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DNA Damage by Low-Energy Electron Impact: Dependence on Guanine Content

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Single-stranded DNA oligonucleotides (33-mers) containing different numbers of guanines ($n = 1-4$) were tethered to a gold surface and exposed to 1 eV electrons. The electrons induced DNA damage, which was analyzed with fluorescence and infrared spectroscopy methods. The damage was identified as strand breaks and found to correlate linearly with the number of guanines in the sequence. This sequence dependence indicates that the electron capture by the DNA bases plays an important role in the damage reaction mechanism.

Introduction

The interaction of low-energy electrons (LEE) with living matter at energies below the ionization threshold (about 7.5 eV for DNA) is of increasing importance from the fundamental scientific as well as from technological points of view. The intrinsic energy-resonant nature of the DNA−LEE interaction leads to effects such as DNA strand breaks,[1,2] sequence-dependent electron capture,[3,4] or nucleoside bond and energy-selective fragmentation.[5] Recent reviews[6,7] of these processes are available. However, despite ongoing efforts, it is fair to say that the mechanism by which LEE induces strand breaks in DNA is presently controversially discussed and remains elusive. Experimental and theoretical evidence is presented for the initiation of energy-resonant processes (dissociative electron attachment, DEA) at nucleobase,[1,8−10] phosphate,[11,12] or sugar[13] units of DNA. Clarifying the mechanism of the DNA−LEE interaction is important because the low-energy secondary electrons are the most abundant radiolysis species generated upon impact of high-energy radiation[14] and therefore highly pertinent to issues such as the development of radio therapy or the estimation of public health risks associated with low doses of continuous exposure to radiation and radiation-induced "bystander" effects.[15]

Experimental Section

Experimental details have been presented elsewhere.[16,17] Additional data can be found in the Supporting Information. Here, we briefly explain the choice of DNA oligonucleotides and the procedures to prepare the single-stranded DNA monolayers on gold and to derive the DNA damage. HPLC-purified single-stranded DNA oligonucleotides were obtained from Thermo Fischer Scientific. Their sequences are shown in Table 1.

The oligonucleotides comprise two distinct parts. One can be described as a short 9-mer adenine unit in which $n = 1$, 2, 3, or 4 adenines are replaced by guanines ($A_{9-n}G_n$). This is followed by the $(dT)_{24}$ sequence. The adenine−guanine part is positioned at the end which is tethered to the surface. Its sequence is too short to provide a stable duplex by itself through the hybridization reaction at room temperature (see Supporting Information). This has an important consequence for the interpretation of the data. A strand break occurring at any of the four guanine positions leads to a DNA fragment left on the surface which is too short to form the duplex, and therefore, irrespective of the position within the A-G part of the oligonucleotides, the guanines contribute with equal weight to the DNA damage as detected by the hybridization−fluorescence method described below.

TABLE 1: Oligonucleotides Used in the Experiments and Their Abbreviations

(A_8G_1)	5′ HS-C_6-AGA AAA AAA-TTT TTT TTT TTT TTT TTT TTT TTT
(A_7G_2)	5′ HS-C_6-AGA GAA AAA-TTT TTT TTT TTT TTT TTT TTT TTT
(A_6G_3)	5′ HS-C_6-AGA GAG AAA-TTT TTT TTT TTT TTT TTT TTT TTT
(A_5G_4)	5′ HS-C_6-AGA GAG AGA-TTT TTT TTT TTT TTT TTT TTT TTT

Self-assembled DNA monolayers were prepared by tethering the DNA oligonucleotides at the thiolated 5′-end onto gold-coated glass chips (Arrandee, Germany). The chips were transferred into an UHV chamber for exposure to a well-defined 1 eV electron beam of an energy resolution of about 0.5 eV. The electron beam energy is defined as the voltage drop from the filament to the chip surface. For the electron exposure, the chips were positioned behind a mask with a 2 mm diameter orifice. The exposure was controlled by a shutter, and the dose was determined using a Keithley 6485 instrument with an NI 6014 card and Labview software. Following the electron bombardment, DNA probes on the chips were hybridized by an overnight exposure to the corresponding Cy5-labeled complementary 33-mer strands (5′-Cy5-$(dA)_{24}$-$(T_{9-n}C_n)$. Fluorescence images were obtained using a confocal scanner (Affimetrix 418) and analyzed with GenePixPro6 software. Infrared data were obtained with a FTIR spectrometer (Nicolet 6700) equipped with the external reflection accessory (85° grazing angle).

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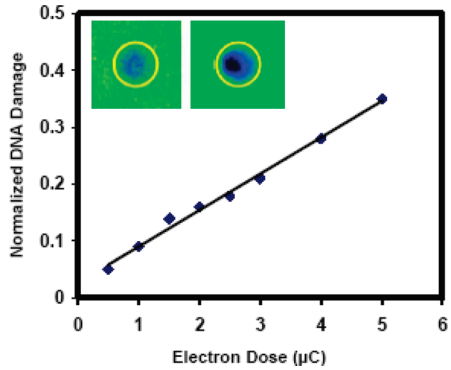

Figure 1. Normalized electron damage to $(dT)_{33}$ oligonucleotides as a function of the dose of 1 eV electrons. Inset: Examples of images used to evaluate the damage (at increasing exposure from left to right). The total fluorescence intensity within the exposed area (circle) is compared to the background intensity (see text).

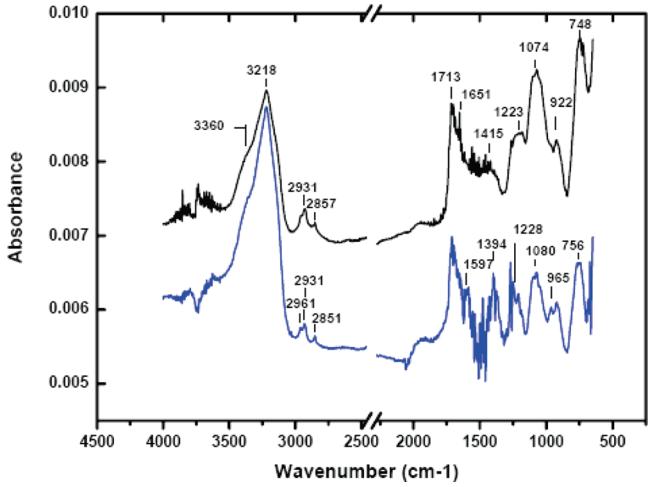

Figure 2. Reflection−absorption infrared spectra of the $d(T)_{33}$ monolayer on gold before (upper spectrum) and after (lower spectrum) exposure to low-energy electrons. The spectra are shifted vertically for clarity.

Results and Discussion

Representative fluorescence images are shown in the inset of Figure 1. DNA damage due to the impact of 1 eV electrons impedes the hybridization ability (duplex formation) and is detected as a reduction in the fluorescence intensity. Thereby, we define the normalized damage as $(1 - I/B)$, where I is the total intensity within the exposed area and B the total intensity of the local background. The local background is defined as the area outside of the exposed area and within a diameter 3 times larger than the exposed area. First, the range of the linear damage−charge correlation for the impact of 1 eV electrons was determined. As shown in Figure 1, below 5 μC, the damage of DNA can be described as a linear function of the electron charge passed.

The damage involves the formation of strand breaks. This is demonstrated by the data (Figure 2) which compare the infrared signatures of a DNA monolayer before and after exposure to low-energy electrons. Although these data were obtained by using a homooligonucleotide, $d(T)_{33}$, one can expect a similar type of process to be operative in other oligonucleotides. The assignments of the bands in Figure 2 can be made based on published work.[18] Of particular interest are the bands at 1074 and 1223 cm^{-1}, which are the symmetric and antisymmetric stretching vibrations of the phosphate group, respectively. The

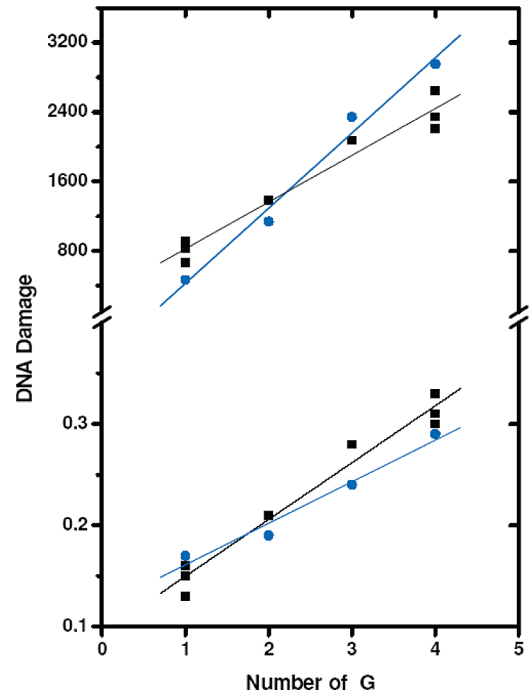

Figure 3. Dependence of the electron damage to $A_{9-n}G_n$ oligonucleotides on the number of guanines (G). The charge passed at 1 eV was 3 μC. The black and blue points represent two separate experiments in which the samples were processed in parallel. The lower data: Damage normalized to the background, $(1 - I/B)$. The upper data: Absolute decrease in the fluorescent intensity compared to the background, $(B - I)$; see text. The first approach eliminates any variation in the fluorescence detection efficiency between different samples, and the second one is insensitive toward any variation in the composition of the DNA monolayer. The black and blue lines are the corresponding linear fits.

intensity of these bands decreases significantly upon the bombardment with low-energy electrons, indicating electron-induced strand breaks and the removal of DNA from the surface upon washing. A similar conclusion was reached based on scanning electron microscopy (SEM) data with a similar system.[17]

The dependence of the electron damage on the guanine content of the $(A_{9-n}G_n)$ $(n = 1-4)$ oligonucleotides is presented in Figure 3. The background normalized damage $(1 - I/B)$ and the absolute decrease in the fluorescent intensity $(B - I)$ caused by impact of a constant dose (3 μC) of 1 eV electrons are shown. The first approach eliminates any variation in the fluorescence detection efficiency between the different samples, and the second one is insensitive toward any variation in the composition of the DNA monolayer due to immobilization/washing steps.

The data in Figure 3 show two different sets of experiments within which four or eight different chips have been processed simultaneously, that is, the DNA immobilization, hybridization, washing, and other processing steps were carried out in a parallel fashion. There is a clear linear relationship between the number of guanines within the sequence and the amount of damage observed with the fluorescent method. We note here that each of the four guanines contributes equally to the damage (see above), and if the damage involves an enhanced formation of strand breaks at guanines, one would expect a linear relationship as shown in Figure 3. The enhanced LEE damage to guanine-rich DNA sequences is somewhat surprising in light of the low ionization potential of guanine in DNA oligonucleotides.[19] Nevertheless, experimental[3,4,10] and theoretical[20,21] studies exist pointing toward a special case of guanine, as compared to other

nucleotides, with respect to the interaction with LEE. Zheng at al.[10] have shown that DNA strand breaks by LEE are strongly suppressed when guanine is removed from the short single-strand tetramers of DNA, GCAT. It has however to be pointed out here that the sequence studied involves only four nucleotides.

Recent calculations[20] of Schyman and Laaksonen indicate that DNA strand breaks would most likely occur when a LEE is attached to the guanine nucleotide. Also, a theoretical study of Gu et al.[21] showed that the electron is attached onto the nucleobase for all DNA nucleotides in aqueous solution except for guanine, where the electron is dipole-bound to the guanine and is also situated near the phosphate moiety.

The data presented in Figure 3 provide clear evidence for the enhancement of the low-energy electron damage to DNA by guanine for oligonucleotides longer than a few bases. In conjunction with the recent data on the electron capture by DNA,[3,4] they also provide direct information on the mechanism of the damage. Naaman et. al[3,4] have recently measured the low-energy electron (<2 eV) transmission yield through self-assembled monolayers of single-stranded DNA oligonucleotides. The authors have found that the electron-capturing probability scales with the number of G bases and that once captured, the electrons do not reside on the bases but in the DNA backbone.[3,4] Considering these data together with the data presented here, which show that the low-energy (1 eV) electron damage to DNA also correlates with the number of G bases, the damage mechanism previously postulated as a result of the experimental[1,10] and the theoretical work[8] is strongly corroborated. Briefly, as described in detail by Simons et. al,[8] very low energy (0.1−2 eV) free electrons attach to base π^* orbitals to form shape resonances, this being followed by an interesting through-bond electron-transfer process resulting in cleavage of sugar−phosphate C−O σ bonds. Hereby, the thermodynamic driving force is the large EA of the phosphate radical.[8] Such a mechanism might also be responsible for the observed protection of single-stranded DNA in the reaction with LEE by proteins such as SSB,[22] which is known to contact DNA directly at the bases.[23]

In conclusion, we provide fluorescence-based evidence for the enhancement of the low-energy electron damage to single-stranded DNA by guanine. The data support the damage reaction channel involving an initial LEE interaction with the DNA bases followed by electron transfer to the phosphate group.

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Supporting Information Available: Measured and calculated hybridization efficiencies of oligonucleotides as a function of their length and general experimental procedure. This material is available free of charge via the Internet at http://pubs.acs.org.

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