THE JOURNAL OF PHYSICAL CHEMISTRY B

Article

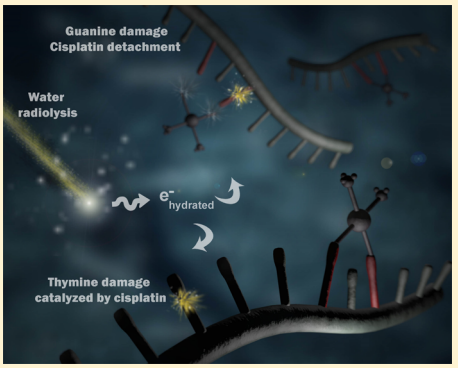
pubs.acs.org/JPCB

Cisplatin Intrastrand Adducts Sensitize DNA to Base Damage by Hydrated Electrons

B. Behmand,* J. R. Wagner, L. Sanche, and D. J. Hunting

Groupe en sciences des radiations, Faculté de médicine et des sciences de la santé, Université de Sherbrooke, Sherbrooke, Québec, Canada J1H 5N4

ABSTRACT: The oligonucleotide TTTTTGTGTTT with or without a cisplatin adduct was reacted with hydrated electrons generated by ionizing radiation. Hydroxyl radicals were quenched with ethylenediaminetetraacetic acid (EDTA), and the solutions were bubbled with wet nitrogen to eliminate oxygen, a scavenger of hydrated electrons. Prior to irradiation, the structure of the initial cisplatin adduct was identified by mass spectrometry as G-cisplatin-G. Radiation damage to DNA bases was quantified by high-performance liquid chromatography (HPLC), after enzymatic digestion of the TTTTTGTGTTT-cisplatin complex to deoxyribonucleosides. The masses of the platinum adducts following digestion and separation by HPLC were measured by mass spectrometry. Our results demonstrate that hydrated electrons induce damage to thymines as well as detachment of the cisplatin moiety from both guanines in the oligonucleotide. This detachment regenerates both unmodified guanine and damaged guanine, in equimolar amounts. At 1000 Gy, a net average of 2.5 thymines and 1 guanine are damaged for each platinum lost from the oligonucleotide. Given the extensive base damage that occurs for each cisplatin adduct lost, it is clear that, prior to undergoing detachment, these adducts must catalyze several cycles of reactions of hydrated electrons with DNA bases. It is likely that a single reaction leads to the loss of the cisplatin adduct and the damage observed on the guanine base; however, the damage to the thymine bases must require the continued presence of the cisplatin adduct, acting as a catalyst. To our knowledge, this is the first time that platinum-DNA adducts have been shown to have catalytic activity. We propose two pathways for the interaction of hydrated electrons with TTTTTGTGTTT-cisplatin: (1) the hydrated electron is initially captured by a thymine base and transferred by base to base electron hopping to the guanine site, where the cisplatin moiety detaches from the oligonucleotide via dissociative electron attachment, and (2) the hydrated electron interacts directly with the platinum-guanine adduct and induces detachment of the cisplatin moiety via dissociative electron attachment. Although the precise mechanism remains to be elucidated, our results provide important insights into the radiosensitization of DNA by cisplatin.



INTRODUCTION

Concomitant chemotherapy and radiotherapy have substantially enhanced tumor control and the survival of patients.[1−4] Radiosensitization by chemotherapeutic drugs is probably due to several factors including an increase in DNA damage, inhibition of DNA repair, diminution of the apoptosis threshold, and perturbation of the cell cycle such that cells remain in the sensitive phases for a longer time.[5] *cis*-Diamminedichloroplatinum(II) or cisplatin is a chemotherapeutic drug used in the treatment of ovary, testicular, lung, head, and neck cancers.[6−8] It binds to the N7 purine sites in DNA, preferentially on guanine, and creates the structural and chemical anomalies which lead to the effects listed above.[9] When nonthermal electrons interact with this chemotherapeutic agent, a shape resonance appears near 0 eV in the gas phase, which leads to dissociative electron attachment (DEA).[10] In a polar medium, such as water, this resonance energy is lower by about 1 eV.[11] Cleavage of the bond between the cisplatin moiety (cisPt) and guanine is also observed on the basis of the fragmentation pattern of Pt-DNA negative ion adducts during electrospray mass spectrometry.[12] Furthermore, electron transfer along the DNA strand, toward the site of the attachment of cisPt adduct, is expected to increase the cross section for DEA at the Pt site.[13]

The damage induced by ionizing radiation results from both the direct and indirect effects. The direct effect is the interaction of either the incident particle or secondary electrons (SE) generated by the primary radiation with DNA and can produce single and double DNA strand breaks or base damage. The vast majority of SE have energies of less than 20 eV.[14,15] Zheng et al.[16] recently studied the effect of monoenergetic electron beams (1, 10, 100, 60 000 eV) on thin films of lyophilized DNA with and without cisPt. They found an enhancement of both single and double strand breaks (SSB and DSB) in DNA modified by cisPt. Since the major component of cells is water (70−80%), the indirect effect of radiation is also important and gives rise to about 50% of the total DNA damage.[17,18] In the indirect effect, ionizing radiation interacts with water molecules and creates the reactive chemical species $^{\bullet}OH$, $H^{\bullet}$, and e_{hyd}^- that can react with DNA and create

Received: February 11, 2014
Revised: April 12, 2014
Published: April 16, 2014

   dx.doi.org/10.1021/jp5014913 | *J. Phys. Chem. B* 2014, 118, 4803−4808

damage. As SE slow down, they start polarizing the electronic orbitals of the surrounding molecules. As they continue to lose energy below ~1 eV, they increasingly polarize the vibrational modes of these molecules, and the phonon modes, to reach a prehydrated state and become a polaron.[19] In this prehydrated state, which lasts for less than a picosecond, the electron becomes hydrated by surrounding water molecules and is trapped in an average potential well of ~ −3.2 eV. It has been shown that the hydrated electron cannot induce strand breaks in unmodified DNA.[20,21] However, according to the results of Behmand et al., based on an electrophoretic assay, hydrated electrons can induce the detachment of cisplatin, when it is bound to an oligonucleotide.[22]

Here, we study the effects of the hydrated electrons generated by γ rays on an oligonucleotide-cisPt complex. Base damage is measured by HPLC following enzymatic digestion of the oligonucleotide. Platinum DNA adducts are measured by HPLC, mass spectrometry (MS), and electrospray ionization tandem mass spectrometry (ESI-MS/MS). Our results show that the presence of cisPt adducts greatly increases the damage to guanine and thymine induced by radiation. In addition to confirmation by HPLC analysis that cisPt detaches from the DNA, our results also indicate that equal amounts of damaged and undamaged guanine are generated for each cisPt adduct that detaches from the oligonucleotide.

MATERIALS AND METHODS

The single strand TTTTTGTGTTT (ODN-GTG) was purchased from the DNA synthesis lab (University of Calgary, Alberta, Canada). The details of the reaction of ODN-GTG with cisplatin and purification giving the ODN-GTG-cisPt complex have been reported elsewhere.[22] Although in cells treated with cisplatin, cisPt-GG cross-links outnumber GTG cross-links, we chose to study the latter adduct because it causes greater DNA structural deformation.

Experimental Conditions. The concentration of the oligonucleotide was approximately 14 μM. On the basis of a rate constant calculation, 99.99% of $^{\bullet}$OH radicals were expected to be eliminated by 25 mM EDTA as a scavenger. The oligonucleotide solutions were bubbled for 1 min with wet nitrogen gas (purity of 99.998%) to minimize the oxygen. Given the low concentration of oligonucleotide in our solutions (14 μM) and the short half-life of prehydrated electrons, we assume that any reaction of prehydrated electrons is negligible.

Irradiation. The samples were irradiated with doses ranging from 250 to 1000 Gy using a cesium-137 irradiator (Best Theratronics, Ottawa, Ontario, Canada).

Digestion. The oligonucleotides (concentration 14 μM) with and without cisplatin were irradiated and digested at 37 °C to deoxyribonucleosides with three enzymes: first, 1 h with nuclease P1, followed by 1 h with snake venom phosphodiesterase I; finally, alkaline phosphatase was added to the sample for 1 h at 37 °C. The reaction was stopped with phosphoric acid and left at 4 °C for HPLC analysis, which was performed with a linear gradient (0−15% acetonitrile in ammonium acetate) and a column (5 μm ODS A 250 × 6 mm; YMC) for the separation.

Extinction Coefficient. The extinction coefficient of the dG-cisPt-dG adduct (23 050 M^{-1} cm^{-1}) at 260 nm was calculated on the basis of the extinction coefficients of deoxyguanosine (11 500 M^{-1} cm^{-1}) and cisPt (50 M^{-1} cm^{-1}).

Mass Spectrometry. The masses of the platinum adducts following digestion were measured by LC-MS/MS. This machine consists of a Shimadzu HPLC system with an auto sampler (SIL-HTc), degasser (DGU-14A), column oven (CTO-10ASvp), binary pumps (LC-10ADvp), UV/vis detector (SPD-20A), and API 3000 tandem mass spectrometer with a turbo-ion spray source (MDS Sciex, Applied Biosystems). A syringe infusion pump (Harvard Model 22) was used to optimize the detection conditions, set at a flow rate of 20 μL/min. The cone voltage was 4500 V, and the collision energy was 45 V. Measurements were made in the negative ion mode.

RESULTS

Analysis of the Damage to DNA Bases. In order to investigate the radiosensibilization of a single stranded oligonucleotide by the presence of a single cisplatin adduct, the oligonucleotide with or without cisPt was irradiated at several doses of ionizing radiation. Analysis of the damage was performed by HPLC following digestion with P1 nuclease and snake venom phosphodiesterase, which cleave phosphodiester bonds to generate mononucleotides, and alkaline phosphatase to remove the phosphate from the free nucleotides. Figure 1a shows the HPLC profile for the ODN-GTG, following enzymatic digestion to deoxyribonucleosides, without and with cisPt prior to irradiation. The profile with cisPt shows the absence of dG and an additional peak corresponding to dG-cisPt-dG as confirmed by MS and discussed below.

Parts b and c of Figure 1 show the HPLC results without and with cisPt, respectively, as a function of irradiation dose. In the absence of cisPt, the thymidine (dT) peak decreased gradually with increasing irradiation dose, reflecting the damage to thymine induced by the hydrated electron, whereas dG remained virtually unchanged. In contrast, with cisPt, both the thymidine and the dG-cisPt-dG peaks decreased markedly with increasing radiation dose. The dG-cisPt-dG peak disappeared at ~1000 Gy, while the dG peak, which was absent at 0 Gy, increased as a function of irradiation dose as a result of the loss of cisPt from guanine in the oligonucleotide. A quantitative analysis of this process is shown in Figure 2. In this analysis, we used a value for the extinction coefficient of dG-cisPt-dG equal to twice that of dG (see Materials and Methods). The black squares (■) in the figure correspond to the percentage of dG formation from cisPt detachment, while the red circles (●) correspond to the percentage of the remaining dG attached to the cisPt. The difference between these two forms of dG indicates the quantity of the dG which underwent damage as a result of the reaction of hydrated electrons with the ODN-GTG-cisPt (green triangles (▲) on the graph). Thus, the dose-dependent disappearance of the dG-cisPt-dG peak from the HPLC profile corresponds to the generation of approximately equal amounts of damaged and undamaged dG (Figure 2).

A comparison of the damage induced to dG as a function of the dose with and without cisPt is shown in Figure 3a, where the potent radiosensitizing effect of the chemotherapeutic agent is particularly evident. Even at the maximum dose of 1000 Gy, there is no detectable damage to dG by radiation in the absence of cisPt, whereas, at this dose, approximately 50% of dG is damaged in the presence of cisPt (Figure 3a). Interestingly, the radiation damage to dT is enhanced approximately 2.5-fold by the presence of the cisPt adduct on dG, as shown in Figure 3b.

Mass Spectrometry Results. The peaks at 17.7 and 19.7 min (Figure 1c) correspond to dG and dT, respectively, based on comparison of HPLC and MS properties with authentic standards. The peak at 19.1 min was identified by more detailed

dx.doi.org/10.1021/jp5014913 | J. Phys. Chem. B 2014, 118, 4803−4808

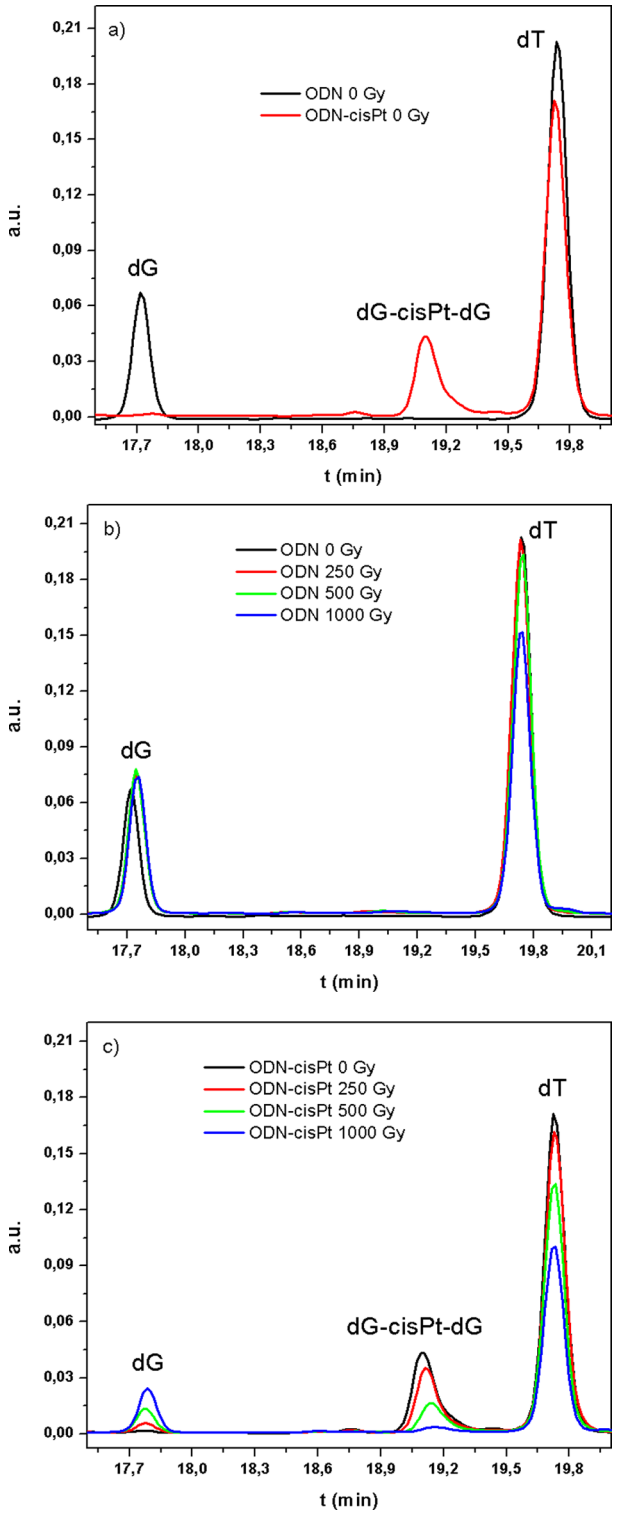


Figure 1. HPLC traces for deoxyribonucleosides from the oligonucleotides following reaction with hydrated electrons, generated by ionizing radiation. The oligonucleotides were irradiated with doses from 0 to 1000 Gy under conditions which quenched •OH radicals and favored hydrated electrons (i.e., reduced O_2) (see Materials and Methods). Afterward, the oligonucleotides were digested to deoxyribonucleosides (see Materials and Methods): (a) no irradiation with and without cisPt, (b) irradiation of the oligonucleotide without cisPt, and (c) with cisPt with different doses.

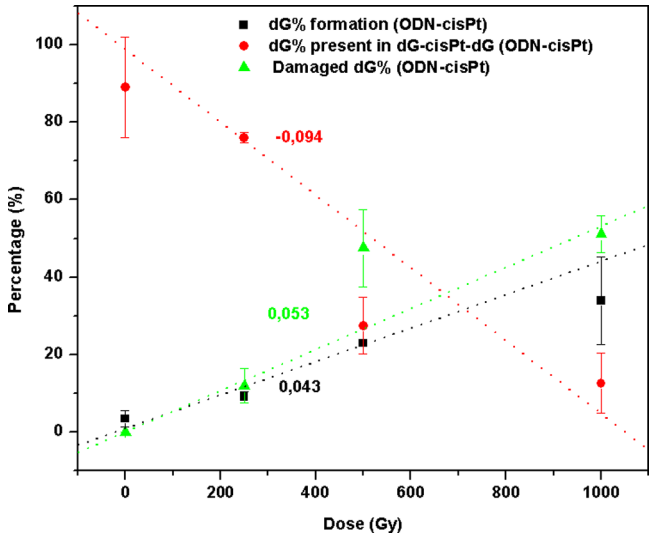


Figure 2. Radiation-induced loss of the dG-cisPt-dG adduct (red ●) and resulting formation of undamaged dG (black ■) and modified dG (green ▲) as revealed by digestion of the oligonucleotide to deoxyribonucleosides followed by HPLC analysis.

analysis. The same peak (at 19.1 min) with identical retention and UV properties at 220 and 260 nm was obtained when either ODN-GTG or GTG was treated with cisplatin, and then digested by P1 nuclease, phosphodiesterase, and alkaline phosphatase, a protocol known to digest DNA to the corresponding 2′-deoxyribonucleosides.[12,23] The MS spectrum of this peak gave a series of three strong negative ion peaks (m/z 760 (100%); m/z 759 (80%); m/z 761 (60%)) indicative of the presence of Pt with its multiple stable isotopes (Figure 5). The fragmentation spectrum of m/z 760 obtained by ESI-MS/MS analysis displayed two fragment ions centered at m/z 726 and 743 (Figure 6), which is characteristic of the sequential loss of two ammonia substituents from dG and dA containing dimeric adducts containing a Pt.[24] In addition, the loss of negatively charged dG ($-m/z$ 266) from the molecular ion was a major process in the fragmentation spectrum. The above results are consistent with the proposed structure (dG-cisPt-dG of Figure 4). Furthermore, this structure is expected to be the major product when DNA containing tandem GG or those separated by a pyrimidine (GTG) are treated with cisPt.[24,12,25]

■ DISCUSSION

Our results demonstrate that irradiation of the ODN-GTG-cisPt complex in solution, under conditions which quench •OH and favor hydrated electrons, leads to extensive thymine base damage as well as to detachment of the cisPt adduct. Interestingly, the loss of the cisPt, which forms an intrastrand cross-link between two guanines separated by a thymine, generates roughly equal amounts of undamaged and damaged guanine (Figure 2). This strongly suggests that one of the guanines in each oligonucleotide is damaged while the other is regenerated during the loss of cisPt. It remains to be seen if the same guanine (e.g., 5′ or 3′) always sustains the damage. No cisPt monomers (G-cisPt) were observed in the HPLC profiles, indicating that the detachment of the cisPt from both guanines occurred in a single, all or nothing step. Our results show that hydrated electrons damage thymine bases in the absence of cisPt; however, the presence of cisPt on the two guanine bases greatly enhances the damage to thymine. An average of four

dx.doi.org/10.1021/jp5014913 | J. Phys. Chem. B 2014, 118, 4803−4808

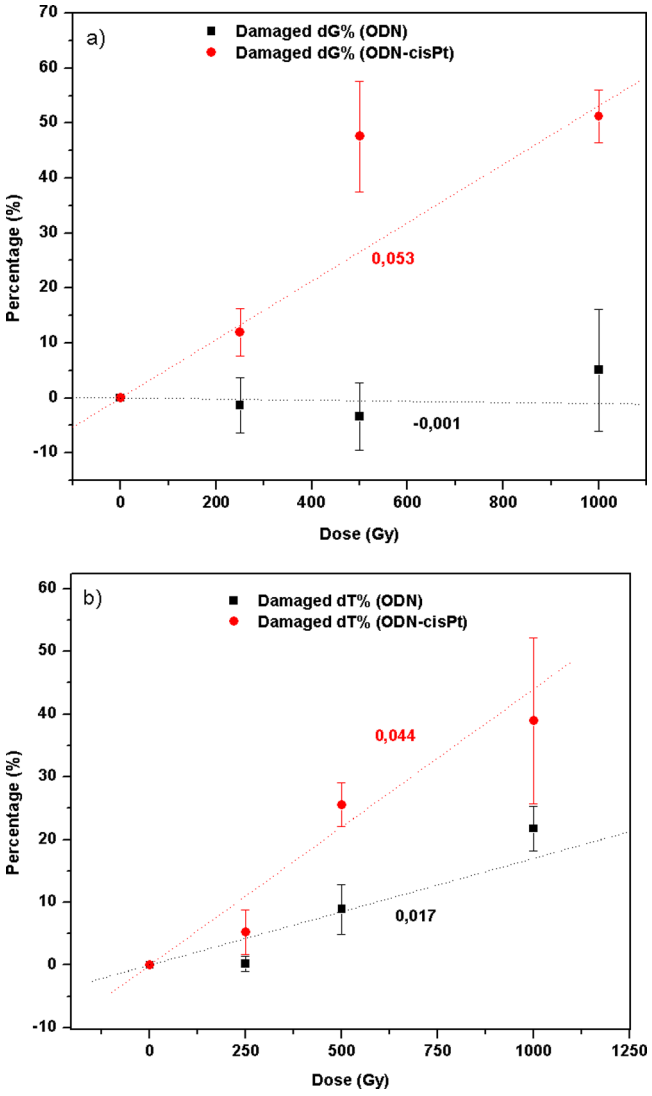


Figure 3. Percentage of modified (a) dG and (b) dT as a function of irradiation dose for ODN-GTG (black ■) and ODN-GTG-cisPt (red ●) following digestion to nucleosides.

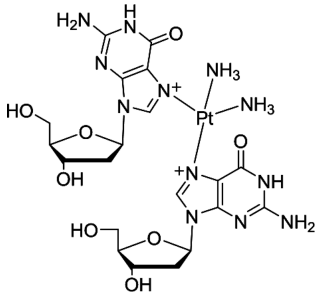


Figure 4. Structure of dG-cisPt-dG following the digestion.

thymines are damaged per ODN-GTG-cisPt at 1000 Gy while only 1.5 thymines are damaged in the oligonucleotide without cisplatin. Thus, 2.5 of the damaged thymines can be attributed to the effect of the presence of cisPt during irradiation.

Fundamental Mechanism. A proposed mechanism must account for the fact that cisplatin increases base damage not only to the guanines to which it is attached but also to adjacent thymines. In the gas phase, Chernyoshova et al. have shown that the collision of low energy electrons with thymine leads to

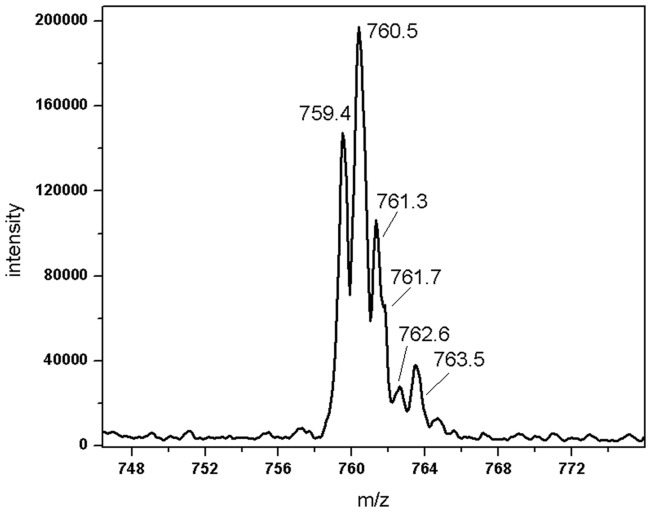


Figure 5. ESI-MS spectrum of dG-cisPt-dG, in negative mode.

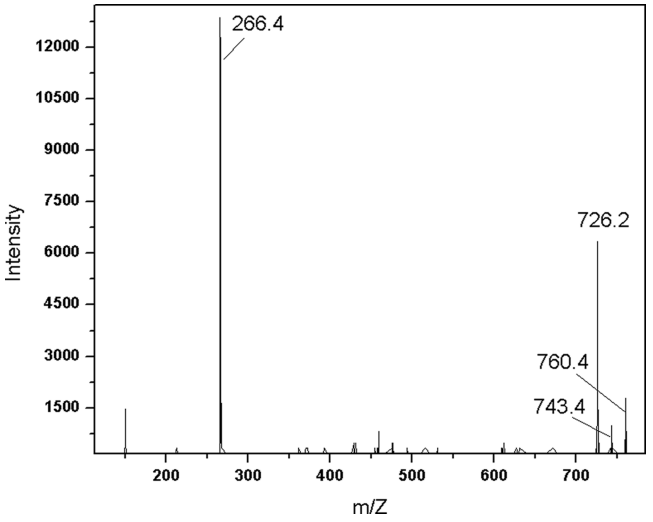


Figure 6. dG-cisPt-dG: ESI-MS/MS spectrum of the peak at 760 in Figure 5 in negative mode.

dissociative electron attachment and to thymine base damage by deprotonation at one of its nitrogens, resulting in an anion $[T\text{-}H]^-$.[26] In contrast, in water, hydrated electrons can react with thymine to yield radical anions followed by either protonation or reaction with other radical.[27] Here, cisPt detachment from the oligonucleotide must be reconciled with the extensive base damage. We propose that the high electron affinity of cisplatin (≥2.9 eV)[11] attracts hydrated electrons and leads to dissociative electron transfer (DET).[22] In our experiments, thymines in oligonucleotides without cisPt adducts also undergo damage albeit at a lower level, but no guanine damage was observed. The strong electron affinity of the cisPt adduct may extend the field of electron attraction beyond the two bases to which it is attached. In addition to direct capture of the hydrated electron by the cisPt leading to the dissociative attachment, hydrated electrons may also reach the chemotherapeutic agent through a base to base transfer mechanism following capture by a thymine base.

The mechanism of electron transfer in DNA has been extensively studied using double stranded DNA by generating either positive hole transfer or excess electron transfer between specific sites of DNA base sequences.[28−34] In general, electron

dx.doi.org/10.1021/jp5014913 | J. Phys. Chem. B 2014, 118, 4803−4808

transfer depends on the difference in energy and the distance between donor and acceptor molecules, such that the efficiency of transfer decreases exponentially with distance. In the case of DNA, several models have been proposed to account for electron transfer.[31−33] A consensus today is that transfer takes place mainly by a hopping process in which charge is distributed on one or more DNA bases and transfer to other sites is strongly dependent on base properties and sequence.[33,34]

Excess electron transfer is base sequence dependent, since some bases are more subject to protonation (e.g., cytosine) than others (e.g., thymine).[29,35] DNA concentration also has an impact on electron migration distance, since high concentrations provide DNA to DNA electron transfer.[30] In contrast, the latter is independent of concentration when DNA in water solution is cooled to form ice.[36] Electron spin resonance (ESR) has been used[29,30,36] to probe excess electron transfer, following gamma ray irradiation from double stranded DNA to the interspersed DNA intercalator mitoxantrone (MX), as a function of temperature and concentration. It was concluded that tunneling operates mainly at low temperature (<190 K), while, at higher temperatures, thermally activated hopping is the prevailing process giving a faster migration rate. In contrast to double stranded DNA, the electron transfer efficiency was only weakly affected by interbase distance in single stranded DNA.[28] To explain this phenomenon, the authors invoked the flexibility of the single stranded oligonucleotide, which allows changes in conformation to decrease the distance between the charge donor and acceptor and thus transfer by bypassing several thymine bases.

In theory, a hydrated electron could be captured by a thymidine base and transferred between thymidine bases. We suggest that the initial polaron (i.e., the hydrated electron) interacts with any of the bases to transform into a base polaron. In other words, the energy of the polarization well of the water around the electron is partly transferred into structural deformation energy around a base.[37] When the charge is thus localized on one base, two channels may be become available: base damage by protonation at C6 or migration to the next base by thermally assisted hopping.

Park et al. have shown the existence of hopping through consecutive thymines,[38] and that phenomenon could also explain electron transfer to cisPt. Otherwise, following the cleavage of the bond between N7 of guanine and the Pt, two pathways are possible: the bond will break and the extra electron will either go to N7 of guanine (path 1) or to Pt (path 2).The first pathway will lead to nonmodified guanine and a reactive cisPt radical. The second will lead to a potentially reactive Pt(I) species that could react with guanine and thymine to produce additional damage. It has been demonstrated by Richmond et al. that Pt(I) reduce the hydroxyl radical adducts of thymine ($^{\bullet}$TOH).[39] This phenomenon could occur in hypoxic cells, where bases are damaged by hydrated electrons followed by their reaction with Pt(I). The unexpected observation of damage to bases outside the Pt attchment sites may provide an important key to understanding the effect of concomitant radiotherapy and chemotherapy treatment, where the cisPt acts as a true catalyst leading to multiple cycles of long-range DNA damage followed, independently, by detachment of the cisPt adduct and damage to one of the guanine bases to which it was attached.

CONCLUSION

The presence of cisPt greatly increases the damage to DNA bases by hydrated electrons produced by ionizing radiation. This damage is found at the Pt attachment sites (guanines) but also at other sites (thymine). These results demonstrate the long-range interaction of cisPt with hydrated electrons possibly owing to its high electron affinity. We propose that cisPt, with its very high electron affinity, attracts electrons leading to DET and loss of the cisPt. In addition, thymine bases can also serve as entry points for hydrated electrons into the DNA, which subsequently undergo base to base electron hopping to the cisPt. This electron transfer process has a certain probability of being interrupted by either DET of the cisPt or protonation of the bases. Moreover, the reactivity of the detached cisPt one electron reduced may create DNA damage and lead to cell death. The reactions described here provide a mechanism to explain the radiosensitization of DNA by cisPt adducts.

AUTHOR INFORMATION

Corresponding Author

*E-mail: behnaz.behmand@usherbrooke.ca. Address: Université de Sherbrooke, Faculté de médecine, Département de médecine nucléaire et de radiobiologie, 3001, 12e Avenue Nord, Sherbrooke, QC, Canada, J1H 5N4. Phone: 1 819 820 6868. Fax: 1 819 564-5442.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

Financial support for this work was provided by the Canadian Institute of Health Research (CIHR).

REFERENCES

(1) Samant, S.; Kumar, P.; Wan, J.; Hanchett, C.; Vieira, F.; Murry, T.; Wong, F. S. H.; Robbins, K. T. Concomitant Radiation Therapy and Targeted Cisplatin Chemotherapy for the Treatment of Advanced Pyriform Sinus Carcinoma: Disease Control and Preservation of Organ Function. *Head Neck* **1999**, *21*, 595−601.

(2) Boscolo-Rizzo, P.; Gava, A.; Marchiori, C.; Baggio, V.; da Mosto, M. C. Functional Organ Preservation in Patients with Locoregionally Advanced Head and Neck Squamous Cell Carcinoma Treated by Platinum-Based Multidrug Induction Chemotherapy and Concurrent Chemoradiotherapy. *Ann. Oncol.* **2011**, *22*, 1894−1901.

(3) Peters, W. A., III; Liu, P. Y.; Barrett, R. J., II; Stock, R. J.; Monk, B. J.; Berek, J. S.; Souhami, L.; Grigsby, P.; Gordon, W.; Alberts, D. S. Concurrent Chemotherapy and Pelvic Radiation Therapy Compared with Pelvic Radiation Therapy Alone as Adjuvant Therapy after Radical Surgery in High-Risk Early-Stage Cancer of the Cervix. *J. Clin. Oncol.* **2000**, *18*, 1606−1613.

(4) Candelaria, M.; Garcia-Arias, A.; Cetina, L.; Dueñas-Gonzalez, A. Radiosensitizers in Cervical Cancer. Cisplatin and Beyond. *Radiat. Oncol.* **2006**, *1*, 1−17.

(5) Choy, H.; Mac Rae, R. M. *Chemoradiation in Cancer Therapy*; Humana Press: Totowa, NJ, 2003.

(6) Kelland, L. The Resurgence of Platinum-Based Cancer Chemotherapy. *Nat. Rev. Cancer* **2007**, *7*, 573−584.

(7) Go, R. S.; Adjei, A. A. Review of the Comparative Pharmacology and Clinical Activity of Cisplatin and Carboplatin. *J. Clin. Oncol.* **1999**, *17*, 409−422.

(8) Burger, A. M.; Double, J. A.; Newell, D. R. Inhibition of Telomerase Activity by Cisplatin in Human Testicular Cancer Cells. *Eur. J. Cancer* **1997**, *33*, 638−644.

(9) Wozniak, K.; Blasiak, J. Recognition and Repair of DNA-Cisplatin Adducts. *Acta Biochim. Polym.* **2002**, *49*, 583−596.

(10) Kopyra, J.; Koenig-Lehmann, C.; Bald, I.; Illenberger, E. A Single Slow Electron Triggers the Loss of Both Chlorine Atoms from the Anticancer Drug Cisplatin: Implications for Chemoradiation Therapy. *Angew. Chem., Int. Ed.* **2009**, *48*, 7904−7907.
(11) Arumainayagam, C. R.; Lee, H. L.; Nelson, R. B.; Haines, D. R.; Gunawardane, R. P. Low-Energy Electron-Induced Reactions in Condensed Matter. *Surf. Sci. Rep.* **2010**, *65*, 1−44.
(12) Williams, R. T.; Nalbandian, J. N.; Tu, A.; Wang, Y. Nuclease Digestion and Mass Spectrometric Characterization of Oligodeoxyribonucleotides Containing 1,2-GpG, 1,2-ApG, and 1,3-GpXpG Cisplatin Intrastrand Cross-links. *Clin. Chim. Acta* **2013**, *420*, 160−170.
(13) For a review, see: Sanche, L. In *Radiation Induced Molecular Phenomena in Nucleic Acid*; Shukla, M. K., Leszczynski, J., Eds.; Springer: Dordrecht, The Netherlands, 2008; Chapter 19.
(14) LaVerne, J. A.; Pimblott, M. Electron Energy-Loss Distributions in Solid, Dry DNA. *Radiat. Res.* **1995**, *141*, 208−215.
(15) Cobut, V.; Frongillo, Y.; Patau, J. P.; Goulet, T.; Fraser, M.-J.; Jay-Gerin, J.-P. Monte Carlo Simulation of Fast Electron and Proton Tracks in Liquid Water-I. Physical and Physicochemical Aspects. *Radiat. Phys. Chem.* **1998**, *51*, 229−243.
(16) Zheng, Y.; Hunting, D. J.; Ayotte, P.; Sanche, L. Role of Secondary Low-Energy Electrons in the Concomitant Chemoradiation Therapy of Cancer. *Phys. Rev. Lett.* **2008**, *100*, 198101-1−198101-4.
(17) Nikjoo, H.; Bolton, C. E.; Watanabe, R.; Terrisol, M.; O'Neill, P.; Goodhead, D. T. Modelling of DNA Damage Induced by Energetic Electrons (100 eV to 100 keV). *Radiat. Prot. Dosim.* **2002**, *99*, 77−80.
(18) DeLara, C. M.; Jenner, T. J.; Townsend, K. M. S.; Marsden, S. J.; O'Neill, P. The Effect of Dimethyl Sulfoxide on the Induction of DNA Double-Strand Breaks in V79-4 Mammalian Cells by Alpha Particles. *Radiat. Res.* **1995**, *144*, 43−49.
(19) Alizadeh, E.; Sanche, L. Precursors of Solvated Electrons in Radiobiological Physics and Chemistry. *Chem. Rev.* **2012**, *112*, 5578−5602.
(20) Kuipers, G. K.; Lafleur, M. V. M. Characterization of DNA Damage Induced by Gamma-Radiation-Derived Water radicals, Using DNA Repair Enzymes. *Int. J. Radiat. Biol.* **1998**, *74*, 511−519.
(21) Kumar, A.; Sevilla, M. D. Role of Excited States in Low-Energy Electron (LEE) Induced Strand Breaks in DNA Model Systems: Influence of Aqueous Environment. *ChemPhysChem* **2009**, *10*, 1426−30.
(22) Behmand, B.; Cloutier, P.; Girouard, S.; Wagner, J. R.; Sanche, L.; Hunting, D. J. Hydrated Electrons React with High Specificity with Cisplatin Bound to Single-Stranded DNA. *J. Phys. Chem. B* **2013**, *117*, 15994−15999.
(23) Samson-Thibault, F.; Madugundu, G. S.; Gao, S.; Cadet, J.; Wagner, J. R. Profiling Cytosine Oxidation in DNA by LC-MS/MS. *Chem. Res. Toxicol.* **2012**, *25*, 1902−1911.
(24) Iijima, H.; Patrzyc, H. B.; Dawidzik, J. B.; Budzinski, E. E.; Cheng, H. C.; Freund, H. G.; Box, H. C. Measurement of DNA Adducts in Cells Exposed to Cisplatin. *Anal. Biochem.* **2004**, *333*, 65−71.
(25) Jamieson, E. R.; Lippard, S. J. Structure, Recognition, and Processing of Cisplatin-DNA Adducts. *Chem. Rev.* **1999**, *99*, 2467−2498.
(26) Chernyshova, I. V.; Kontros, J. E.; Shpenik, O. B.; Tamuliene, J.; Balevicius, M. L. Low-Energy Electron Collisions with Gas-Phase Thymine Molecule. *Mater. Sci.* **2011**, *17*, 322−325.
(27) Von Sonntag, C. *The Chemical Basis of Radiation Biology*; Taylor and Francis: London, 1987.
(28) Giese, B. Long-Distance Charge Transport in DNA: The Hopping Mechanism. *Acc. Chem. Res.* **2000**, *33*, 631−636.
(29) Cai, Z.; Li, X.; Sevilla, M. D. Excess Electron Transfer in DNA: Effect of Base Sequence and Proton Transfer. *J. Phys. Chem. B* **2002**, *106*, 2755−2762.
(30) Cai, Z.; Sevilla, M. D. Studies of Excess Electron and Hole Transfer in DNA at Low Temperatures. *Top. Curr. Chem.* **2004**, *237*, 103−127.
(31) Ly, D.; Sanii, L.; Schuster, G. B. Mechanism of Charge Transport in DNA: Internally-Linked Anthraquinone Conjugates Support Phonon-Assisted Polaron Hopping. *J. Am. Chem. Soc.* **1999**, *121*, 9400−9410.
(32) Wan, C.; Fiebig, T.; Kelley, S. O.; Treadway, C. R.; Barton, J. K.; Zewail, A. H. Femtosecond Dynamics of DNA-Mediated Electron Transfer. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 6014−6019.
(33) Schuster, G. B. Long-Range Charge Transfer in DNA: Transient Structural Distortions Control the Distance Dependence. *Acc. Chem. Res.* **2000**, *33*, 253−260.
(34) Jortner, J.; Bixon, M.; Langenbacher, T.; Michel-Beyerle, M. Charge Transfer and Transport in DNA. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 12759−12765.
(35) Ito, T.; Rokita, S. E. Criteria for Efficient Transport of Excess Electrons in DNA. *Angew. Chem.* **2004**, *116*, 1875−1878.
(36) Cai, Z.; Sevilla, M. D. Electron Spin Resonance Study of Electron Transfer in DNA: Inter-Double-Strand Tunneling Processes. *J. Phys. Chem. B* **2000**, *104*, 6942−6949.
(37) Rezaee, M.; Sanche, L.; Hunting, D. J. Cisplatin Enhances the Formation of DNA Single- and Double-Strand Breaks by Hydrated Electrons and Hydroxyl Radicals. *Radiat. Res.* **2013**, *179*, 323−331.
(38) Park, M. J.; Fujitsuka, M.; Kawai, K.; Majima, T. Direct Measurement of the Dynamics of Excess Electron Transfer through Consecutive Thymine Sequence in DNA. *J. Am. Chem. Soc.* **2011**, *133*, 15320−15323.
(39) Richmond, R. C.; Simic, M. G. Effect of Radiation on Cis-Dichlorodiammineplatinum (II) and DNA in Aqueous Solution. *Br. J. Cancer* **1978**, *37* (Suppl. III), 20−23.