Mechanism and Site of Attack for Direct Damage to DNA by Low-Energy Electrons

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We report results on the desorption of OH^- induced by 0–19 eV electrons incident on self-assembled monolayer films made of single and double DNA strands of different orientations with respect to a gold substrate. Such measurements make it possible to deduce the mechanism and site of OH^- formation within a biomolecule as complex as DNA. This type of damage is attributed to dissociative electron attachment to the phosphate group of DNA, when it contains the counterion H^+.

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Mutagenic, genotoxic, and other potentially lethal lesions induced by high energy radiation in biological cells are usually produced by the reaction of DNA with secondary species generated along radiation tracks [1]. Secondary electrons with energies below 20 eV are the most abundant of these secondary species [2]. It is therefore crucial to understand the effects of low-energy electrons (LEEs) on the DNA molecule to obtain a nanoscopic view of radiobiological damage. The experiments of Boudaiffa *et al.* [3] clearly showed that electrons of 5–20 eV contribute significantly to single and double-strand breaks (SSBs and DSBs) in DNA. Below 5 eV only SSBs were observed [4]. Later Pan *et al.* [5] investigated the LEE induced desorption of H^-, O^-, and OH^- from physisorbed DNA films. They demonstrated that the dissociative electron attachment (DEA) mechanism is involved in the bond breaking process responsible for strand breaks. The abundant H^- yield observed in their experiments was assigned to the dissociation of temporary anions formed by the capture of the incident electron by the deoxyribose unit and/or the bases, whereas O^- production arose from temporary electron localization on the phosphate group. Since in their experiment, no basic subunit inside DNA contained OH radicals, they suggested that reactive scattering of O^- may be involved in the release of OH^- [5].

In this Letter, we report experimental results on electron damage induced to self-assembled monolayer (SAM) films of DNA. Because of this advance in DNA film deposition, we can investigate LEE interactions with pure and well defined films of single and double stranded DNA of different orientation with respect to the supporting substrate. From these results, we determine both the mechanism and site of OH^- production [6]. The present results demonstrate that the phosphate-counterion portion of DNA plays an important role in LEE damage induced to DNA, and provide new insight about LEE-DNA interactions.

The experiments were performed with phosphothioated DNA obtained from substitution by sulfur of the oxygen doubly bonded to phosphorous. The following four different samples were prepared with the 40-mers oligonucleotides 5'-GGT ACC AGG CCT ACT ACG ATT TAC GAG TAT AGC GAG CTC G-3' (G indicates the base guanine, C the cytosine, A the adenine, and T the thymine) with and without their complementary strands. These samples were purchased from University Core DNA services at the University of Calgary. A sulfur (1S) was substituted at one end of one backbone in the single (ss) and double (ds) stranded configurations (1S-ssDNA and 1S-dsDNA) and 5 sulfur atoms (5S) were substituted in the backbone in the ss and ds configurations (5S-ssDNA and 5S-dsDNA). Figure 1 shows the structure of 1S-ssDNA and the complementary strand in the 1S-ds DNA configuration.

The SAMs were chemisorbed by the sulfur atoms on gold substrates supplied by Arrandee, Werther, Germany. The substrates were cleaned by (1) rinsing in ethanol and pure (i.e., doubly distilled and deionized) water, (2) exposition to ozone for 30 min, (3) rinsing with pure water to remove the impurities due to reactions with ozone, (4) exposition to ozone for another 30 min, and finally (5) rinsing

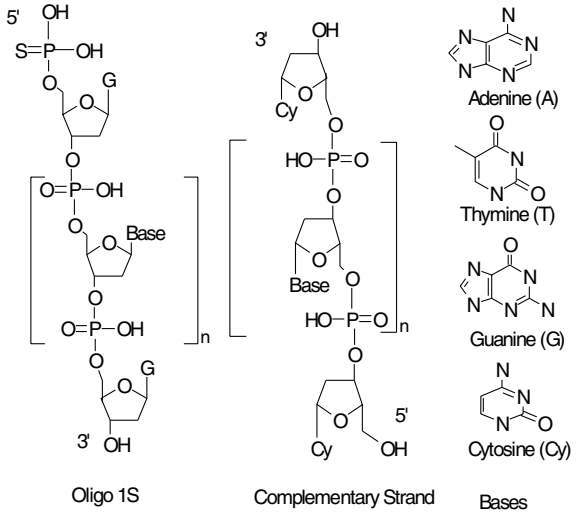

FIG. 1. The molecular structure of a 40-mer oligonucleotide (1S) and complementary strand. The bracket represents the repeated portion of the strand with different bases ($n = 38$.)

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in pure water. DNA SAM samples were prepared by immersing the cleaned gold substrates in a solution of DNA [7] for at least 12 h. The DNA concentrations were 2.7, 12.7, 3.5, and 3.5 μM for 5S-ssDNA, 1S-ssDNA, 5S-dsDNA, and 1S-dsDNA, respectively. The samples were rinsed thoroughly with pure water and dried under a stream of N_2 gas. As shown in Fig. 2, the 1S-ss and 1S-ds SAMs have a tendency to stand perpendicular to the gold surface [8], whereas the 5S-ss and 5S-ds SAMs being anchored on the surface at five different positions lie parallel to the surface [9]. According to the molecular structure in Fig. 1, the ss SAMs [Figs. 2(a) and 2(c)] have a terminal sugar with OH at the 3′ position, whereas in the ds SAMs [Figs. 2(b) and 2(d)] one chain is terminated with OH′s at the 3′ and 5′ positions of the sugar and the other has only one terminal sugar with OH at the 3′ position.

Twenty DNA samples were placed in a sample holder which was inserted in a load-lock vacuum system ($\sim 1.0 \times 10^{-8}$ torr). After evacuation for 12 h, the samples were transferred to an ultrahigh vacuum (UHV) chamber ($\sim 2.0 \times 10^{-10}$ torr) one at a time (via a gate valve). The apparatus has been described in detail elsewhere [10]. Once in the UHV system, the surface of the DNA sample was positioned perpendicular to a mass spectrometer (Extrell 150-QC). An electron beam produced by a Kimball Physics ELG-2 gun, with an energy resolution of 0.5 eV, was focused on a 4 mm^2 spot on the SAM, at an incident angle of 70° to the surface normal. The incident electron energy (0–19 eV) dependence of the magnitude of the OH^- yield (i.e., the yield function) and the time dependence of the OH^- signal at a fixed incident electron energy were measured with an electron current of 200 nA at the target. All experiments were performed at room temperature. The incident electron energy was calibrated within ±0.3 eV by taking 0 eV as the onset of electron transmission through the film.

The behavior of the time dependence of the OH^- signal is independent of electron energy and similar for all SAM configurations. A typical dose-response curve recorded at 7.0 eV is shown in the inset of Fig. 3. The yield functions of OH^- for the four different DNA SAM configurations are shown in curves *A* to *D* of Fig. 3. They all have a threshold about 2.0 eV, the lowest value among all anions (i.e., H^-, O^-, OH^-, $CH2^-$, $CH3^-$, CN^-, OCN^-, $OCNH^-$) detected in this type of experiment [11]. The 1S SAM yield functions (curves *A* and *B* of Fig. 3) consist essentially of a broad maximum located about 7 eV, whereas for the 5S SAMs (curves *C* and *D* of Fig. 3) superposition of peaks lying at about 5.5 and 6.7 eV (see Fig. 3) followed by a very broad structure extending from 8 to 14 eV is observed. Such features in electron-stimulated anion yield functions are characteristic of the formation of transient anions, which dissociate into a neutral fragment and a stable anion (i.e., DEA). Thus, the results of Fig. 3 indicate the formation of such anions in DNA, where they have been shown to result from temporary electron localization on basic subunits (i.e., the bases, deoxyribose, and phosphate groups) [12]. In principle, OH^- could also arise from H_2O molecules retained by DNA. However, purposely condensing H_2O molecules on our SAMs considerably diminished the OH^- signal. As seen from curve *E* in Fig. 3, previous measurements [13] have shown that OD^- electron-stimulated yields from condensed D_2O films are negligible. The OH^- signal could also arise from DEA to a molecule synthesized by the electron beam during the bombardment. In this case, however, the OH^- signal would increase as a function of time contrary to observation (see inset of Fig. 3). As previously suggested [5], reactive scattering [14] could also occur from a reaction

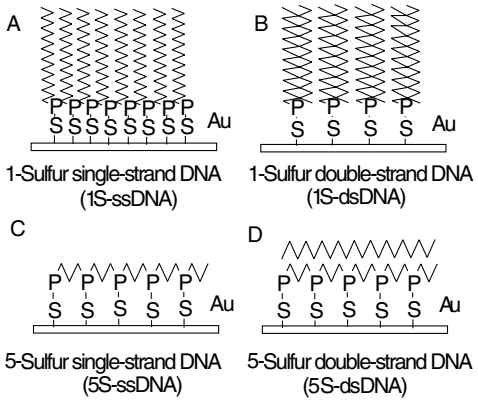


FIG. 2. Self-assembled monolayer (SAM) samples of DNA chemisorbed on a gold substrate.

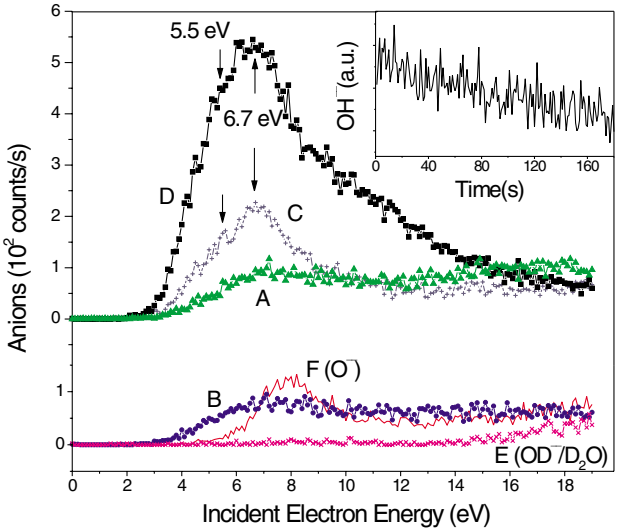
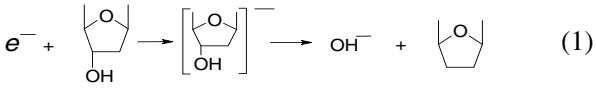


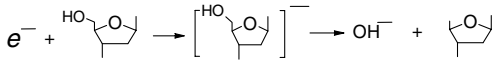
FIG. 3 (color online). Dependence of the OH^- yields on incident electron energy for a SAM of *A*, 1S-ssDNA; *B*, 1S-dsDNA; *C*, 5S-ssDNA; and *D*, 5S-dsDNA. *E* represents the yield of desorbed OD^- from a six monolayers water film on Pt [13] and F, the desorbed O^- yield from a 5S-dsDNA film [11]. The inset shows the time dependence of the OH^- signal from a 1S-ssDNA film recorded at an incident electron energy of 7 eV.

between the O^-, produced via DEA to the phosphate group, and the adjacent deoxyribose unit. In this case, the OH^- yield function would bear a resemblance to that for O^- production from which it is derived [14]. However, the O^- yield functions recorded from the type of SAMs investigated in the present experiment (e.g., curve *F* in Fig. 3) [11] and for condensed multilayer films of DNA under similar conditions [5] are different from those shown in curves *A* to *D* of Fig. 3; the yield function from a multilayer film exhibits a single broad peak centered about 9.5 eV followed by a monotonic rising signal beyond 14 eV. Furthermore, in the experiments of Refs. [5,11], the threshold for O^- formation lies close to 5 eV, whereas that for the OH^- signal from SAMs lies at 2.0 ± 0.3 eV. *This finding discards the possibility of reactive scattering below* 5 eV. We therefore suggest that *the* OH^- *signal arises essentially from direct DEA.* Since OH is present only in DNA at the terminal sugars and phosphate groups of the backbone (see Fig. 1), we are left with the possibility of dissociation of a local transient anion at these two positions, i.e., DEA via the reactions



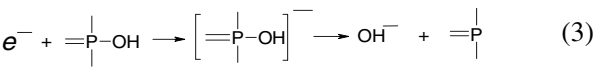
(1)

at the $3'$ end,



(2)

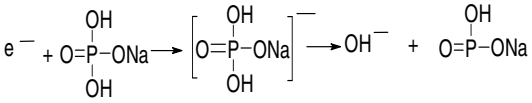
at the $5'$ end, and



(3)

within the backbone.

We can determine which of these reactions produces the OH^- desorption by considering film composition and orientation and anion escape probability [15]. As shown previously [16], with the exception [15] of F^-, anions desorbed by LEE impact on molecular solid films arise principally from the surface region, i.e., from within about 1 nm from the vacuum-solid interface [15–17]. This surface sensitivity, which in our case depends on OH density near the vacuum-DNA interface, is due to the short penetration depth of the anions [15]. Considering close-packed assembly of the strands, the surface density of terminal deoxyribose with OH is about $5 \times 10^{13}/\text{cm}^2$ in configurations 2(a) and 2(b), whereas in Figs. 2(c) and 2(d), it is approximately $5 \times 10^{12}/\text{cm}^2$ and $10^{13}/\text{cm}^2$, respectively. Thus, if the majority of the OH^- signal arose from reactions (1) and (2), the yield from configurations with high surface sugar density [i.e., 2(a) or 2(b) should be much larger compared to the one from configurations 2(c) and 2(d)]. Experimentally, the opposite is observed: at 6.5 eV the signal decreases by a factor of 2.6 in going from 2(c) to 2(a) or 2(b) and by a factor of 6.5 in going from 2(d) to 2(a) or 2(b). Furthermore, according to curves *A* and *B* in Fig. 3, the magnitude of the OH^- signal and the yield functions from the surface region of 1S-ss and 1S-ds DNA are the same as if the source of DEA were the same. In both of these SAMs, the phosphate group density near the surface is the same, but configuration 2(a) has only sugars with OH in the $3'$ position, whereas configuration 2(b) has an equal admixture of sugars with OH at the $3'$ and $5'$ positions. Since OH^- desorption via DEA at the end of a chain and in a ring structure are expected to be different, it would be highly fortuitous that these different OH positions produce *the same yield function* with the *same magnitude. Therefore, if the* OH^- *signals from* $3'$ *and* $5'$ *positions are different, they must be small compared to the signal from the phosphate groups in order to produce curves A and B in Fig.* 3, *which are identical.* In fact, in experiments on solid films of the two different sugar molecules (i.e., 3-hydroxytetrahydrofuran and α-tetrahydrofurfuryl alcohol) corresponding to the sugar moieties of reactions (1) and (2), *no* OH^- *anion* was observed to desorb by electron impact [17]. Similarly, no OH^- ions are formed by electron impact on gaseous deoxyribose [18]. Thus, *present results strongly suggest that the source of* OH^- *in our DNA arises from the phosphate group.* In configurations 2(a) and 2(b), this group lies beneath the surface sugars, so that OH^- ions produced at this location are likely to scatter on surrounding DNA components, lose kinetic energy, and often end up with insufficient energy to escape the induced polarization potential and emerge into a vacuum. However, in 2(c) and 2(d), the OH′s of the phosphate groups lie closer to the DNA-vacuum interface. In this case, OH^- can escape more easily into vacuum causing an increase in the signal as seen in Fig. 3, curves *C* and *D*. The increase in OH^- yields in going from *C* to *D* may be partly due to the increase of the number of phosphate groups, but reduction of the average image force of the metal on departing OH^- in the larger DNA should also contribute to this gain in magnitude. In curves *A* and *B* of Fig. 3, the energy shift of the main peak and disappearance of the structure seen in curves *C* and *D* may be attributable to OH^- scattering prior to desorption and the dependence of the capture cross section on the angle of incidence of the electron beam with respect to the axis of DNA. *Thus, all of our results are consistent with reaction* (3). Furthermore, the DEA reaction



(4)

has recently been observed [11]. The OH^- yield formation exhibits a single broad peak with a maximum about 8 eV and thus confirms the existence of an intermediate anion state analog to that in Eq. (3), leading to OH^- production. In each of these cases, the additional electron could localize temporarily in antibonding σ^* orbitals of the molecule. Furthermore, in trimethylphosphate [4], a surrogate for the

DNA phosphate group, and in recent density functional theory studies of electron attachment to a sugar-phosphate-sugar unit of DNA [19], electron localization into the lowest antibonding σ^* orbitals was found to lead to DEA. We therefore suggest that the 5.5 and 6.7 eV resonances in curves *C* and *D* of Fig. 3 result from core-excited resonances formed by a positive ion core binding two electrons in σ^* orbitals.

Finally, we note that in our previous electron impact experiments [5] on *physisorbed* DNA films, OH^- formation via direct DEA to the phosphate unit was shut off by replacing the counterion H^+ at the oxygen position by Na^+. However, OH^- desorption was still observed, but the yield function was different from any of those shown in Fig. 3. It resembled that of the O^- yield and had a threshold at 5 eV. It was suggestive of a two step process: formation of O^- via DEA to the phosphate group followed by reactive scattering of the O^- ion with the nearby deoxyribose unit.

In summary, we have shown that below 19 eV electron impact on DNA with OH in the phosphate unit produces OH^- via DEA to this unit in the backbone. Between 2 and 5 eV, this process occurs exclusively via direct DEA [i.e., reaction (3)]. Above 5 eV, direct DEA to the phosphate unit is still the dominant mechanism, but we cannot completely rule out a possible contribution to the OH^- yield arising from reactive scattering [5,14] of O^-. We therefore conclude that the phosphate-counterion part of DNA plays a significant role in LEE induced DNA damage. More generally, present knowledge indicates a dependence of DNA fragmentation on the method of preparation of the molecule and provides some clues into the task ahead to deduce, from experiments performed under well defined laboratory conditions, the effects of LEEs on DNA under physiological conditions.

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