DNA Cleavage

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Probing Biomolecules by Laser-Induced Acoustic Desorption: Electrons at Near Zero Electron Volts Trigger Sugar–Phosphate Cleavage**

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Secondary electrons with low energy (< 10 eV) are produced in large quantities along the ionization pathway when high-energy quanta interact with biological material.[1] These electrons are able to induce strand breaks in plasmid DNA[2] at energies near 0 eV.[3,4] The underlying molecular mechanisms are still under debate, but it is well established that dissociative electron attachment (DEA) plays a pivotal role.[5,6] The single building blocks of DNA (nucleobases, sugars, and phosphates) have been well studied.[5,7–9] To obtain information on the molecular mechanisms of strand breaks, however, it is crucial to know how a negative charge evolves in a biomolecular system that is composed of more than one subunit of DNA. For example, it was proposed from a theoretical study that an initial capture of an electron by a nucleobase and subsequent transfer of the negative charge to the DNA backbone results in cleavage of a phosphate–sugar bond.[10–13] A competing pathway is direct DEA to the phosphate group[9,14] or to the sugar unit.[8,15] The experimental investigation of relevant molecular systems such as nucleosides, sugar phosphates, and nucleotides is particularly challenging since they are thermally labile and nonvolatile. To overcome the experimental shortcomings and to enable a completely new range of experiments we applied laser-induced acoustic desorption (LIAD)[16] of neutral biomolecules to study dissociative electron attachment in the gas phase. Here we present results on DEA to the nucleoside thymidine (Td) and to D-ribose-5-phosphate (RP), the latter serves as a model compound for the DNA or RNA backbone. We present the first experimental evidence that electrons with energies close to 0 eV are resonantly captured by RP in the gas phase with subsequent cleavage of the sugar–phosphate linkage.

It was shown previously that LIAD is suitable for transfering neutral intact biomolecules into the gas phase.[16,17] A detailed description of the currently used apparatus is given elsewhere.[18] In brief, a solution of sample molecules in methanol is deposited on a thin titanium foil (12.7 μm, Alfa Aesar), and the solvent quickly removed at low pressure to form a uniform layer. The coated titanium foil is then introduced into a high vacuum chamber and irradiated from the reverse side with a pulsed Nd:YAG laser (532 nm, 3 mJ pulse^{-1}, repetition rate 15 Hz). The short laser pulse (2–6 ns) generates a shock wave that propagates through the metal to the opposite surface, ultimately leading exclusively to a gentle desorption of neutral and intact molecules. The desorbed molecules then interact with an electron beam of defined energy, and the generated anions are then analyzed by means of a quadrupole mass spectrometer. The sample holder is moveable in one direction so that the irradiated spot can be moved along the entire foil.

The electron beam is generated by a simple electron gun composed of a tungsten filament and four molybdenum electrodes. The electrons are guided along a homogeneous magnetic field generated by a pair of Helmholtz coils, thereby resulting in a well-defined electron beam at an energy resolution of 0.8–1.0 eV. The electron energy scale is calibrated by means of the well-known SF_6^-/SF_6 resonance near 0 eV. The flow of SF_6 was switched off prior to each measurement on Td or RP so that no remaining SF_6^- signal was observed. Both the Td and RP samples were purchased from Sigma Aldrich (stated purity ≥ 99 and ≥ 98 %, respectively) and used without any further purification.

We first provide evidence that the LIAD method generates intact gas-phase compounds of thymidine. It was shown recently that attachment of electrons to gas-phase thymidine leads to cleavage of the glycosidic bond to yield the closed shell $(T\text{-}H)^-$ fragment ion.[19] This reaction was observed within a strong low-energy resonance (1–3 eV) and a comparatively weaker resonance in the range 6–8 eV. However, in this study, thermal desorption was employed, which led to partial decomposition of the Td, and consequently formation of the pure nucleobase thymine through the picking-up of hydrogen atoms. The authors concluded that the resonance at 1–3 eV is due exclusively to electron attachment to thymine,[20] and hence only the $(T\text{-}H)^-$ signal in the range 6–8 eV is due to DEA to intact Td.[19]

Figure 1 shows the ion yield curve of $(T\text{-}H)^-$ generated by DEA to Td desorbed by LIAD. No ion production is

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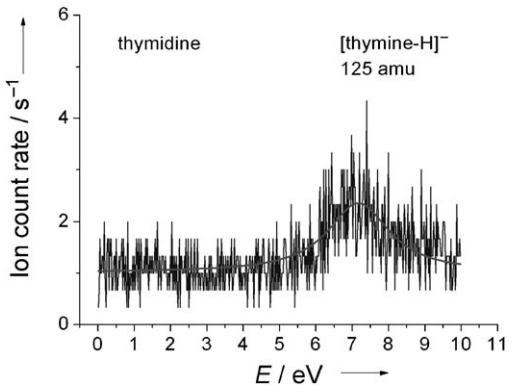

Figure 1. Ion yield of the [thymine-H]⁻ ion generated by DEA to thymidine. Thymidine was transferred into the gas phase by laser-induced acoustic desorption (LIAD).

observed at low energy, but exclusively at higher energy (between 6 and 9 eV). A comparison with the previous ion yield curve obtained using thermal desorption[19] clearly shows that exclusively intact Td molecules are in fact present in the gas phase. The low-energy resonance in thymine (1–3 eV) which triggers the loss of a neutral hydrogen atom from N1 clearly does not lead to a rupture of the glycosidic bond in thymidine. Nevertheless, Figure 1 shows that cleavage of a glycosidic bond is induced by electrons with energies of 6–9 eV when electronic excited states are involved.

Several studies have shown that low-energy electrons efficiently attach to the nucleobases,[5,7] and it was also demonstrated that electron capture and the generation of a strand break depend on the sequence of nucleobases.[6,21] However, the most straightforward mechanism of electron-induced strand breakage would be a direct attachment to the backbone followed by a C–O or P–O bond cleavage. Recent high-resolution electron energy loss spectroscopy (HREELS) measurements on DNA suggested that low-energy electrons mainly interact with the DNA backbone.[22] Previous investigations on electron attachment to isolated sugar moieties[8,15] and model compounds for the phosphate group[9] indicated effective DEA reactions already at very low energies (down to 0 eV). Herein we study DEA to a sugar–phosphate unit, which serves as a better model for the DNA backbone. The question is, whether the sensitivity of the sugar and phosphate moieties towards low-energy DEA is preserved in D-ribose-5-phosphate (RP) and which bonds are involved.

The structural integrity of the RP molecules desorbed by LIAD was determined by collecting evaporated molecules on an aluminum foil. The condensed molecules were analyzed by electrospray ionization mass spectrometry and compared to a fresh sample. The mass spectra are nearly identical and are dominated by the deprotonated molecule ([RP-H]⁻ at 229 amu; the spectra are shown in the Supporting Information).

Figure 2 shows that RP in fact undergoes DEA at very low energies. The two main fragment anions are observed at 97 and 149 amu and can be ascribed to the DEA reactions shown in Equations (1) and (2).

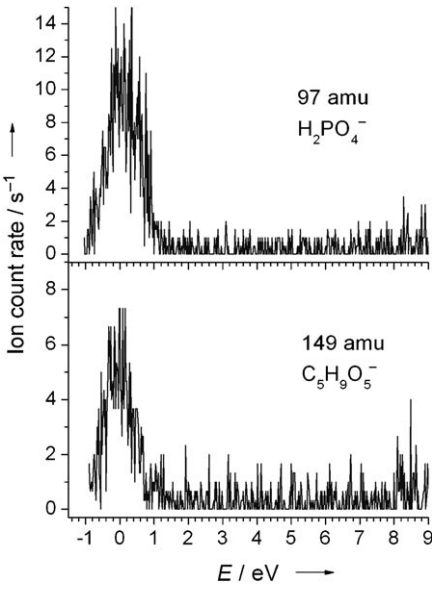

Figure 2. Ion yields of the phosphate ion $H_2PO_4^-$ and the sugar ion $C_5H_9O_5^-$ generated by DEA to D-ribose-5-phosphate.

$$e^- + RP \rightarrow RP^{\#-} \rightarrow H_2PO_4^- \ (97\ amu) + C_5H_9O_4 \quad (1)$$

$$e^- + RP \rightarrow RP^{\#-} \rightarrow C_5H_9O_5^- \ (149\ amu) + H_2PO_3 \quad (2)$$

Both reactions represent cleavage between the phosphate and sugar units. The initial step is the formation of a transient negative ion $RP^{\#-}$ close to 0 eV which dissociates at the C–O or P–O bond, with the negative charge remaining on either the phosphate or the sugar moiety (Figure 3). Both reactions correspond to a strand break in DNA (or RNA). The $C_5H_9O_5^-$ ion at 149 amu is a closed-shell dehydrogenated D-ribose anion. The parent ion of RP or the parent ion after loss of a hydrogen atom was not observed in the present experiment.

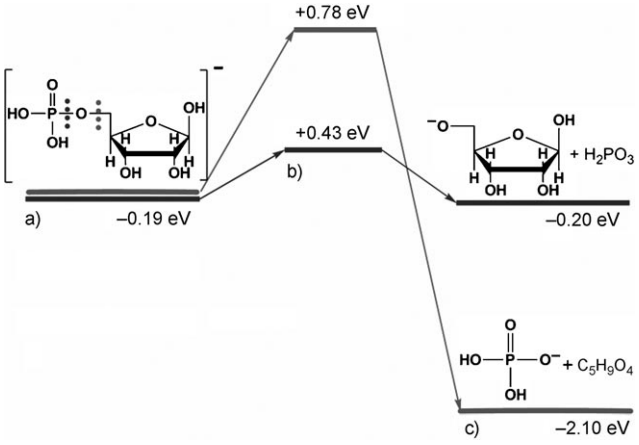

Figure 3. Activation barriers for both fragmentation pathways. All the molecules were fully optimized at the B3LYP/6-31++G** level of theory and the given energies are in respect to the neutral RP (not shown). a) Substrate anion, b) transition states, and c) the sum of isolated products.

One would intuitively expect a low-lying π* resonance located on the phosphate group of the precursor anion of Reactions (1) and (2). However, recent theoretical studies on nucleotides,[10,11,13] trimethyl phosphate,[23] and other compounds with P=O groups[23] have indicated not a local π* resonance, but a series of transient anions where the extra electron resides in rather delocalized combinations of σ* orbitals, with the lowest negative ion state located at 1.9 eV.[23] In view of this proposal, the character of the resonance associated with Reactions (1) and (2) remains under question, and dipole-supported states may play a role as entry into DEA. We note that phosphate esters showed DEA resonances below 2 eV[9] which were associated with the cleavage of the P–O and the C–O bonds, the latter generating $H_2PO_4^-$ ions. The electron affinity of H_2PO_4 is very large (4.57 eV[24]) and exceeds the bond dissociation energy of the C–O bond, thus making the reaction thermodynamically accessible at 0 eV. We further note that isolated D-ribose dissociatively captures electrons close to 0 eV.[8,15]

Computationally determined barriers (Figure 3) further reveal that Reaction (1) is a thermodynamically driven process, which leads to the creation of stable products. In contrast, the sum of the energies of the products in Reaction (2) is barely lower than the energy of the parent anion, but the kinetic barrier to surmount a transition state is significantly lower than in the case of Reaction (1), thus making it a kinetically controlled reaction. The reaction barriers for Reaction (1) are very close to those observed earlier for sugar–phosphate–sugar compounds.[25]

Our calculations at the B3LYP/6-31++G** level of theory predict a slightly bound RP^- ion (0.198 eV), with the excess electron occupying an appreciably extended molecular orbital. Depending on the geometry of RP, the appearance of a dipole bound state is highly probable. It remains to be explored whether a dipole-supported state may act as a doorway for DEA in electron attachment.[26]

Single-strand breaks of plasmid DNA were observed at electron energies near 0 eV.[4] The present experiments demonstrate that a sugar–phosphate compound undergoes DEA near 0 eV and is associated with the cleavage of the sugar–phosphate linkage. The experiments suggest that the breakage of DNA strands by electrons at very low energies is triggered by DEA directly to the DNA/RNA backbone. Experiments using whole nucleotides in the gas phase are currently in progress to evaluate how electron localization at the sugar–phosphate backbone competes with transient electron capture by the nucleobases and how this influences the strand break.

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