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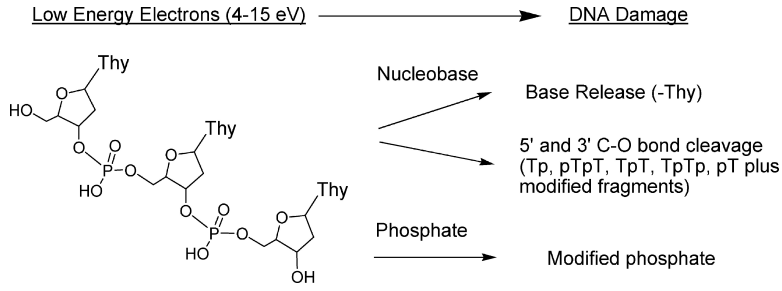

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Low Energy Electron Induced DNA Damage: Effects of Terminal Phosphate and Base Moieties on the Distribution of Damage

Zejun Li, Yi Zheng, Pierre Cloutier, Léon Sanche, and J. Richard Wagner*

Department of Nuclear Medicine and Radiobiology, Faculty of Medicine, Université de Sherbrooke, Sherbrooke, Québec, Canada J1H 5N4

Received October 2, 2007; E-mail: richard.wagner@usherbrooke.ca

The transfer of energy from ionizing radiation to molecules generates large quantities ($\sim 10^5$/MeV) of low energy electrons (LEE, <30 eV).[1] These species react with DNA and its components, leading to various ionic and radical fragments and strand breaks as one type of final damage.[2] Previously, we showed that LEE induces *N*-glycosidic (N−C) cleavage, leading to base release (e.g., release of thymine from thymidine), and phosphodiester−sugar (C−O) cleavage, leading to fragments containing a terminal phosphate group and as yet unidentified sugar fragments.[3,4] The most likely step leading to C−O cleavage involves the decay of transient radical anions of the phosphate P═O π^* orbital to the strongly dissociative C−O σ^* orbital.[5] It is unclear, however, whether initial phosphate P═O π^* radical anions are created from (1) initial electron capture by the base moiety followed by transfer to the phosphate or (2) direct electron capture by the phosphate.[6,7] Here, we show that the presence of terminal phosphate groups enhances total damage but decreases C−N and C−O cleavage, suggesting that the latter steps in the formation of damage occur by initial electron capture by the base rather than the phosphate group.

In this study, we focus on LEE induced damage within a series of small DNA model compounds (dThd, pT, Tp, pTp, TpT, pTpT, TpTp, pTpTp, and TpTpT; counterion = Na^+; Figure 1). Details of the irradiation system have been described.[8] Briefly, the above compounds were spin-coated on the inside surface of tantalum cylinders (3.2 cm × 2.5 cm diameter) to obtain a thin solid film (2.5 nm) of 4 to 5 monolayers. The compounds were then bombarded with $\sim 10^{16}$ monoenergetic electrons at ambient temperature under a vacuum of 10^{-9} Torr. The electron energy was selected to coincide with the peak of resonance (11 eV) using a dose within the linear regime of damage as shown for dThd (Figures S1 and S2). After irradiation, the samples were transferred to a N_2-purged glovebox, dissolved in aqueous solution, and lyophilized to dryness under vacuum. The parent and modified compounds were quantified by HPLC−UV as shown for TpT (Figure S3). The identity and amount of products were based on comparison of their retention and absorption properties with authentic standards. In addition, the analysis of some products was confirmed by treatment with alkaline phosphatase.

The yield of LEE induced products is given in Table 1. The results show that the addition of terminal phosphates causes considerable increases in total damage as estimated by HPLC−UV (note that total damage includes all losses of initial molecules). The total damage for monomers increased from 47 to 129 to 162 for thymine containing zero, one, and two terminal phosphate groups, respectively. A similar trend was observed for the series of dinucleotides. The total damage for dinucleotides increases from 141 to 267 to 321 for TpT, pTpT, and pTpTp, respectively.

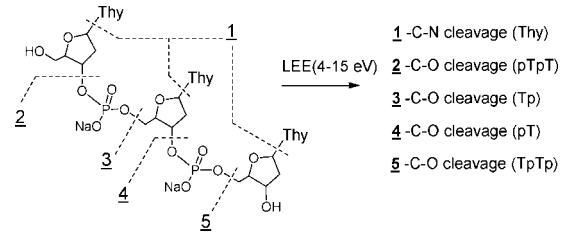

Figure 1. Structure of 5′-TpTpT-3′ and position(s) of cleavage for *N*-glycosidic (N−C) (**1**) and phosphodiester−sugar (C−O) bonds (**2**−**5**). The structure of other DNA model components consists of monomers (pT, Tp, pTp) and dimers (pTpT, TpTp, pTpTp). Na was the counterion.

These results indicate that terminal phosphate groups efficiently capture 11 eV electrons and that these events cause considerable damage. This effect may be related to the primary structure of phosphate or to other factors, such as changes in the conformation of molecules, changes in the intermolecular ordering of molecules, or to the presence of H_2O bonded to phosphate groups. Recent experiments with thin films of NaH_2PO_4 and tetrahydrofuran have shown that the phosphate group has a very large cross section (10^{-15} cm^2)[9] for LEE (7−12 eV) induced fragmentation, which is much larger than that of the furyl ring.[10,11] Thus, the increase in total damage upon the addition of terminal phosphates may be attributed to increases in the initial electron capture by phosphate.

In sharp contrast to total damage, the presence of terminal phosphate leads to a marked decrease in base release (N−C) and phosphodiester−sugar (C−O) cleavage. For example, N−C cleavage decreased from 9.5 to 4.9 to 0.9 in monomers and from 23.4 to 6.4 to 1.7 in dimers for zero, one, and two phosphate groups (Table 1). Similarly, the presence of terminal phosphate decreased C−O cleavage in dimers (12.9 to 9.4 to 3.8). The same trend was observed taking values of damage normalized to the size of the molecule (Table 1; values in parentheses). Interestingly, a similar decrease in N−C bond cleavage occurs for other nucleosides and mononucleotides of DNA (dAdo and dAMP, dCyd and dCMP, and dGuo and dGMP; unpublished results).

The finding that the addition of terminal phosphate increases total damage *but does not* increase base release (N−C) and phosphodiester−sugar (C−O) cleavage indicates that initial electron capture by terminal phosphate leads to a separate pathway of damage. In other words, electron transfer does not take place between initial transient anions of the terminal phosphate group and species that are thought to give rise to N−C and C−O cleavage (i.e., base π^* or P═O π^* anions).[4−6] On the contrary, the addition of terminal phosphate sharply

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Table 1. Yield of Products from LEE Induced DNA Damage

sample[a]	total damage[b]	Thy[b]	%[c]	pT[b]	Tp[b]	pTp[b]	pTpT[b]	TpTp[b]	sum[b]	%[d]	ratio[e]
dThd(242)	47(0.117)	9.5(0.024)	20.2								
pT(366)	129(0.212)	4.9(0.008)	3.8								
Tp(366)	123(0.202)	4.5(0.007)	3.7								
pTp(490)	162(0.199)	0.9(0.001)	0.6	n.d.[f]	n.d.						
TpT(568)	141(0.149)	23.2(0.025)	16.5	7.2	5.7				12.9(0.014)	9.1	1.80
pTpT(692)	267(0.232)	6.4(0.006)	2.4	5.1	n.d.	4.3			9.4(0.008)	3.5	0.68
TpTp(692)	272(0.237)	6.1(0.005)	2.2	n.d.	4.9	6.2			11.1(0.010)	4.1	0.55
pTpTp(816)	321(0.237)	1.7(0.001)	0.5	n.d.	n.d.	3.8	n.d.	n.d.	3.8(0.003)	1.2	0.45
TpTpT(895)	155(0.104)	29.5(0.020)	19.0	2.9	2.5	n.d.	10.3	6.8	22.5(0.015)	14.5	1.31

[a] DNA samples and fragments written from 5′ to 3′ with p indicating the position of terminal phosphate groups (MW in parentheses). [b] Total damage includes all losses of initial targeted molecules based on HPLC−UV analysis (see Supporting Information for details). The values are expressed as a ratio of damaged molecules to 1000 initial target molecules and are the average of three independent experiments; SD = ±20%; the yields in parentheses represent the corresponding molecules of damage per LEE (note that these values take into account the size or MW of the target molecule). [c] Percentage of N−C cleavage based on total damage. [d] Percentage of C−O cleavage based on total damage. [e] Ratio of N−C to C−O cleavage. [f] Not detected.

decreases damage resulting from N−C and C−O cleavage. Such a diminishing effect may be explained by the ability of transient base anions to efficiently undergo electron transfer to the terminal phosphate (in addition to transfer to the internal phosphate). It should be noted that transfer to the terminal phosphate leading to C−O cleavage and the associated fragments, that is, free phosphate and unknown DNA fragments, was not determined in our analyses. Thus, as predicted theoretically[5,12] and supported by experiments,[6,7,14] it appears that the main pathway leading to base release and phosphodiester−sugar cleavage originates from the initial attachment of electrons to the base moiety.

The difference between dimers (TpT) and the trimer (TpTpT) is particularly interesting. When we consider the number of damaged molecules per 1000 molecules of the target, the yield of damage for TpT is comparable to that for TpTpT (141 and 155, respectively; Table 1). In contrast, when we take values that are normalized to the size of the molecule, the yield of damage for TpT was significantly larger than that of TpTpT (0.149 and 0.104, respectively; Table 1; values in parentheses). Moreover, in the case of TpTpT, a greater percentage of the total damage was channeled to N−C and C−O cleavage. The combined yield of products arising from N−C and C−O cleavage was 33.5% of the total damage for TpTpT (19 and 14.5%) compared to 25.6% for TpT, 5.9% for pTpT, 6.3% for TpTp, and 1.7% for pTpTp (Table 1). This indicates that the percentage of damage channeled to N−C and C−O cleavage increases with the number of nucleotides in the molecule. On the basis of HPLC−UV analysis, we conclude that LEE induced DNA damage is an efficient process, generating one damaged molecule of TpTpT per 10 LEE, and that 1/3 of this damage flows to base release and phosphodiester−sugar cleavage in short fragments of DNA.

Changes in the profile of damage in going from monomers to trimers may be explained in part by considering the effect of base stacking on LEE mediated damage. Enhanced overlap of π orbitals during base stacking may lead to greater delocalization of the initial electron wave,[14] allowing more thermodynamically favorable and selective pathways[7,15] for the formation and decay of transient anions of the subunit. Last, base release and phosphodiester−sugar cleavage was about 8-fold greater at the 5′ and 3′ termini (46.6) of TpTpT compared to that at the central position (5.4).[16] This effect may also be related to stacking of the three bases such that external bases physically protect the internal base from initial LEE attack.[4,6]

This work has several implications for LEE induced DNA damage. First, electron capture by the base followed by electron transfer to the phosphodiester bond (P═O) and cleavage of the phosphodiester−sugar bond (C−O) appears to be a major pathway of strand break formation in DNA. At this time, we cannot rule out the possibility that electron capture directly by the phosphate also leads to strand breaks; however, this pathway does not afford the same profile of products as the former pathway via C−O cleavage (i.e., fragments with a terminal phosphate and a modified sugar). Second, assuming that base release and phosphodiester−sugar cleavage arise from the same transient base anions, the pathway involving phosphodiester−sugar cleavage should be greatly favored in continuous stretches of DNA because they do not have terminal phosphate groups (which enhance other pathways of damage). Thus, we predict that immediate base release is negligible, whereas phosphodiester−sugar cleavage is an overwhelming process in LEE reactions with long strands of DNA.

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Supporting Information Available: Experimental procedures, graphs of damage versus electron energy and irradiation time, and representative HPLC analysis of TpT. This material is available free of charge via the Internet at http://pubs.acs.org.

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(16) We assume that base release from the middle T is close to zero and that from 5′ and 3′ ends is approximately equal (see ref 4); in addition, the release of uracil (U) from TpUpT was negligible (unpublished results). Thus, the total of damage from the termini (46.6) includes base release (29.5) and C−O cleavage (10.3 + 6.8).

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