particular site. E_0 refers to the energy threshold of the reaction at 0 K. The experimental threshold energy (the appearance energy of $(M-H)^-$) can be different from E_0 as a result of two main effects: 1) the transient negative ion is formed by means of a Franck–Condon transition, which can be viewed as the activation energy for the DEA reaction with the tendency that the fragment ion is observed above E_0; and 2) the internal energy of the target molecule tends to lower the appearance energy.

Since there are no experimental numbers available for the different C–H and N–H bond dissociation energies in the nucleobases, we refer to recent high-level ab initio studies on T and U. With the G2MP2 method the following data were calculated for thymine:[6] D(N1-H) = 4.4 eV/EA(T-H) = 3.5 eV; D(N3-H) = 5.8 eV/EA(T-H) = 4.5 eV; D(CH_2-H) = 4.5 eV/EA(T-H) = 2.9 eV; D(C6-H) = 4.9 eV/EA(T-H) = 2.7 eV. From that the following energetic thresholds are obtained: E_0(N1-H) = 0.9 eV, E_0(N3-H) = 1.3 eV, E_0(CH_2-H) = 1.6 eV, and E_0(C6-H) = 2.2 eV.

In U the corresponding numbers are (also calculated with the G2MP2 method[2]): D(N1-H) = 4.4 eV/EA(U-H) = 3.6 eV; D(N3-H) = 5.4 eV/EA(U-H) = 4.0 eV; D(C5-H) = 5.2 eV/EA(U-H) = 2.5 eV; D(C6-H) = 5.0 eV/EA(U-H) = 2.8 eV leading to the following energy thresholds: E_0(N1-H) = 0.8 eV, E_0(N3-H) = 1.4 eV, E_0(C5-H) = 2.7 eV, and E_0-(C6-H) = 2.2 eV.

On this basis, we arrive at the following conclusions:

a) Although H loss from the C sites is energetically accessible above 1.6 eV it is not observed within the entire resonant feature extending to more than 3 eV.
b) At energies below 1.4 eV the loss of H occurs exclusively from the N1 position, since the competing channel (loss from N3) is energetically only accessible above 1.4 eV.
c) At energies above 1.4 eV hydrogen loss takes place from both the N1 and N3 positions.

The fact that H loss from N1 occurs preferentially at low energy (1 eV) with respect to the range above 1.4 eV may be due to the different mechanisms contributing to reaction (1). Owing to the large dipole moment of uracil ($\approx$4.5 D), it was recently proposed that a dipole-bound (DB) state could act as a doorway state for DEA.[13] Supported by ab initio calculations, the sharp peak at 1 eV was assigned as a dipole-bound vibrational Feshbach resonance (exciting three quanta of the N1-H stretch mode), which couples to the repulsive σ^* (N1-H) valence state. The underlying broader feature would then be due to the π_2^* shape resonance,[14] which can lead to DEA through vibronic mixing of the π states with the repulsive σ^* anion states. The overall DEA reaction is then more effective via the initial DB state in comparison to the initial π^* shape resonance leading to the dominant peak at 1 eV.

The fact that the loss of CH_3 is not observed when N1–H is replaced by N1–CH_3 has significant consequences for the molecular description of strand breaks by low-energy electrons. In a recent theoretical study[15] (modeling a section of DNA containing cytosine, the sugar ring, and the (neutralized) phosphate group), a low-lying anionic curve was predicted which connects the initial π^* anion state of the base to a σ^* state in the backbone. An electron captured by a DNA base may thereby be transferred to the backbone, which may lead to rupture of the C–O bond between the phosphate and the sugar. In DNA the N1 position is coupled to the sugar moiety, and according to the present findings transfer of charge and energy via the N1–C bond will not take place. This is also strongly supported by very recent gas-phase experiments[16] on thymidine (representing thymine coupled to a sugar moiety) which definitely exclude the transfer of electrons initially localized on thymine to the sugar moiety.

We therefore conclude that the direct migration of the excess charge from the π^* anions of the nucleobases to the DNA backbone can be excluded as a mechanism leading to strand breaks. Instead electrons near 2 eV induce H loss at N3, which, in a biological environment, may initiate further reactions eventually leading to strand breaks. In addition, experiments on isolated deoxyribose show effective low-energy DEA processes.[17] If these DEA reactions are preserved for deoxyribose coupled within the DNA network, such processes will be of considerable importance for strand breaks.

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