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Hydrated Electrons React with High Specificity with Cisplatin Bound to Single-Stranded DNA

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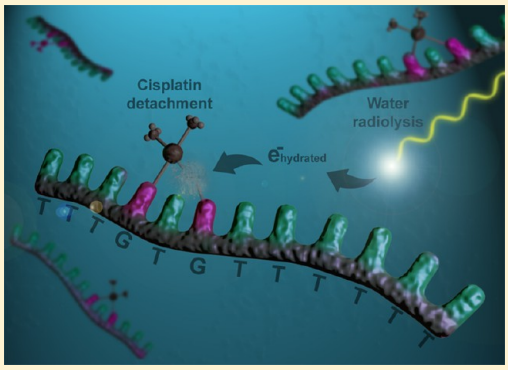
ABSTRACT: Short oligonucleotides TTTTTGTGTTT and TTTTTTTGTTT in solution with and without cisplatin (cisPt) bound to the guanine bases were irradiated with γ-rays at doses varying from 0 to 2500 Gy. To determine the effect of hydrated electrons from water radiolysis on the oligonucleotides, we quenched $^{\bullet}$OH radicals with ethylenediaminetetraacetic acid (EDTA) and displaced oxygen, which reacts with hydrated electrons, by bubbling the solution with wet nitrogen. DNA strand breaks and platinum detachment were quantified by gel electrophoresis. Our results demonstrate that hydrated electrons react almost exclusively at the position of the cisPt adduct, where they induce cisPt detachment from one or both guanines in the oligonucleotide. Given the high yield of hydrated electrons in irradiated tissues, this reaction may be an important step in the mechanism of radiosensitization of DNA by cisPt.



INTRODUCTION

Cisplatin (cisPt) or *cis*-diamminedichloroplatinum(II) is a chemotherapeutic agent widely used in the treatment of testicular, ovary, neck, lung, and head cancers.[1−3] cisPt is composed of a single platinum atom bound to two chlorine atoms and two ammonium groups. In blood and interstitial fluid, the chloride concentration is high (104 mM), and thus, cisPt conserves its chlorine atoms and remains intact. In contrast, inside cells, the chloride concentration is considerably reduced (5 mM), which causes the loss of chlorine atoms by hydrolysis, yielding $[Pt(NH_3)_2Cl(H_2O)]^+$ and $[Pt(NH_3)_2(H_2O)_2]^{2+}$. These products react with DNA, RNA, and proteins as well as with phospholipids in the cell membrane.[4−6] Of these reactions, the binding of cisPt to DNA is the dominant process.[5,7] cisPt binds to the N7 purine sites, preferentially on guanine, yielding 60−65% intrastrand GG cross-links, 25% intrastrand AG cross-links, with a small percentage of intrastrand GNG cross-links.[8] Such binding results in structural DNA anomalies such as a 23° unwinding and a 33° bending angle for the case of GNG intrastrand cross-links. The latter inhibits DNA replication and transcription, which can prevent cancer cell growth and lead to apoptosis.[9]

Clinical studies have shown that concomitant chemotherapy and radiotherapy of cancer can enhance the survival rate of patients compared to nonsynchronous treatments.[10−12] Two nonexclusive mechanisms, related to the binding of cisPt in vivo with DNA, have been proposed to explain these results. Studies in cultured cells[13] and tumor-bearing mice[14] suggest that the binding of HMG1 to cisPt adducts may inhibit repair of radiation damage.[7] There is also evidence that the presence of the chemotherapeutic agent bound to DNA can increase the initial yield of radiation damage.[15]

This initial damage to DNA in cells proceeds via two mechanisms referred to as the direct and the indirect effects. In the direct effect, the incident particle interacts directly with DNA components (bases, sugar, and phosphate) to produce radical cations and secondary electrons (SE). In addition, water molecules tightly bound to the DNA undergo ionization but rapidly transfer the electron-deficient "hole" to DNA bases. The vast majority of SE have energies below 20 eV,[16,17] and they in turn can produce large quantities of highly reactive excited molecules, radicals, cations, and anions.[19] The direct effect induced by low-energy electrons (LEEs) has been elegantly demonstrated in experiments that measured the yield of DNA single (SSBs) and double (DSBs) strand breaks as a function of the energy of electrons in the range of 1−20 eV. Under dry conditions, it was found that below 15 eV, the damage results from the formation of transient negative ions.[20−22] More recently, experiments with lyophilized supercoiled plasmid DNA irradiated by LEEs have shown that the yields of SSB and DSB were considerably enhanced in the presence of cisPt.[23] In the indirect effect, radiation produces reactive species in the vicinity of DNA that diffuse and react with it.[24] Because water is the principal component of cells, most of these reactive species arise from water radiolysis. Given that at least 50% of the total damage caused to DNA in irradiated cells arises from the indirect effect,[25] this type of damage is important for understanding cisPt radiosensitization. Experiments with aqueous and dry irradiated plasmids have shown that damages caused by the indirect effect are at least as important as those resulting from the direct effect.[26] The most abundant radicals at 10^{-6} s following irradiation of water are $^{\bullet}$OH, hydrated

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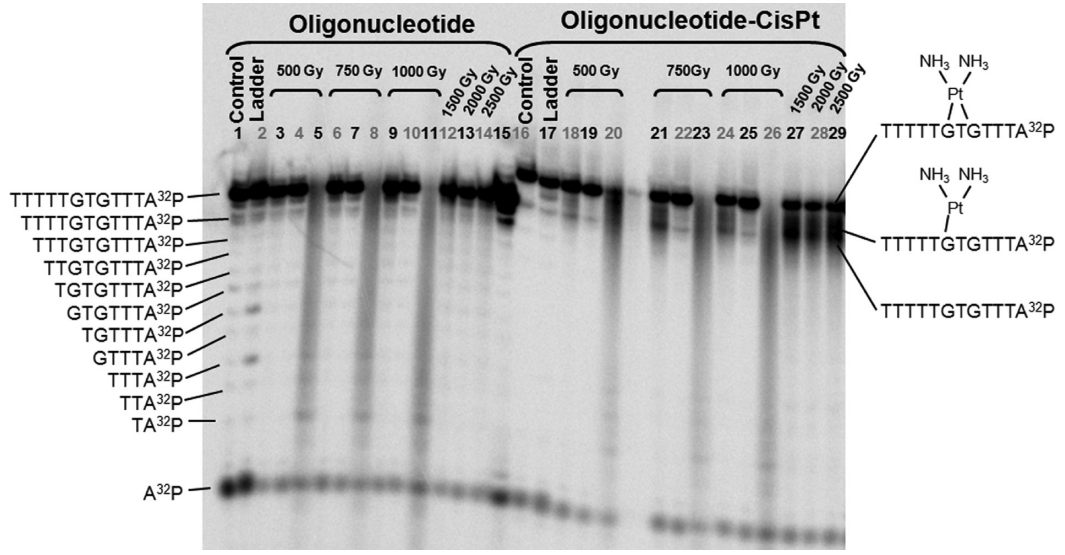

Figure 1. Gel electrophoresis showing the effect of •OH and hydrated electrons on oligonucleotides, with and without the cisPt adduct, as a function of irradiation dose. The first lanes (3, 6, 9, 12, 13, 14, 18, 21, 24, 27, 28, 29) for each radiation dose show the effects of hydrated electrons because the •OH radicals were captured by the scavenger EDTA and O_2 was displaced by bubbling the solution with wet nitrogen. In the second lanes for each dose (4, 7, 10, 19, 22, 25), both •OH radicals and hydrated electrons were scavenged in the presence of EDTA by bubbling the solution with wet N_2O. The third lanes for each dose (5, 8, 11, 20, 23, 26) show the effect of •OH radicals, with hydrated electrons being scavenged by O_2. Lane 15 is not pertinent.

electrons, and •H with G values of 0.24, 0.28, and 0.06 μmol/J, respectively.[27]

•OH radicals are the most reactive species for the induction of DNA damage via the indirect effect.[24] However, before becoming hydrated, LEEs enter in a prehydrated state[19] when their energies become lower than that of the conduction level. In the prehydrated state, those electrons, with lifetimes less than 1 ps, can also react with and damage DNA[18,19] through dissociative electron transfer (DET).[28] Time-resolved femtosecond laser spectroscopy experiments have shown that the purine bases, particularly guanine, are more sensitive to DET than pyrimidine bases.[28] With similar experiments, Lu et al.[8,29] have shown a high DET reactivity of prehydrated electrons with cisPt.

Hydrated electrons are trapped in potential energy wells (−3.2 eV), but they still react with DNA to induce damage on the nucleobases.[30,31] However, they do not produce SSBs or DSBs in normal, unmodified DNA.[32] Nevertheless, hydrated electrons have been found to induce SSBs and interstrand cross-links in double-stranded oligonucleotides having the radiosensitizer 5-bromodeoxyuridine incorporated into mismatch structures.[30,31]

DNA damage induced by cisPt in mouse fibroblast cells has been investigated by mass spectroscopy. Iijima et al. observed the lesion principally at the G-G and A-G sites.[33] This technique has also been used to show the interaction of LEEs with cisPt, which leads to the loss of chlorine from cisPt in a one- and two-step process via dissociative electron attachment.[34]

In the present work, the damages induced by hydrated electrons, generated by water radiolysis, on olignucleotide−cisPt complexes are investigated for cisPt mono- and diadducts.

MATERIALS AND METHODS

Reaction of Oligonucleotides with CisPt. The single-strand oligonucleotides ODN-GTG and ODN-TGT were purchased from the DNA synthesis laboratory at the University of Calgary, Alberta, Canada. CisPt was first dissolved in water at a concentration of 220 μM. The oligonucleotide was then mixed with a solution of 44 μM cisPt to give a ratio of oligonucleotide/cisPt of 1:5. The solution was kept at room temperature for ∼46 h to allow reaction of cisPt with the guanines.

Oligonucleotide−CisPt Purification. The oligonucleotide was purified on a G-25 Sephadex microcolumn followed by high-performance liquid chromatography (HPLC) to separate the different products (oligonucleotide and oligonucleotide−cisPt complex). A linear gradient (0−15% acetonitrile in ammonium acetate) and a column (5 μm ODS A 250 × 6 mm; YMC) were used for the separation.

3′ End Labeling of Oligonucleotides. The oligonucleotides were end-labeled with γ-^{32}P ddATP using terminal deoxynucleotidyl transferase (DTD) and buffer. Afterward, they were purified with a G-25 Sephadex microcolumn.

Experimental Conditions. The final concentration of the oligonucleotide was 0.03 μM in phosphate buffer (pH 7.0, 10 mM). •OH radicals were scavenged with ethylenediaminetetraacetic acid (25 mM EDTA). N_2O was also used to scavenge the hydrated electrons. To minimize scavenging of hydrated electrons by oxygen, the oligonucleotide solutions were bubbled with wet nitrogen gas (purity of 99.998%) for 1 min. Considering the short lifetime of the prehydrated electron (∼10^{-13} s) and the very low oligonucleotide concentration (∼10^{-8} M) relative to the high amount of the hydrated electrons generated over the irradiation time (2.4×10^{-3} M for a dose of 1000 Gy), we expect hydrated electron reactions to be dominant in our solution, while the action of prehydrated electrons is considered negligible. Furthermore, if the prehydrated electron has sufficient time to diffuse and react with the DNA, then the •OH should also be able to react with DNA before being captured by EDTA, and this is not the case, as demonstrated by the absence of DNA strand breaks.

Irradiation. Oligonucleotide solutions were irradiated in a cesium-137 Gammacell with the dose varying between 500 and 2500 Gy (11.6 Gy/min).

Denaturing Gel Electrophoresis. Oligonucleotides were loaded on a 7 M urea denaturing 20% polyacrylamide gel.

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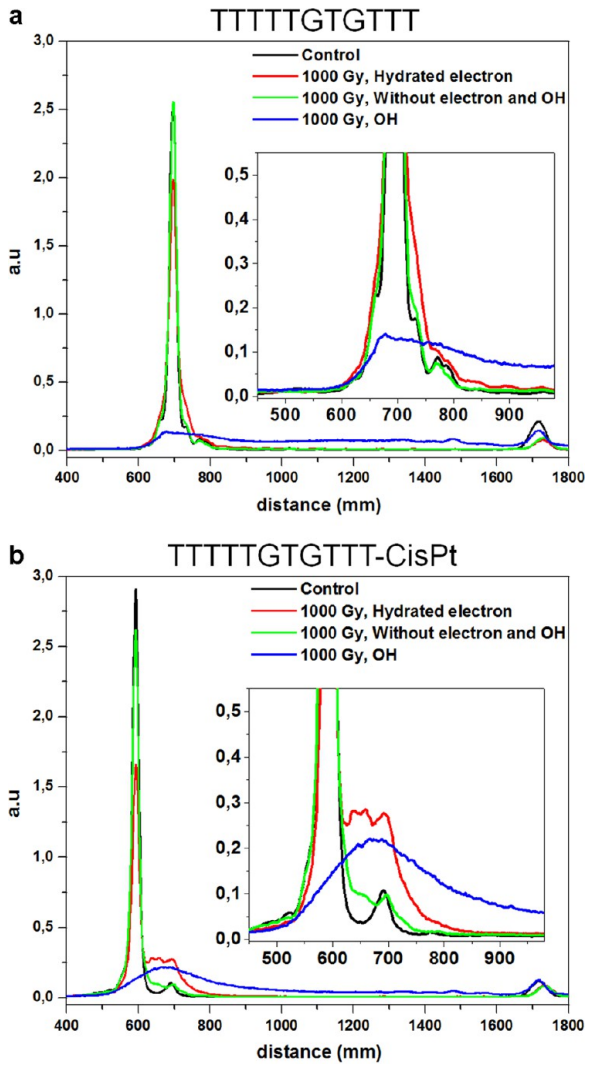

Figure 2. Gel electrophoresis profiles showing the effect of •OH radicals and hydrated electrons on ODN-GTG (a) and ODN-GTG−cisPt (b), following irradiation with1000 Gy. Note the red curve in panel b, which shows the two-step loss of cisPt induced by hydrated electrons.

A molecular weight ladder was produced by Maxam and Gilbert sequencing treatment. An electric field was applied to the electrophoresis gel for 2.5 h. The phosphor screen cassette (Molecular Dynamics Inc.) was exposed to the gel for 16 h and scanned with a Storm fluorescence scanner (Molecular Dynamics Inc.).

RESULTS

Damages Induced to Oligonucleotides, with and without CisPt, by OH Radicals and Hydrated Electrons. Figure 1 shows the action of •OH radicals and hydrated electrons on the single-stranded ODN-GTG and the oligonucleotide−cisPt complex (ODN-GTG−cisPt) in lanes 1−14 and lanes 16−29, respectively. As can be seen, the band corresponding to the intact ODN-GTG−cisPt (lanes 16−29) migrates slower than the band for the unmodified oligonucleotide (lanes 1−14). This shift results from a different charge and geometry of the oligonucleotide containing a cisPt adduct. In lane 1, corresponding to the control (0 Gy) with no cisPt adduct, there are strand breaks (8.8%) that are probably induced by electron emission from ^{32}P. Strikingly, no such damage was observed for the ODN-GTG−cisPt at 0 Gy, even though this complex was labeled with ^{32}P at the same specific activity as the control oligonucleotide.

Figure 2 is a graphical representation of the gel in Figure 1 for an irradiation dose of 1000 Gy. The black curves in (a) and (b) show the control oligonucleotide (700 mm of migration) and ODN-GTG−cisPt (600 mm of migration), respectively, prior to irradiation. In the latter, a small amount of the oligonucleotide (3.1%) lacking the cisPt adduct is observed as a satellite peak at 700 mm. The blue curves in (a) and (b) show that extensive DNA damage is induced by the •OH radical as the principal peak vanishes to give rise to fragments. This occurs for both the oligonucleotides without and with the cisPt adduct. In contrast, the red curves show that the oligonucleotide damage induced by the hydrated electron does not give rise to strand breaks but rather to rupture of the cisPt bond. As shown in Figure 2b, this effect results in two satellite peaks. Our interpretation is that the peak at 650 mm of migration corresponds to an oligonucleotide with one cisPt bond broken and the second peak at 700 mm to the oligonucleotide without cisPt. This suggests an interaction between the hydrated electron and cisPt leading to bond rupture (one or two bonds). Presumably, the liberated cisPt then reacts with water in an exothermic process.[34]

The green curves, where both •OH radicals and hydrated electrons are captured, are similar to the control spectra at 0 Gy (black curves), as expected, but also bear some slight resemblance to the hydrated electron curve, presumably because of the small amount of nonscavenged hydrated electrons. We observe residual breakage of the cisPt bond, corresponding to about 0.13% for one bond broken and 1.4% for two bonds broken.

CisPt Monoadduct Detachment from ODN-TGT Induced by Hydrated Electrons. Figure 3 shows the effect

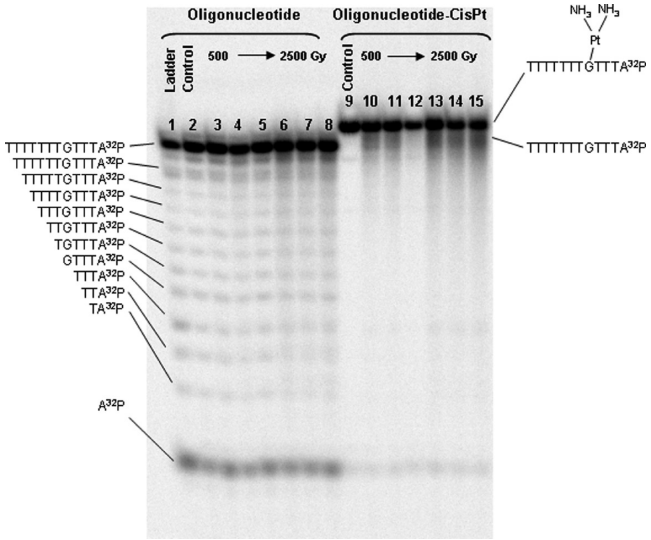

Figure 3. Gel electrophoresis showing the effect of hydrated electrons as a function of the dose of radiation given to the ODN-TGT (lanes 3−8 correspond to 500, 750, 1000, 1500, 2000, and 2500 Gy, respectively) and given to ODN-TGT−cisPt (lanes 10−15 correspond to 500, 750, 1000, 1500, 2000, and 2500 Gy, respectively).

of hydrated electrons for different irradiation doses (0−2500 Gy) on the ODN-TGT (lanes 3−8) and ODN-TGT−cisPt (lanes 10−15). In the oligonucleotide containing a cisPt adduct, we observe as a function of dose a rising signal next to the parental oligonucleotide peak, which appears at the same position as the

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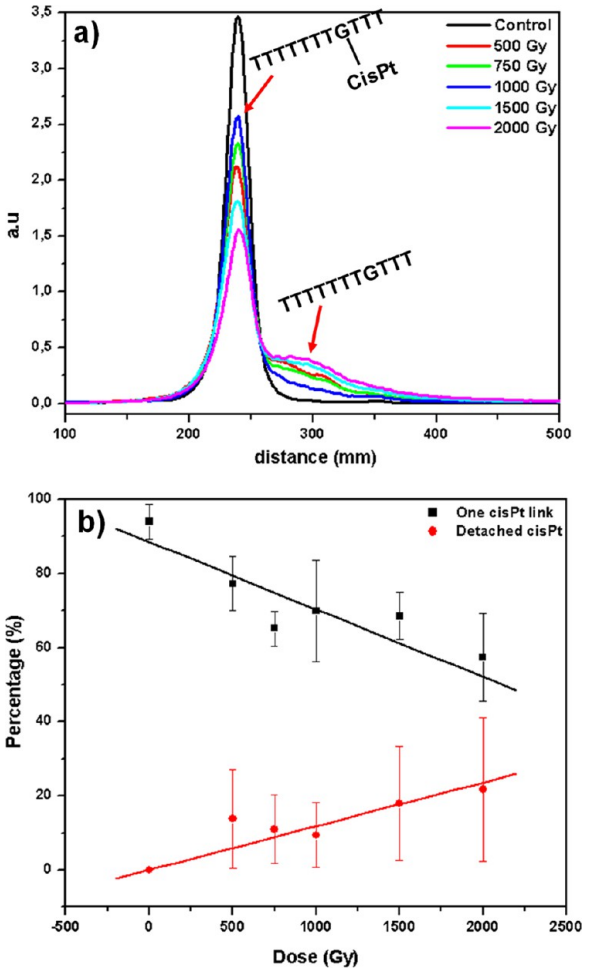

Figure 4. Gel electrophoresis profiles showing the effect of hydrated electrons as a function of the dose on the ODN-TGT−cisPt (a) and the evolution of the ODN-TGT−cisPt and the formation of ODN-TGT as a percentage of total (b).

unmodified oligonucleotide. This signal is therefore attributed to the loss of cisPt from the oligonucleotide. A graphical representation of this detachment is presented in Figure 4a, where the peak at 300 mm of migration represents the oligonucleotide without cisPt. Because there is only a single guanine for attachment of cisPt in this oligonucleotide, only one satellite peak is associated with the rupture of this bond. The quantitative analysis of the total yield of cisPt detachment as a function of the dose was carried out using Lorentzian−Gaussian fit, and the result is shown in Figure 4b.

The loss of the cisPt adduct from the oligonucleotide and the formation of the ODN-GTG are linear functions of the radiation dose. Furthermore, the total amount of oligonucleotide (ODN-GTG plus ODN-GTG−cisPt) declines between 0 and 2000 Gy by only 15%, indicating that very little oligonucleotide is destroyed by the radiation, as would be expected if SSBs were formed.

Effect of CisPt Detachment from the ODN-GTG As a Function of the Irradiation Dose: Contribution of Hydrated Electrons. Figure 5 shows the effect of hydrated electrons for different doses of radiation (0−2000 Gy) on the ODN-GTG (lanes 1−6) and ODN-GTG−csisPt (lanes 7−12). As a function of the dose, we observe a rising signal next to the parental peak resulting from the loss of cisPt from the oligonucleotide. A graphical representation of this signal is presented in Figure 6a. Because there are two guanines serving

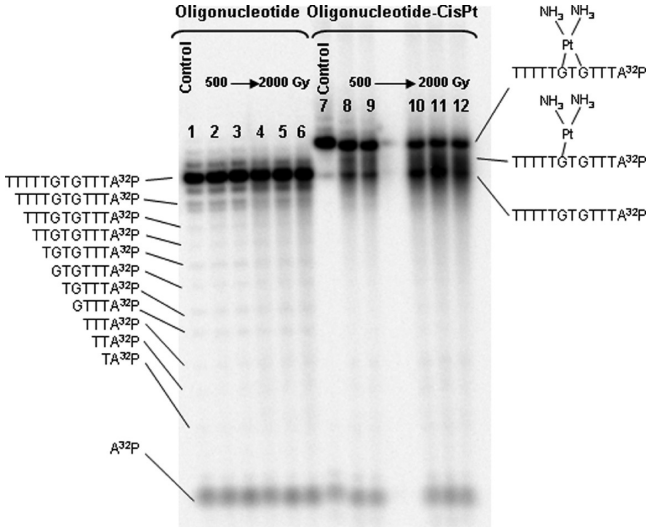

Figure 5. Gel electrophoresis showing the effect of hydrated electrons as a function of the dose to ODN-GTG (lanes 2−6 correspond to 500, 750, 1000, 1500, and 2000, respectively) and ODN-GTG−cisPt (lanes 8−12 correspond to 500, 750, 1000, 1500, and 2000, respectively).

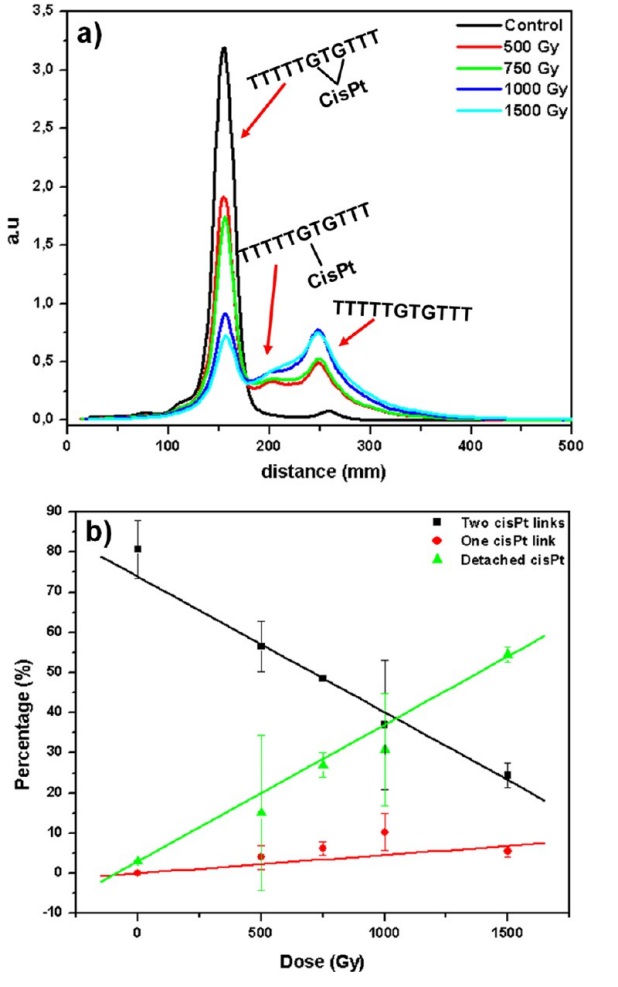

Figure 6. Gel electrophoresis profile showing the effect of hydrated electrons as a function of the dose to ODN-GTG−cisPt (a) and the evolution of the ODN-GTG−cisPt and the formation of ODN-GTG in percentage (b).

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as attachment sites for cisPt, two satellite peaks are associated with the rupture of one or both of these bonds. The quantitative analysis of the total yield of cisPt detachment as a function of the dose is shown in Figure 6b. At doses up to 1500 Gy, the parental peak decreases linearly, and product peaks increase linearly. Very little oligonucleotide containing a single oligonucleotide−cisPt bond was observed. As shown in Figure 6b, the slope of the dose−response curve for the rupture of one of the two cisPt−guanine bonds in the ODN-GTG is 7-fold smaller than the slope for the rupture of both cisPt bonds. Interestingly, the parental peak associated with the complex ODN-GTG−cisPt decreases faster than the one associated with the ODN-TGT−cisPt in Figure 4, indicating that it is easier to detach cisPt when it is doubly bonded to the oligonucleotide than when it is singly bonded.

DISCUSSION

The main effect observed by irradiating the ODN-GTG−cisPt and ODN-TGT−cisPt in solution is the detachment of the cisPt adduct from the oligonucleotide. In the case of ODN-GTG−cisPt, this detachment can occur in either a one- or two-step process. Interestingly, the yield of total cisPt detachment from the ODN-GTG is 3-fold higher than that for the ODN-TGT. This may indicate less bonding stability in the two-bond cisPt adduct with the ODN-GTG. The departure of cisPt does not induce strand break formation even at the sites of attachment of the cisPt. Because the present results can detect only strand breakage and cisPt loss, we cannot rule out possible base damage induced by cisPt detachment. In addition, the reduced cisPt released from the oligonucleotide is expected to be reactive and may also cause base damage.

DET[28] involving the hydrated electron is proposed as the mechanism responsible for the rupture of cisPt−guanine bonds. As presented in the scheme in Figure 7, the hydrated electron is

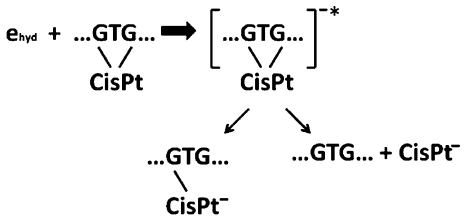

Figure 7. Dissociative electron-transfer mechanism, involving a hydrated electron, resulting in bond cleavage between cisPt and its complex.

captured by the cisPt complex in its lowest unoccupied molecular orbital (LUMO). From ab initio calculations, those orbitals have a repulsive σ^* character along all cisPt coordination axes.[34] This results in possible channels for one or two cisPt−guanine bond cleavage, if the time of electron occupation in that state is sufficiently long (i.e., of the order of a vibrational period of a cisPt−guanine bond).[35] From the rotovibrational ground state, the anion needs a minimum energy to dissociate called the thermodynamic threshold (ΔH) given by the bond dissociation energy, D(A−B), minus the electron affinity (EA) of the fragment on which the electron stabilizes[35]

$$\Delta H_0(B^-) = D(A{-}B) - EA(B) \quad (1)$$

Thus, for DET to occur, the potential energy surfaces of the transient anion along the cisPt−guanine bonds must at least partly lie above ΔH in the Franck−Condon region. During the separation of cisPt from guanine, the fragment with the greater EA is expected to keep the extra electron because it corresponds to the lowest thermodynamic threshold according to the above equation. In Figure 6b, the conversion of the ODN-GTG−cisPt to the ODN-GTG occurs as a linear function of dose, which suggests that a single hydrated electron induces the simultaneous breakage of two cisPt−guanine bonds. A priori, this double bond break in ODN-GTG−cisPt can occur in two ways: either a cisPt−guanine bond is first broken and the remaining fragments react with and dissociate the other cisPt−guanine bond, or the transient anion formed via DET dissociates by simultaneously breaking the two cisPt−guanine bonds. The latter mechanism has been observed by Kopyra et al.[34] using a mass spectrometer as a detector, in LEE impact experiments on gaseous cisPt, containing its original chlorine atoms. They found that a transient anion formed at ∼0 eV dissociates by simultaneously breaking the bonds between Pt and the chlorine atoms. The platinated fragment has a much higher probability to keep the extra electron due to its high electron affinity. This possibility within a ODN-GTG−cisPt is represented in the scheme of Figure 7. Judging from the huge magnitude of cisPt bond scission with respect to that of other bonds within the oligonucleotide, observed in our experiment, hydrated electrons appear to localize preferentially at the site of cisPt, where they undergo DET. This high affinity for hydrated electrons is probably due to the high electron affinity of the cisPt adduct.

CONCLUSION

The present results using a γ-irradiated oligonucleotide−cisPt solution have demonstrated a strong interaction between hydrated electrons and the cisPt monoadduct at the guanine base and the intrastrand adduct bonded between two guanine bases separated by thymine. This interaction leads to hydrated electron capture by the oligonucleotide almost exclusively at the site of binding of cisPt. The transient anion formed by this attachment of the hydrated electron dissociates by breaking one or two cisPt−guanine bonds. In the latter case, cisPt detaches from the oligonucleotide. Although the reaction of cisPt adducts with hydrated electrons does not lead to strand break formation when cisPt detaches from DNA, it may induce base damage at adjacent sites and thus be potentially cytotoxic especially in hypoxic tumor cells because oxygen quenches hydrated electrons.

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Notes

The authors declare no competing financial interest.

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