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Electron stimulated desorption of anions containing Oxygen and Nitrogen from self-assembled monolayers of DNA

N Mirsaleh-Kohan¹, A D Bass¹, P Cloutier¹, L Sanche¹,²

¹Département de médecine nucléaire et radiobiologie, Faculté de médecine et les sciences de santé, Université de Sherbrooke, Sherbrooke, QC J1H 5N4 Canada
²Department of Physics and Astronomy, The Open University, Walton Hall, Milton Keynes, MK7 6AA United Kingdom

Andrew.Bass@Usherbrooke.ca

Abstract. The electron stimulated desorption of anions from self-assembled monolayers of double stranded DNA is reported. Desorption of the oxygen and nitrogen containing anions O\(^{-}\), OH\(^{-}\), CN\(^{-}\), OCN\(^{-}\), and OCNH\(^{-}\) is induced by the impact of 0.1-20 eV electrons. The anion desorption yields, measured as a function of incident electron energy exhibit pronounced maxima that can be attributed to dissociative electron attachment (DEA) to basic DNA units. Above 15 eV, desorption is attributed to dipolar dissociation (DD). This study further indicates that electrons with energy as low as 2.5 ± 0.3 eV can not only cause damage to DNA but also produce fragments with considerable kinetic energy.

1. Introduction

Although the vast majority of investigations have focused on interactions of high energy radiation with living cells and DNA molecules, it is well established that the genotoxic effects of high energy particles are effectuated by the secondary species generated by the primary particles. These secondary species can cause mutagenic, recombinogenic, and other potentially lethal DNA lesions such as single and double strand breaks [1]. The most abundant of these species are secondary electrons with typical energies below 30 eV [2]. Therefore, understanding the mechanisms of low-energy electron (LEE) induced damage in DNA is highly relevant to radiobiology.

Boudaïfa et al. [3] investigated strand break formation in plasmid DNA by 3-20eV electrons. Single and double strand breaks were observed even at electron energies well below ionization thresholds (7.5 eV to 10 eV) [4]. Thresholds for these breaks were measured to be near 3-5 eV with a maximum at about 10 eV. The authors concluded that these breaks occur as a result of electron attachment to DNA subunits to form a transient negative ion (TNI), and subsequent bond dissociation. The threshold for strand-breaks in their work differs from that for damage induced by photons, where the onset lies at about 7 eV [5].

To better understand the mechanisms underlying DNA damage through electron attachment, studies have examined interactions of LEE with various components of DNA such as the bases [6-10], deoxyribose analogues [11,12], and the phosphate group [13] of the backbone in gas and solid phases. For all of the DNA components [6-13], the formation of resonances (i.e., TNI) that dissociate into a stable anionic and radical fragments was observed. One of the remaining challenges is to identify, in a long DNA chain, which TNI state of the different basic constituents (the bases, sugar and phosphate
group and structural water) lead to specific damages (e.g. which TNI on what basic units lead to DNA strand breaks).

Pan et al. [14] investigated the electron stimulated desorption (ESD) of anions induced by 3-20 eV electron impact on lyophilized thin films of linear and supercoiled DNA. Their results suggested that dissociative electron attachment (DEA) is the process that leads to the formation of the observed desorbed anions (H¯, O¯, and OH¯).

Techniques developed to form self-assembled monolayers (SAMs) of alkanethiols on gold substrates [15] are also applicable to thiolated DNA [16]. The high order and purity of the resulting DNA SAMs offer distinct advantages over other techniques (such as lyophilization) when preparing thin film samples of DNA. In this paper we report the electron stimulated desorption (ESD) of anions from SAM films of double stranded thiolated DNA on clean gold substrates using 40-mer oligonucleotides. A long chain of DNA was used to increase the complexity of our DNA model. Anion desorption was analyzed as a function of incident electron energy (0-20 eV) and irradiation time at a fixed electron energy employing a high-sensitivity time-of-flight mass spectrometer. This study provides information on the TNI states responsible for damage to specific basic units of DNA.

2. Experimental

Phosphothiolated oligonucleotides of double stranded DNA were prepared with the 40-base sequence 5’-GGTAC, CAGGC, CTACT, ACGAT, TTACG, AGTAT, AGCGA, GCTCG-3’, where G stands for guanine, T for thymine, A for adenine, and C for cytosine (see Figure 1). The thiolated oligonucleotide and its complimentary strand were purchased from University Core DNA services at the University of Calgary. Thiolation was performed by substitution of five oxygens doubly bonded to phosphorus with sulfur atoms (5S-dsDNA). The 5S-dsDNA is assumed to lie parallel to the surface [17] as shown in Figure 1. Gold plates were used as substrates and purchased from Arrandee, Germany. They were cleaned, prior to chemisorption of the DNA, by multiple exposure to UV/ozone and rinsing with ethanol and doubly-distilled, deionized water (pure water).

![Figure 1](a) Part of double stranded DNA (b) Schematic drawing of self-assembled monolayer (SAM) of double stranded DNA chemisorbed on a gold substrate

SAM DNA films were prepared by immersing the cleaned gold substrate in the DNA solution [18] for 12 hours. The plates then were immersed in pure water three times for 20 minutes to remove any unbound molecules. Finally, the plates were dried under a stream of N2 gas and placed into a load-lock vacuum system (<1×10^-8 torr) to facilitate transfer into the ultrahigh vacuum chamber (~5×10^-10 torr) for LEE bombardment.
Anions desorbed by LEE impact were measured by a time-of-flight (TOF) mass analyzer. The ESD system and its specifications have been discussed in detail previously [19]. Recently, a newly designed load-lock has been coupled to the TOF mass analyzer, and permits us to introduce and to study samples prepared under ambient conditions. After evacuation for a minimum of about 4 hours, the films can be transferred, sequentially into the main chamber for irradiation.

The DNA SAM films were bombarded by LEE from an electron gun (Kimball Physics ELG-2). The beam was at an incident angle 45° with respect to the substrate and focused into an estimated spot-size of 1mm². The electron gun was operated in a pulsed mode with 800 ns duration at a rate of 5 kHz. The films were irradiated from 0.1 to 20 eV with 4nA time-averaged transmitted current measured at 10 eV. The energy scale of the incident electron is estimated to have uncertainty of ±0.3 eV and the full-width, half-maximum (FWHM) of the electron energy is approximately 0.5 eV. A short time (~10 ns) after the electron beam pulse, desorbed anions were repelled from the films by a negative potential pulse (rise/fall time 30 ns, pulse width of 2 µs) applied to the gold substrate, directing the anions into the entrance optics of a reflectron TOF mass analyzer positioned at a distance of 10mm from the film surface.

Three series of ESD experiments were performed: (a) the mass spectrum of each SAM film was measured at a fixed electron energy (b) the variation of the desorbed ions signal was monitored as a function of incident electron energy to obtain the yield function for each desorbed ion, and (c) the time-dependence of ion signal was recorded at a fixed incident electron energy.

To examine the efficiency of the DNA adsorption on the gold substrates, X-ray Photoelectron Spectroscopy (XPS) was performed using our previously described XPS spectrometer [20]. The X-ray source (15kV, 350 W) uses an Al anode. The X-ray beam impinged onto the sample surface at an incident angle of 72° relative to the substrate while the hemispherical electron energy analyzer was positioned normal to the sample surface. The pass energy was set to 11.75 eV which results in a resolution of between 2 to 3 eV. XPS spectra for Nitrogen, N1s (binding energy of ~400 eV) and Phosphorous, P2p (binding energy of ~133 eV) peaks were recorded for a 5S-dsDNA film as shown in Figure 2.

Figure 2 XPS measurements of N1s (binding energy of ~400eV) and P2p (binding energy of ~133eV) peaks for a 5S-dsDNA film where intensity is proportional to the amount of DNA chemisorbed on a gold substrate.

3. Results
We report in this section the desorption of the anions O¯ /NH2¯ (16amu), OH¯ (17amu), CN¯ (26amu), OCN¯ (42amu), and OCNH¯ (43amu) induced by LEE impact onto 5S-dsDNA SAM films. No anions more massive than 43amu were observed. This may in part be due to heavier fragments tending to possess insufficient kinetic energy to overcome attractive polarization and charge-image forces induced in the film and metal substrates by their negative charge [21].
The dependence of the desorbed anion yields on incident electron energy (yield functions) for a 5S-dsDNA SAM is shown in Figure 3. The anion yield functions exhibit energy thresholds of 4-5 eV, except for OH\(^{−}\) which displays a threshold closer to 2.5 eV. Most yield functions show resonance structure near 7-8.5 eV, but OH\(^{−}\) has a maximum resonance near 6.5 eV. The yield function for OCN\(^{−}\) does not show as strong a signal, threshold or resonance maximum as can be seen for the other anions. However, irradiation for an extended period of time [22] shows that this ion has a threshold of about 5 eV and a maximum around 6.7 ±1 eV. All anion signals show a monotonic increase at energies above 14-15 eV. Variations of the desorbed anion signals as a function of time, at incident energy of 8 eV were also recorded and are reproduced in Figure 4 for anions O\(^{−}\), OH\(^{−}\), CN\(^{−}\), OCN\(^{−}\) and OCNH\(^{−}\).

Figure 3 Incident electron energy dependence of desorbed anions yields from a 5S-dsDNA SAM.

Figure 4 Time dependence of desorbed anions from a 5S-dsDNA film recorded at incident electron energy of 8 eV.
4. Discussion

Formation of a stable negative ion by LEE impact usually occurs through DEA for electron energies less than 15 eV and/or dipolar dissociation (DD) for electron energies above 15 eV. In the DEA process, a TNI dissociates into a stable negative ion and one or more neutral species. Alternatively, in the case of large molecules, dissociation of a TNI can break a bond leaving the excess electron on one side of the broken bond and forming a radical on the other side. Since the initial step in the DEA process is electron attachment to form a TNI, the yield function of the anion fragment or bond breaking exhibits pronounced maxima at the resonance energy. A negative ion can also be produced through the DD process where the ion pair is a result of dissociation of an electronically excited molecule. In the DD process, the anion yield function always has a non-resonant behavior to which can be superimposed a resonant line shape; the non-resonant portion of the signal increases monotonically with electron energy. Anion desorption may also arise from the reactive scattering [23] of a primary anion (produced by for example, DEA) with surrounding molecules. Thus, the yield function of the secondary anion usually carries the resonance signature of the primary ion yield, but with a much smaller magnitude. Stable anions may also be formed through other reactions of primary anions, but such species do not possess sufficient energy to desorb. However, any new chemical species synthesized during electron impact may also undergo DEA and DD and contribute to the desorption signal, but this may only become apparent at long irradiation times.

In the present experiments, the energy dependence of all desorbed anions shows a resonance feature around 7-8.5 eV that is a typical of DEA. The continuous rise in the anions signals above 15 eV is typical of the non-resonant, dipolar dissociation process. Thus interaction of LEE with DNA SAMs in our experiment produces fragment anions via both DEA to DNA and DD as previously reported [14]. The time dependence of anion desorption signals at a fixed incident electron energy reflects the processes responsible for the production of each anion. In the case of DEA and reactive scattering from intact molecules, the anion desorption signal is expected to decrease exponentially with time, whereas the signal deriving from molecules synthesized by the electron beam should increase as a function of time. As shown in Figure 4, time dependence of O⁻/NH₂⁻ shows an exponential decay. The mass resolution of our apparatus does not allow us to differentiate between O⁻ and NH₂⁻ for mass 16amu. However, more detailed investigation on the latter revealed that 16amu desorbed from SAM films is more likely to be oxygen [22, 7]. In the ESD experiment presented here, O⁻ appears to arise from DEA of DNA components since the time dependence of this ion shows an exponential decay. Previously, Pan et al.[14] concluded that desorption of O⁻ signal from linear and supercoiled DNA results of DEA to the phosphate group of DNA, more specifically from P=O bond of the phosphate group. Furthermore, electron-stimulated desorption of O⁻ from thin films of phosphate film (NaH₂PO₄) was investigated [13]. In this latter experiment, the yield function of oxygen anion presented a single broad peak at 8.0 eV and a threshold of 4.7 eV in good agreement with the threshold (5 eV) and maximum (7.9 eV) seen in our experiment (the difference in energies is within our experimental uncertainty). Thus, the signal of O⁻ in our ESD experiment is most likely produced from DEA to the phosphate group of DNA.

The OH⁻ yield function exhibits a threshold of about 2.5 eV with a maximum around 6.5 eV (Figure 3). The time dependence of OH⁻ signal exhibits an exponential decay with time as shown in Figure 4, indicating that the observed OH⁻ desorption arises from DEA to the DNA and/or a reactive scattering processes. In the case of reactive scattering, OH⁻ can be generated via reactive scattering of O⁻, with for example, hydrocarbon species [24]. However the present OH⁻ yield function does not greatly resemble that of O⁻ and is of much higher intensity, indicating that OH⁻ ions do not originate from reactive scattering of O⁻. We thus attribute the OH⁻ ions signal to DEA and DD to the DNA components.

Anions containing nitrogen, CN⁻, OCN⁻, and OCNH⁻ in the present experiment show very weak signals compared to other observed anions. This weakness may indicate that these anions are unable to easily escape from the surface due to higher mass and their (presumed) low kinetic energies. Nitrogen-based fragment anions necessarily originate from the DNA bases, since only bases contain nitrogen.
Abdoul-Carime et al. [7] investigated nitrogen-containing anions desorbed from solid films of the four DNA bases. In each case, the cyanided anion, CN\(^-\), was observed to desorb, while OCN\(^-\) desorption was only observed from thymine films. Interestingly, no OCNH\(^-\) was detected from any of the bases. Several studies have also investigated fragmentation of gas-phase DNA bases by LEE [6, 8, 9]. In the gas phase, CN\(^-\) was detected from all DNA bases [6, 8, 9], whilst OCN\(^-\) was observed from only guanine, thymine and cytosine [6, 8, 9]. OCNH\(^-\) was only detected from fragmentation of thymine [8]. CN\(^-\) and OCN\(^-\) were also observed to desorb from thin films of short oligonucleotide GCAT by impact of LEE [25]. The time dependence of these anions in Figure 4 shows a slightly increase as a function of irradiation time, suggesting that these ions are generated from LEE-induced species. This hypothesis could also partially explain why the signal from these anions is initially so low.

In summary, LEE (0.1-20 eV) impact on 5S-dsDNA films produces the anion fragments O\(^-\), OH\(^-\), CN\(^-\), OCN\(^-\), and OCNH\(^-\) as shown. From the dependence of the desorbed anion signals on incident electron energy, it is seen that the desorbed species arise from direct dissociative electron attachment to- and dipolar dissociation of DNA basic units. In our experiment, mass 16amu can be attributed to the oxygen ion and arises as a result of DEA to phosphate group of DNA. Nitrogen-containing ions, which have been observed previously in ESD experiments from thin films of the DNA bases, were here observed to also desorb from a long DNA chain (40-mer). Thus it is likely that damage processes observed for the pure bases remain effective once the molecules are bonded with the sugar in DNA. However the increase in signal with irradiation-time suggests that the yields of these species are greater from electron degraded bases.

Finally, according to our ESD results, desorption of anions induced by LEE occurs at a threshold energy as low as 2.5 eV (OH\(^-\)) in a good agreement with the results on single and double stranded breaks of DNA [3]. Here, electrons with energy as low as 2.5 eV can induce anion fragments from DNA with sufficient kinetic energy (~ 1eV) to escape the induced polarization potential. This threshold is well below that reported for the DNA damage induced by UV irradiation [26] emphasizing the different damage mechanisms induced by LEE and photons. Furthermore, one can speculate that following DEA, the radical left on the DNA could lead to a strand break while reactive scattering by the dissociating anions with the other strand might produce further damage. Although the probability of such subsequent processes is expected to be low, it nevertheless offers a mechanism for double strand breaks with electrons of energy as low as 2.5 eV.

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