Dissociative electron attachment to hydrated single DNA strands

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The present experiments concern electron interactions with a film of short single strands of DNA covered by 3 monolayers of water, which corresponds to 5.25 water molecules per nucleotide. We report on the desorption of H^-, O^-, OH^- from this target induced by 3–20 eV electrons. Below 15 eV, these anions emanate principally from a new type of dissociative core-excited transient anions formed via electron capture by a DNA-H_2O complex. A smaller portion of the H^- desorption signal arises from weakly bonded H_2O molecules. The overall anion yield from DNA is increased by a factor of 1.6 owing to the presence of water.

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INTRODUCTION

When high energy particles interact with living cells, they produce large quantities of low energy ($E<30$ eV) electrons (LEE) [1]. For this reason and because of the considerable magnitude of the cross sections for electronic excitation in electron-biomolecule scattering [2], these secondary particles are expected to play an important role in radiobiology and its applications. Thus, to understand in more details the direct effects of radiation, numerous calculations [3,4] and experiments [2] have been performed on LEE-induced damage to DNA and its constituents. It is now established [2] that below 15 eV, strand breaks, base release, and fragmentation producing anion radicals, occur essentially via the formation of transient anions located on specific DNA components (i.e., bases, sugar, and phosphate group). These transient states can dissociate (i.e., decay by dissociative electron attachment, DEA) or emit the additional electron, leaving the molecular site unaltered or in an electronically excited state. If the latter state is dissociative, bond scission can occur, so that both decay channels lead to damaged DNA. Furthermore, the interaction leading to DEA has been shown to be fairly independent of DNA chain length and hydrogen bonding between DNA strands [5–8]. Information obtained on DEA to short single DNA strands can therefore be transferred to more complex configurations of the molecule.

The experiments that led to our comprehension of the mechanisms giving rise to DNA damage were performed under dry high vacuum conditions, which do not take into consideration the hydrated and aerobic environment of the cell. It is therefore crucial, if we are to apply our knowledge of LEE-DNA interactions to practical problems in radiation protection and therapy [2,9], to show how these fundamental mechanisms are affected and modified in the presence of vital cellular components, particularly H_2O, O_2 and the histone proteins, which are in contact with DNA. As the first step toward this goal, we present here the results of LEE-induced desorption of H^-, O^-, and OH^- from a thin film of a short DNA strand into which water molecules have been absorbed.

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EXPERIMENTAL SETUP

The investigations are performed with the tetramer GCAT, composed of the bases guanine (G), cytosine (C), adenine (A), and thymine (T). This compound was purchased from Alpha DNA (Montreal, QC) and purified by high performance liquid chromatography (with standard deviation of 10%). The aqueous solution of the sample is deposited on a clean tantalum substrate, frozen, lyophilized with a hydrocarbon-free sorption pump and transferred into an ultrahigh vacuum system. The average thickness of the film is calculated to be about 1.6 (±0.3) nm (i.e., about 2 monolayers) which corresponds to a surface density of $\sim 6\times 10^{14}$ nucleosides/cm^2. The DNA solution is prepared without any added salt, so that the negative charge on one of the oxygens of the phosphate group is mainly counterbalanced by a proton (H^+ from H_2O) [6,8].

A detailed description of sample preparation and the present experimental arrangement can be found in Ref. [8]. Here only a brief description is given. The apparatus consists of two chambers: a load-lock chamber ($\sim 1\times 10^{-9}$ Torr) with a multiple-sample holder, to which 16 samples can be mounted and transported onto a rotary feedthrough in the main chamber ($\sim 2\times 10^{-10}$ Torr). In the latter, a LEE beam produced by a modified electron gun (Kimball Physics Inc. ELG-2) is focused on a 1.5 mm^2 spot with an energy resolution of 0.5 eV full width at half-maximum (FWHM) and an electron current of about 10 nA. Electrons with kinetic energy below 20 eV impinge onto the sample in the horizontal plane at an incident angle of 70° to the surface normal. The electron energy scale is calibrated within an estimated error about ±0.3 eV by taking 0 eV as the onset of electron transmission through the film [10]. Desorbed anions are analyzed by a quadrupole mass spectrometer (Extrel 150-QC), which is positioned perpendicular to the film surface.

The water vapor (i.e., H_2O, D_2O or $H_2\,{}^{18}O$) is introduced through a stainless-steel tube connected to a gas-handling manifold and adsorbed on a film of oligonucleotides cooled at 90 K by liquid nitrogen. The manifold consists of a precision-leak valve connected to small expansion volume, where the absolute pressure can be measured by a barometer. This volume is further connected via an admission valve to a small tube having an opening located in front of the sub-

strate. The number of molecules which condensed on the substrate is determined within ±50% by calibrating the differential pressure drop in the volume required to form a 1 monolayer, assuming a sticking coefficient of unity and no change in growth mode for adlayers. One monolayer (ML) of H_2O is a hydrogen-bonded bilayer with a surface density of $\sim 1.05 \times 10^{15}$ cm^{-2} [11]. The water samples consist of triply distilled water degassed by three freeze-thaw cycles under vacuum.

RESULTS AND DISCUSSION

In previous work, we reported detailed studies on LEE impact on a thin solid film of GCAT oligomers [8,12]. Below 15 eV, anion desorption by electron impact occurred via the formation of dissociative core-excited transient anions on specific subunits of the tetramer, which produced broad peaks in the H^-, O^-, OH^- CN^-, and OCN^- yield functions. Above 15 eV, nonresonant dipolar dissociation (DD) dominated the desorption yields. The H^- ion was by far the most abundant anion fragment desorbed from GCAT. The origin of the H^-, O^-, and OH^- yields was established from comparison of gas- and condensed-phase anion desorption measurements on isolated components of DNA and longer single and double stranded DNA [2,6,7] with those from GCAT [8]. A pronounced peak at 9.2 eV in the H^- yield function was ascribed to H^- production at the carbon site of nucleobases [8]. The O^- signal originated from the doubly bonded oxygen of the phosphate group and the OH^- signal from the protonated phosphate group and the terminal OH's on the sugar moiety [8].

The energy dependence of DEA to H_2O, including the distribution of H^- kinetic energies, is well characterized, both in the gas [13] and condensed [14] phases. The ground electronic state ($^1A_1$) of isolated water molecule has the $(1a_1)^2$ $(2a_1)^2$ $(1b_2)^2$ $(3a_1)^2$ $(1a_1)^2$ $(1b_1)^2$ configuration. The lowest unoccupied molecular orbital ($4a_1$) lies near 7 eV above the ground state, so that the probability of electron attachment to a water molecule to create a one-particle (shape) resonance, is relatively low. The formation of core-excited resonances (two-particle one-hole states), where incoming electron forms a $(4a_1)^2$ electron pair by promoting another electron from an occupied orbital is preferred [15]. The calculated and measured gas-phase excitation energies are 6.65 eV, 9.25 eV, and 12.75 eV (±0.3 eV) for the configurations $^2B_1$: $(1b_1)^{-1}(4a_1)^2$, $^2A_1$: $(3a_1)^{-1}(4a_1)^2$ and $^2B_2$: $(1b_2)^{-1}(4a_1)^2$, respectively. Each of these states generates H^-, O^-, and OH^- [13].

The desorption of H^-/D^- has been also observed in thin films of condensed water [14]; the yield of the $^2B_1$ anion peaks at 7.4 eV, as seen from Fig. 1(a). The $^2A_1$ state appears with a shoulder around 9 eV. The higher energy of the $^2B_1$ state was ascribed to perturbation of electronic structure of water upon condensation [14]. The desorption of O^- and OD^- anions from amorphous D_2O films was observed only at electron doses ($>7.5 \times 10^{14}$ electrons/cm^2) [16] much larger than those administered in the present experiment. These anion yields exhibit a very weak broad structure between 5 and 12 eV, mainly as a result of DEA to molecules

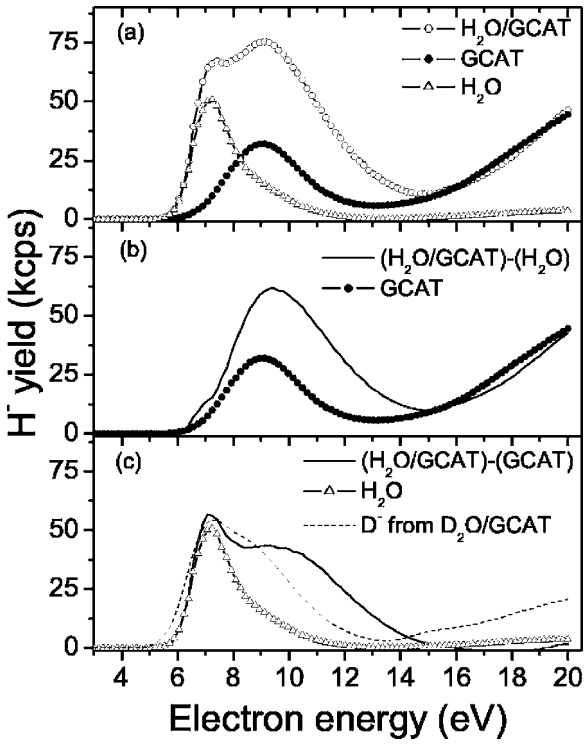


FIG. 1. (a) The H^- ion yield functions obtained from GCAT (solid circle), 3 ML of water (open triangle) and two-component films, H_2O/GCAT (open circle). (b) The H^- ion yield function from a H_2O/GCAT film from which the ion yield of H^- recorded for H_2O is subtracted. (c) The H^- ion yield function from a H_2O/GCAT film from which the ion yield of H^- recorded for GCAT is subtracted. The dashed curve in (c) represents the yield function of D^- desorption from D_2O/GCAT film.

synthesized during electron impact. At higher energies, all anion desorption yields show a monotonic increase due to DD.

The hydration of DNA is considered to be composed of two regimes: primary hydration involving strongly bonded water molecules (<9 water molecules per nucleotide) and secondary hydration shells involving more loosely bonded water molecules (from 9 up to 20 water molecules per nucleotide) [17]. Monitoring the vibrational modes, affected by water in infrared absorption experiments on DNA films, showed that the attachment of water molecules is located at many different positions with different binding strengths around DNA [18]. The strongest binding sites are located at the oxygens of the phosphate group, another one lies near the oxygen on the sugar ring and the weakest one resides on the bases [18]. Although the ionic phosphate group is responsible for the majority of the first layer of hydration, adenine and guanine can also absorb some of the water of primary hydration [19].

In the present work 3 ML of water are deposited on GCAT; this gives an average of 5.25 H_2O molecules per nucleotide adsorbed on oligomer films. This number does not include the 2.5 structural H_2O molecules per nucleotide, which cannot be removed from DNA under vacuum conditions [17]. Thus, assuming a uniform water distribution, our two-component films represent DNA with the addition of 60% of the first hydration shell.

Figure 1(a) presents H^- ion yields obtained from a pure film of the GCAT oligomer, a 3 ML thin film of H_2O and a two-component target consisting of water and GCAT. The ion yields obtained from the homogeneous films are in excellent agreement with previous experimental data [8]. The yield function of H^- observed from the H_2O/GCAT film displays two prominent peaks that are separated from one another by about 2 eV. The largest peak at 9.3 eV appears to be associated with the signal arising from GCAT, which exhibits a smaller peak also at 9.3 eV. The first feature peaking at 7.3 eV can be associated to DEA via resonant capture of the electron in the $^2B_1$ state of H_2O. Such characteristics arise from H_2O molecules embedded in an amorphous water ice environment [14]. In the present experiments, this was confirmed by two independent measurements showing that the H^- signal at 7.3 eV (1) rises with increasing H_2O coverage and (2) diminishes much faster than the other signals with bombardment time, presumably owing to desorption via intramolecular vibrational excitation coupling to the H_2O-H_2O surface bonds [20]. The existence of such "weakly bonded" water molecules suggests that some regions of the DNA, absorb larger quantities of water molecules than others.

Figures 1(b) and 1(c) present the anion yield functions obtained by subtraction of the H^- desorption signal observed for pure H_2O and GCAT films, respectively, from the H_2O/GCAT curve in Fig. 1(a). The yield functions of GCAT and H_2O are also traced in Figs. 1(b) and 1(c), respectively, for comparison. Subtraction of the H_2O signal from that of the mixture film should have led to the yield function of GCAT, if the resulting signal arose from a linear combination of desorption yields of both components. This is not the case; the resulting differential yield function has a larger magnitude and extends to higher energies, indicating that it arises completely or partly from another type of dissociative transient anion. The latter can be seen as a perturbation of the original anion formed with a base of GCAT, or as a perturbation of the original H_2O^- anion, by the interaction of H_2O with the oligomers; it can also be seen as a new type of anion whose parent is a complex resulting from the interaction of H_2O with DNA. For simplicity, we adopt this latter concept and now refer to the parent state of this new anion as the GCAT·H_2O complex. Similarly, subtracting the GCAT signal from that of the mixture film does not entirely reproduce the H_2O yield function, but results in a yield function having an additional broad peak around 10 eV. This peak represents the signal arising from the GCAT·H_2O complex, since any contribution from intact GCAT has necessarily been subtracted. This new core-excited resonance, lying in the 9–10 eV region, is different in magnitude and width from the 9.3 eV resonance in pure GCAT. Its existence is not too surprising in view of the strong hydrogen bonds between nucleobases and gaseous water, as inferred from infrared laser spectroscopy studies and *ab initio* calculation [21]. Additionally, the average enthalpy and the activation energy for desorption of the water strongly bounded to CsDNA was obtained by differential scanning calorimetry [22]. The enthalpy, which is a measure of the energy difference between the bound and unbound states of the water molecule, was measured to be 0.32 ± 0.10 eV/H_2O. The activation energy,

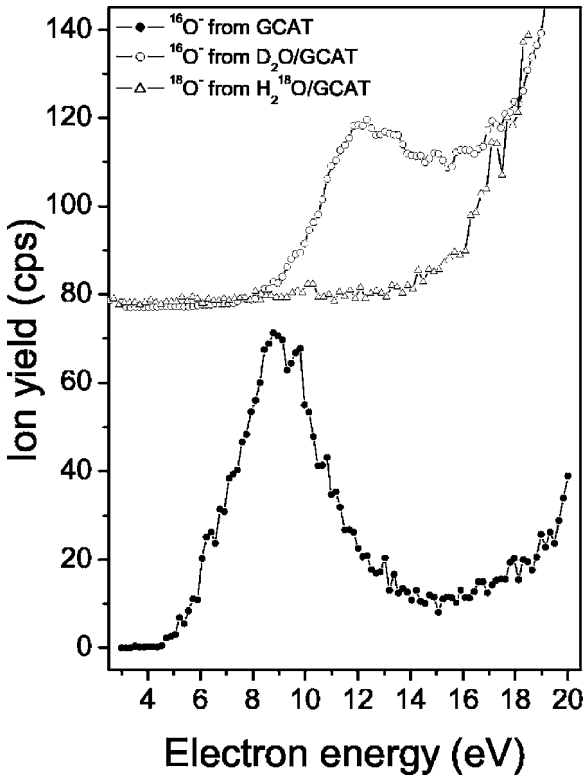


FIG. 2. The O^- ion yield as a function of incident electron energy obtained from a pure GCAT (a lower curve) and D_2O/GCAT and $H_2{}^{18}O$/GCAT films. The curve baselines for anions desorbed from H_2O/GCAT films have been shifted vertically for clarity.

which is a measure of the height of the energy barrier the water molecule must overcome for desorption, was estimated to be 0.63 ± 0.04 eV/H_2O. These measurements demonstrate the existence of water strongly bonded to DNA.

The formation of a GCAT·H_2O complex is expected not only to influence H^- desorption from the bases of GCAT, but also H^- desorption from the water molecule. The contribution arising exclusively from the water adlayers and the perturbation to the hydrogen anion yield induced by GCAT can be seen by condensing D_2O instead of H_2O onto the GCAT film. The D^- signal arising from a mixture film in which H_2O has been replaced by D_2O can be seen as a broad feature near 9 eV in the dashed line in Fig. 1(c). Normally, D_2O and H_2O films produce the same yield functions [16]; as seen from Fig. 1(c) the D^- signal is appreciably modified by contact with GCAT.

The formation of transient anions from GCAT·H_2O complexes is even more obvious in the O^- and OH^- yield functions. In Figs. 2 and 3, we compare the yield function for O^- and OH^- anions desorbed from pure GCAT and isotopically labeled H_2O/GCAT mixture films. In both cases, the 9-eV resonance is replaced by a new one peaking near 11–12 eV and having a reduced width (i.e., 3.3 eV FWHM for the O^- peak and 5.2 eV FWHM for the OH^- peak) compared to 4 and 6.1 eV FWHM peaks in the yield function for O^- and OH^- desorption from GCAT, respectively. The possibility that O^- and OH^- desorb from weakly bonded water molecules can be excluded owing to the fact that (1) the yield

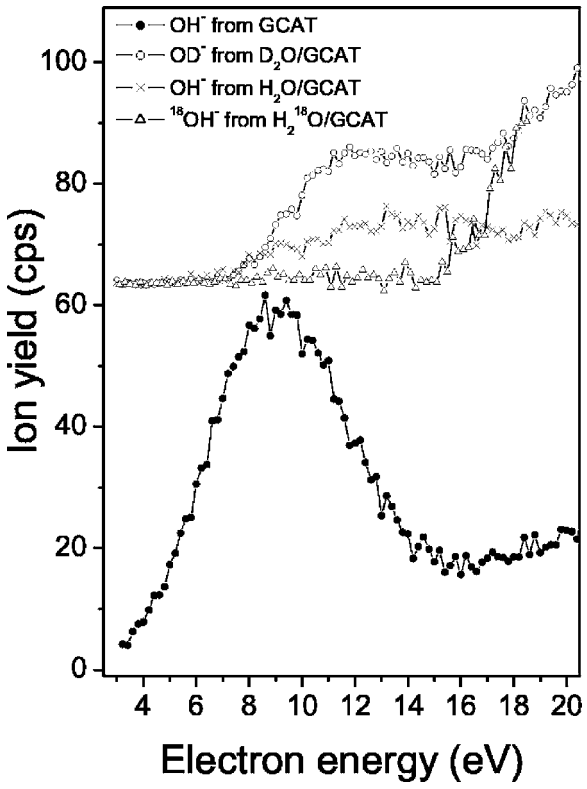


FIG. 3. The OH^- ion yield as a function of incident electron energy obtained from a pure GCAT film (lower curve). The upper curves show the OD^-, OH^-, and $^{18}OH^-$ energy dependences, respectively, obtained from a D_2O, H_2O, and $H_2\,^{18}O$/GCAT films. The curve baselines for anions desorbed from H_2O/GCAT films have been shifted vertically for clarity.

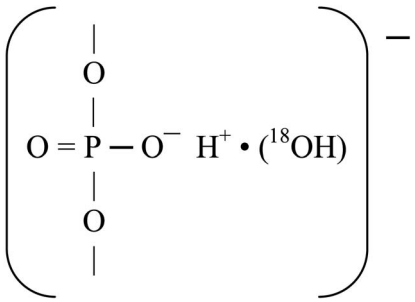
functions for the H_2O/GCAT film do not resemble those observed from pure films of H_2O and D_2O [14,16]; (2) the signals from mixture films decrease with exposure to the electron beam as opposed to that of pure water ice films [16]; and (3) the magnitude of the signals [16] is much larger in the case of mixture films.

Since the O^- yield function from H_2O (or D_2O)/GCAT films is very different than that from the pure GCAT films (Fig. 2), it is probable that the O^- signal also arises principally from either perturbed water in the complex or from the perturbed phosphate group of GCAT, but as described below that signal could be strongly suppressed. It is known that addition of water to dry DNA results first in the binding of H_2O to the phosphate group [18]. The O^- signal, which emanates exclusively from this phosphate unit [6,7], could therefore be considerably attenuated by additional water clustering at this location. On the other hand, since the O^- scattering cross section from H_2O is much larger than that for H^-, energy losses due to scattering, which decrease desorption yields, should play a much less important role in H^- desorption. Furthermore, the bases constitute the last sites to be filled by H_2O in DNA [18]. Aggregation of water molecules is therefore not expected to be significant on the bases from which a considerable portion of the H^- signal emanates [6–8].

To elucidate this question we have performed an experiment where the GCAT molecules were covered with $H_2\,^{18}O$. The upper panel in Fig. 2 compares the signal of $^{18}O^-$ desorption from the GCAT·$H_2\,^{18}O$ complex with one from the corresponding process in H_2O/GCAT film. The present $^{18}O^-$ yield function resembles exactly those observed for O^- from pure film of water [16]. Thus it is immediately obvious that oxygen anion desorption from water is completely restricted to the signal above 14 eV, while the signal occurring in the 11–12 eV region arises from transient anions formed via electron capture by the GCAT·H_2O complex. Moreover, the O^- signal arises from the GCAT portion of this complex.

The two upper curves in Fig. 3 were recorded with a film of GCAT covered by 3 ML of D_2O containing 20% H_2O. As seen from these curves, GCAT covered with D_2O molecules exhibits a OD^- desorption signal similar to that of OH^- from H_2O coverage [16], thus indicating once again that the signal arises mainly from H_2O/GCAT complexes. Furthermore, the OD^- signal is about 3 times higher than the OH^- signal, which is close to the ratio of 4:1 in the amount of condensed D_2O and H_2O. The measured lower ratio from the yields is probably due to the smaller mass of H^-, which provides more kinetic energy for desorption, and the different sensitivity of the mass spectrometer to H^- and D^- ions. These effects are also reflected in the higher DD yield in OD^- compared to that in OH^- above 15 eV. Thus, within the OH^- ion desorbing from the complex, the hydrogen atom essentially arises from H_2O bonded to GCAT. On the contrary, oxygen atoms emanating from GCAT·H_2O have their origin in the GCAT portion of the complex.

Further evidence that oxygen atoms emanate from the GCAT portion is provided by the curve in Fig. 3, which exhibits the $^{18}OH^-$ signal desorbed from $H_2\,^{18}O$ condensed on a GCAT film; the absence of the 12 eV resonance in this yield function indicates that the oxygen in OH^- ion desorbing from H_2O/GCAT mixture films comes from GCAT. On the other hand, the OD^- data in Fig. 3 establishes that the hydrogen in OH^- desorption from H_2O/GCAT has its origin in the H_2O of GCAT·H_2O. We are therefore led to the conclusion that the transient anion of the complex decays on a potential energy surface, which involves a hydrogen atom from H_2O and oxygen from GCAT. This result is not surprising, since it is already known that LEE impact on dry desalted DNA results in OH^- desorption from the protonated phosphate group [2,7]. In other words, in the presence of $H_2\,^{18}O$ that group forms the transient anion



and it is the OH^- from the counter ion which desorbs into vacuum. In any case, the bonding between O^-H^+ and ^{18}OH would be insufficient to produce a dissociative potential energy surface capable of imparting sufficient kinetic energy

to overcome the induced polarization potential and propel $^{18}OH^-$ in vacuum. On the other hand, formation of a core-excited resonance involving a σ^* bond on the phosphate group of DNA [2,7] can impart eV's of energy to OH^-. Thus, the 12 eV resonance is not observed with ^{18}O labeled water, but when D_2O binds to the phosphate group the desorption of OD^- from the dissociative P-OD σ^* bond is observed.

CONCLUSION

We have performed anion desorption experiments stimulated by the impact of 3–20 eV electrons on a short single strand of DNA composed of the four bases. Two monolayers of the tetramer were successively covered with 3 ML of amorphous ice of different isotope content ($H_2\,^{16}O$, $D_2\,^{16}O$, and $H_2\,^{18}O$). Comparison of H^-, O^-, and OH^- desorption data from the pure tetramer with signals from tetramers covered with water and isotopically labeled water made it possible to postulate the formation of a transient anion whose parent is a complex made of the tetramer and a water molecule. As expected from the experiment of Falk *et al.* [18,19] on the binding energy of water to DNA, the binding site of this complex is located at the negatively charged oxygen of the phosphate group. Such a complex permits the formation of a transient anion located on the phosphate group, which decays by O^- desorption, and more specifically, OH^- desorption by rupture of the P-O^- bond. H^- desorbs upon LEE impact by dissociation of a transient anion of the complex which causes bond cleavage on the H_2O portion. Thus, both H_2O and the tetramer are perturbed by their mutual binding interaction and consequently the anions resulting from temporary electron attachment. The signature of the perturbation imposed on the tetramer portion is seen in the O^- and OH^- yield functions, whereas that imposed on the H_2O molecule appears in the H^- signal.

In summary, DNA damage via DEA induced by LEE is increased by a factor of about 1.6 when an amount of water corresponding to 60% of the first hydration layer is added to vacuum-dried DNA. This enhancement is induced by the formation of new dissociative transient anions, which arise from the interaction between H_2O and DNA. Although the magnitude of this enhancement is significant, it is much smaller than the modification in various yields of products caused by the first hydration layer of DNA during the radiochemical events [23] that follow the deposition of the energy of LEE in irradiated cells.

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