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Radiation-Induced Formation of 2′,3′-Dideoxyribonucleosides in DNA: A Potential Signature of Low-Energy Electrons

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Supporting Information

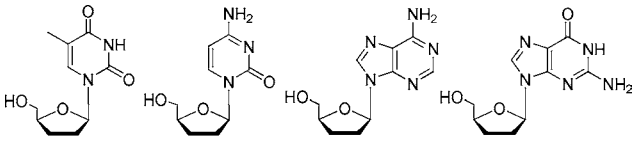
ABSTRACT: We have identified a series of modifications of the 2′-deoxyribose moiety of DNA arising from the exposure of isolated and cellular DNA to ionizing radiation. The modifications consist of 2′,3′-dideoxyribonucleoside derivatives of T, C, A, and G, as identified by enzymatic digestion and LC-MS/MS. Under dry conditions, the yield of these products was 6- to 44-fold lower than the yield of 8-oxo-7,8-dihydroguanine. We propose that 2′,3′-dideoxyribonucleosides are generated from the reaction of low-energy electrons with DNA, leading to cleavage of the C3′−O bond and formation of the corresponding C3′-deoxyribose radical.

It is well established that the interaction of high-energy ionizing radiations (X-ray, charged particles) with DNA generates diverse DNA damage including base damage, base release, strand breaks, and DNA−DNA or DNA−protein cross-links.[1,2] In general, this damage is attributed to both the direct ionization or excitation of DNA components (the direct effect) and the radiolysis of water leading to reactive species, i.e., hydroxyl radicals (•OH) that subsequently react with DNA (the indirect effect). The mechanism of radiation-induced DNA damage, however, is complicated by the formation of numerous intermediate species including positive and negative ions, organic radicals, secondary low-energy electrons (LEEs), and reactive oxygen species. When ionizing radiation interacts with biological material, the energy is deposited in spurs that contain one or more ion pairs and can result in clustered damage.[3] Formation of clustered damage explains why ionizing radiation is much more lethal and mutagenic compared to other agents that generate reactive species in a more homogeneous manner.[3−5]

The direct effect has been given less attention than the indirect effect. Initially, a high-energy photoelectron, or any type of incident fast charged particle, produces a large quantity of ions and lower energy secondary electrons along its path. For example, absorption of a 1 MeV photon in biological tissues leads to the generation of a comparatively enormous number of secondary electrons (4×10^4) with a most probable energy of about 10 eV and a distribution lying essentially below 30 eV.[6,7] These LEEs strongly interact with biological molecules, leading to their ionization and excitation.[7] On the basis of electron spin resonance (ESR), the products of DNA ionization (base radical cations and base radical anions) redistribute on DNA bases such that the radical cation localizes on the base with the lowest oxidation potential, guanine, whereas the electron predominantly resides on bases with the highest electron affinity, thymine and cytosine.[8] This is consistent with the distribution of base damage when isolated DNA is exposed to ionizing radiation in the solid state showing the oxidation of guanine to 8-oxo-7,8-dihydroguanine and the reduction of thymine and cytosine to the corresponding 5,6-dihydropyrimidine.[9] In addition, the direct effect can lead to the formation of single- and double-strand breaks when solid DNA is exposed to X-rays, such that prompt breaks represent about 20% of the total of base and sugar damage.[10] Ultimately, the distribution of final base and sugar products is dependent on the extent of hydration and base sequence.[9−11] Despite continued efforts to understand radiation-induced DNA damage, there is a lack of information about the structure of products and the mechanism of formation of this damage in cellular DNA. Here, we demonstrate the formation of four radiation-induced products likely arising from the initial interaction of LEEs.

The formation of four radiation-induced products was observed in calf thymus (CT) DNA following γ-irradiation, enzymatic digestion, and analysis by LC-MS/MS (Chart 1; experimental details in SI). The products co-eluted on reversed-phase chromatography and displayed MS properties identical to those of authentic standards (Figures 1 and S1−S4). In MS analyses, the products and standards displayed a major fragment corresponding to the nucleobase moiety (MH^+ minus dideoxyribose (m/z 100)) and a minor fragment corresponding to the dideoxyribose moiety (MH^+ minus Thy, Cyt, Ade, Gua). For both products and standards, the ratios of the two multiple reaction monitoring (MRM) transitions were identical. Thus, we conclude that the products observed in irradiated DNA are 2′,3′-dideoxyribonucleosides (ddG, ddA, ddC, ddT). The MRM signal of 2′,3′-dideoxyribonucleosides in pure solution was linear over 5 orders of magnitude with

Chart 1. Structure of the Four 2′,3′-Dideoxyribonucleosides



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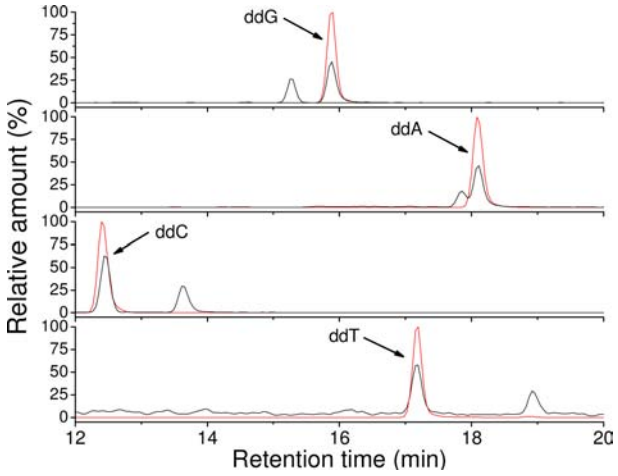


Figure 1. LC-MS/MS analysis of 2′,3′-dideoxyribonucleosides. Isolated CT-DNA (100 μg, dry) was exposed to γ-ray (4 kGy) and digested by enzymes, and DNA damage (in black) was quantified by multiple reaction monitoring (MRM) in comparison with authentic standards (in red) using specific molecular and fragment ions in MRM mode: ddT, *m/z* 227→127; ddC, *m/z* 212→112; ddA, *m/z* 236→136; ddG, *m/z* 252→152. The minor peaks at 18.9, 13.6, 17.8, and 15.2 min in the analyses of ddT, ddC ddA, and ddG, respectively, in DNA are tentatively identified to the 2′,5′-dideoxyribonucleoside derivatives.

respect to the amount of injected standard (2 fmol to 200 pmol; $r^2 \geq 0.99$). The signal-to-noise (S/N) ratio for the injection of 2 fmol was 8, 24, 42, and 3 for ddG, ddA, ddC, and ddT, respectively ($n = 3$).

Radiation-induced yields of 2′,3′-dideoxyribonucleosides (ddC, ddG, ddA, ddT) and 8-oxo-7,8-dihydroguanine (8oxoG) are given in Table 1. The formation of all products

Table 1. Radiation-Induced Yields of 2′,3′-Dideoxyribonucleosides[a]

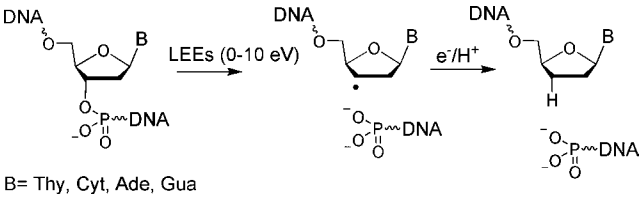
	DNA damage PER 10^9 bases (Gy)					
	ddT	ddC	ddA	ddG	sum	8oxoG
CT (dry)	0.28	0.30	0.25	0.23	1.06	47.1
CT (wet)	0.30	0.23	0.13	0.16	0.82	7930
cells (dry)	0.28	0.35	0.17	0.08	0.88	5.4
cells (wet)	0.12	0.11	0.06	0.03	0.32	9.4

[a]Isolated CT-DNA (100 μg) was irradiated dry (10 Torr for 30 min) and wet (1 μg/μL in aerated solution). F98 glioma cells (5×10^6) were irradiated dry (as above) and wet (as intact cells). Yields were obtained from a linear regression ($r^2 \approx 0.98$) of a graph of lesions/10^9 bases vs dose in Gy; dose range = 0−4.2 kGy; dose rate = 11.3 Gy/min; SD ≈ 10% (Figures S5−S8). ddC and ddT were greater than ddG and ddA ($P < 0.05$).

was linear as a function of radiation dose (0−4 kGy; Figures S5−S8), indicating that they are primary products of ionizing radiation. The yields of 8oxoG in isolated and cellular DNA compared well with those reported previously.[12,13] In comparison to 8oxoG, the yield of 2′,3′-dideoxy products was 44-fold lower in isolated dry DNA and 29-fold lower in the DNA of intact cells. Similarly, the yield of 2′,3′-dideoxy products appear to be lower by 10-fold or more compared to that reported for 2-deoxyribose oxidation products (e.g., 2-deoxyribonolactone).[14,15] Interestingly, 8oxoG increased over 100-fold on going from dry to wet DNA, while 2′,3′-dideoxy products decreased slightly in comparison. Thus, one can rule out $^{\bullet}$OH as a precursor to 2′,3′-dideoxy products. The sharp increase of 8oxoG upon irradiation of a dilute aqueous solution of DNA can be attributed to the initial generation of $^{\bullet}$OH followed by subsequent reactions with DNA. In addition, the yield of 2′,3′-dideoxy products was only on average 3-fold lower in intact cellular DNA compared to isolated dry DNA, whereas one would expect a higher degree of protection for diffusible species such as $^{\bullet}$OH.[15]

We propose that LEEs generated from the interaction of radiation within or near DNA are the precursors of 2′,3′-dideoxyribonucleosides (Scheme 1). The study of LEE

Scheme 1. Proposed Mechanism for the Formation of 2′,3′-Dideoxyribonucleosides by Ionizing Radiation



reactions with DNA model systems are carried out in the condensed phase under ultrahigh vacuum. In initial experiments, bombardment of dThd with LEEs of 0−10 eV produced the release of free nucleobase, Thy, together with sugar fragments including 1,2-dideoxyribose.[16] In subsequent studies, we showed that LEEs induce cleavage of the phosphodiester bond in short oligomers giving fragments with a terminal phosphate group.[17−22] This is consistent with the proposed mechanism of strand breaks via the formation of 2′,3′-dideoxyribonucleosides (Scheme 1). These studies,[16−22] accompanied with numerous theoretical investigations,[23−27] suggest that LEEs of 0−3 eV are initially captured by the nucleobase giving a transient negative ion and that the electron transfers to the phosphate group through interaction with the antibonding orbital of the C−O bond. Once localized on the latter orbital, the C−O bond can rupture via a process known as dissociative electron attachment. Electrons of 10 eV, used in the present experiment, can follow a similar pathway by first losing 6−8 eV to the nucleobase by electronic excitation and then, with only 2−3 eV, undergoing transfer to the C−O bond.[20,21,27] Alternatively, 10 eV electrons can directly localize on the P−O bond, forming an electronically excited transient anion, which can dissociate, leading also to cleavage of the C−O bond.[7] Both processes occur in similar proportions. These processes are characterized by a resonant structure appearing in the yield function of formation of products that is *below* the ionization threshold of DNA components.[27]

LEE-induced cleavage of the C−O bond results in the formation of a C3′-centered radical of 2-deoxyribose and a fragment with a terminal phosphate (Scheme 1). C3′-centered radicals of 2-deoxyribose have been observed from ESR analyses of irradiated hydrated DNA at 77 K and MS studies investigating the fragmentation of oligonucleotides by atmospheric pressure negative ion photoionization.[28,29] In both studies, LEEs were suggested to be the primary precursor of C3′-centered radicals as depicted in Scheme 1. Recently, the chemistry of C3′-deoxy-3′-thymidinyl radicals was explored by using photoactive 3′-derivatized nucleosides as a source of radicals in deaerated aqueous solution; the results indicated efficient conversion of C3′-radicals to ddT in the presence of a

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hydrogen donor.[30] This pathway is supported in the present work by the effect of oxygen. The yield of ddT in solutions of DNA increased from 0.30 ± 0.01 in O_2-saturated solutions to 1.5 ± 0.17 (5-fold) in solutions that were depleted of O_2 prior to irradiation (Figure S6d,e). This suggests that O_2 reacts with intermediate C3′-radicals, thereby diverting the C3′-radical away from the pathway leading to the formation of 2′,3′-dideoxyribonucleosides. The difference between dry and wet cells may also reflect the ability of O_2 to trap C3′-radicals.

There were other interesting aspects about the formation of 2′,3′-dideoxyribonucleosides in isolated and cellular DNA. First, the yield of pyrimidine products (ddT, ddC) was significantly higher than that of purine products (ddA, ddG). The same order of damage was observed upon exposure of short oligomers to LEEs, which is related to the electron affinity of nucleobases.[20] Second, our analyses show the formation of another product with identical MRM parameters and very similar retention times on HPLC with respect to 2′,3′-dideoxyribonucleoside (Figure 1). One may tentatively identify the minor peak in each chromatogram as 2′,5′-dideoxyribonucleosides. These products can be formed by C−O bond cleavage similar to that in Scheme 1 except that cleavage takes place at the 5′ rather than the 3′ side of the nucleotide. Similar to studies of oligomers, the formation of 2′,5′-dideoxy products appears to be lower than that of the corresponding 2′,3′-dideoxy products.[20,26]

In summary, we have identified novel radiation-induced sugar modifications (ddT, ddC, ddA, and ddG). These products are likely formed by the reaction of LEEs with DNA leading to C−O bond cleavage and strand breaks. Although the yield of 2′,3′-dideoxyribonucleosides is modest, they may be a signature of LEE-induced DNA damage. Indeed, the formation of 2′,3′-dideoxy products may only be a fraction of the total damage induced by LEEs, and because single reactions can induce multiple bond cleavage events,[20,21] they may contribute in good part to the formation of highly deleterious clustered damage.

■ ASSOCIATED CONTENT

Supporting Information

Experimental details; MS analyses of ddC, ddG, ddA, and ddT; graphs depicting formation of 2′,3′-dideoxyribonucleosides vs radiation dose under different conditions (data for Table 1). This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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