Bond- and Site-Selective Loss of H^- from Pyrimidine Bases

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Electron attachment to gas phase thymine and uracil leads to H^- loss within a broad and structured feature in the energy range between about 5 and 12 eV consisting of 4 overlapping resonances. By using thymine and uracil methylated at the N1 and N3 positions, respectively, and taking into account recent results from partly deuterated thymine, we find that by tuning the electron energy, H^- loss turns out to be not only bond selective, i.e., (C-H) versus (N-H) bonds, but also site selective (N1 versus N3 site). Such a bond and site selectivity by energy has not been observed before in dissociative electron attachment. Implications for the mechanism of strand breaks observed in plasmid DNA are considered.

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The study of reactions induced by low-energy electrons in DNA nucleobases constitutes an essential step towards an understanding of radiation damage on a molecular basis [1,2]. The interaction of high-energy quanta with living cells creates exceeding amounts of low-energy electrons along the ionization track [3]. The interaction of these secondary electrons with the vital components of a cell like DNA, water, and its surroundings hence represents a key issue relevant for radiation damage, but also for the operation of radio sensitizers used in cancer therapy. It has been demonstrated that in plasmid DNA electrons below the ionization threshold induce both single strand breaks (SSBs) and double strand breaks (DSBs) [4]. Very recently it was shown that even subexcitation electrons (below the threshold for electronic excitation, 0–4 eV) induce SSBs [5]. Since the quantum yields for DSBs and SSBs show a resonant behavior with electron energy, it was suggested that electron capture is the initial step. To date, however, the molecular mechanism is not clear how excess electrons in DNA induce strand breaks. In order to decipher possible pathways, different research groups established programs to study electron induced reactions in building blocks of DNA including the nucleobases [5–9], the deoxyribose molecule [10], and thymidine [11] (representing a thymine bound to the sugar). These gas phase studies showed that all nucleobases (NBs) undergo dissociative electron attachment (DEA) at subexcitation energies (<3 eV) leading to dehydrogenation according to

$$e^- + \mathrm{NB} \rightarrow \mathrm{NB}^{*-} \rightarrow (\mathrm{NB\text{-}H})^- + \mathrm{H} \qquad (1)$$

with NB^{*-} the transitory negative ion of the corresponding nucleobase and $(NB\text{-}H)^-$ the closed shell anion formed by the ejection of a neutral hydrogen radical. The reaction is energetically driven by the appreciable electron affinity of the (NB-H) radicals, which is in the range between 3 and 4 eV [6]. Experiments with partly deuterated thymine [9] demonstrated that reaction (1) is exclusively operative at the N sites. By using thymine, methylated at the N1 position, and uracil, methylated at the N3 position, it was found very recently in our laboratory [12] that neutral hydrogen loss at subexcitation energies can be made site selective. By properly adjusting the electron energy, one can switch between H ejection from the N1 site to loss from the N3 position. In addition to this dominant low-energy feature [exclusively leading to dehydrogenation (1)] recent gas phase experiments on NBs identified a series of different negatively charged fragment ions appearing at resonant features in the energy range above 5 eV [6,7]. One of the prominent ions generated in this energy domain is the fragment anion H^- with an ion yield consisting of a series of four overlapping resonances between 5 and 13 eV (Fig. 1). The associated DEA reaction can be considered as the complement to (1) with respect to the excess charge, viz.,

$$e^- + \mathrm{NB} \rightarrow \mathrm{NB}^{*-} \rightarrow (\mathrm{NB\text{-}H}) + \mathrm{H}^-. \qquad (2)$$

By using partly deuterated thymine (T_D) it was very recently shown in our laboratory that one can switch between H^- loss from the C bonds to H^- loss from the N bonds by tuning the electron energy accordingly [13]. Here we show by using thymine and uracil methylated at the N1 and N3 positions, respectively, that each of the four resonances leads to H^- loss from one particular position (N1, N3, CH_2-H, C6). Reaction (2) is hence bond and site selective by properly tuning the electron energy.

The present investigation is performed in a crossed electron–molecule beams device previously described in detail [14]. The electron beam is formed in a hemispherical electron monochromator, in the present experiments operated at an energy resolution of between 110 and 130 meV (FWHM) and an electron current of 5–8 nA. The molecular beam emanates from a source consisting of a temperature regulated oven and a capillary. The sample is heated up to 400 K in the case of the methylated nucleobases and to 450 K for uracil and thymine, thus resulting in sufficient pressure to form an effusive beam of intact molecules, which is then crossed perpendicularly by the electron beam. Negative ions formed in the collision zone are

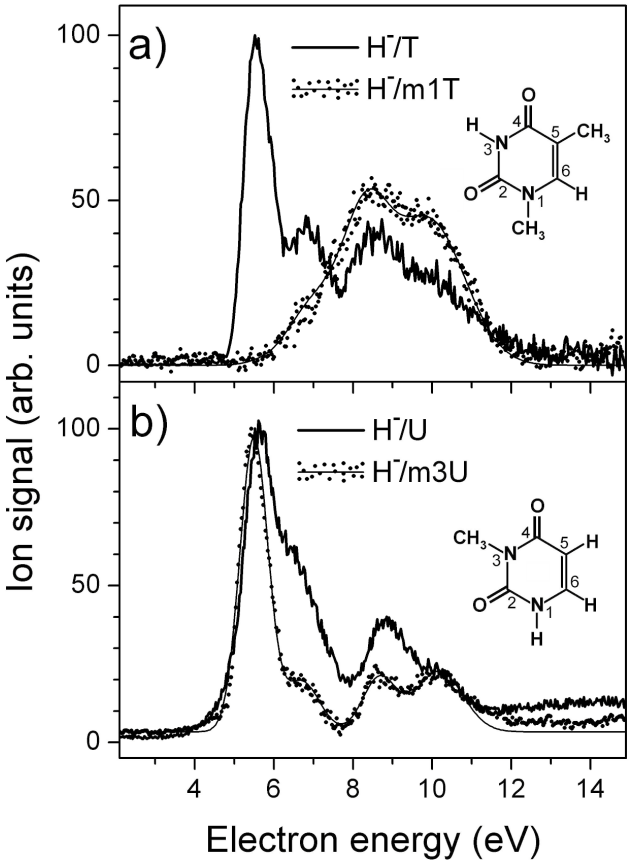

FIG. 1. (a) H^- formation from thymine (T) and thymine methylated on the N1 position (m1T). (b) H^- formation from uracil (U) and uracil methylated on the N3 position (m3U). In case of the methylated molecules the points correspond to the experimental data and the thin line is a multiple Gaussian fit of the data. The intensities $H^-/m1T$ and $H^-/m3U$ are arbitrarily adjusted to roughly match those of H^-/T and H^-/U, respectively.

extracted by a weak electric field towards the entrance of the quadrupole mass spectrometer and detected by a channeltron using a single pulse counting technique. The intensity of a particular mass-selected negative ion is then recorded as a function of the electron energy. The electron energy scale is calibrated using the well known Cl^-/CCl_4 specifics of the cross section. All samples were purchased from Sigma-Aldrich at a stated purity of >97%. H^- is a product anion of many molecules such as H_2O and various hydrocarbons that are present even in the UHV apparatus and, in case of water, even as moisture in the sample. Thus the H^- signal originating from the biomolecules of interest has to be deduced via subtraction of two cross sections, i.e., one measured at a sufficiently high temperature that contains H^- from the biomolecule of interest and all the other possible sources, and one measured at a temperature of about 30 K less that contains contributions mainly from the residual gas and moisture in the sample. This procedure is not necessary in the case of D^- from partially deuterated thymine, which explains the clear data situation discussed below for this reaction.

Figure 1(a) shows a comparison of the H^- ion yield between methylated thymine (m1T) and nonlabeled thymine (T). Obviously, methylation at the N1 position changes drastically the cross section shape, i.e., formation of H^- is strongly suppressed in the low-energy part of the H^- distribution arising from electron attachment to nonlabeled thymine. We therefore interpret the H^- signal arising from m1T as H^- loss mainly originating from the N3 and the carbon positions. At the same time the presence of a second methyl group increases the chance for H^- loss from CH_3 (see also below) and is responsible for the relative increase of the resonance at about 10 eV when going from the H^-/T to the $H^-/m1T$ cross section curve.

In Fig. 1(b) we compare the H^- ion yield measured upon DEA to uracil (U) and uracil methylated at the N3 position (m3U). The strong suppression of the second resonance at an electron energy of 6.8 eV in the case of m3U indicates that H^- loss from the N3 position is almost exclusively contributing to this resonance. The remaining signal at the 6.8 eV resonance in the case of $H^-/m3U$ can be explained by the presence of contaminating thymine or other isomers of m3U in the effusive beam or as a problem in the correction of the background H^- originating from water and hydrocarbon molecules (see above). It is interesting to note that in both cases, thymine (see above) and also uracil, the methylated samples show a relative increase at the 10 eV resonance indicating that this resonance is connected to loss from the CH_3 position.

To elucidate in more detail this surprising result, we have briefly to recall our very recent findings on H^- loss from thymine deuterated at the C positions (T_D) [13]. Figure 2 compares H^- formation from thymine (H^-/T) with the corresponding reactions in the deuterated compound, namely, (D^-/T_D) and (H^-/T_D). The H^-/T yield is characterized by three resonances peaking at 5.5, 6.8, and 8.5 eV with a distinct shoulder at 10 eV. From Fig. 2 it is immediately obvious that H^- or D^- loss from the C positions is completely restricted to the features above 7 eV, while H^- loss from the N sites essentially occurs from the two prominent resonances at 5.5 and 6.8 eV. The small contribution on the D^- yield below 7 eV [as seen on the expanded scale in Fig. 2(b)] is due to the limited isotope purity (98%) which was also experimentally confirmed by recording the $(T_D\text{-}H)^-$ and $(T_D\text{-}D)^-$ ion at 1 eV. The small contribution on the H^-/T_D yield above about 7 eV is due to the background H^- signal that cannot be corrected for by the subtraction process mentioned.

H^- loss from the C positions can further be specified by comparing T and U in Fig. 1 and noting that T represents U methylated at the C5 position (T = m5U). From the fact that the shape of the cross section for T and U is fairly similar (taking into account that the energy resolution in case of U was worse than that in case of T) and does not show the characteristic differences as exhibited by m1T

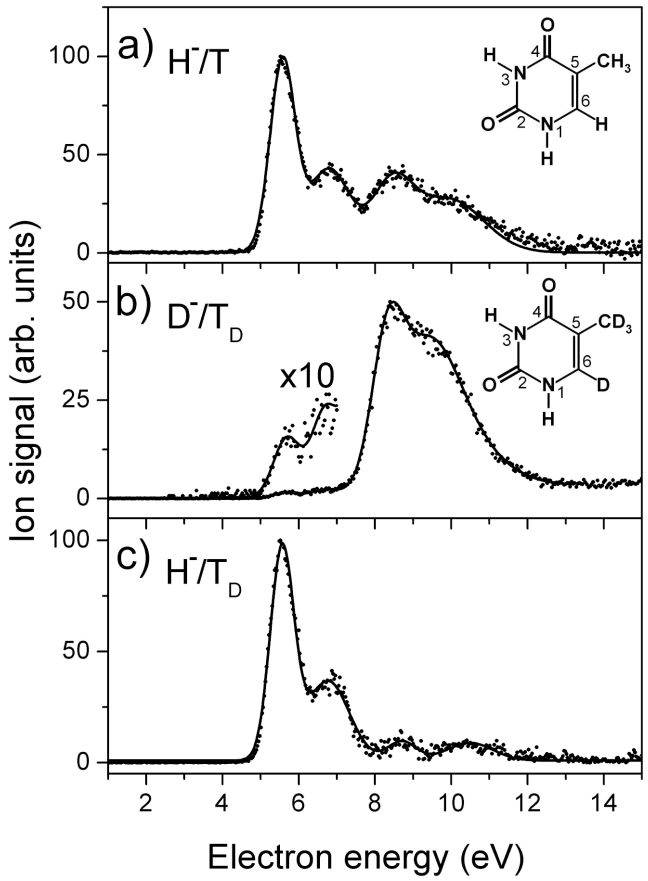

FIG. 2. H^- formation from thymine (T) compared to H^- and D^- formation from thymine deuterated at the C positions (T_D). The points correspond to the measurements, and the lines are multiple Gaussian fits to the data. The intensity scale is arbitrary but comparable between the three products.

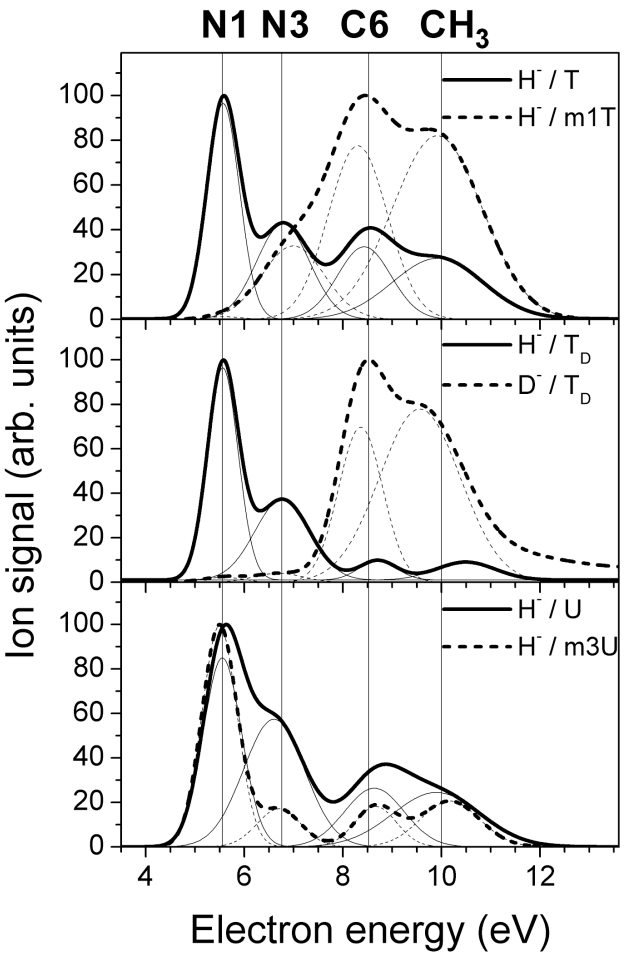

FIG. 3. Multiple Gaussian fits of the data shown in Figs. 1 and 2. The vertical dotted lines indicate the mean values of the position of the center of the 4 different resonances.

and m3U, we can conclude that H^- loss from the C positions is essentially due to C6.

In summarizing the present findings and also for confirming the conclusions drawn, we have fitted multiple Gaussians to the measured anion efficiency curves of H^- and D^- formed upon DEA to T, m1T, T_D, U, and m3U shown in Figs. 1 and 2. The corresponding fits are shown in Fig. 3, and the overriding conclusion is that in all cases the attachment spectra can be described by the same four resonances (vertical dashed lines indicating the average positions of these four resonances). The label at the upper end of the lines indicates the site from which the H^- originates at this specific resonance according to the discussion above.

From this follows the remarkable fact that for all hydrogen anions formed upon DEA for the presently investigated pyrimidine bases we observe four resonances that are practically common in their position and width. Only their relative intensities depend strongly on the structure of the precursor molecule, i.e., methylation at the nitrogen sites or deuteration on the carbon sites. Therefore in view of the present finding we arrive at the following interpretation of the various cross section shapes and their origins: The narrow resonance peaking at 5.5 eV exclusively is due to H^- loss from the N1 position. The resonance peaking at 6.8 eV predominantly is due to H^- loss from the N3 position. The higher energy features primarily are caused by H^- loss from the C atoms. Moreover, methylation at any of the positions (either C5 when going from U to T or the N3 position when going from U to 3mU) leads to a relative increase of the 10 eV resonance compared to the 8.5 eV resonance indicating that the 10 eV resonance is very likely caused predominantly by H^- loss from CH_3.

It has to be noted that, in light of current efforts to control the cleavage of particular bonds by the use of tailored ultrashort laser pulses (coherent control) [15], (i) such a bond and site selectivity in terms of electron energy is a very remarkable observation. It must be noted that this bond and site selectivity does not result from any particular energy constraints (which is, for example, the case for the site selectivity of neutral H loss [12]). A recent

high level *ab initio* study [16] predicts the binding energies (BE) in T as BE(N1-H) = 4.4 eV, BE(N3-H) = 5.8 eV, BE(C6-H) = 4.9 eV, and BE(CH_2-H) = 4.5 eV. (ii) The minimum energy required for reaction (2) is then obtained by subtracting the electron affinity of H (0.75 eV) [17]. (iii) It is apparent that in all cases for energies above about 4 to 5 eV H^- loss is energetically possible from any site. In addition, it shows that most reactions must be accompanied by the release of considerable excess energy (amounting to several eV).

As energy constraints cannot explain this site selectivity, other reasons must hence be responsible, e.g., the particular electronic structure of the associated transient precursor ions accessed by electrons of different energies. While the low-energy anion states in T (at subexcitation energies) can be characterized as one particle shape resonances creating π^* anions [18], the resonances at energies above 5 eV are so far not well explored. A quantum-dynamics scattering calculation in uracil [19] predicts a one particle shape resonance at 9.1 eV with strong antibonding C5-H character. The question is to which degree at energies as high as 9 eV are single particle shape resonances formed. At these energies, electron collisions may rather generate core excited resonances. In that case the incoming electron induces electronic excitation in the target and is then captured in the field of the excited molecule.

Finally, we note that in plasmid DNA both SSBs and DSBs have been observed recently in the energy range between about 6 and 12 eV [2,4]. The present results show that in the energy range below about 6 eV H^- loss exclusively occurs from the N1 site. In DNA, thymine is coupled to the sugar by the (N1-C) bond. The present findings show that H^- loss is inhibited at electron energies below 6 eV when N1-H is replaced by N1-CH_3, and this should also apply if a sugar molecule is connected to the N1 site. Thus the present results rather point to the fact that the strongest H^- resonance observed in the pyrimidines (i.e., at about 5.5 eV) is suppressed in plasmid DNA and may not contribute to strand breaks. Conversely, at energies above about 7 eV H^- loss from the C atoms dominates. Moreover, from the present results information on the relative contribution of H^- originating from CH_3 and the C6 position can be derived. In the first case a C-centered radical is formed, which in organic chemistry is known to be very reactive and hence is expected to lead to strand breaks.

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