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Low-energy electron-induced DNA damage: product analysis and mechanistic studies of damage in short oligonucleotides

By

Zejun Li

Department of Nuclear Medicine and Radiobiology

Thesis presented to the Faculty of Medicine and Health Sciences to obtain a diploma of Philosophy Doctorate (Ph. D.) in Radiobiology

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Évaluateurs

Léon Sanche, co-directeur, département de médecine nucléaire et de radiobiologie

J. Richard Wagner, co-directeur, département de médecine nucléaire et de radiobiologie

Michael Huels, président de jury, département de médecine nucléaire et de radiobiologie

Jean Lessard, évaluateur externe au programme, département de chimie, Université de Sherbrooke

Christopher Wilds, évaluateur externe à l'Université, Department of Chemistry and biochemistry, Concordia University

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Dommages à l'ADN induits par les électrons de basse énergie : l'analyse des produits et études des mécanismes de dommage dans des oligonucleotides courts

Par
Zejun Li
Département de médecine nucléaire et de radiobiologie

Thèse présentée à la Faculté de médecine et des sciences de la santé en vue de l'obtention du diplôme de philosophiae doctor (Ph.D.) en radiobiologie, Faculté de médecine et des sciences de la santé, Université de Sherbrooke, Sherbrooke, Québec, Canada, J1H 5N4

Résumé

L'objectif majeur de notre groupe de recherche est de comprendre le mécanisme de dommage à l'ADN induit par les électrons de basse énergie "secondaire" provenant de radiations ionisantes et sa relation avec la radiosensibilité et la radiothérapie. Le professeur Sanche a développé un nouveau système d'irradiation d'électron de basse énergie avec lequel une relativement grande surface constituée de couches minces d'ADN peut être irradiée avec des électrons mono-énergétiques sous hyper-vide. Ceci permet l'irradiation de l'ADN ciblé et la formation d'une quantité suffisamment grande d'ADN endommagé pour effectuer des analyses chimiques (HPLC, GC/MS et LC/MS-MS) sur les produits générés et ainsi élucider le mécanisme de dommage à l'ADN par les électrons de basse énergie. Mon projet se concentre sur les systèmes simples, dans lesquels des nucléosides (dThd), nucléotides (pT, Tp et pTp), oligonucléotides (TT et TTT) et des oligonucléotides modifiés (T5BrUT) sont exposés à des électrons de basse énergie. Les réactions subséquentes sont étudiées par analyses chimiques des produits formés.

Mes études ont révélé trois mécanismes de fragmentation dans l'ADN induits par les électrons de basse énergie : 1) le groupe phosphate terminal a une plus large section efficace dans les dommages induits par les électrons de basse énergie; 2) la capture initiale des électrons de basse énergie et le bris de lien subséquent dans l'anion intermédiaire dépend de la séquence et de l'affinité des électrons de la base; 3) à 10 eV, un électron peut induire un événement double. La présente étude fourni une base chimique de la formation de bris par la réaction des électrons de basse énergie avec l'ADN.

Mots clés: dommage à l'ADN, mécanisme, électrons de basse énergie

Low-energy electron-induced DNA damage: product analysis and mechanistic studies of damage in short oligonucleotides

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Abstract

The major objective of our group is to understand the mechanism of DNA damage induced by secondary low-energy electrons (LEE) arising from ionizing radiation and its relationship to radiosensitization and radiotherapy. Prof. Sanche has developed a novel low-energy electron irradiation system in which a relatively large area of thin films of DNA constituents can be irradiated with mono-energetic electrons under ultra high vacuum. This permits the irradiation of target DNA and the formation of sufficient degraded material to allow for chemical analysis (HPLC, GC/MS, and LC/MS/MS) of products remaining on the target surface, so as to elucidate the mechanism of LEE-induced DNA damage. My project focuses on simple systems, in which small DNA components nucleosides (dThd), nucleotides (pT, Tp, pTp), oligonucleotides (TT and TTT) and modified oligonucleotides (T5BrUT) are exposed to low-energy electrons, and the subsequent reactions are studied by chemical analysis of the products.

My studies revealed three mechanisms of LEE-induced fragmentation reactions in DNA: 1) the terminal phosphate group has a larger cross-section in LEE-induced DNA damage; 2) initial LEE capture and subsequent bond breaking within the intermediate anion depend on the sequence and electron affinity of the bases; and 3) at 10 eV, one electron might induce double events. This study provides a chemical basis for the formation of DNA strand breaks by the interaction of LEE with DNA.

Key words: DNA damage, mechanism, low-energy electrons

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Zejun Li, Yi Zheng, Pierre Cloutier, Léon Sanche and J. Richard Wagner.
Journal of the American Chemical Society, **2008**, *130*(17), 5612–5613.
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Low-Energy Electron-Induced DNA Damage: Effect of Base Sequence in Oligonucleotide Trimers.
Zejun Li, Pierre Cloutier, Léon Sanche, and J. Richard Wagner.
Journal of the American Chemical Society, **2010**, *132*(15), 5422–5427.
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List of abbreviations

A	Adenine
Ade	Adenine
a.m.u	Atomic mass unit
AM1	Austin model 1
AN	Acetonitrile
Ap	Adenosine-3'-monophosphate
aug-cc-pVDZ	Augmented, correlation consistent polarized valence double zeta
B3LYP	Becke, three-parameter, Lee-Yang-Parr
BrU	Bromouracil
5BrU	5-Bromouracil
5BrUT	5'-5BrUpT-3'
C	Cytosine
5ClU	5-Chlorouracil
cm	Centimeter
Cp	Cytidine-3'-monophosphate
Cyt	Cytosine
dA	2'-Deoxyadenosine
dAdo	2'-Deoxyadenosine
dAMP	2'-Deoxyadenosine monophosphate
dC	2'-Deoxycytidine
dCyd	2'-Deoxycytidine
dCMP	2'-Deoxycytidine monophosphate
DD	Dipolar dissociation
DE	Dissociative excitation
DEA	Dissociative electron attachment
DFT	Density functional theory
dG	2'-Deoxyguanosine
dGuo	2'-Deoxyguanosine
dGMP	2'-Deoxyguanosine monophosphate
DMF	*N*, *N*-dimethylformamide
DNA	Deoxyribonucleic acid
dNTPs	Deoxy-ribonucleoside triphosphates
DSB	Double strand break(s)
dT	Thymidine
3', 5'-dTDP	Thymidine-3',5'-diphosphate
dThd	Thymidine
3'-dTMP	Thymidine-3'-monophosphate
5'-dTMP	Thymidine-5'-monophosphate
DZP++	Double-zeta plus polarization
E	Electron energy
E_o	Initial electron energy
ΔE	Transferred electron energy

E	Energy barrier / activation energy
ε	Dielectric constant
EA	Electron affinity
e^{-}_{aq}	Solvated electrons/hydrated electrons
E_B	Binding energy
e_c^{-}	Electron re-emitted into the continuum
e^{-}_{hyd}	Hydrated electrons
E_{ox}	Oxidation potential
e^{-}_{pre}	Prehydrated electrons
ESD	Electron stimulated desorption
e_t^{-}	Electron transfer from the base to the phosphate unit
5FU	5-Fluorouracil
FWHM	Full width at half maximum
G	Guanine
GC/MS	Gas chromatography/ mass spectrometry
Gp	Guanosine-5'-monophosphate
Gua	Guanine
HOMO	Highest occupied molecular orbital
HPLC	High-performance liquid chromatography
kcal/mol	Kilo calorie per mole
KE	Kinetic energy
MeV	Mega electron volts
mol	Mole
MW	Molecular weight
LC	Liquid chromatography
LC/MS/MS	Liquid chromatography/mass spectrometry/mass spectrometry
LEE	Low energy electron(s) (0-30 eV)
LUMO	Lowest unoccupied molecular orbital
min	Minute
mL	Milliliter
ML	Monolayer(s)
mm	Millimeter
mM	Milli mole per liter
MP2	Second order Møller-Plesset perturbation theory
MS	Mass spectrometry
M. W.	Molecular weight
N_2	Nitrogen gas
NHE	Normal hydrogen electrode
nm	Nanometer
oligos	oligonucleotides
pA	Adenosine-5'-monophosphate
pAp	Adenosine-3',5'-diphosphate
pAT	5'-pApT-3'
p5BrUT	5'-p5BrUpT-3'

pC	Cytidine-5'-monophosphate
PCM	Polarizable continuum model
pCT	5'-pCpT-3'
PES	Potential energy surface
pG	Guanosine-5'-monophosphate
pGp	Guanosine-3',5'-diphosphate
pGT	5'-pGpT-3'
pT	Thymidine-5'-monophosphate
pTp	Thymidine-3',5'-diphosphate
pTpTp	5'-pTpTp-3'
pTT	5'-pTpT-3'
pUT	5'-pUpT-3'
pXT	5'-pXpT-3' (X= T, C, A, G)
SCF	Self-consistent field
SE	Secondary electron(s)
SSB	Single strand break(s)
T	Thymine
TAp	5'-TpAp-3'
TAT	5'-TpApT-3'
T5BrU	5'-Tp5BrU-3'
T5BrUp	5'-Tp5BrUp-3'
T5BrUT	5'-Tp5BrUpT-3'
TCp	5'-TpCp-3'
TCT	5'-TpCpT-3'
TGp	5'-TpGp-3'
TGT	5'-TpGpT-3'
Thy	Thymine
TMA	Transient molecular anion
TNI	Transient negative ion
Tp	Thymidine-3'-monophosphate
TT	5'-TpT-3'
TTp	5'-TpTp-3'
TTT	5'-TpTpT-3'
TTXT	5'-TpTpXpT-3' (X= T, C, A, G)
TU	5'-TpU-3'
TUp	5'-TpUp-3'
TUT	5'-TpUpT-3'
TXp	5'-TpXp-3' (X= T, C, A, G)
TXTT	5'-TpXpTpT-3' (X= T, C, A, G)
U	Uracil
UHV	Ultra high vacuum
UT	5'-UpT-3'

Chapter I – Introduction

I.1 Radiation and people

Ever since people knew what were radiations and radioisotopes more than 100 years ago, people started using them. Nowadays, radiations and radioisotopes are widely used not only in medical examination and treatment, but also in many other fields such as weaponry, energy, material manufacture, food processing, etc. However, people also suffered greatly from radiation, e.g., the atomic bomb explosion in Hiroshima and Nagasaki in 1945 and the release of radioactive material in Chernobyl nuclear power plant in 1986. So radiation has both good and bad effects. It is useful in many fields but using it is dangerous and it has harmful effects on people and the environment.

It is well-known that radiation is bad to the health of people because it damages living cells. But people still use radiations in the medical field and other fields. So understanding the mechanisms of radiation action on cells and tissues is crucial for using radiation and estimating the risk.

I.2 How radiation reacts with the cells

Earlier experiments have shown that the primary sensible sites for cell lethality induced by ionizing radiation are focused in the nucleus. Thus, when ionizing radiation passes through a cell, DNA is the critical target (Steel, 2002; Hall and Giaccia, 2006). DNA is the vehicle for inheritance in cellular life and DNA damage is the critical event in radiation cell killing and mutation. For this reason understanding the processes (chemical reactions) that damage DNA and cause mutations takes on great importance. In this thesis, we deal with damage to DNA induced by secondary LEE arising from ionizing radiation.

DNA is genetic material in the cell that stores hereditary information. When ionizing radiation irradiates the cell, the most significant damage is in DNA. If the damaged DNA is properly repaired, the cell survives and remains a healthy parent cell which gives healthy offspring cells. If the damaged DNA is not properly repaired, it may not properly replicate and may transmit the deficient traits from one generation to another; the cell can die or mutate and induce cancer (von Sonntag, 1987, 2006). However, there are vast differences in the time scale involved in these various events, which lead to cell death. For example, the physical process takes place in less than 10^{-15} s. The following physical-chemical processes have lifetimes of about 10^{-10} to 10^{-9} s. Finally, the biological expression takes place in a longer time from a few seconds or minutes to a few years. For example, the oncogenic effect of ionizing radiation may be delayed many years. Radiobiology is the study of the effect of ionizing radiation on biological molecules and living organism, e.g., DNA damage, cell death, mutation, carcinogenesis, etc. The experiments can be performed through chemical methods, physical methods, in vivo or in vitro biological methods, or even through theoretical calculations to predict the processes involved.

I.3 Excitation and ionization of radiation

Any kind of radiation delivers energy to the materials absorbing the radiation. Energy absorption by the material occurs via changes in the molecule or the atom. There are two possible changes when the energy is absorbed by the material. The first is excitation and the second is ionization. If the electron of the outer shell of the atom is raised to a higher energy level, this process is said to be electronic excitation. If sufficient energy is absorbed to overcome the binding energy of the electron and release one or more electrons from the atom, then ionization happens. When the radiation is of sufficient energy to produce ionization, it is called ionizing radiation. In the process of ionization, in solid matter by high energy radiation, many secondary electrons (SE) are produced. If the SE also have enough energy to excite or ionize molecules in the medium, they can further excite or ionize them before they

are thermalized, i.e., before they give their energies to the environment. In the whole process, a large number of LEE (0-30 eV) are produced. It is reported that about $\sim 4\times 10^4$ LEE are produced along the radiation track per MeV of deposited energy (LaVerne and Pimblott, 1995a, 1995b; Pimblott and LaVerne, 2007). The secondary LEE arising from ionizing radiation induced damage to DNA will be the major theme of this thesis.

I.4 Production of secondary LEE

We take two kinds of ionizing radiation to explain the production of secondary LEE: an x-ray (or γ-ray) photon and a fast charged particle which represents the electromagnetic radiation and particulate radiation.

First, there are two processes which dominate when x-ray photons are absorbed. At high energies (~ 0.7-10 MeV), the Compton process dominates. In this process, the photon gives part of its energy to an almost free electron (which means that the binding energy of this electron is very small in comparison with the energy of the photon, i.e., almost negligible) as kinetic energy resulting in a fast electron and a photon with reduced energy. The photon takes part in further interactions. As a result, a large number of relatively fast electrons are produced, and they can further excite or ionize other atoms in the medium and give a large amount of the secondary LEE. At lower energies ($E< 0.5$ MeV), usually it is the photoelectric absorption processes that become dominant. The electron is ejected from an inner shell of an atom and the photon energy is used to overcome the binding energy of the atomic electron and provide kinetic energy to this ejected electron. Thus, the energy of the incident photon ($h\nu$) is shared with the kinetic energy (KE) of the ejected electron and the binding energy of the electron in its orbit (E_B), i.e., $KE=h\nu - E_B$. Whether the absorption process is the Compton or the photoelectric process, much of the energy of the absorbed photon is converted into kinetic energy of fast secondary electrons and subsequent secondary LEE.

When the fast Compton or photoelectron or particulate radiation, such as a fast charged particle, passes near molecules, it produces a fast varying electric field and magnetic field on this molecule. This perturbation does not change the energy and momentum of the fast particle very much; thus the energy transfer is like the absorption of electromagnetic radiation by the molecules of the medium as mentioned above. The primary charged particle loses part of its energy, with highest probability of 22 eV (LaVerne and Pimblott, 1995a, 1995b; Pimblott and LaVerne, 2007; Srdoc et al., 1995). The emission of such low-energy quanta (22 eV) successions represents the energy loss and energy deposition of the charged particle to the molecules. This process can lead to excitation and ionization. The possibility of excitation and ionization should be the same but about 20% of the energy deposited by fast charged particles in organic matter may lead to excitation, and the rest, i.e., 80%, may lead to ionization. The ionization energy is shared as potential energy of the cation and the kinetic energy of secondary LEE. As a result, the largest portion of the energy of primary particle changes to the energy of the secondary electrons (LaVerne and Pimblott, 1995a, 1995b; Pimblott and LaVerne, 2007). Statistically, about 40% of the energy of the primary radiation goes to the energy of the secondary LEE.

Overall, along the radiation track, many species are created which are composed of excited atoms and molecules, cations, radicals, and secondary electrons. Secondary electrons are produced abundantly ($\sim 4\times 10^4$ per MeV of deposited energy), through energy deposit from the electromagnetic radiations and particulate radiations such as x-rays, γ-rays, high-energy electrons, protons, alpha-particles, heavy charged particles, etc. (Cobut et al., 1998). The energy of the secondary electrons has a distribution that lies essentially below 70 eV with a most probable energy below 10 eV (LaVerne and Pimblott, 1995a, 1995b; Pimblott and LaVerne, 2007; Henke et al., 1977, 1981); these are referred to as secondary LEE.

I.5 Electron interaction with molecules

At low energies, electron-molecule collisions can be divided into two main types: direct and indirect interactions (Figure 1). The direct interaction may also be referred to as non-resonant interaction and the indirect interaction may also be referred to as resonant interaction. Direct interaction has short interaction times. Direct interaction usually occurs at higher electron energies above the ionization threshold of the molecules.

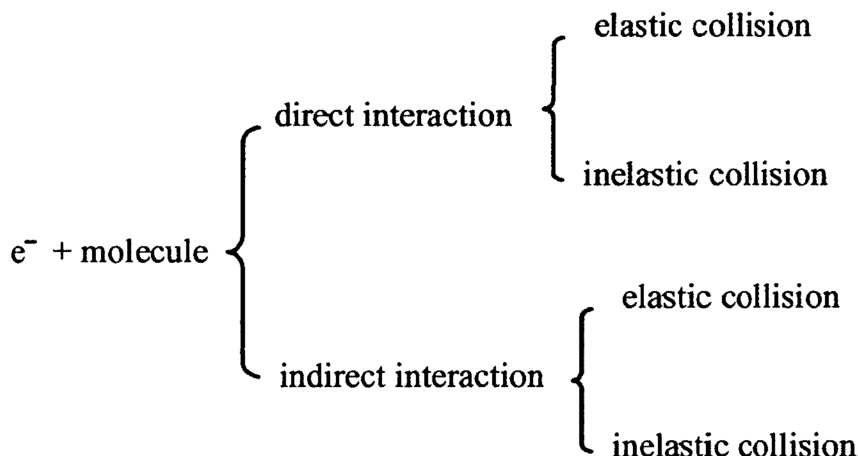


Figure 1. Schematic diagram of electron-molecule collisions.

The direct and indirect interaction can be divided as elastic and inelastic according to the amount of energy transferred from the electron to the target. In elastic collisions, the loss of electron energy to the target is negligible. In the case of inelastic collision, the loss of electron energy to the target is not negligible. In this case, electronically excited states may be created and followed by subsequent reactions.

If the incoming electron is bound to the target for a relatively longer time than the usual direct interaction time, resonant electron interaction takes place (Palmer and Rous, 1992; Sanche, 1991; Sanche, 1995; Sanche, 2000; Sanche, 2002). In this case, a transient negative ion (TNI) or transient molecular anion (TMA) is formed. This resonant state may be regarded as a previously unfilled orbital of the molecule temporarily occupied by an electron. This process can be described as following: e^- $(E_0) + M \rightarrow M^{*-}$. At specific energies, resonance interaction takes place which

corresponds to the energies of transient anions. At this specific energy, i.e., at the resonance energy, usually an increased product yield is observed.

I.6 LEE interaction with a molecule XY

There are three main pathways for a diatomic molecule XY to interact with a LEE (Sanche, 2002; Bass and Sanche, 2004).

First, an excited neutral state of the molecule XY* may be created by pathway "I" through the direct electron interaction (inelastic interaction). This process is illustrated in Figure 2. XY* may disperse its excess energy by emitting a photon and/or transferring its energy to the surrounding environment (pathway Ia). When the electronically excited neutral state XY* is dissociative, the molecule dissociates in ground state (X + Y) or excited fragments (X + Y*) (pathway Ib). Dipolar dissociation (DD) produces a cation ($Y^{+(*)}$) and an anion (X^-) (pathway Ic) if the electron energy is higher than a certain energy threshold (~14-16 eV).

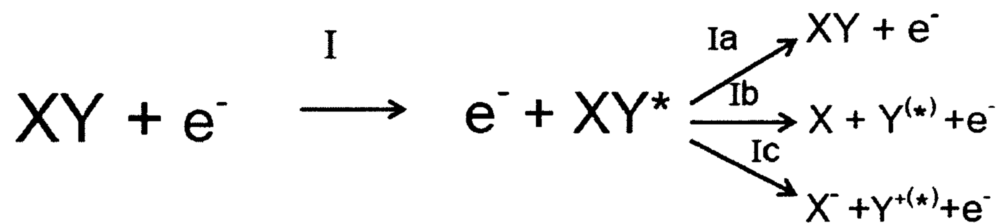


Figure 2 (reprinted from Sanche, 2002). Pathway "I" when an electron interacts with a molecule XY.

The second major pathway is a resonant interaction. This process is illustrated in Figure 3 (pathway "II"). Through pathway "II", an incident electron temporarily attaches to the molecule and a "TNI" or a "TMA" ($XY^{-(*)}$) is produced. Through pathway IIc, the resulting "TNI" or "TMA" may autoionize, which means the electron leaves the molecule and an excited neutral state of the molecule XY* is

produced which may decay through pathway Ia, Ib, or Ic. The other process, which is also the most important process, is called **dissociative electron attachment (DEA)**. In this process, the anion may dissociate into a neutral fragment in its excited (Y^*) or ground state (Y) and a stable anion (X^-) (pathway IIa). It is accepted that the DEA process happens when a LEE interacts with a DNA molecule. During its lifetime, the "TNI" or "TMA" can stabilize by giving its energy (ΔE) to another system (for example, by photon emission to a surrounding medium or by collisional action with another molecule in the medium (process IIb) and result in a stabilized XY anion, XY^-.

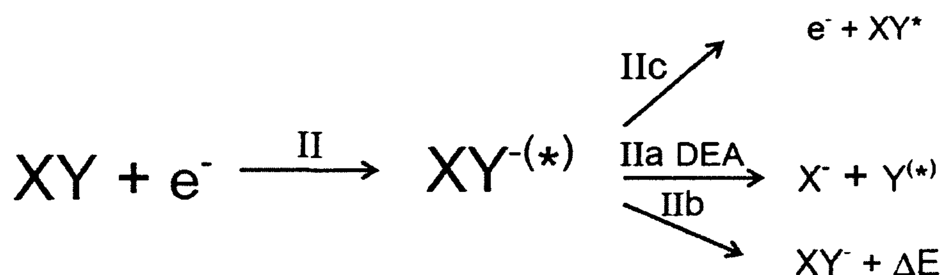


Figure 3 (reprinted from Sanche, 2002). Pathway "II" when an electron interacts with a molecule XY.

The third pathway is "III". When the incoming electron can directly ionize the molecule resulting in a dissociative cation ($XY^{+(*)}$), it may break up as shown through reaction IIIa ($X + Y^{+(*)}$). This process is illustrated in Figure 4 and usually this dissociation channel pathway is non-resonant.

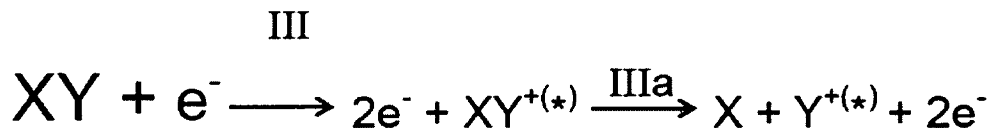


Figure 4 (reprinted from Sanche, 2002). Pathway "III" when an electron interacts with a molecule XY.

I.7 "Shape" resonances and "core-excited" resonances

In the resonant process of LEE interaction with a molecule XY, there are two major types of resonances or transient anions involved (Schulz, 1973a, 1973b; Sanche, 2002): one is called "shape" resonance and the other is called "core-excited" resonance. The "shape" resonance is also called a single-particle resonance which means that when the additional electron, usually at lower energies ($E < 3eV$), goes to a previously unfilled orbital (such as LUMO) of the target (Figure 5). When previously unfilled orbitals (such as LUMO) are occupied by two electrons and the transitory anion is formed, this resonance is termed "core-excited" (Figure 5). In the "core-excited" resonance, the first step is that one core electron is excited from the ground state (because the incident electron has a higher energy, usually $E > 3eV$), usually resulting in an electron in the LUMO and a hole in the HOMO. Simultaneously, a core-excited anion is produced by capturing the exciting electron in the LUMO (Sanche, 1991). It may be also referred to as a one-hole, two-particle state (Figure 5). Also, "core-excited" electron resonance located around 10 eV may play an important role in the fragmentation of DNA during the process of dissociative electron attachment to DNA, because studies have shown that the yield of products are highest with electron energies around 10 eV (Pan et al., 2003; Zheng et al., 2006).

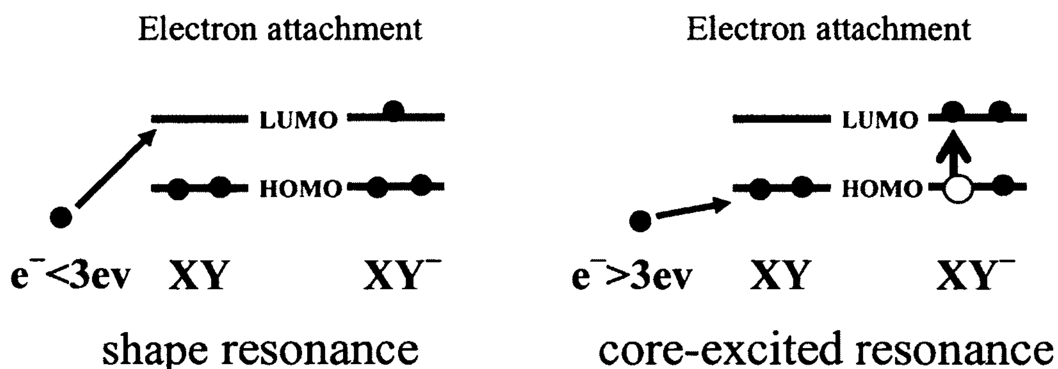
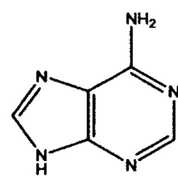


Figure 5. "Shape" resonance and "core-excited" resonance

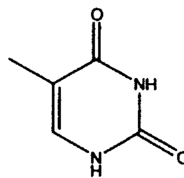
Due to limitation of the apparatus in the present study, all the DNA experiments with LEE irradiation are done at the "core-excited" resonance energy region, i.e., around 10 eV. However, even at energies around 10 eV, three decay channels take place, i.e., elastic collision, DEA, and inelastic collision. This will be also discussed in Chapter III. In future experiments, we will focus on the shape resonance energy region, i.e., using 1 eV electrons, which are near the energy of thermalized electron in aqueous solution.

I.8 DNA damage induced by ionizing radiation

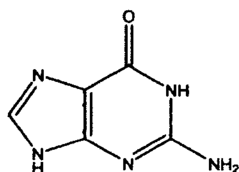
DNA molecule (Deoxyribonucleic acid molecule) is a polymeric macromolecule. The macromolecule consists of nucleotides. A nucleotide is a nucleoside and a phosphate group. A nucleoside consists of a 5-carbon sugar (2-deoxyribose) and a nitrogen containing base, i.e., nucleobase, attached to the sugar. There are four different base moieties referred to as nucleobases in DNA: adenine (A), thymine (T), guanine (G) and cytosine (C). The structures of the four nucleobases A, T, G, C are illustrated in Figure 6.



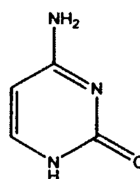
Adenine



Thymine



Guanine



Cytosine

Figure 6. Structures of the four nucleobases: A, T, G, C.

Consequently, there are four fundamental types of nucleosides (dA, dT, dG, dC) and eight fundamental types of nucleotides (pA or Ap, pT or Tp, pG or Gp, pC or Cp). Taking nucleobase thymine as an example, the nucleoside is called thymidine (dT, dThd) and if the phosphate is connected to the 5' of the sugar, the nucleotide is called thymidine-5'-monophosphate (pT) and if the phosphate is connected to the 3' of the sugar, the nucleotide is called thymidine-3'-monophosphate (Tp). The structure of thymine-3'-monophosphate, i.e., Tp, is illustrated in Figure 7.

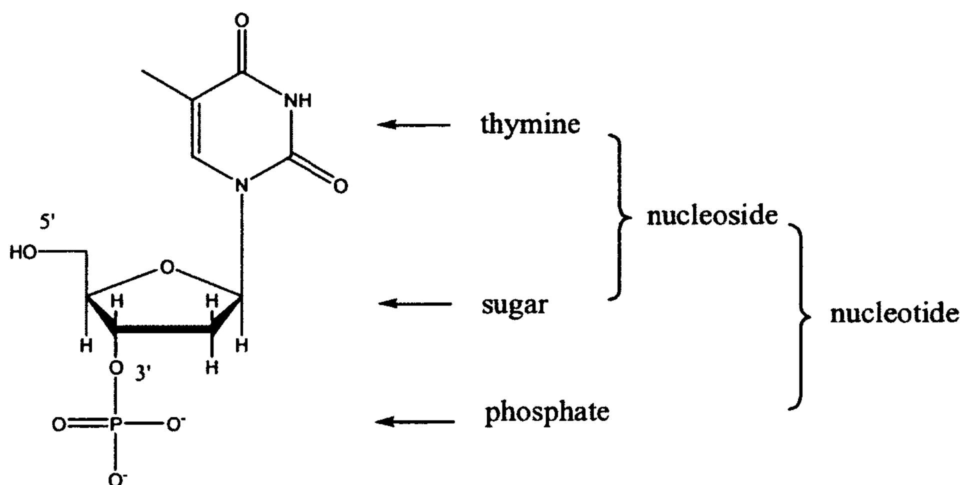


Figure 7. Structure of a nucleotide, thymine-3'-monophosphate, i.e., Tp.

A DNA macromolecule consists of two antiparallel single stands of repeated mononucleotides. The repeated mononucleotides are connected one to the next through phosphodiester bonds. The two antiparallel single strands are connected together through hydrogen bonds between A-T and G-C base pairs having the form of a right-handed helix. The base to base π stacking between adjacent bases on the same strand may also be attributed to the stability of the polymer.

DNA molecules are the most important functional and genetic molecules in the cell nucleus. So if DNA is damaged, there will be disastrous results such as cell death and mutation. As mentioned above (Chapter I.2), DNA is the critical target for ionizing radiation. So the study of radiation induced DNA damage takes on great importance in medicine and radiobiology. Two well-know types of damage induced by ionizing radiation include single-strand break (SSB) and double-strand break (DSB). A break in one strand of the DNA is called a single-strand break. Single-strand breaks are usually repaired rapidly using the opposite strand as a template, and thus, they are relatively benign. The well separated DNA single-strand breaks on both strands can also be readily repaired and also be thought of as single-strand breaks. But, if the breaks in the two strands are very near, for example,

within one turn of the helix or ten base pairs, or opposite to each other, this case is referred to as a double-strand break (DSB). Because there is no template for the repair, double-strand breaks are believed to be much more lethal than single-strand breaks induced by ionizing radiation. In addition, many other kinds of damages happen such as base damage, base release, DNA-DNA cross-links, DNA-protein cross-links, etc. The combined damage within ten base pairs is the most lethal, which is also called clustered damage or locally multiply damaged sites (LMDS). If the damaged DNA is repaired rapidly and correctly, the cell survives. If the repair is not within sufficient time, for example, within a cell cycle, or is incorrectly repaired (mis-repair), it may result in cell killing, mutation, or carcinogenesis.

Cells also protect themselves through an adaptive response, which means when large radiation exposure is preceded by a small dose, the effect of the large dose is sometimes diminished. This is a protective mechanism because small doses of radiation appear to stimulate protective responses, triggering DNA repair mechanisms and the elimination of severely damaged cells. However, all levels of radiation are considered harmful. For instance, there is no threshold (a level, e.g., a radiation dose, below which there is no observable biological effect) for induction of cancer by radiation. In addition, the phenomenon of bystander effect happens which means that induction of biological effects in cells that are not directly traversed by a charged particle but are in close proximity to cells that are. Thus radiation-induced damage to DNA is a complicated process and it is hard to predict the results. Besides DNA, there are other important targets in cells. For example, radiation damage to cell membrane may also be important because the membrane damage is possibly triggering apoptosis

Both direct ionization and water radiolysis products may lead to the formation of the types of strand breaks, which are called direct action and indirect action of radiation-induced DNA damage. In the direct action, a secondary electron can be produced from absorption of a photon and interaction with the DNA to induce a modification. In the indirect action, the secondary electrons can interact with water

molecules to produce hydroxyl radicals (OH•) or other species such as solvated electrons and H-atoms, which in turn produce DNA damage.

I.9 DNA damage induced by LEE

We know that secondary LEE are the most abundant species induced by ionizing radiation; thus the study of LEE-induced damage to DNA takes on great importance. In 2009, Wang et al. reported their remarkable discovery in the *Journal of the American Chemical Society*: even free electrons that have lost all of their kinetic energy also interact with the DNA building blocks (Wang et al., 2009). This work was highlighted in a News & Views article in *Nature* (Sanche, 2009). By using femtosecond time-resolved laser spectroscopy, the researchers have revealed that adenine and cytosine can effectively trap an prehydrated electron (e^{-}_{pre}) to form stable anions in aqueous solution, and demonstrated that thymidine and especially guanine are highly susceptible to dissociative electron transfer of e^{-}_{pre}, leading to bond dissociation in DNA in aqueous solution. This challenges conventional ideas about radiation-induced DNA damage which suggest that the molecular source of genomic damage induced by the indirect effect in irradiated cells can be attributed to hydroxyl radicals (O'Neill and Fielden, 1993; von Sonntag, 1987; Sanche, 2009). Hydroxyl radicals usually form from the interaction of the water molecules in the cell with high-energy photons or charged particles. This finding indicated another important product of water's radiolysis, i.e., the prehydrated electron, might also be the source of DNA damage and serve as another important culprit.

Thus one can propose that the biological effects of radiation on living organism result not only from the impact of primary quanta on DNA, but also from secondary species that are produced by the primary interactions along the radiation track. Among the secondary species, LEEs are the most abundant, so it is crucial to determine their action on DNA in cells. One can expect that LEEs will produce novel modifications on DNA molecules because these electrons have a kinetic energy that

can induce resonant interactions, such as the process of dissociative electron attachment (DEA). Different from damage to DNA induced by hydroxyl radicals, which leads to oxidation, LEEs initiate a reductive step at first, followed by reactions that will result in DNA damage.

I.10 Experimental design to model LEE-induced damage to DNA

For the experimental design to study the LEE-induced DNA damage, usually five parts are needed. The first part is an electron gun, which is the most important part and is used for the generation of the LEE beam. By setting different parameters on it, it can emit electrons with a wide range of energies (from a few eV to hundreds of eV and obtain an absolute energy spread of 0.5 eV full width at half maximum). The second part is a chamber, which is used to host the electron gun; an oil-free turbomolecular pump is used to pump the chamber rapidly from atmospheric pressure to the ultra high vacuum (UHV) range ($\sim 10^{-9}$ Torr). UHV is necessary to protect the electron gun when it is working and keep away any contaminants. The third part is the LEE bombardment target. Usually it is composed of condensed DNA molecules or their components in the gas or condensed phases. In the case of condensed-phase experiments, the fourth part is the sample holder, which is usually a metal substrate. Up to now, many kinds of metals have been tested but the best and most widely used are either tantalum or gold because experimental results have indicated that DNA was stable on tantalum or gold substrates. For the gas phase experiments, the DNA basic component must be vaporized to interact with the electrons emitted in the chamber. The drawback of the gas phase experiment is the possible decomposition of the sample during the heating process. Also, some big molecules cannot be vaporized so that the system is limited to the study of small molecules. For the solid phase experiments, the sample must be lyophilized on the metal substrate or chemisorbed on a gold substrate, e.g., oligonucleotide self-assembled monolayers (SAM). The fifth part is the product analysis instrument after LEE bombardment. It can either be located inside or outside the UHV chamber. Many methods of analysis have been

used; for example, mass spectrometry (MS), high performance liquid chromatography (HPLC), gas chromatography-mass spectrometry (GC/MS), liquid chromatography-mass spectrometry-mass spectrometry (LC/MS/MS), electrophoresis, etc.

As for the LEE bombardment target, oligonucleotide DNA and plasmid DNA are widely studied using the method mentioned above (Zheng et al., 2005; Li et al., 2010; Boudaïffa et al., 2000) because they are similar to natural DNA macromolecules and their damage products are easy to detect and analyze. Oligonucleotide DNA is synthesized artificially. It can be short or long, in single strand form or double strand form. Also, several modified bases, e.g. 5BrU, can be introduced into the DNA strand. Plasmid DNA is usually extracted from microbes (e.g., E. coli) and it is a natural macromolecule with 2000 ~ 150,000 base pairs. Short oligonucleotides and their stable decomposition products can be easily analyzed and quantified by HPLC/UV. The structure of decomposition products can be identified by GC/MS and LC/MS/MS. Long oligonucleotide DNA strands and their strand break products can be easily analyzed and quantified by polyacrylamide gel electrophoresis (PAGE) through Maxam-Gilbert sequencing. The smaller the strand break product, the faster it moves in the polyacrylamide gel. Plasmid DNA and its strand break products can be easily analyzed and quantified by agarose gel electrophoresis. There are three forms of plasmid DNA: supercoiled DNA (scDNA), open circular DNA (ocDNA), and linear DNA. The natural form of plasmid DNA is supercoiled DNA. When a single strand breaks, it becomes open circular DNA (ocDNA) and when the double strand break happens, it becomes linear DNA. In agarose gel, the order of migration is supercoiled DNA (scDNA) > linear DNA > open circular DNA (ocDNA). In my experiments, I use short oligonucleotide DNA molecules and HPLC/UV analysis for the products.

For now, short DNA single strands (oligonucleotide DNA) can be synthesized by the method of solid-phase synthesis of DNA or the polymer chain reaction (PCR)

using dNTPs (deoxy-ribonucleoside triphosphates: dATP, dGTP, dTTP, dCTP) and enzymes. The short DNA strand is called oligonucleotide. The oligonucleotide can be in its single-stranded or double-stranded configuration (the association of at least six to ten complementary nucleotide base pairs depending on the sequence is required for the stable formation of DNA duplexes in water). The double-stranded DNA achieves contact through hydrogen bonds between A-T and G-C base pairs. By heating or adding some chemical agents, such as urea, the hydrogen bonds between A-T and G-C break, and the double-stranded oligonucleotide denatures leading to a single-stranded oligonucleotide. By cooling down, the denatured single-stranded DNA can be renatured into double-stranded DNA. The oligonucleotide is a good model for studying DNA damage induced by ionizing radiation because it is easy to get and the damage products are easy to analyze. In my experiments, I use short, single-stranded oligonucleotides such as trimers.

I.11 Study of LEE-induced DNA damage in the past

In the past, much experimental effort focused on the interactions of LEE with simple DNA components or DNA macromolecules such as plasmid DNA. These studies include investigation of the basic interactions of LEE with nucleobases (Aflatooni et al., 1998; Scheer et al., 2004; Ptasińska et al., 2005; Gohlke et al., 2003; Abouaf et al., 2003; Hanel et al., 2003; Feil et al., 2004; Huels et al., 1998; Aboul-Carime et al., 2001b, 2004; Denifl et al., 2004; Klyachko et al., 1999), ribose derivatives (Antic et al., 1999; Antic et al., 2000; Breton et al., 2004; Huels et al., 2004; Lepage et al., 1998; Ptasińska et al. 2004, Park et al., 2006), oligonucleotides and plasmid DNA (Dugal et al., 2000; Aboul-Carime et al., 2000a; Huels et al., 2003; Pan et al., 2003; Martin et al., 2004; Ray et al., 2005; Folkard et al., 1993; Nogues et al., 2004). Meanwhile, theoretical simulations using ab initio or density functional theory (DFT) calculations have also been performed to predict the process involved (Anusiewicz et al., 2004; Bao et al., 2006; Barrios et al., 2002; Berdys et al., 2004; Gu et al., 2005, 2006, 2007, 2009, 2010; Kobytecka et al., 2008, 2009; Kumar and Sevilla,

2007; Li et al., 2002, 2003, 2006, 2007; Richardson et al., 2004, Schyman and Laaksonen, 2008; Simons, 2006, 2007; Voityuk et al., 2001; Voityuk, 2009; Wesolowski et al., 2001; Wetmore et al., 2001). These studies support the ability of LEE bombardment to induce DNA damage and suggest that a resonance process, i.e., dissociative electron attachment (DEA), happens, and that a stable anion forms followed by fragmentation of the anion and formation of radical fragments. The technique of electron stimulated desorption (ESD) has been used to study the reaction of LEE with condensed-phase DNA components together with mass spectrometry (MS) detection of small primary radicals and ions (<100 a.m.u.) desorbing from the surface of the solid target upon LEE bombardment under ultra high vacuum (UHV) (Huels et al., 1998, 2003; Aboul-Carime et al., 2001b, 2004; Denifl et al., 2004; Klyachko et al., 1999; Antic et al., 2000; Ptasińska et al., 2004; Dugal et al., 2000; Pan et al., 2003). The existing LEE techniques are only capable of analyzing degradation products trapped in or desorbed from a condensed film, i.e., positive ion, negative ion, and neutral species can be measured. However, most of the non-volatile radiation products, which remain on the surface, have not been identified, e.g., strand break products, base reduction products, etc. There is a missing link to elucidate the chemical steps from the initial electron-initiated reactions to the final damage. Therefore, it is necessary by coupling chemical analysis with LEE irradiation to obtain information of the structure of the final products. The objective of this research project is to study chemical modifications of DNA induced by LEE, starting with basic DNA components.

For these reasons, a novel sample spin coating and LEE irradiator system has been set up in our group that can prepare a uniform film of biomolecules and used to bombard the inside surface of a cylinder target. With this configuration, we are able to irradiate a surface of a hundred times (10-50 μg) larger than with a conventional electron gun, and therefore, provide a sufficient amount of degraded material for preliminary chemical analysis of nonvolatile products outside the vacuum. After recovery from the substrate, the sample can be analyzed by high-pressure liquid

chromatography (HPLC)/UV, GC/MS, or LC/MS/MS for detection of both the strand break products (Zheng et al., 2005; Li et al., 2010) and the base reduction products (Park et al., 2010), so as to elucidate the mechanism of LEE-induced DNA damage.

I.12 The new LEE irradiator system in our lab

Recently, a novel spin coating and LEE gun irradiator system was developed in our lab (Zheng et al., 2004b). The difference of this system is that a large area of thin film of the sample surface (26 cm^2) and a relatively large amount of biomolecules can be bombarded (10-50 μg) with LEE, providing sufficient degraded material for chemical analysis of the nonvolatile products. This novel system includes a spin coating system and an electron gun irradiator and is described briefly below.

The biomolecules to be irradiated are spin coated onto the inner surface of the tantalum cylinders (Figure 8). Seven tantalum cylinders are packed together with Teflon spacers. The sample solution is injected independently onto the inner surface of each cylinder through the apertures between the spacers. The sleeve of cylinders was put into a chamber and rotated magnetically outside the chamber to an angular velocity up to around 1500 rpm. At this speed the liquid is distributed onto the inner surface of each cylinder uniformly. With pumping, the pressure inside the chamber rapidly drops from atmospheric to 2-5 Torr. During this procedure, the liquid freezes onto the tantalum substrate. When the pressure drops below the 2-5 Torr range, sublimation can be assumed to be completed and then the pressure rapidly reaches 100 mTorr. Usually, this step takes between 30 and 60 minutes depending on the solvent used. During this procedure, the sample can be assumed to be distributed uniformly onto the inner surface of the cylinders.

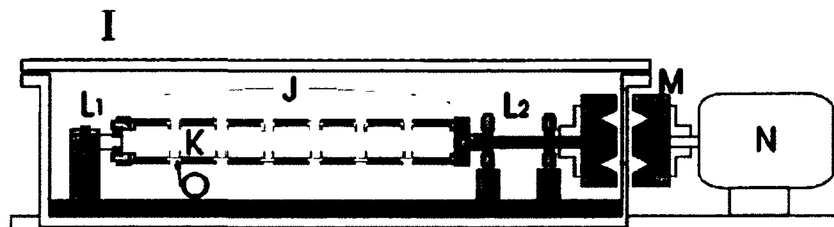


Figure 8. Schematic diagram of the spin coating system. *I*—vacuum chamber, *J*—tube holder, *K*—sample substrate, L_1 and L_2—ball-bearing shafts, *M*—magnetic coupling, *N*—electric motor, *O*—Teflon spacer.

The LEE gun irradiator was put in an ultra high vacuum (UHV) chamber driven by an oil-free turbomolecular pump allowing the chamber to be pumped rapidly from atmospheric pressure to the 10^{-9} Torr range without significant hydrocarbon contamination (Figure 9). The LEE gun irradiator can irradiate the inner surface (26 cm^2) of a tantalum cylinder with 3-130 eV electrons having an absolute energy spread of 0.5 eV full width at half maximum. The cylinders are mounted on a rotatable circular platform. Rotation of the cylinders secured to the platform allows each sample to be bombarded by the electron gun at a specific energy and current for a given time. The UHV chamber can be opened by a quick access port to a dry N_2-purged glove box for the recovery of the sample in it. After irradiation, the tantalum cylinders are mounted with Teflon caps for recovery with water. Finally, the resulting solution is analyzed by HPLC/UV, GC/MS, or LC/MS/MS.

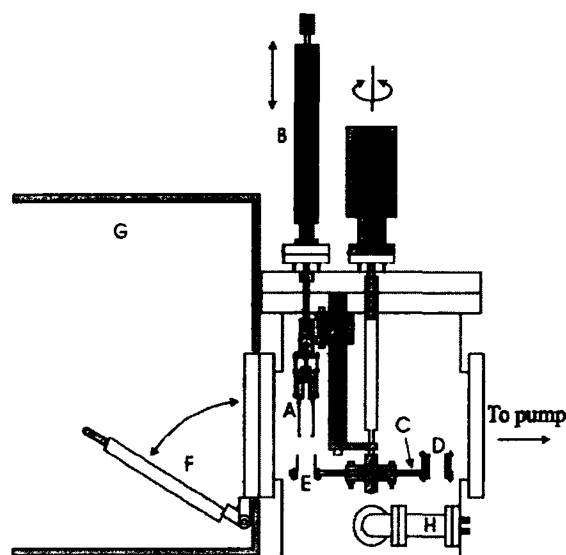


Figure 9. General view of UHV electron irradiator chamber. *A*—electron gun, *B*—linear drive, *C*—rotatable disk used as cylinder support, *D*—electron current detector, *E*—cylindrical sample substrate, *F*—quick access port, *G*—glove box sealed under a N_2 atmosphere, *H*—ion gauge.

I.13 Previous results in our lab

Using this system and HPLC/UV, GC/MS analysis, Zheng et al. in our lab first irradiated a relatively simple DNA nucleoside, thymidine (Zheng et al., 2004a). The results show that LEE irradiation efficiently induces *N*-glycosidic bond cleavage in thymidine and gives thymine as a major product.

Furthermore, two oligonucleotide tetramers (CGTA and GCAT) were irradiated with monoenergetic LEE. The products included non-modified nucleobase, nucleoside and nucleotide fragments resulting from the cleavage of phosphodiester and *N*-glycosidic bonds of each tetramer (Zheng et al., 2005). This is consistent from results with thymidine. In addition, the distribution of non-modified products suggests a mechanism of damage involving initial electron attachment to nucleobase moieties,

followed by electron transfer to the sugar-phosphate backbone, and subsequent dissociation of the phosphodiester bond. These results demonstrate that the phosphodiester bond breaks by a distinct pathway in which the negative charge localizes on the phosphodiester bond giving rise to non-modified fragments with an intact terminal phosphate group. These studies support electron transfer of LEE from the base moiety to the sugar-phosphate backbone in DNA as suggested by theoretical studies (Simons, 2006). Such a mechanism of damage suggests that the capture of non-thermalized electrons by DNA bases followed by the formation of strand breaks may be an important factor in radiation induced DNA damage in living cells.

I.14 Description of this research project

The main objective of the present research project is to elucidate the chemical aspects of the mechanism of DNA single strand breaks induced by LEE using the newly-developed LEE gun irradiator system and small DNA components as model substrates and direct chemical analysis (HPLC/UV analysis) of the products remaining on the substrate as the basis for DNA damage.

Our initial study examines the effect of terminal phosphate and base moieties on LEE-induced DNA damage, which constitutes the first article (chapter II). The DNA model compounds are monomers (pT, Tp, pTp) and dimers (pTpT, TpTp, pTpTp) and a trimer TpTpT. First, we find that the presence of terminal phosphate groups in monomers (pT, Tp, pTp) and dimers (pTpT, TpTp, pTpTp) increases overall damage by 2−3-fold while it decreases N−C and C−O bond cleavage by 2−10-fold. This suggests that the capture of LEE directly by the terminal phosphate does not contribute to N−C and C−O bond cleavage. Second, we find that terminal bases appear to shield the internal base from damage, resulting in a bias of damage toward the termini. In summary, the presence of terminal phosphate base moieties greatly affects the distribution of LEE-induced damage in DNA model compounds.

Furthermore, the effect of base sequence in oligonucleotide trimers was

investigated; it constitutes the second article (chapter III). The model compounds include TXT, where X represents one of the four normal bases of DNA (thymine (T), cytosine (C), adenine (A), and guanine (G)). The results indicated that when trimers were irradiated with LEE (10 eV), the total damage decreased 2-fold in the following order: TTT > TCT > TAT > TGT. The release of nonmodified nucleobases mainly occurred from the terminal sites of trimers (i.e., T) whereas the release of central nucleobases was minor (C) or not at all detected (A and G). This study indicates that the initial LEE capture and subsequent bond breaking within the intermediate anion depend on the sequence and electron affinity of the bases, with the most damage attributed to the most electronegative base, T.

In chapter IV, the model is still the oligonucleotide trimer TXT, but X represents 5-bromouracil, a potential radiosensitizer, i.e., a 5-bromouracil substituted trimer TTT in the second place. The results show that, when T5BrUT is irradiated with LEE (10 eV), TUT is the major product. Substitution of 5-bromouracil with the central thymine of TTT increases the total damage to trimer by at least 50%. This change leads to an increase in both C–N bond and C–O bond cleavage compared to TTT. These findings support the hypothesis that 5-bromouracil is a strong electron captor and LEE is initially captured by the base and then transferred from the base either to the C–N bond leading to base release or to the C–O bond leading to phosphodiester bond cleavage. Most importantly, a relatively large percentage of fragments arising from N–C and C–O bond cleavage contained uracil rather than 5-bromouracil (e.g., uracil, pUT and TUp) are detected, indicating that a single 10 eV electron induces double events, i.e., Br–C and C–N cleavage or Br–C and C–O cleavage. These findings suggest that LEE from ionizing radiation induce multiply damaged sites in DNA especially when it contains modified bases such as 5-bromouracil that have a high cross-section for electron capture.

Overall, my studies have helped elucidate the mechanism of LEE-induced fragmentation reactions in DNA: the terminal phosphate group has a larger

cross-section in LEE-induced DNA damage; initial LEE capture and subsequent bond breaking within the intermediate anion depend on the sequence and electron affinity of the bases; at 10 eV, one electron can induce double events. The present study provides a chemical basis for the formation of strand breaks by the reaction of LEE with DNA.

Chapter II – First article

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.

Journal of the American Chemical Society, **2008**, *130*(17), 5612–5613.

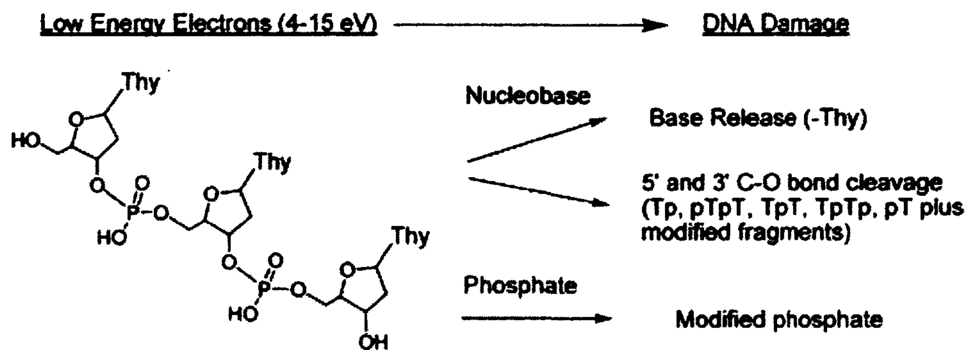
I did all the experiments in this paper and wrote the first version of the manuscript. Dr. Yi Zheng taught me how to use the machine and Mr. Pierre Cloutier helped me to repair the machine when it had problems. Prof. Léon Sanche and Prof. J. Richard Wagner are my joint supervisors and helped me to revise the manuscript and to submit it.

Résumé

Les électrons de basse énergie (EBE) induits des lésions à l'ADN par attachement dissociatif, ce qui implique la libération d'une base (rupture de la liaison N-glycosidique (N-C), et la formation de cassures (rupture de la liaison phosphodiester du sucre (C-O). La détermination de l'effet des EBE au phosphate terminal et aux bases a été évaluée par une exposition à des composés modèle d'ADN dans la phase condensée, suivie d'une analyse par HPLC-UV des produits restant à la surface. Tout d'abord, la présence de groupes terminaux de phosphate dans les monomères (PT, Tp, PTP) et des dimères (pTpT, TPTP, pTpTp) augmente en général les dommages de l'ordre de 2-3 fois, tandis qu'elle diminue 2-10 fois la rupture de la liaison N-C et la liaison C-O. Ceci suggère que la capture directement de l'EBE par le phosphate terminal ne contribue pas à la rupture de la liaison N-C et la liaison C-O. Deuxièmement, les bases terminales semblent protéger la base interne de dommages, entraînant un biais de dommages vers le terminus. En résumé, la présence de la fraction terminale de base de phosphate affecte sérieusement la distribution des dommages dans les composés modèle d'ADN induite par les EBE.

Mots clés: dommage à l'ADN, électrons de basse énergie, HPLC–UV

SYNOPSIS TOC



ABSTRACT

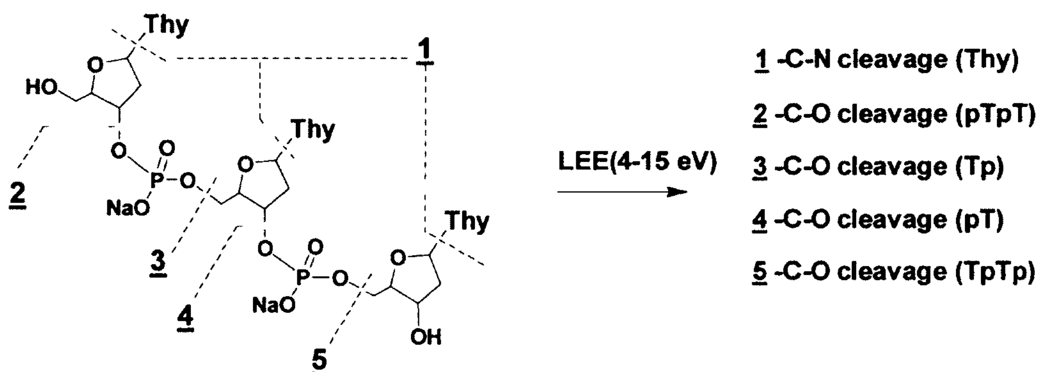
Low energy electrons (LEE) induce DNA damage by dissociative electron attachment, which involves base release (*N*-glycosidic bond (N−C) cleavage) and the formation of strand breaks (phosphodiester−sugar bond (C−O) cleavage). The effect of terminal phosphate and base moieties was assessed by exposing DNA model compounds to LEE in the condensed phase followed by HPLC−UV analysis of products remaining on the surface. First, we report that the presence of terminal phosphate groups in monomers (pT, Tp, pTp) and dimers (pTpT, TpTp, pTpTp) increases overall damage by 2−3-fold while it decreases N−C and C−O bond cleavage by 2−10-fold. This suggests that the capture of LEE directly by the terminal phosphate does not contribute to N−C and C−O bond cleavage. Second, we report that terminal bases appear to shield the internal base from damage, resulting in a bias of damage toward the termini. In summary, the presence of terminal phosphate base moieties greatly affects the distribution of LEE induced damage in DNA model compounds.

Key words: DNA damage, low-energy electrons, HPLC−UV

The transfer of energy from ionizing radiation to molecules generates large quantities (~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 ~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

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.



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.

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).

Table 1. Yield of Products from LEE Induced DNA Damage

sample[a] (M. W.)	total damage[b]	products									
		base release		phosphoester cleavage							ratio[e]
		Thy[b]	%[c]	pT[b]	Tp[b]	pTp[b]	pTpT[b]	TpTp[b]	sum[b]	%[d]	
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.

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 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,[6,7,13] 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).

Chapter III – Second article

Low-Energy Electron-Induced DNA Damage: Effect of Base Sequence in Oligonucleotide Trimers.

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

Journal of the American Chemical Society, **2010**, *132*(15), 5422–5427.

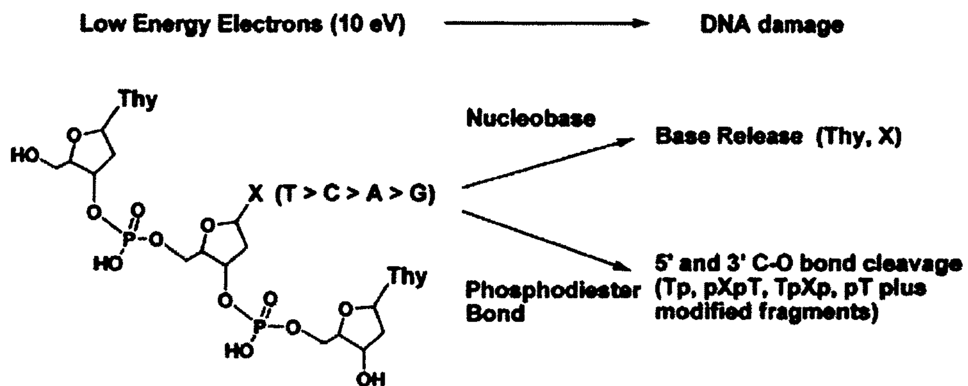
I did all the experiments in this paper and wrote the first version of the manuscript. Mr. Pierre Cloutier helped me to repair the machine when it had problems. Prof. Léon Sanche and Prof. J. Richard Wagner are my joint supervisors and helped me to revise the manuscript and to submit it.

Résumé

Le dommage à l'ADN induit par les électrons de basse énergie (EBE) a attiré une attention considérable ces dernières années puisqu'ils représentent un pourcentage élevé de l'énergie totale déposée par les rayonnements ionisants et parce que les EBE peuvent endommager les composants de l'ADN. Dans cet article, nous avons étudié l'effet de séquences de bases dans une série de trimères d'oligonucléotides par l'analyse des dommages retrouvés dans la phase non volatile condensée après irradiation par EBE. Les composés modèles comprennent TXT, où X représente l'une des quatre bases normales de l'ADN (la thymine (T), cytosine (C), adénine (A), et la guanine (G)). En utilisant l'analyse par HPLC-UV, plusieurs fragments connus formés par la libération de nucléobases non modifiées (T et X) ainsi que par la rupture de la liaison phosphodiester C-O (pT, pXT, Tp, et TXp) ont été quantifiés. La totalite des dommages a été estimée par la disparition des pics parents dans le chromatogramme des échantillons non irradiés et irradiés. Lorsque les trimères ont été irradiés avec EBE (10 eV), le dommage total a été diminué d'un facteur de 2 dans l'ordre suivant: TTT> TCT> TAT> TGT. La libération de nuclobases non modifiées (donnant de 17 à 24% du total des produits) s'est principalement produite à partir des terminaux de trimères (i.e.,T), alors que la libération de nucléobases centrales était mineure (C) ou pas du tout détectée (A et G). En comparaison, la formation de produits résultant de la rupture de la liaison phosphodiester représentait 9 à 20% du dommage total et ceux-ci étaient distribués entre les quatre sites possibles de rupture dans les trimères. Cette étude indique que l'étape initiale de capture d' EBE et la rupture subséquente de la liaison au sein de l'anion transitoire dépend de la séquence et de l'affinité électronique des bases, avec la plupart des dommages étant attribués à la base la plus électronégative, i.e., T.

Mots clés: rayonnements ionisants, analyse chimique, phase condensée

SYNOPSIS TOC



ABSTRACT

DNA damage induced by low-energy electrons (LEEs) has attracted considerable attention in recent years because LEEs represent a large percentage of the total energy deposited by ionizing radiation and because LEEs have been shown to damage DNA components. In this article, we have studied the effect of base sequences in a series of oligonucleotide trimers by the analysis of damage remaining within the nonvolatile condensed phase after LEE irradiation. The model compounds include TXT, where X represents one of the four normal bases of DNA (thymine (T), cytosine (C), adenine (A), and guanine (G)). Using HPLC-UV analysis, several known fragments were quantified from the release of nonmodified nucleobases (T and X) as well as from phosphodiester C–O bond cleavage (pT, pXT, Tp, and TXp). The total damage was estimated by the disappearance of the parent peaks in the chromatogram of nonirradiated and irradiated samples. When trimers were irradiated with LEE (10 eV), the total damage decreased 2-fold in the following order: TTT > TCT > TAT > TGT. The release of nonmodified nuclobases (giving from 17 to 24% of the total products) mainly occurred from the terminal sites of trimers (i.e., T) whereas the release of central nucleobases was minor (C) or not at all detected (A and G). In comparison, the formation of products arising from phosphodiester bond cleavage accounted for 9 to 20% of the total damage and it partitioned to the four possible sites of cleavage present in trimers. This study indicates that the initial LEE capture and subsequent bond breaking within the intermediate anion depend on the sequence and electron affinity of the bases, with the most damage attributed to the most electronegative base, T.

Key words: Ionizing radiation, chemical analysis, condensed phase.

Introduction

The reaction of low-energy electrons (LEEs) with DNA is relevant to radiobiology because the transfer of energy from ionizing radiation to molecules generates large quantities (~3×10^4/MeV) of secondary low-energy electrons (energy $E_0 < 30$ eV).[1] There is a paucity of knowledge about the chemistry of anionic and neutral species resulting from the capture of LEEs by DNA components. In recent years, DNA damage induced by electron attachment has been shown to occur with elementary DNA components (i.e., nucleobases, deoxyribose, and phosphate), nucleosides, nucleotides, oligonucleotides, and plasmid DNA.[2,3] LEEs efficiently attach to DNA components where they form transient anions that dissociate into highly reactive neutral and anion radicals from electronically excited states or by dissociative electron attachment (DEA). Below the energy threshold of electronic excitation, only the dissociation of transient anions involving no electronic excitation (i.e., shape resonances) can create reactive species (i.e., a neutral radical and a stable anion). The heterocyclic and aromatic nature of DNA bases with their relatively low energy π^* orbitals allows them to be particularly efficient in excess electron capture.[4] At low energies ($E < 3$ eV), shape resonances cause electron capture in π^* valence molecular orbitals,[5] giving rise to valence anions. At higher energies (i.e., 10 eV), core excited resonances are formed, which can also decay by DEA. Core-excited resonances result from electron capture by the positive electron affinity of an electronic excited state. Hence, they lie below the parent excited state. When they lie above, they are called core-excited shape resonances. Although all of these processes induce DNA damage, the chemical steps leading to the final modification of the DNA remain to be established.

The reaction of LEEs with condensed-phase DNA components has been studied by the technique of electron-stimulated desorption, which allows for the detection of small primary radicals and ions (<100 amu) that undergo desorption from the surface of a solid target upon LEE impact under ultrahigh vacuum (UHV).[3,6] In addition, a number of studies demonstrate the formation of single- and double-strand breaks (SSBs and DSBs) by postirradiation analysis of damaged plasmids using gel electrophoresis.[3,7] Recently, we developed a novel irradiator in which relatively large quantities of biological molecules (10–50 μg) can be bombarded with LEEs.[8] This

system provides sufficient degraded material for the preliminary chemical analysis of nonvolatile products. Thus, the present technology allows one to investigate LEE-induced damage of condensed-phase oligonucleotides with subsequent analysis of the residues after irradiation.

Previously, we showed that LEE efficiently induce two types of bond dissociation in model DNA leading to stable nonvolatile products: (1) cleavage of the N-glycosidic bond leading to the release of nonmodified nucleobases (e.g., release of thymine from thymidine) and (2) cleavage of the phosphodiester C–O bond leading to the formation of a fragment with an intact terminal phosphate group and unknown modified fragments.[2b, 2d] The latter cleavage pathway was based on theoretical predictions. Theoretical studies indicate that below about 3 eV, electrons can cleave the C–O bond of the backbone at the 3′ and 5′ positions not only via direct capture by a phosphate group,[9] but also via electron transfer.[10] In the latter process, an incoming electron captured by one of the lowest π^*-resonance states of the nucleobase transfers to the sugar–phosphate group of the molecule, where it resides for a sufficiently long time to cause C–O σ-bond rupture. The hypothesis of electron transfer from a base to the phosphate group was first supported experimentally by measurements of SSBs in plasmid DNA in the 0–5 eV range.[11] Later, Zheng et al. bombarded thin molecular films of single-stranded tetramers of DNA with electrons having energies between 4 and 15 eV.[12] They found that both shape and core-excited resonances formed by the initial attachment of the electron to the nucleobase lead to the cleavage of either the N-glycosidic or C–O bonds.

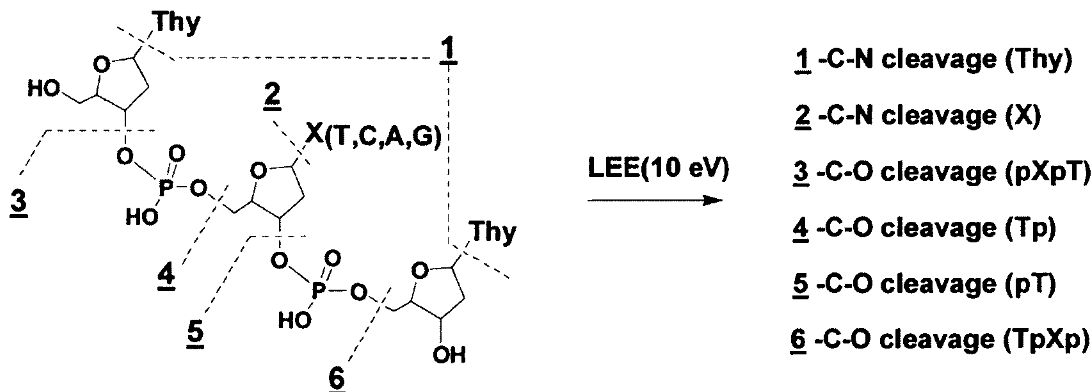
In the present work, we continue our chemical analyses of nonvolatile damage remaining within LEE-irradiated DNA samples. These studies are complementary to the electron-stimulated desorption of anions and strand-breaking analyses, with the goal of identifying all possible reactions. We focus on the analysis of LEE-induced damage in a series of oligonucleotide trimers by HPLC-UV because the analysis of larger fragments obtained from the bombardment of longer oligonucleotides is complicated by the greater number and lower overall yield of products. The model compounds include TpXpT, where p represents the phosphate unit, X represents one of the four normal nucleobases in DNA, connected to a 2-deoxyribose moiety, and T corresponds to thymine (Scheme 1). Each trimer contains three sites for *N*-glycosidic

bond cleavage, resulting in base release (producing T and X), and four sites for phosphodiester bond cleavage (producing pT, pXT, Tp, and TXp), leading to the potential formation of six possible nonmodified fragments.

Experimental Section

Sample Irradiation. Experimental details of the LEE irradiator and the procedure to irradiate samples have been reported elsewhere.[8] Briefly, approximately 80 μg of an HPLC-purified compound was dissolved in 5 mL of nanopure-grade H_2O (Milli-Q water system, 18 MΩ·cm, 25 °C), and the solution was deposited by spin coating onto the inner surface of seven chemically clean tantalum cylinders (3.2 cm × 2.5 cm diameter). The average thickness of the film on the cylinder was 2.5 ± 0.1 nm

Scheme 1. Structure of 5'-TpXpT-3' and Position(s) of Cleavage for the *N*-glycosidic Bond (1, 2) and Phosphodiester bond (3-6).



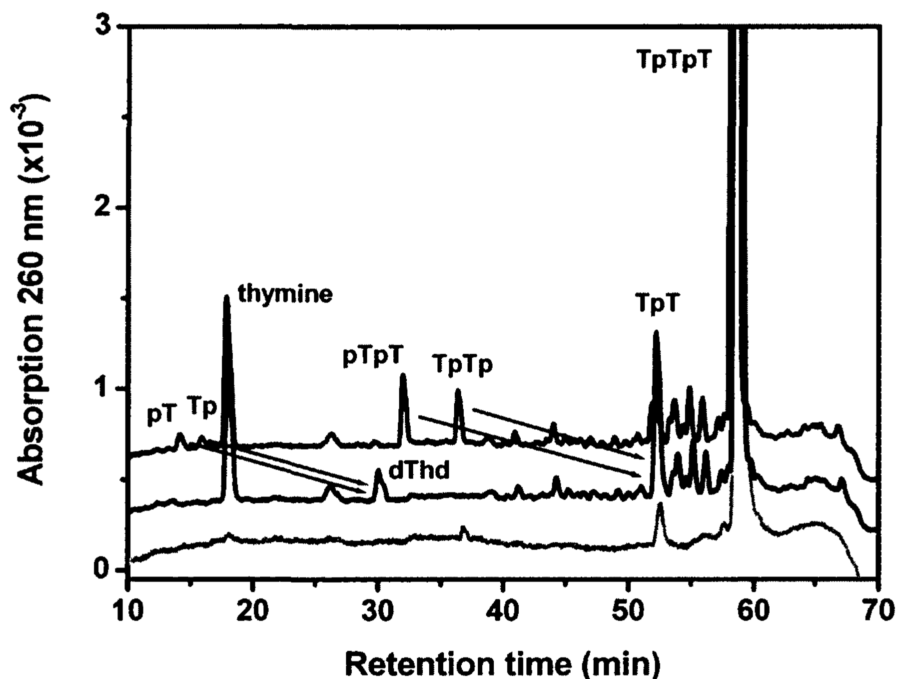
(four to five monolayers (ML)), assuming that the molecules with a density of 1.7 g cm^{-3} [13] are uniformly distributed on the surface of the cylinder. All manipulations of samples, before and immediately after irradiation, were carried out in a sealed glovebox containing an atmosphere of dry nitrogen. The samples were transferred from the glovebox directly to the LEE irradiation chamber. The latter was subsequently evacuated for ~24 h to reach a pressure of about 10^{-9} Torr at ambient temperature. The irradiator generated a uniform electron beam over the entire sample surface of the cylinder with an energy resolution of 0.5 eV FWHM. Each cylinder containing the sample was irradiated individually with a constant irradiation time, beam current, and incident electron energy. Under the present conditions, the time of irradiation was 2.5 min, giving a total exposure of approximately 1.0×10^{16} electrons per cylinder. The current and irradiation time were adjusted to give an exposure well within the linear regime of the dose–response curve. Within this linear regime, the film does not accumulate sufficient excess charge to modify the electron energy and induced processes.[2d] The average thickness of the film (2.5 nm) is smaller than both the penetration depth of 10 eV electrons in liquid water or amorphous ice and the inelastic mean free path in biological solids.[14, 15] Therefore, one can expect that electrons impinging on the film undergo single inelastic scattering events with the molecules.

HPLC Analysis. After irradiation with LEE, the samples were transferred from ultrahigh vacuum to the dry-nitrogen-purged glovebox. The compounds and their radiation products were recovered from the surface of tantalum cylinders by the addition of 12 mL of nanopure-grade H_2O (Milli-Q water system, 18 MΩ·cm, 25 °C). The nonirradiated samples (three cylinders) and the irradiated samples (four cylinders) were pooled, frozen, and lyophilized to dryness. The nonirradiated and irradiated samples were dissolved in 150 and 200 μL of nanopure grade H_2O, respectively, in order to have an equal amount of product per volume in each sample. Half of the sample was analyzed by HPLC. The other half was first treated with alkaline phosphatase (1 unit, Roche Applied Science) for 1 h at 37 °C to remove the terminal phosphate group of nucleotides and then analyzed by HPLC under the same conditions as for the nontreated sample. In the chromatogram, the identity of DNA fragments containing a terminal phosphate group was supported by their conversion to derivatives without a terminal phosphate group upon treating with alkaline

phosphatase (Figure 1). The HPLC system consisted of a Waters alliance HT system (model 2795) equipped with a refrigerated autosampler and a dual-wavelength absorbance detector (model 2487). The separation of products was achieved using an analytical YMC-Pack ODS-A column (250 × 6 mm^2) maintained at 30 °C, using a linear gradient from 1 to 10% acetonitrile in buffer containing 25 mM NaH_2PO_4 (pH 5.7) over an interval of 60 min and at a flow rate of 1.0 mL/min. All products were detected at 210 and 260 nm. The yield of damage in terms of the number of damaged molecules per 1000 target molecules was estimated by comparing the peak for the nonmodified trimer in the chromatograms of irradiated and nonirradiated samples. The yield of LEE-induced products was determined by calibration with authentic reference compounds.

Reference Compounds. Thymine (T), cytosine (C), adenine (A), guanine (G), thymidine (dThd), and mononucleotides (pT and Tp) were purchased from Sigma-Aldrich (St. Louis, MO). In addition, several standard dinucleotide fragments of TpXpT containing either a 3′ or 5′ terminal phosphate group were prepared by the enzymatic digestion of the corresponding trimers with micrococcal nuclease (Roche Applied Science, giving TXp) and with P1 nuclease (MP Biomedical, giving pXT).[16] TpXpT was purchased from Alpha DNA (Montreal, QC) and UCDNA Services (Calgary, AB). All samples were purified by HPLC-UV using the same methods as described above for irradiated samples except that a volatile buffer solution (i.e., triethylammonium acetate (20 mM, pH 7)) was used. The purified solution was lyophilized to dryness and redissolved in nanopure-grade H_2O before spin-coating and irradiation. The yield of LEE-induced DNA fragments was determined by comparison of the peak area at 260 nm with the peak area of standard solutions prepared from commercially available compounds (nucleobases, 2′-deoxynucleosides, and mononucleotides (pT and Tp)). In addition, other fragments containing a terminal phosphate (pXpT and TpXp) were obtained by the partial digestion of oligonucleotide trimers with nucleases together with HPLC purification of the fragments. The concentration of standard solutions of trimers and their fragments was estimated by their optical absorption at 260 nm taking the given molar absorptivity of DNA bases (http://www.basic.northwestern.edu/biotools/oligocalc.html). The optical density was measured using a spectrophotometer (Hitachi U-2000).

Figure 1. Analysis of TpTpT radiation products by HPLC/UV detected at 260nm. The trimer was exposed to 10^{16} electrons with an energy of 10 eV. The lower chromatogram (green) depicts the analysis of a nonirradiated sample. The upper and middle chromatograms show the corresponding irradiated sample, which was divided in two parts: one was treated with alkaline phosphatase (middle; black) and the other received no treatment (upper; red). Blue arrows illustrate the conversion of products with a terminal phosphate to that without.



Results and Discussion

This work focuses on LEE-induced damage that arises from the cleavage of the *N*-glycosidic bond and phosphodiester bonds of oligonucleotide trimers containing T at the terminal positions and different DNA bases at the central position. The analysis of damage was limited to nonmodified fragments of TXT, including nucleobases, mononucleotides, and dinucleotides. After the irradiation of trimers with 10 eV electrons, six known fragments were identified and quantified by HPLC-UV (Figures 1 and 1S-3S; Table 1). These results, as well as those from previous studies,[2] indicate that small DNA components and oligonucleotides undergo two major reactions with LEEs (Scheme 1): cleavage of the C–N bond to give nonmodified nucleobases (i.e., base release of T or X from TXT) and cleavage of the C–O phosphodiester bond to give nonmodified fragments with a terminal phosphate (i.e., pT, Tp, pXT, and TXp from TTT). The remaining products include modified trimers and their fragments, which have not yet been identified in our studies. Previously, we reported a higher amount of total damage for TTT in a series of experiments with small DNA components containing T (155 (previous work) instead of 92 (this work) per 1000 initial molecules).[2c] The difference may be attributed to variations in the uniformity of the film and electron density along the cylinder, which are set for a given series of experiments and are difficult to control between one series of experiments and another. In other words, the error in the values of the total number of degradation products is large from one set of experiments to another but within a given set the error in the relative values is small.

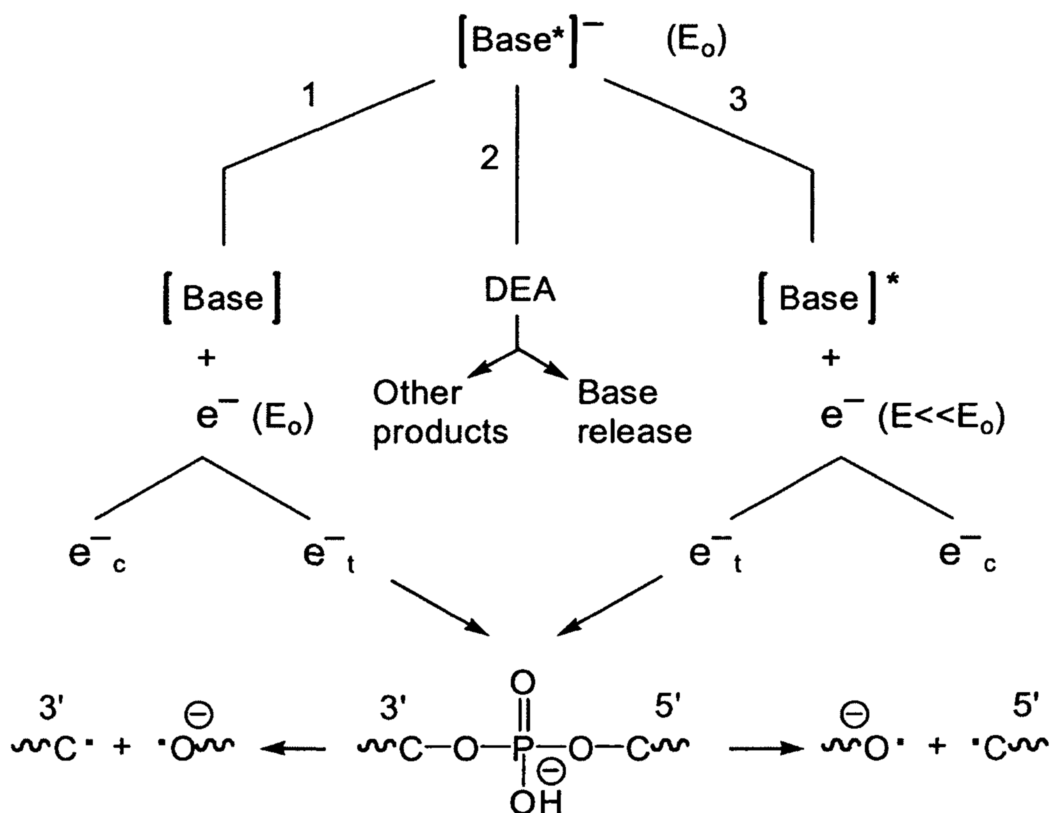
To help interpret our results, we propose a model in which the initial interactions of very low energy electrons (<3 eV) are considered to be similar to those of higher-energy (3–15 eV) electrons (Scheme 2). This model illustrates the pathways leading to base release and C–O bond cleavage following initial electron capture by the nucleobase. In the case of our experiments (i.e., E_o = 10 eV), the incoming electron first forms either a core-excited or a core-excited shape resonance on the nucleobase. The transient anion can decay into three channels: (1) the elastic channel on the left where the electron is re-emitted with the same energy (E_o); (2) the direct DEA channel leading to fragmentation of the parent nucleobase or base release; and (3) the electronically inelastic channel, which can lead to the electronic excitation of a

Table 1. Yield of Products from LEE-Induced DNA Damage

sample[a]	damage[b]	base release				phosphodiester bond cleavage						
TXT		thymine	X	sum	%[c]	pT	Tp	pXT	TXp	sum	%[d]	ratio[e]
X=thymine	92	17.0		17.0	18.5	1.6	1.2	4.3	2.7	9.8	10.7	1.73
X=cytosine	76	12.3	0.4	12.7	16.7	1.5	1.0	2.5	2.1	7.1	9.3	1.79
X=adenine	56	10.7	n.d[f]	10.7	19.1	1.4	1.2	2.0	2.0	6.6	11.8	1.62
X=guanine	37	8.9	n.d[f]	8.9	24.1	1.8[g]	1.4[g]	2.1	1.9	7.2	19.5	1.24

[a] DNA samples and fragments written from 5' to 3' with p indicating the position of terminal phosphate groups. [b] Total damage includes all losses of initial targeted molecules based on HPLC-UV analysis. The values are expressed as the ratio of damaged molecules to 1000 initial target molecules and are the average of three independent experiments; SD= ± 20%. [c] Percentage of C–N cleavage based on the total damage. [d] Percentage of C–O cleavage based on the total damage. [e] Ratio of C–N to C–O cleavage. [f] Not detected. [g] The sum of pT and Tp is significantly different for TGT compared to that of the other trimers (independent *t* test $P < 0.05$).

Scheme 2. Decay Channels of Transient Negative Ions of DNA Bases Formed at an Initial Electron Energy of E_o[a].



[a]Pathways 1–3 represent the elastic ($E = E_o$), dissociative electron attachment (DEA), and electronically inelastic ($E \ll E_o$) channels, respectively. e_c^- represents the electron re-emitted into the continuum. e_t^- represents the transfer of an electron from the base to the phosphate unit. The transient anion formed on the phosphate unit dissociates, leading to C–O bond cleavage.

base and the release of a very low energy electron (E = 0−3 eV). In cases 1 and 3, the electron can be re-emitted into the continuum (e_c^-) or transfer (e_t^-) within DNA. When the extra electron transfers to and localizes on the sugar−phosphate group, the C−O σ bond breaks via DEA. According to the previously mentioned investigations and recent theoretical calculation on electron diffraction,[17] it is reasonable to assume that breakage of the C−O bond within DNA occurs principally via pathway 3 for E_o = 10 eV. Thus, LEE-induced damage near 10 eV can be discussed in terms of theoretical models for electrons of lower energies (0−3 eV).

Sensitivity of DNA Bases to LEE-Induced Damage. The introduction of cytosine, adenine, and guanine at the central position of oligonucleotide trimers resulted in a considerable decrease in total damage as estimated by HPLC-UV analysis (Table 1). This corresponds to about a 2.5-fold decrease in total damage as a result of changing the central thymine moiety to a guanine moiety. Thus, the sensitivity of DNA bases to LEEs decreases in the following order: T > C > A > G. Interestingly, the trend correlates with the electron affinity of DNA bases, as determined experimentally[18] and as supported by theoretical calculations.[19, 20] Seidel et al. measured the reduction potentials (V vs NHE) of the nucleosides in acetonitrile (AN) and *N,N*-dimethylformamide (DMF) solution. The results showed that the experimental reduction potentials of the 2′-deoxyribonucleosides follow the order of dThd (−2.18 V) > dCyd (−2.35 V) > dAdo (−2.52 V) > dGuo (<−2.76 V), indicating that dThd is the easiest 2′-deoxyribonucleoside to reduce and dGuo is the hardest 2′-deoxyribonucleoside to reduce.[18] In other words, near zero eV electrons will preferentially flow to dThd. Using DFT calculations, Richardson et al. predicted that the electron affinities of 2′-deoxyribonucleosides also followed the same order with dThd (0.44 eV) > dCyd (0.33 eV) > dGuo (0.09 eV) ≈ dAdo (0.06 eV).[19] Li et al. calculated the electron affinities of different nucleobases by using DFT methods, and they predicted the same order of electron affinity for nucleic acid bases (T > C > A > G).[20] Our results suggest that LEE, which loses most of its energy via pathway 3 in Scheme 2, may preferentially cause damage to pyrimidine bases in DNA. Similarly, the same trend is observed for solvated electrons such that T and C are the most susceptible DNA bases.[21] The effect of electron affinity on LEE-induced damage is likely related to the formation of initial transient negative ions and electron transfer between DNA bases and from the base to the phosphate group (as discussed below).

Interaction of LEE with DNA Base Arrays (Trimers). The total damage decreases from 9.2 to 3.7% of the total number of bombarded molecules when the middle DNA base of TTT is substituted with alternative nucleobases (Table 1). From a classical point of view, these results are surprising. Even if we assume that the replaced base does not participate any more in the damage, the maximum reduction of damage should be a third of the original damage, giving 7.4% damaged molecules instead of 3.7%. These results suggest that the initial interaction of LEEs or the subsequent chemistry of bases in a trimeric configuration is greatly influenced by the presence of neighboring bases. This effect may be related to the electron affinity of the bases as a determining factor in the capture of LEEs. For example, the electron affinity of trimeric models of XBZ has been examined through theoretical calculations[22, 23] indicating that the electron affinity of DNA bases (B in XBZ) is greatly influenced by the adjacent bases. When X and Z are pyrimidine bases, the trimeric XBZ anion is adiabatically stabilized such that the electron affinity of B increases. In contrast, when X and Z are purine bases, the trimeric XBZ anion becomes adiabatically unstable, leading to a decrease in the electron affinity of B.

The ability of trimers to develop intramolecular base stacking or form aggregates upon evaporation into the condensed phase may lead to changes in the energy levels of trimers and the probability of electron transfer between the bases. In particular, base stacking allows the orbitals of neighboring bases to overlap and form novel ground to excited-state transitions, which may affect the initial formation of transient ions from LEE attachment. Furthermore, one expects an overlap of the extra electron wave function of DNA bases in oligonucleotide trimers. This overlap is likely to be larger in the homotrinucleotides, providing a maximum exchange of captured electrons between bases. If the wave functions are coherent at the resonance energies or at lower energies in pathway 3 (Scheme 2), then they can considerably increase the electron capture cross section of the entire oligonucleotide. Replacing T with another base in the trimer would tend to break this coherence and reduce the electron-capture probability and possibly the electron-transfer probability in pathway 3. In fact, in recent model calculations [17a, b] it has been shown that, because its wavelength is longer than the interunit distances in DNA at low energies ($E_o \leq 3$ eV), the electron scattered within DNA has a high probability of being delocalized in the molecule. Thus, in the case of pathway 3, the inelastically scattered electron is first likely to

undergo multiple intersite transfers before being captured at the phosphate unit in a resonant state. As shown theoretically, the partial wave content of the electron wave function inside DNA then becomes dominated by constructive interference below 6 eV; this condition considerably enhances (i.e., up to at least an order of magnitude) the transfer probability.[17a, b] It is therefore highly probable that there exists in TTT a strong coherence enhancement of the electron wave function owing to the homogeneity and periodicity in the positions of the T bases. When this periodicity and coherence of the extra electron wave function are broken in TXT, a reduction in electron transfer will occur with a corresponding reduction in C–O bond cleavage and base release. On the basis of these considerations, for nearly thermalized electrons (pathway 3), the exchange of electrons between the bases is expected to be proportional to the electron affinity of the middle base. The exchange of such electrons would therefore be in the order of TTT > TCT > TAT > TGT, which corresponds to the order of decreasing DNA damage observed experimentally.

Base Release at the Terminal Positions. The reactions of LEE with oligonucleotide trimers led to the release of nonmodified nucleobases with a strong bias for the release of thymine from the terminal positions of TXT (17–24% of the total damage). This result is also supported by the inefficient release of bases from the central position of mixed trimers in comparison to the release of thymine from terminal positions. For example, the release of cytosine was 15-fold less than the release of thymine from TCT (0.4 compared to 6.15 (12.3/2) molecules per initial target molecule; Table 1). The release of purine bases from the central position (i.e., TAT and TGT) could not even be detected in our analyses, indicating that it is even less than that of cytosine. These results may simply reflect the stronger attraction of the extra electron by the deeper potential of T when compared to that of the other bases. However, even in TTT, *N*-glycosidic bond cleavage at the terminal bases is much larger. This phenomenon was also observed in former studies with smaller fragments (T and TT) and oligonucleotide tetramers.[2b-d] Thus, it appears that when LEEs are initially captured by the trimer they efficiently localize at the termini to induce C–N bond cleavage and the release of nonmodified thymine. It is also possible that when the electron is located on the middle base, electron transfer via pathway 3 is favored as deduced previously by the analysis of LEE-induced products from TTT.[2c] In fact, both processes could act synchronously to reduce C–N bond cleavage in the

middle.

In theoretical studies of base release from nucleosides, Li et al. predicted that the activation barrier for the C–N bond-breaking process was about 20 kcal/mol and Gu et al. predicted 18.9–21.6 kcal/mol (gas phase).[24,25] In contrast, a higher-energy barrier for C–N bond cleavage was predicted for the central sugar–cytosine C–N bond of the CCC trimer (43 kcal/mol using very low energy electrons (0.8 eV); calculations made in a gas-phase single-DNA-strand trimer).[26] The higher-energy barrier for C–N bond breaking at the central position may also be related to the lack of C–N bond cleavage at this position.

Effect of G on C–O Bond Cleavage. The introduction of cytosine, adenine, and guanine into the central position of oligonucleotide trimers in general resulted in minor changes in the yield of products arising from C–O bond cleavage (i.e., the release of monomers and dinucleotides from trimers (Table 1)). There was a significant increase in the release of pXT and TXp fragments that resulted from C–O cleavage of the sugar moiety connected to the terminal base; however, this can probably be attributed to enhanced electron localization at the termini as described previously. Interestingly though, there was a significant increase in the yield of pT and Tp fragments arising from C–O cleavage at the internal position for TGT in comparison to that for the other trimers (Table 1). Moreover, the total damage decreased 2-fold in going from TTT to TGT, and thus the presence of guanine in comparison to other bases significantly increased the C–O bond cleavage as a percentage of the total damage. Thus, C–O bond cleavage at positions 4 and 5 of oligonucleotide trimers (Scheme 1) is favored when the central nucleobase is guanine.

The finding that guanine enhances C–O bond cleavage is supported by theoretical studies as related to differences in electron density. In particular, Gu et al. recently showed that 2′-deoxyadenosine-3′,5′-diphosphate (pAp) and 2′-deoxyguanosine-3′,5′-diphosphate (pGp) radical anions formed below 3 eV (i.e., via pathway 3 in Scheme 2 in our case) have low vertical detachment energies and the excess electron, attached to pGp, exclusively resides in the vicinity of the 3′-phosphate group of the nucleotide containing guanine when it is solvated.[27] The high excess electron density on the phosphate group of pGp might result from electron transfer from the guanine moiety to induce 3′ C–O bond cleavage giving pT as the

product. Ray et al. also suggest experimentally that guanine, because of its high dipole moment, could function as a gateway when capturing LEEs and that when the electron is captured by the base it rapidly transfers to the DNA backbone.[28] In contrast, Schyman et al. do not observe the excess electron near the phosphate group in an aqueous solution in their theoretical study of LEE attachment to guanosine 3′-monophosphate, but they obtain a low activation energy for the phosphodiester bond rupture induced by LEEs in both the gas phase and aqueous solution.[29] For LEE attachment, they predicted an energy barrier of 10.3 kcal/mol for the 3′ C–O bond breaking in gas phase and 5.3 kcal/mol in aqueous solution. The energy of 3′ C–O bond breaking for G derivatives was generally lower than that for thymine and cytosine derivatives. (The values obtained from different calculations are summarized in Table 2.) From these and our results, one can expect that DNA strand breaks via pathway 3 will most likely occur when a LEE is initially attached to guanine.[10c,d,29-31] In fact, Solomun et al. recently reported a linear increase in DNA fragmentation as a function of the number of guanine residues upon exposure of self-assembled oligonucleotide monolayers to 1 eV electrons.[32]

Summary

We investigated damage induced by 10 eV electrons on TXT where X = T, C, A, or G. The total damage decreases in the order of T > C > A > G. Considering that base release occurs almost exclusively from the termini, we conjecture that in a long DNA polymer, base release will be suppressed (because of the lack of termini) and C–O cleavage will be favored with respect to total damage. The observed dependence of the yield of various products on the nature of the central base can be explained by the influence of base electronegativity on either initial electron capture or electron transfer between the bases. Constructive interference of the wave function of electron capture by the trimer would favor transient anion formation in TTT whereas substitution by a different base in the middle would have a tendency to destroy this coherence and hence lower the capture probability. Furthermore, when the electron is temporarily located on a base, the transient anion thus formed could transfer within its lifetime to other bases and the backbone. Here again, the nature of the base and the secondary structure of the base combination are expected to play a major role. Indeed,

Table 2. Different Calculations for the Energy Barrier of C–O Bond Rupture after LEE Attachment.

authors	model	method/basis sets	ΔE^{a} gas phase	ΔE^{a} aqueous solution[b]	reference
Schyman et al.	Gp	B3LYP/DZP++	10.28	5.25	29
Gu et al.	Tp	B3LYP/DZP++	7.06	13.73	30a
Gu et al.	Cp	B3LYP/DZP++	6.17	12.82	30a
Bao et al.	pT	B3LYP/DZP++	13.84	17.86	30a,b
Bao et al.	pC	B3LYP/DZP++	14.27	17.97	30a,b
Sevilla et al.	pT	B3LYP/6-31G*	14.8	28.9	31
Simons et al.	Tp	SCF/6-31+G*	8.26–13.01[c]	6.71–24.53[c]	10c,26
Simons et al.	Cp	SCF/6-31+G*	8.38–15.6[d]	5.1–28.1[d]	10d,26

[a]ΔE = Energy barrier for 3′C–O or 5′C–O bond breaking (in kcal/mol). [b]Polarizable continuum model (PCM) using water as a solvent with the dielectric constant of $\varepsilon = 78.39$. [c]Electron energy E (eV) between 0.25 and 1.0. [d]Electron energy E (eV) between 0.2 and 1.5.

the sensitivity of T compared to that of other bases suggests that sequences containing consecutive pyrimidines are probably preferential sites for LEE-induced DNA damage.[23] Paradoxically, though, the pathway leading to C−O bond cleavage is enhanced when the C−O bond is next to G in comparison to other bases.

Supporting Information Available. Analysis of LEE-induced damage to TCT, TAT, and TGT by HPLC-UV. This material is available free of charge via the Internet at http://pubs.acs.org.

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Chapter IV – Third article

Low-Energy Electron-Induced DNA Damage: One Electron Induces Double Events in the 5-Bromouracil Substituted Oligonucleotide Trimer 5'-TpTpT-3'.

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

To be submitted.

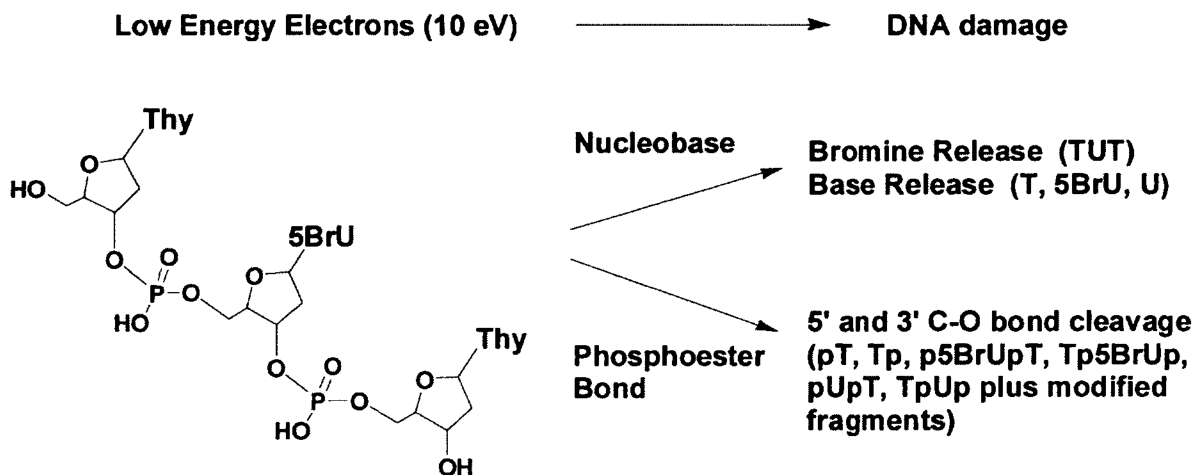
I did all the experiments in this paper and wrote the first version of the manuscript. Mr. Pierre Cloutier helped me to repair the machine when it had problems. Prof. Léon Sanche and Prof. J. Richard Wagner are my joint supervisors and helped me to revise the manuscript and to submit it.

Résumé

Ce travail porte sur la réaction des EBE avec les trimères 5'-TpXpT-3', ou X représente la thymine ou le 5-bromouracile, un radiosensibilisateur potentiel. L'analyse HPLC-UV des trimères irradiés par des EBE de 10 eV permet de quantifier six fragments connus, qui résultent de la libération de nucléobases non-modifiées (T et 5BrU) et la rupture de la liaison C-O (pT, Tp, pXT, TXp), ainsi que la production de TUT. Ce dernier composé résulte de la débromation du T5BrUT. Les résultats montrent que, lorsque T5BrUT est irradié par des EBE, le TUT est le produit principal. La substitution de la thymine centrale de TTT par le 5-bromouracile augmente le total des dommages au trimère d'au moins 50%. Ce changement entraîne une augmentation de la rupture dans les liaisons C-N et C-O par rapport à TTT. Ces résultats soutiennent l'hypothèse que le 5-bromouracile est un capteur électronique fort et que les EBE sont d'abord captés par la base et sont ensuite transférés de la base vers la liaison C-N, conduisant ainsi à la libération de la base. Alternativement, les EBE peuvent être transférés à la liaison C-O ce qui conduit à la rupture de la liaison phosphodiester. Plus important encore, un pourcentage relativement grand de fragments provenant de la rupture de la liaison N-C et la liaison C-O qui comportent l'uracile au lieu de 5-bromouracile (par exemple, l'uracile, pUT et TUp), indiquent qu'un seul électron de 10 eV induit des événements doubles, i,e., la rupture des liaisons Br-C et C-N ou les liaisons Br-C et C-O. Ces résultats suggèrent que les EBE provenant des rayonnements ionisants induisent de multiples dommages dans l'ADN surtout s'il contient des bases modifiées ayant une grande section efficace de capture d'électrons telles que le 5-bromouracile.

Mots clés: 5-bromouracile, radiosensibilisant, événements doubles, électrons

SYNOPSIS TOC



ABSTRACT

This work addresses the reaction of low energy electrons (LEE) with oligonucleotide trimers 5'-TpXpT-3' where X represents thymine and 5-bromouracil, a potential radiosensitizer. Analysis of the trimers irradiated with 10 eV electrons by HPLC-UV permits the quantification of six known fragments, which arise from the release of non-modified nucleobases (T and 5BrU) and C-O bond cleavage (pT, Tp, pXT, TXp), as well as TUT, which arises from debromination of T5BrUT. The results show that, when T5BrUT is irradiated with LEE, TUT is the major product. Substitution of the central thymine of TTT with 5-bromouracil increases the total damage to the trimer by at least 50%. This change leads to an increase in both C-N bond and C-O bond cleavage compared to TTT. These findings support the hypothesis that 5-bromouracil is a strong electron acceptor and that LEE are initially captured by the base and then transferred from the base to either the C-N bond leading to base release or to the C-O bond leading to phosphodiester bond cleavage. Most importantly, a relatively large percentage of fragments arising from N-C and C-O bond cleavage contained uracil rather than 5-bromouracil (e.g., uracil, pUT and TUp), indicating that a single 10 eV electron induces double events, i.e., Br-C and C-N cleavage or Br-C and C-O cleavage. These findings suggest that LEE from ionizing radiation induce multiply damaged sites in DNA especially when it contains modified bases such as 5-bromouracil that have a high cross-section for electron capture.

Key words: 5-Bromouracil, radiosensitizer, double events, electrons

Introduction

Radiotherapy is the most common nonsurgical treatment of patients with malignant tumors. Such treatments rely on the lethal and mutagenic effects of ionizing radiation on cancer cells which, classically, can be divided between reactions of radicals resulting from the ionization of water (hydroxyl radicals, solvated electrons, H-atoms; referred to as the indirect effect) and the reaction of organic radical cations resulting from the direct ionization of DNA components (the direct effect).[1] In addition, there is a third pathway of damage, which has been given relatively little attention in the past. This pathway involves the reaction of secondary low energy electrons (LEE; < 30 eV) arising from the initial deposition of the energy of the primary electrons.[2,3] For example, the absorption of 1 MeV of ionizing radiation leads to the generation of $\sim 3 \times 10^4$ of potentially damaging secondary LEE.[4] Indeed, LEE is largely responsible for the ionization of water and DNA components upon exposure to ionizing radiation. However, LEE may also contribute to additional reactions, which occur below the ionization threshold of DNA, and subsequently may lead to damage. Our overall goal is to elucidate the mechanism of LEE-induced damage in chemical terms in order to develop new modalities of radiotherapy and combined chemo/radiotherapy.[5]

The interaction of LEE with DNA has been investigated using simple DNA components (bases, nucleosides, nucleotides), oligonucleotides, and plasmids.[6,7] The majority of these studies report the formation of small fragments (<100 a.m.u.) upon irradiation with LEE under ultra high vacuum.[7] In addition, a number of studies demonstrate the formation of single and double strand breaks by post-irradiation analysis of DNA damage using gel electrophoresis.[6e,8] Together, the above studies support a general mechanism of LEE-induced DNA damage in which LEE forms a transient molecular ion that dissociates into radicals through a resonance process, referred to as dissociative electron attachment (DEA). This mechanism is supported by theoretical studies involving quantum theory simulation, in particular, density functional theory (DFT).[9] These studies however are restricted to the interaction of small DNA components, for example, dinucleoside phosphate systems are among the largest studied by reliable theoretical methods.[10] On the basis of theoretical studies, an electron transfer model has been proposed in which LEE is initially captured by the

base and then transferred to either the *N*-glycosidic bond (C-N) leading to base release or the phosphodiester-sugar (C-O) bond leading to strand cleavage.[11] Despite much experimental and theoretical efforts, the chemical steps involved in the formation of DNA damage induced by LEE remain poorly understood.

The atom of bromine has a very similar van der Waals radius to that of the methyl group CH_3, and thus, 5-bromouracil resembles thymine in double stranded DNA. Indeed, 5-bromouracil may be incorporated into cellular DNA in place of thymine upon incubation of cells with the corresponding 2'-deoxyribonucleoside. The incorporation of 5-bromouracil does not significantly change the DNA structure or base coding properties. The presence of 5-bromouracil (5BrU) in cellular DNA however sensitizes cells to radiation-induced DNA damage and may lead to cell death.[12] Thus, 5-bromouracil and its derivatives have been seriously considered as potential radiosensitizers in radiation therapy.[13] The well-accepted mechanism of radiosensitization involves the reaction of solvated (i.e., thermalized) electrons with 5-bromouracil leading to heterolytic cleavage of the C-Br bond and the formation of bromide anions and highly reactive uracil-5-yl π radicals. Subsequently, uracil-5-yl radicals can abstract a hydrogen atom from the sugar moiety of the neighboring base giving products that lead to phosphate-sugar cleavage in double stranded DNA.[14,15] On the other hand, it is reasonable to propose that nonthermal LEE are also involved in the mechanism of radiosensitization by 5-bromouracil.[16] From studies in the gas phase, the reaction of LEE and 5-bromouracil induces the desorption of bromide anions and uracil-5-yl radicals (i.e., 5BrU + e^- (~0 eV) → $5BrU^-$ → (U-yl)• + Br^-), and to a lesser extent, bromide radicals and uracil-5-yl anions.[16a,b] This process shows a resonance at very low electron energies (< 2.0 eV) indicating that it occurs by dissociative electron attachment (DEA).[16a,b] In addition, the reaction of LEE with 5-bromouracil leads to the production of other small molecular weight ions, which include OCN^- and $H_2C_3NO^-$, resulting from bond dissociation.[16b]

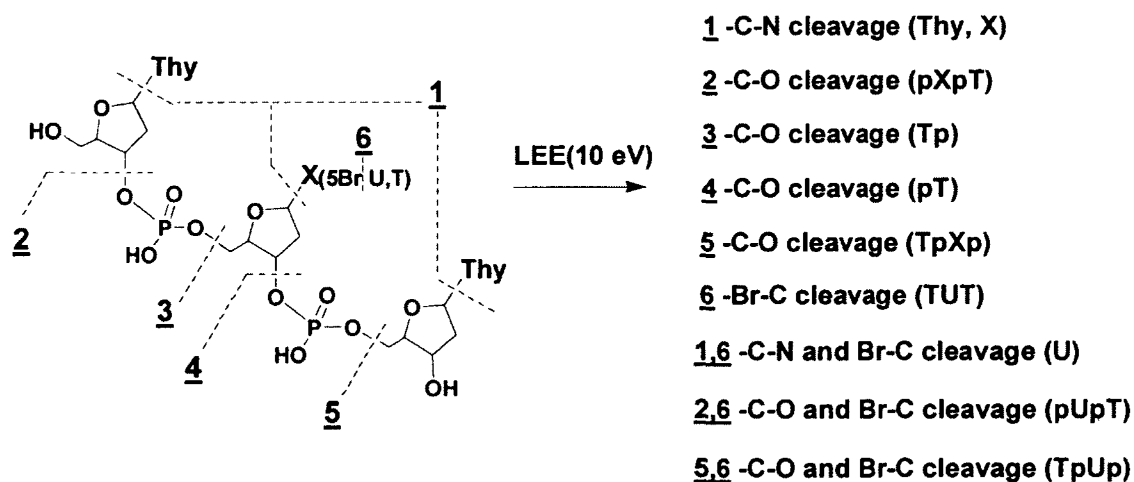
In the present study, the reaction of LEE with oligonucleotides containing 5-bromouracil is examined by direct chemical analysis of the products remaining after irradiation with LEE of films deposited on a metallic substrate. Our model system is an oligonucleotide trimer (TTT) in which the central thymine is substituted for either uracil or 5-bromouracil (Scheme 1). Our results show that 10 eV electrons induce

C-Br cleavage resulting in TUT as the major product. In addition, the releases of fragments containing uracil (pUT and TUp) and non-modified uracil indicate that an electron with only 10 eV induces double events, i.e., Br-C and C-N cleavage or Br-C and C-O cleavage, in single T5BrUT molecules.

Experimental Section

Sample Irradiation. Experimental details of the LEE irradiator and the procedure to irradiate thin films of oligonucleotides have been reported elsewhere.[17] Briefly, approximately 80 μg of HPLC purified compound was dissolved in 5 mL of nanopure grade H_2O (Milli-Q water system, 18 MΩ·cm, 25°C) and the solution was deposited by spin-coating onto the inner surface of seven chemically clean tantalum

Scheme 1. Structure of 5'-TpXpT-3' and position(s) of cleavage for the *N*-glycosidic bond (1), phosphodiester bond (2-5), and C-Br bond cleavage (6).



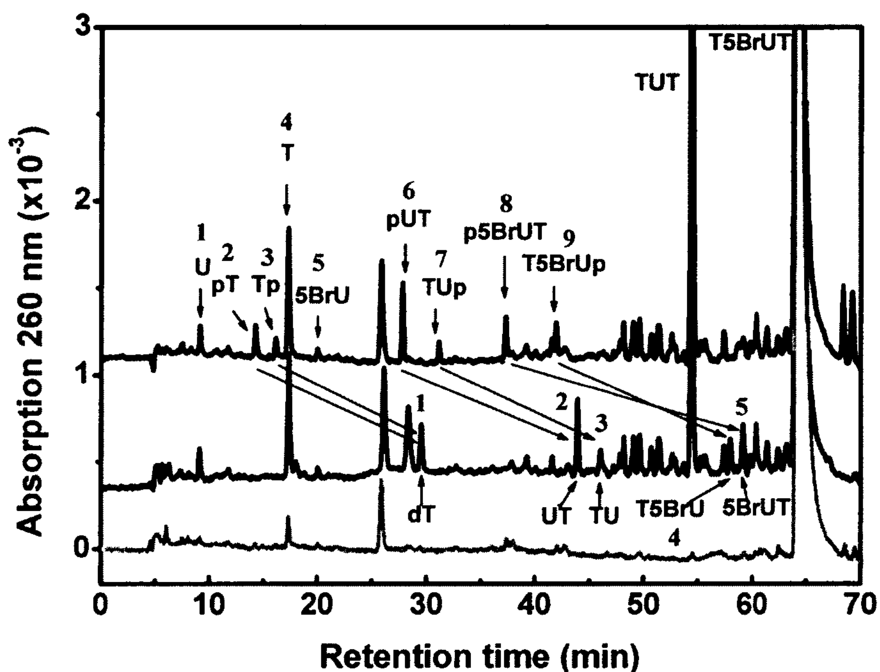
cylinders (3.2 cm × 2.5 cm diameter). The average thickness of the film on the cylinder was 2.5 ± 0.1 nm (4 to 5 monolayers (ML)), assuming that the molecules are uniformly distributed onto the inner surface of the cylinder and that the average density of DNA is 1.7 g cm^{-3}.[18] All manipulations of samples, before and immediately after irradiation, were carried out in a sealed glove box under an atmosphere of dry nitrogen. After spin-coating, the samples were transferred to the LEE irradiation chamber, which was subsequently evacuated for ~24 h to reach a pressure of about 10^{-9} Torr at ambient temperature. The irradiator generated a uniform electron beam over the entire sample surface of the cylinder with an energy resolution of 0.5 eV full width at half-maximum (FWHM). Each cylinder containing a sample was irradiated individually with constant irradiation time, beam current, and incident electron energy. Under present conditions, the time of irradiation was 2.5 min giving a total exposure of approximately 10^{16} electrons per cylinder. The current and irradiation time were adjusted to give an exposure well within the linear regime of the dose response curve. Within this linear regime, we can assume that the film does not accumulate excess charge.[6c] The average thickness of the film (2.5 nm) was considerably smaller than the penetration depth (5-20nm) of 10 eV electrons in either liquid water or amorphous ice.[19] Because the penetration depth is in fact smaller than the inelastic mean free path for electronic excitation of biological solids (9-28nm), electrons impinging on the film will be transmitted to the metal substrate and will, at most, lead to single inelastic scattering events with the molecules.[20]

HPLC Analysis. After irradiation with LEE, the samples were removed from ultra high vacuum and placed into a dry nitrogen-purged glove box. The compounds and their radiation products were recovered from the surface of the tantalum cylinders by the addition of 12 mL of nanopure grade H_2O (Milli-Q water system, 18 MΩ·cm, 25°C). The nonirradiated samples (three cylinders) and the irradiated samples (four cylinders) were pooled, frozen and lyophilized to dryness. The nonirradiated and irradiated samples were dissolved in 150 μL and 200 μL of nanopure grade H_2O, respectively, in order to have an equal amount of product per volume in each sample. Half of the sample was analyzed by HPLC. The other half was first treated with alkaline phosphatase (1 unit, Roche Applied Science) for 1 h at 37°C to remove the terminal phosphate group of nucleotides, and then analyzed by HPLC under the same conditions as the non-treated sample. In the chromatogram, the identity of DNA

fragments containing a terminal phosphate group was supported by their conversion to derivatives without a terminal phosphate group upon treating with alkaline phosphatase (see Figure 1). The HPLC system consisted of a Waters alliance HT system equipped with a refrigerated autosampler, a 2795 separations module and a 2487 dual wavelength absorbance detector. The separation of products was achieved using an analytical YMC-Pack ODS-A column (250 × 6 mm), maintained at 30°C, using a linear gradient from 1% to 10% acetonitrile in buffer containing 25 mM NaH_2PO_4 (pH 5.7) over an interval of 60 min and at a flow rate of 1.0 mL/min. All products were detected at 210 and 260 nm. The total yield of damage in terms of the number of damaged molecules per 1000 target molecules was estimated by the amount of nonmodified trimer in irradiated and nonirradiated samples. The yield of LEE-induced products was determined by calibration with authentic reference compounds.

Reference Compounds. TTT and TUT were purchased from Alpha DNA (Montreal, QC) and T5BrUT was purchased from UCDNA Services (Calgary, AB). 5-Bromouracil (5BrU), uracil (U), thymine (T), thymidine (dThd), and mononucleotides (pT and Tp) were purchased from Sigma-Aldrich (St. Louis, MO). In addition, several standard dinucleotide fragments of TpXpT (X represents thymine, uracil, 5-bromouracil respectively) containing either a 3' or 5' terminal phosphate group were prepared by enzymatic digestion of the corresponding trimers with micrococcal nuclease (Roche Applied Science, giving TXp) and with P1 nuclease (MP Biomedical, giving pXT).[21] All samples were purified by HPLC-UV using the same methods as described above for irradiated samples except that a volatile buffer solution, i.e., triethylamine acetate (20 mM, pH 7) was used. The purified solution was lyophilized to dryness and redissolved in nanopure grade H_2O before spin-coating and irradiation. The yield of LEE-induced DNA fragments was determined by comparison of the peak area at 260 nm with the peak area of standard solutions prepared from commercially available compounds (nucleobases, 2'-deoxynucleosides, and mononucleotides (pT, Tp)). In addition, other fragments containing a terminal phosphate (pXpT and TpXp) were obtained by the partial digestion of oligonucleotide trimers with nucleases together with HPLC purification of the fragments. The concentration of standard solutions of trimers and their fragments was estimated by their optical absorption at 260 nm taking the given molar absorptivity of DNA bases

Figure 1. Analysis of Tp5BrUpT radiation products by HPLC/UV detected at 260nm. The trimer was exposed to 10^{16} electrons with an energy of 10 eV. The lower chromatogram (green) depicts the analysis of a nonirradiated sample. The upper and middle chromatograms show the corresponding irradiated sample, which was divided in two parts: one was treated with alkaline phosphatase (middle; red) and the other received no treatment (upper; black). Blue arrows illustrate the conversion of products with a terminal phosphate to that without.



(http://www.basic.northwestern.edu/biotools/oligocalc.html). The optical density was measured using a spectrophotometer (Hitachi U-2000).

Results

The reaction products remaining in the film after irradiation of Tp5BrUpT by LEE were detected and quantified by HPLC-UV (see Figure 1). Their yields are listed in Table 1. As much as 75% of the compounds produced were accounted for in our analyses. The products identified in this work arise from cleavage of the *N*-glycosidic bond (T and 5BrU) and phosphodiester bonds of oligonucleotide trimers (pT, Tp, pTT, TTp, p5BrUT, T5BrUp) together with cleavage of bromine-carbon (Br-C) bond resulting in products containing uracil (TUT, U, pUT, TUp). Our results show that 10 eV electrons induce C-Br cleavage resulting in TUT as the major product. In addition, the release of fragments containing uracil (pUT and TUp) and non-modified uracil indicate that a single 10 eV electron might induce two lesions, i.e., Br-C and C-N cleavage or Br-C and C-O cleavage, in single T5BrUT molecules.

In Figure 1, the lower chromatogram (green) depicts the analysis of a nonirradiated sample which underwent the same procedure as the irradiated sample. The chromatogram of the nonirradiated sample is clean compared with that of the irradiated sample (the upper one; black) except for a peak around 26 min and a small peak around 17 min, which indicated that the manipulations during spin-coating, pumping of the chamber, and lyophilization did not damage the sample very much. The peak around 26 min might come from a contaminant from the solution, or the sample holder. The small peak around 17 min is thymine. It might be from the depyrimidination from the manipulations. Therefore, under our conditions, we consider that most products arise from LEE bombardment.

In Figure 1, the upper and middle chromatograms show the corresponding irradiated sample, which was divided in two parts: one was treated with alkaline phosphatase (the middle one; red) and the other received no treatment (the upper one; black). Blue arrows illustrate the conversion of products with a terminal phosphate to that without. In the upper chromatogram, the intense peak around 55 min is TUT, which indicates that when T5BrUT is irradiated with 10 eV electrons, TUT is the

Table 1. Yield of Products from LEE Induced DNA Damage

Sample[a]	Damage[b]	Bromine release		Base release				Phosphodiester bond cleavage						
TXT		TUT	%[c]	T	X(U)	Sum	%[d]	pT	Tp	pXT(pUT)	TXp(TUp)	Sum	%[e]	ratio[f]
X=5BrU	138	53.5	38.8	25.8	1.8(4.4)	32.0	23.2	4.3	2.5	3.0(4.5)	2.1(1.3)	17.7	12.8	1.81
X=T	92			17.0		17.0	18.5	1.6	1.2	4.3	2.7	9.8	10.7	1.73
X=U	82			14.8	2.5	17.3	21.1	1.1	0.8	2.4	2.1	6.4	7.8	2.70

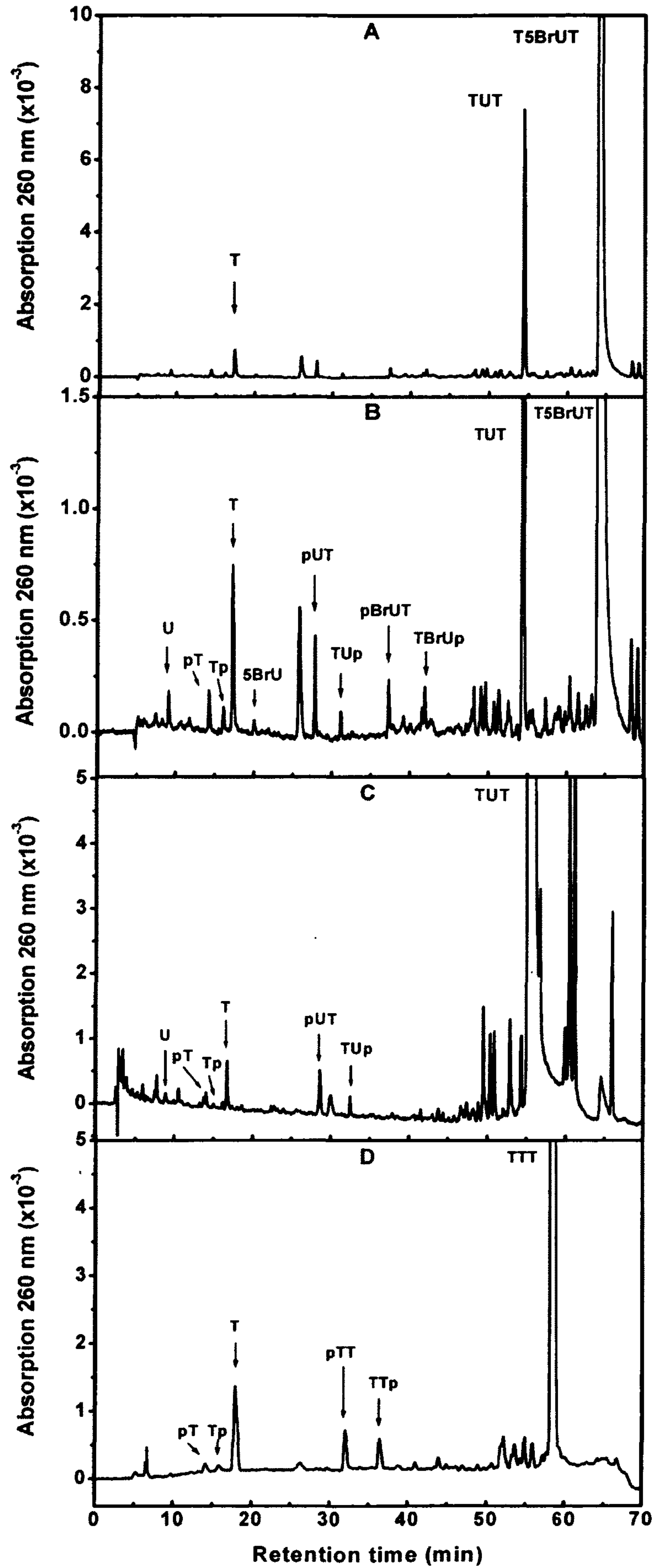
[a] DNA samples and fragments written from 5' to 3' with p indicating the position of terminal phosphate groups. [b] Total damage includes all losses of initial targeted molecules based on HPLC-UV analysis. The values are expressed as a ratio of damaged molecules to 1000 initial target molecules and are the average of three independent experiments; SD= ± 20%. [c] Percentage of Br-C cleavage based on total damage. [d] Percentage of C-N cleavage based on total damage. [e] Percentage of C-O cleavage based on total damage. [f] Ratio of C-N to C-O cleavage.

major product (40%, see yields in Table 1 and Figure 2 A). Peaks 1, 4, and 5 in the upper chromatogram are uracil, thymine, and 5-bromouracil respectively. They do not change after treatment with enzyme. Peaks 2 and 3 are pT (thymidine-5'-monophosphate) and Tp (thymidine-3'-monophosphate) respectively. After treatment with enzyme, they change to dT (thymidine, peak 1 in the middle chromatogram). Peaks 6 and 7 are pUT and TUp (written from 5' to 3' with p indicating the position of terminal phosphate group, the same in the following text) respectively. After treatment with enzyme, they change to UT and TU (peaks 2 and 3 in the middle chromatogram). The amount of pUT is 3 times higher than that of TUp (see Figures 1 and 2 B). Peaks 8 and 9 are p5BrUT and T5BrUp respectively. After treatment with enzyme, they change to 5BrUT and T5BrU (peaks 5 and 4 in the middle chromatogram). The amount of U, pUT, and TUp is comparable to that of T, 5BrU, p5BrUT and T5BrUp but the amount of them is much less compared with that of the major product, TUT. There was a 2-fold greater release of uracil base than of 5-bromouracil, and with respect to C-O bond cleavage, there was 1.5-fold greater release of pUT than of pBrUT.

The total damage of TTT is 92 per 1000 parent molecules. After the introduction of 5-bromouracil to the central site of TTT, i.e., T5BrUT, its damage increases to 138, which corresponds to a 50% increase compared with TTT. This change led to an increase in both thymine release and C-O bond cleavage compared with TTT. The thymine release is increased from 17 to 25.8. The C-O bond cleavage is increased from 9.8 to 17.7. The release of nucleobase from the trimer takes place mainly from the terminal sites, i.e., thymine release, whereas that from the central position is much less pronounced (5-bromouracil, uracil).[22]

We detected the release of non-modified uracil as well as fragments containing uracil, i.e., pUT and TUp (Figure 2 B). The identity of fragments containing uracil was confirmed by comparing the products obtained by exposure to LEE with those obtained by enzymatic nuclease digestion of TUT (Figure 1, see Experimental Section). The yield of products was measured by HPLC-UV as a function of time of bombardment. Within the first 5 min of irradiation, the formation of fragments was linear as a function of dose. Within this regime, the yield of products may be considered to result from a single electron hit, since damage resulting from multiple

Figure 2. Comparison of Tp5BrUpT, TpUpT, and TpTpT radiation products by HPLC/UV detected at 260nm. The trimers were exposed to 10^{16} electrons with an energy of 10 eV. Picture A focuses on the formation of the major product, TpUpT. Picture B, C, and D show the strand break products of Tp5BrUpT, TpUpT and TpTpT.


A
T5BrUT
TUT
T
B
TUT
T5BrUT
T
pUT
pBrUT
U
TBrUp
pT
Tp
5BrU
TUp
C
TUT
T
U
pUT
pT
Tp
TUp
D
TTT
T
pTT
TTp
pT
Tp
Absorption 260 nm (x10^{-3})
Retention time (min)

successive damaging collisions will necessarily be nonlinear with radiation exposure. In our experiments, the current of LEE and irradiation time were adjusted to give the samples an exposure well within the linear regime of the dose response curve. Furthermore, these conditions ensure that the DNA film on the inner surface of the tantalum cylinder does not accumulate any appreciable amount of negative charges. Thus, the products in our experiments result from single collisions of incident electrons with T5BrUT.[6c]

Discussion

Thus far, there have been enormous experimental efforts in our group to elucidate the mechanism of LEE-induced damage to DNA. 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.[6b,c] These results support the hypothesis that LEE is initially captured by the base and then transferred from the base to either the *N*-glycosidic bond leading to base release or the phosphodiester bond leading to C-O bond cleavage.[23] Recently, we reported the effect of different nucleobases on electron-induced damage in trimers in which the middle base is replaced with different DNA bases.[22] These studies show that the sensitivity of DNA damage decreases in the following order TTT>TCT>TAT>TGT, and the reactions of electrons with DNA bases is greatly influenced by base sequence. Furthermore, C-O bond cleavage is favored next to guanine. These results give much insight into the reaction of electrons with DNA and provide experimental support for various theoretical studies. In the present work, we have advanced our previous work to determine the effect of 5-bromouracil on electron-induced damage within an oligonucleotide trimer TXT where X represents thymine or 5-bromouracil.

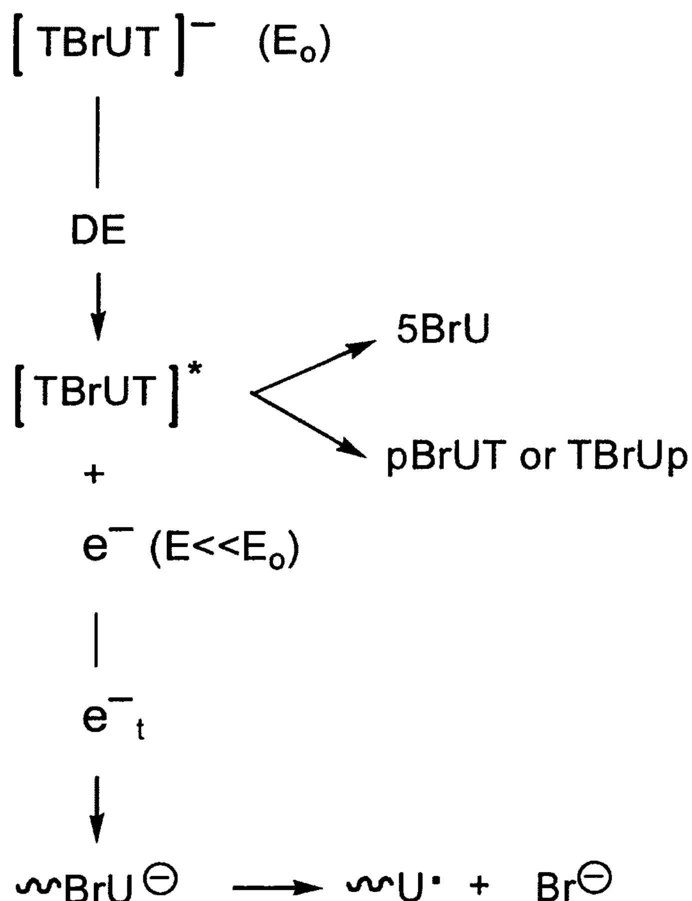
The formation of DNA damage by the reaction of solvated thermalized electrons is enhanced in DNA containing 5-bromouracil because of its high electron affinity(5BrU > 5ClU > 5FU > U ≈ T), and hence its high electron capture cross section, compared to nonmodified bases. This reaction is also enhanced owing to the

strong repulsive character of the dissociative radical anion state of 5-bromouracil, which undergoes dissociation into bromide anions and reactive uracil-5-yl radicals.[24,25] Subsequently, uracil-5-yl radicals react with neighboring DNA bases and the sugar moiety to cause a multitude of DNA damage.[26]

In the present work, we show that nonthermalized LEE undergo a similar reaction involving the formation of uracil. The reaction of LEE likely involves dissociative electron attachment (DEA). The initial capture of LEE by 5-bromouracil has been shown in the gas phase to induce Br-C cleavage leading to bromide anions and uracil-5-yl radicals.[16a,b] The uracil-5-yl radicals may be reduced to anions and protonated by water in the condensed phase, or alternatively, abstract a H-atom from the major target, T5BrUT. During this process the major product, TUT, appears. It is more likely that the uracil-5-yl radical abstracts a H-atom from the same molecule as shown by Sugiyama et al.[27] This finding supports Abdoul-Carime et al.'s prediction[16a,b] for LEE. Through analysis of the spin density on Br along the anion radical PES (potential energy surface) of 5-bromouracil as a function of C-Br distance, Li, et al. reported that the negative charge shifts to Br as the C-Br distance increases and at C-Br = 5 Å, the charge on Br is -0.74 and the spin is 0.25. The tendencies of charge/spin distribution suggest that bond cleavage of the BrU anion is more likely to result in a Br^- ion and a uracil-yl radical, rather than a Br radical and uracil-yl anion.[25a]

In our experiment, the release of uracil together with 5-bromouracil suggests that a 10-eV electron can induce both bromine-carbon Br-C and C-N *N*-glycosidic bond cleavage in the same T5BrUT molecule. The formation of fragments containing uracil (pUT and TUp) together with p5BrUT and T5BrUp also suggest that a 10 eV electron induces double events in one T5BrUT molecule, i.e., Br-C and C-O cleavage. A mechanism is proposed in Scheme 2. In this case, the resonance decays via electronic excitation leaving a nearly thermalized electron in the DNA molecule and an electronically excited state of the C-O or N-C bonds which dissociates.[22] Simultaneously, the remaining electron transfers to the lowest potential (5BrU), where it forms a transient radical anion, which dissociates into Br^- and the uracil-yl radical. If the incoming electron is captured specifically by the brominated base, it could transfer to the phosphate group. In this case, the electron leaves the base in a

Scheme 2. Proposed mechanism of formation of double lesions by the reaction of one low-energy electron with one T5BrUT molecule. This pathway represents the electronically inelastic decay channel of the transient negative ion of DNA formed at initial electron energy of E_o. The dissociative excitation (DE) produces an electron of energy $E << E_o$. Channel. e_t^- represents the transfer of the ejected electron to the BrU unit. The excited molecule [TBrUT]* dissociates, leading to glycosidic N–C bond (giving 5BrU as the product) or phosphoester C–O bond (giving pBrUT or TBrUp as the product) cleavage. The transient anion formed on the BrU unit dissociates, leading to Br–C bond cleavage.



dissociative state, which leads to the products Br^- and uracil-yl. The departing electron transfers to the C-O bond where it forms a transient radical anion. The anion dissociates by breaking the C-O bond.

According to theoretical studies, Br-C cleavage is the lowest energy process (about 1.88 kcal/mol)[25a] whereas the other processes, including 3' or 5' C-O cleavage and C-N *N*-glycosidic bond cleavage are higher by at least 3-fold and 10-fold respectively.[11,28,29] Any of these energies are far lower than the energy of 10 eV electrons (i.e., 10 eV = 230.6 kcal/mol). The 10 eV electron gives most of its energy to excite the C-O or N-C bonds and the energy left over on the nearly thermalized electron would also be sufficient for the electron transfer to the 5BrU group and subsequent cleavage of the Br-C bond (e.g., 1 eV = 23.06 kcal/mol). Thus a 10 eV electron is thermodynamically capable of inducing double events in single collisions with the target molecules. The dissociative excitation (DE) of C-O or N-C bonds together with the transfer of the remaining electron to 5BrU result in the double lesions induced by a single 10 eV electron in one T5BrUT molecule. Similarly, breakage of the C-O bond by electron transfer to the phosphate group after dissociation of the Br-C bond is also accessible energetically to 10 eV electrons.

The total damage increases from 92 to 138 when there is a 5-bromouracil in the central site, i.e., the introduction of 5-bromouracil increased the total damage by 50% compared with TTT. This effect might result from the high electron affinity of 5-bromouracil, which could translate into a higher electron capture cross section by TBrUT, but also from the low activation energy of Br-C bond cleavage after an electron is attached to it.[24,25] The presence of 5-bromouracil also results in increased releases of pT and Tp compared with TTT (Table 1), e.g., pT is increased from 1.6 to 4.3 and Tp is increased from 1.2 to 2.5. This might result from the radiosensitive effect of 5-bromouracil and the electron captured by the central 5-bromouracil is transferred to the other parts of the molecule. Owing to the high electron affinity of 5-bromouracil, electron attachment to the central base is increased where it can transfer to other parts of the molecule, i.e., the C-O bond adjacent to 5-bromouracil (Scheme 1, cleavage sites 3 and 4), is also increased, inducing a higher probability of C-O bond cleavage and resulting in increased releases of pT and Tp. However, at other positions, the C-O bond cleavage is decreased (Scheme 1, cleavage sites 2 and

5), e.g., p5BrUT is decreased from 4.3 to 3.0 and T5BrUp is decreased from 2.7 to 2.1. But, if we compare p5BrUT and pUT, T5BrUp and TUp (aforementioned, an electron may induce double events in one molecule), the cleavage at sites 2 and 5 are 7.5 and 3.4, respectively. That is, the C-O bond cleavage is also increased at the sites that are not adjacent to 5-bromouracil. The release of pUT was 50% higher than that of p5BrUT, which indicated that the one-event damage is less efficient than the double-event damage. There was no production of TU or UT with a terminal OH group, i.e., these products were only formed after treatment with phosphatase (see Figure 1).[6c]

Conclusions

This paper reports the first evidence that a low energy electron can induce double events in brominated single stranded DNA. Irradiation of T5BrUT with low energy electrons results in TUT as the major product. Compared with TTT, T5BrUT is more sensitive to low energy electrons. These findings help us to understand the mechanism of the interaction of 5-bromouracil with ionizing radiation when incorporated into DNA. They also support the experimental results or calculations indicating that 5-bromouracil incorporated in oligonucleotides does greatly sensitize single-stranded DNA and bubbles within double-stranded DNA.[30,31]

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Chapter V – Further discussion

N-glycosidic bond (N-C) cleavage in thymidine: non-ionizing process

By using the novel spin coating and LEE gun irradiator system described in Chapter I, sufficient amount of degraded compound can be produced and prepared for chemical analysis by HPLC/UV or LC/MS/MS. One nucleoside, thymidine (dT, dThd), was the first compound investigated in our system (Chapter II).

Chemical analysis showed the formation of several fragments produced by LEE bombardment. By comparing the retention time and UV spectrum in HPLC analysis with authentic standards, the main product was recognized as thymine (Figure 1). This is consistent with Zheng's result (Zheng et al., 2004a).

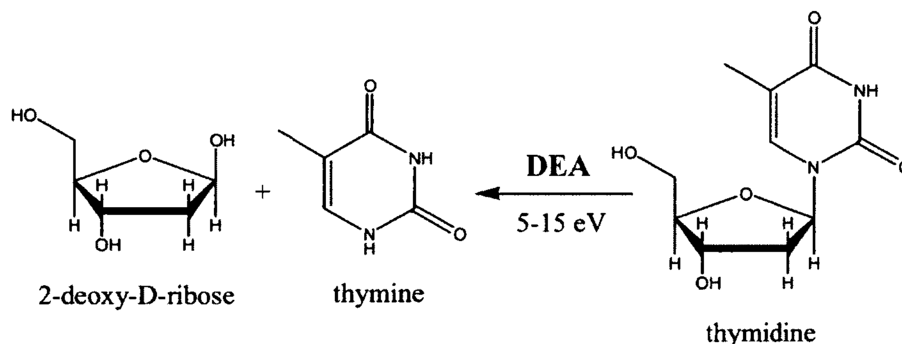
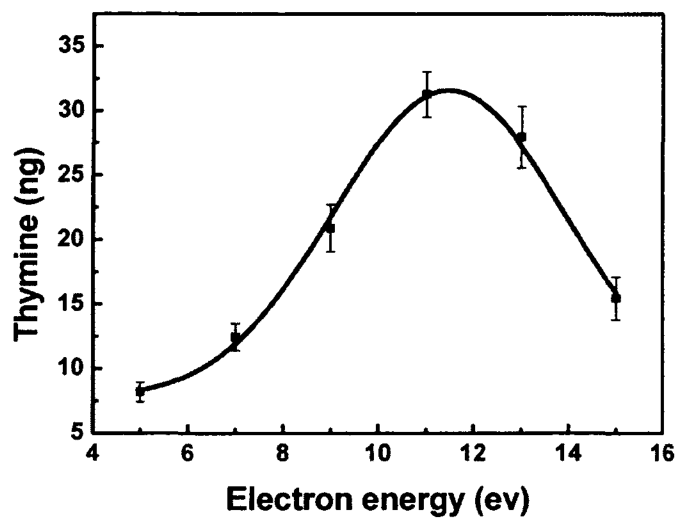


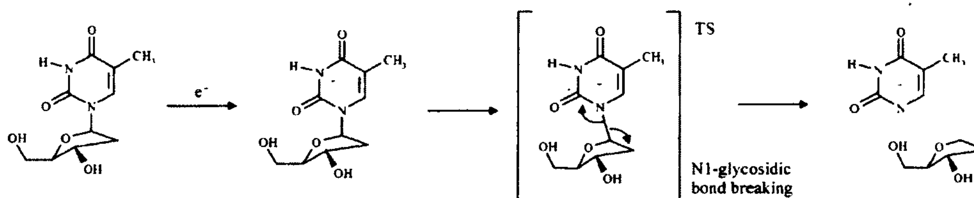
Figure 1. LEE-induced *N*-glycosidic bond cleavage in thymidine.

The formation of thymine from thymidine as a function of electron energy showed a broad resonance centered near 11-12 eV (Figure 2), which occurred principally via the formation of "core-excited" resonances located near this energy. This also supports the idea that the release of thymine from thymidine takes place via a non-ionizing resonant process, i.e., through DEA process (Zheng et al., 2006a).

Figure 2. The release of thymine from thymidine as a function of electron energy. Thymidine was irradiated with 1.0 × 10^{16} electrons at the energies of 5-15 eV. The formation of thymine exhibited a maximum around 11 eV, which occurs principally via the formation of core-excited resonances located near this energy.



DNA bases are heterocyclic and aromatic, and their low energy π*-orbitals allow them to serve as efficient captors of excess electrons (Aflatooni et al., 1998). The π molecular orbitals give rise to valence anions where the excess electron is attached to the valence molecular orbitals (Simons and Jordan, 1987). Through density functional theoretical (DFT) studies, Gu et al. (Gu et al., 2005) predicted that electron capture by the base and subsequent base release are elementary steps of the mechanism of *N*-glycosidic bond (N-C) cleavage in thymidine; i.e., in the first stage, an electron is attached to the thymine moiety and a stable thymidine radical anion is formed, and then the *N*-glycosidic bond ruptures, releasing a thymine anion and a 2-deoxyribose radical. As predicted by theoretical studies, the *N*-glycosidic bond breaking activation energy in the dT anion is low (18 kcal/mol), however, the electron detachment energy for dT anion is high (22 kcal/mol), which means removing the electron from dT anion needs more energy than breaking the *N*-glycosidic bond; thus the cleavage of the *N*-glycosidic bond dominates when an electron is attached to thymidine. The proposed mechanism is illustrated in Scheme 1 (Gu et al., 2005).



Scheme 1 (reprinted from Gu et al., 2005). A proposed mechanism for thymidine *N*-glycosidic bond break by LEE bombardment.

Increased damage in monomers

As the second series of molecules in the present study, three mononucleotides, thymidine 3'-monophosphate (Tp, 3'-dTMP), thymidine 5'-monophosphate (pT, 5'-dTMP), and thymidine 3', 5'-diphosphate (pTp, 3',5'-dTDP) were bombarded with LEE individually to investigate the effect of 3' and 5' phosphate group under the same LEE irradiation condition.

At 11 eV, an electron can temporarily attach to the phosphate group of DNA in two ways: directly or via electron transfer from the base moiety (Zheng et al., 2006a; Pan and Sanche, 2005; Pan and Sanche, 2006; Berdys et al., 2004; Simons, 2006). When electron transfer occurs, the base can be left in the ground state or in an electronically excited state (Zheng et al., 2006a; Ptasinska and Sanche, 2007). In the latter case, the transferring electron lies at a sufficiently low energy to occupy the P=O pi* orbital which crosses the strongly dissociative σ^* orbital responsible for rupture of the C–O bond (Berdys et al., 2004). Any increase in damage due only to the addition of a phosphate group must therefore arise from the direct attachment of 11 eV electrons. Thus, the results in Table 1, Chapter II show that the terminal phosphate group directly and efficiently captures 11-eV electrons and that these events lead to considerable chemical modifications, although the exact nature of some of the resulting products has not been analyzed (there is an increase in the presence of UV absorbing products in the chromatogram). The present results support results by Pan et al., Breton et al., and Park et al.'s showing that phosphate has a larger cross section than furyl ring (Pan and Sanche, 2006; Breton et al., 2004; Park et al., 2006).

Decreased *N*-glycosidic bond (N-C) cleavage in monomers

In sharp contrast to the change in total damage, the addition of terminal phosphate(s) to monomers causes the reverse effect (a marked decrease) in the release of non-modified thymine. We cannot rule out the possibility that some base release arises from sugar radicals generated by C–O bond cleavage or other processes. For example, the majority of carbon centered radicals on the deoxyribose moiety of nucleosides generated by OH• radical mediated H-atom abstraction undergo subsequent chemical reactions that eventually lead to the release of unaltered DNA bases (von Sonntag, 1987; Henle et al., 1995). Contrary to OH• radical mediated pathways of base release, however, we find that heating the sample under conditions that increase OH• radical mediated base release several fold, do not affect the yield of LEE-mediated base release or C–O bond cleavage products (Li et al., 2006). In other words, carbon centered radicals resulting from LEE reactions do not significantly contribute to base release. In addition, the yield of base release is lower than C–O bond cleavage products in most short DNA fragments (except TT and TTT; ratio in Table 1, Chapter II). Thus, similar to the release of thymine from thymidine, the most

likely pathway to explain base release in LEE reactions involves DEA leading to direct N–C bond cleavage (Zheng et al., 2004a; Abdoul-Carime, et al., 2004a; Li et al., 2006; Gu et al., 2005).

Theoretical studies have indicated that the energy barrier required to break the *N*-glycosidic bond in dT radical anion is higher (18.9 kcal/mol) than that required to break either the C5'–O5' σ-bond of pT anion (13.84 kcal/mol) or C3'–O3' σ-bond of Tp anion (7.06 kcal/mol) in the gas phase (Gu et al., 2005; Gu et al., 2006; Bao et al., 2006). Gu and Bao have also indicated that the energy barrier for breaking the *N*-glycosidic bond in dC anion is 21.6 kcal/mol, and it is higher than the energy barrier for breaking either the C5'–O5' σ-bond of pC anion (14.27 kcal/mol) or the C3'–O3' σ-bond of Cp anion (6.17 kcal/mol) in the gas phase (Gu et al., 2005; Gu et al., 2006; Bao et al., 2006). In the model of pGp anion (Gu et al., 2010), the authors found that either in the gas phase or aqueous solution, *N*-glycosidic bond cleavage has a much higher energy barrier (24.08 kcal/mol in gas phase, 9.99 in aqueous solution) than both C5'–O5' σ-bond and C3'–O3' σ-bond cleavage (11.23–12.97 kcal/mol in gas phase, 1.06–3.56 in aqueous solution). These results are illustrated in Table 1 and mean that when an electron is attached to the nucleobase, the *N*-glycosidic bond is harder to break, and electron transfer to the phosphate C–O bond is facilitated, and as a result C–O bond cleavage predominates. Our experimental results support these theoretical predictions.

Table 1. Different calculations on the energy barrier of C–O and N–C bond rupture after LEE attachment.

Authors	model	method/basis sets	ΔE^{a}(C–O)		ΔE^{b}(N–C)	
			gas phase	aqueous solution[c]	gas phase	aqueous solution[c]
Bao et al., 2006; Gu et al., 2005	pT; dT	B3LYP/DZP++	13.84	17.86	18.9	
Gu et al., 2005, 2006	Tp; dT	B3LYP/DZP++	7.06	13.73	18.9	
Bao et al., 2006; Gu et al., 2005	pC; dC	B3LYP/DZP++	14.27	17.97	21.6	
Gu et al., 2005, 2006	Cp; dC	B3LYP/DZP++	6.17	12.82	21.6	
Gu et al., 2010c	pGp	B3LYP/DZP++	11.23–12.97	1.06–3.56	24.08	9.99

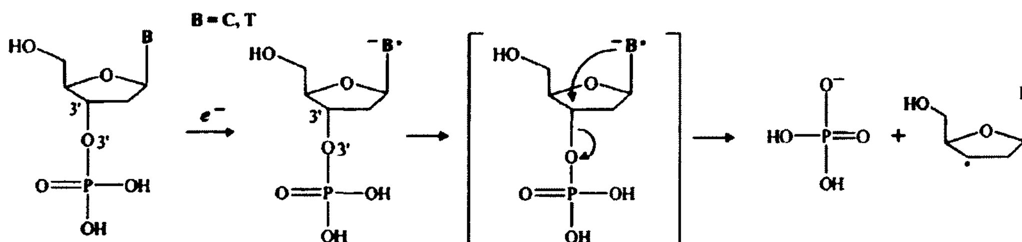
[a]ΔE = Energy barrier for 3' C–O or 5' C–O bond breaking (in kcal/mol) [b]ΔE = Energy barrier for N–C bond breaking (in kcal/mol) [c]Polarizable continuum model (*PCM*), using water as solvent with the dielectric constant ε=78.39.

The process of electron transfer

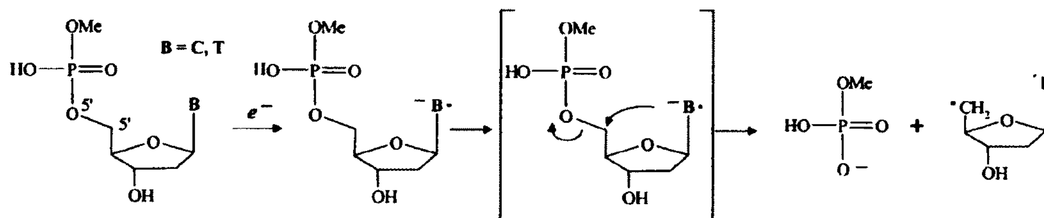
Our results (Table 1, Chapter II) can be explained by a simple model in which the electron is initially captured by a base or by a phosphate group with a strong propensity for the terminal position. The high capture and fragmentation cross sections of the latter group increase total damage which, in counter-part, decreases rupture of the *N*-glycosidic bond and the 5'-phosphate and 3'-phosphate C–O bond. In the absence of a phosphate group, the yield of base release is increased because of the absence of the phosphate group which competes for electron capture. In other words, initial capture by the base is increased and the latter directs the additional electron to the *N*-glycosidic bond or the C–O bond.

Scheme 2 illustrates the results of a density functional theoretical (DFT) study of LEE-induced phosphoester C–O bond cleavage in thymidine nucleotide at 3' (Gu et al., 2006) and Scheme 3 at 5' (Bao et al., 2006). The results indicated that in the first stage, a stable thymidine anion is formed, followed by electron transfer to the phosphate group C–O bond, and then, the phosphoester C–O bond breaks to release a phosphate anion and a 2-deoxyribose radical, which will result in LEE-induced single strand break.

Scheme 2 (reprinted from Gu et al., 2006). A proposed mechanism of the LEE-induced single strand bond cleavage in pyrimidine nucleotide at 3'.

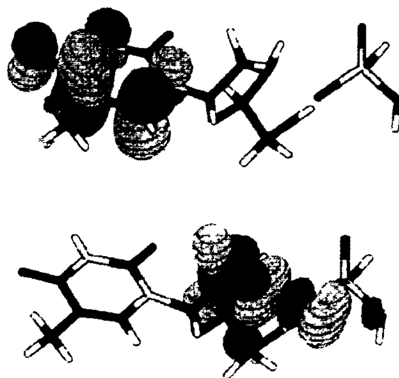


Scheme 3 (reprinted from Bao et al., 2005). A proposed mechanism of the LEE-induced single strand bond cleavage in pyrimidine nucleotide at 5'.



Berdys et al. also studied LEE attachment to the thymine π*-orbital in thymidine nucleotide through ab initio calculations (Berdys et al., 2004c). Their results (Figure 3) show the orbital occupied by the attached electron at two R values (C–O bond length). At the smaller R value, thymine π*-orbital (top) hosts the excess electron, but as R value increases above about 1.9 Å (C–O bond breaks), the excess electron transfers from the thymine moiety through the deoxyribose and onto the phosphate unit (bottom). The results indicated that an excess electron first attaches to the base moiety in thymidine, followed by electron transfer to the phosphate group C–O bond as the C–O bond breaks.

Figure 3 (reprinted from Berdys et al., 2004c). Orbital containing the excess attached electron in thymidine nucleotide for C–O bond length value below 1.9 Å (top) and for C–O bond length value beyond 1.9 Å (bottom).



Our results show that the addition of terminal phosphate increases total damage but decreases base release (N−C) and phosphodiester−sugar (C−O) cleavage (Table1, Chapter II), which indicates that initial electron capture by terminal phosphate does not transfer to the base moiety and give rise to N−C and C−O cleavage or that electron capture by the phosphate does not lead to strand cleavage. Thus, as predicted theoretically (Gu et al., 2006; Bao et al., 2006; Berdys et al., 2004c) and supported by experiments (Zheng et al., 2006a; Zheng et al., 2006b; Martin et al., 2004), 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 and then transfer to the *N*-glycosidic bond (N-C) and phosphoester bond (C-O) to induce their cleavages.

Cleavage at 3' C−O bond and 5' C−O bond in oligonucleotide dimer TT

Interestingly, the products resulting from 3' C−O bond cleavage were higher than those from 5' C−O bond cleavage in TT. As shown in Figure 4, C3'−O3' σ-bond break (site 1, Figure 4) results in pT as the product and C5'−O5' σ-bond break (site 2, Figure 4) results in Tp as the product. The amount of product pT (7.2) is higher than that of Tp (5.7) (Table1, Chapter II). In recent theoretical studies (Table 1, Chapter V), Gu et al. reported that the energy barriers for C3'−O3' σ-bond cleavage of Tp anion in gas phase and aqueous solution are 7.06 kcal/mol and 13.73 kcal/mol, respectively (Gu et al., 2006), while Bao et al. (using the same techniques as Gu et al.) reported that the energy barriers for C5'−O5' σ-bond cleavage of pT anion in gas phase and aqueous solution are 13.84 kcal/mol and 17.86 kcal/mol, respectively (Bao et al., 2006). Therefore, they concluded that the C3'−O3' σ-bond rupture dominates the LEE-induced SSB of DNA. Our results are in agreement with their theoretical studies.

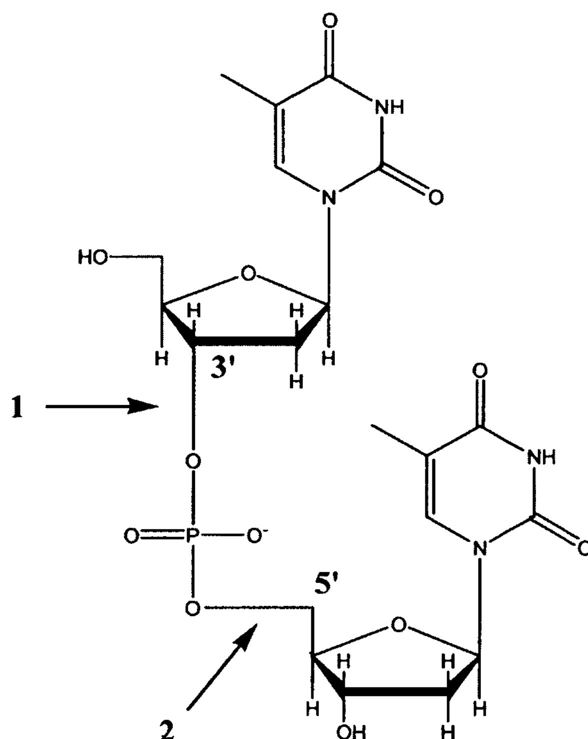
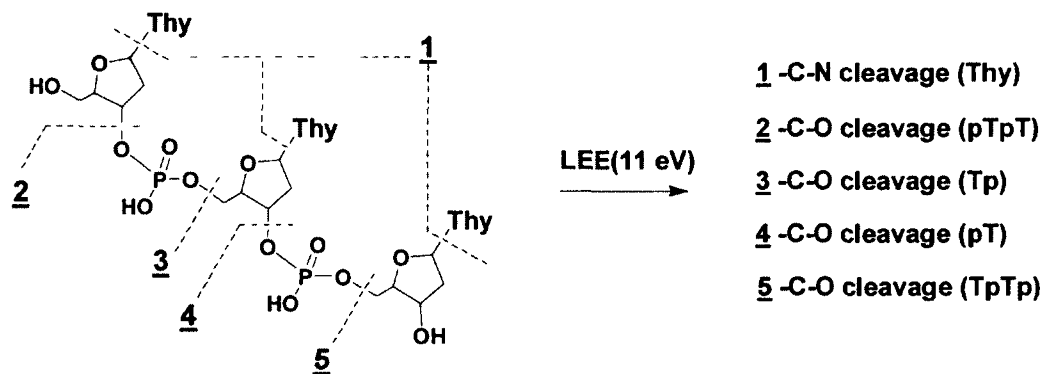


Figure 4. Phosphodiester bond cleavage sites in oligonucleotide TT.

Effect of base stacking in oligonucleotide trimer TTT

A more complex molecule, oligonucleotide trimer TpTpT, was bombarded using our system (Scheme 4). As seen from Table 1, Chapter II, the total yield of products is larger for TTT than TT and its phosphorylated derivatives. Also, TTT yields are the highest for base release and C–O bond cleavage (Table 1, Chapter II). This indicates that LEE are more efficient at creating DEA-mediated damage in TTT than in TT and its phosphorylated derivatives. Thus, base release and C–O bond cleavage appears to be the major pathway of damage in TTT. In other words, other processes including LEE reactions involving the phosphate group, the deoxyribose moiety or the base moiety are minor in comparison to base release and electron transfer to the C–O bond in LEE-mediated damage. This may be attributed to the effect of base stacking in TTT. Lastly, it should be noted that the distribution of damage was strongly skewed toward the ends in TTT. The majority of damage appeared at the 5' (25.1, site 1 plus site 2 in Scheme 4) and 3' (21.6, site 1 plus site 5

in Scheme 4) termini whereas the internal base (2.5 and 2.9, site 3 and site 4 in Scheme 4) suffered 8-fold less damage (We assume that base release from either end is approximately equal and that base release from the middle T is close to zero (Zheng et al., 2005); thus, if the total is 29.5 then each side is approximately 14.75. With these values we estimate the bias to be 25.05 on the 5'-side (14.75 + 10.3, the sum of C–N and C–O bond cleavage) and 21.55 on the 3'-side (14.75 + 6.8)). This feature of damage in TTT may also be explained by considering the effect of base stacking so that the outside bases shield the internal base from initial LEE attack in LEE-mediated damage (Zheng et al., 2005, 2006b).



Scheme 4. Structure of 5'-TpTpT-3' and position(s) of cleavage for the *N*-glycosidic bond (1), phosphodiester bond (2-5). Thy represents thymine. The products resulting from the bond cleavage (1-5) are indicated in the parentheses.

Effect of base stacking on damage in oligonucleotide trimers

The effect of base stacking and base sequence in oligonucleotide trimers (TTT, TCT, TAT, and TGT) was investigated using the same LEE irradiator system (Scheme 1, Chapter III). Our results (Table 1, Chapter III) indicated that the substitution of purine bases, A and G, to the central site of the trimer TTT resulted in a considerable decrease in the total damage of trimers. Assuming all the other experimental procedures are the same, any decrease in the total damage due only to the substitution of a different nucleobase must therefore arise from different base stacking which will

result in different electron affinities of the trimers. Thus, the results of Table 1, Chapter III show that the different base stacking efficiently affect the capture of 10-eV electrons by the trimer and that these events lead to considerable chemical modifications.

The effect of base stacking on electron affinities in DNA trimers was also studied theoretically using semi-empirical or MP2 methods. The effect of base stacking on electron affinities in DNA trimers was first reported early in 2001 by using semi-empirical method AM1 (Voityuk et al., 2001). Their results have indicated 5'-TTT-3' and 5'-TCT-3' probably serve as the strongest electron sinks. In 2008, Kobytecka et al. examined the electron affinity of trimeric models of XTY (T represents thymine, X and Y represent different nucleobases) through theoretical calculations using MP2 level of theory with the aug-cc-pVDZ basis set (Kobytecka et al., 2008). The results indicated that the electron affinity of DNA bases (T in XTY) is greatly influenced by the adjacent bases. Replacing X and Y with pyrimidine bases can stabilize the trimeric XTY anion and as a result the electron affinity of T increases. However, replacing X and Y with purine bases destabilizes the trimeric XTY anion and results in the decreased electron affinity of T. A similar trend was reported by Kobytecka et al. in 2009 while the molecule model is XCY (C represents cytosine, X and Y represent different nucleobases, Kobytecka et al., 2009). Changes in the anion stability may be explained in part by considering the effect of base stacking. The increased anion stability in the homotrinucleotides may result from the enhanced overlap of pi orbitals during base stacking. As a result, the delocalization of the initial electron wave is increased (Caron and Sanche, 2003, 2004, 2005), making more thermodynamically decay pathways (Zheng et al., 2006a; Ptasinska and Sanche, 2007) for the transient anions, which will result in the increased damage. Our experimental results support these theoretical predictions.

Influence of guanine moiety on phosphoester bond (C-O) cleavage

In recent DFT theoretical studies of CpG and GpC dimers (Gu et al., 2010a), investigations of the geometric features, molecular orbital analyses, and the charge distribution for the radical anions of the guanine contained oligonucleotides indicate

that in these anionic systems: *ca.* 80% of the excess electron is located on the cytosine nucleobase moiety; *ca.* 7% of the excess electron is located near the hydration water molecules in these systems; the phosphate group in the oligonucleotide dimers does not host the excess electron. These results may simply reflect the stronger attraction of extra electron by the deeper potential of C when compared to that of G and G is not easy to host an excess electron. But guanine might enhance C-O bond cleavage and serve as a weak link in DNA. The finding that guanine enhances C-O bond cleavage is supported by theoretical and experimental studies (Schyman and Laaksonen, 2008; Solomun et al., 2009). The recent study with pGp anion (Gu et al., 2010b) showed that the cleavage of the N−C *N*-glycosidic bond (site 3 in Figure 5) has a much higher energy barrier (24.08 kcal/mol in gas phase and 9.99 kcal/mol in aqueous solution) than the cleavage of the 3' or 5' C−O bond (sites 1 and 2 in Figure 5, 11.23−12.97 kcal/mol in gas phase and 1.06–3.56 kcal/mol in aqueous solution) which indicated when an electron is attached to the guanine of pGp, the *N*-glycosidic bond is harder to break, i.e., the base release is minor, however, the electron transfer to phosphate C–O bond is facilitated and the C–O bond break is dominated. Our experimental results (Table 1, Chapter III) with trimers indicated that even if the damage was decreased due to the introduction of guanine into the trimer, the percentage of C–O cleavage based on total damage was higher than that for the other bases (thymine, cytosine, and adenine). These results supported the former predictions that guanine might serve as a weak link in DNA (Schyman and Laaksonen, 2008; Solomun et al., 2009).

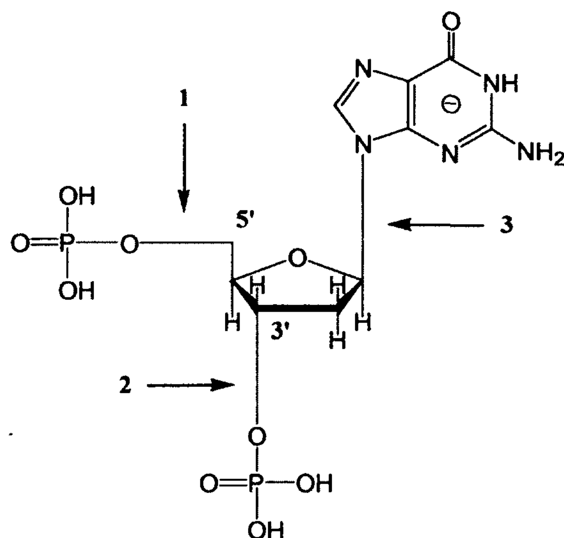


Figure 5. Phosphoester bond (site 1, and 2) and *N*-glycosidic bond (site 3) cleavage sites in molecule model pGp.

Unpublished results with oligonucleotide tetramers

Our unpublished results with oligonucleotide tetramers are listed in Table 2. The change of the total damage depends on the nature of the base, e.g., 168(TTTT) → 117(TTCT) → 77(TTAT) → 47(TTGT), which corresponds to about a 4-fold decrease in total damages as a result of changing the thymine moiety at the central positions to a guanine moiety. This change might be due to changes in initial electron capture and changes in base stacking. These results support experimental and theoretical studies indicating that the electron affinity of DNA bases decreases in the following order: T > C > A > G (Seidel et al., 1996; Wesolowski et al., 2001; Li et al., 2002a; Richardson et al., 2004). This change also affects base release (from 23.4 to 3.2) and phosphoester bond cleavage (from 17.1 to 3.8), however, the percentage of C–O cleavage based on total damage is not affected very much in TTGT and TGTT (Table 2) and this also supports the notion that guanine might serve as a weak link in DNA as studied theoretically and experimentally (Schyman and Laaksonen, 2008; Gu et al.,

2010c; Solomun et al., 2009; Ray et al., 2005; Li et al., 2010). It has been predicted that TTT might serve as the electron sink and GGG might serve as the radical cation sink in DNA (Voityuk et al., 2001; Kobytecka et al., 2008, 2009; Burrows and Muller, 1998; Senthilkumar et al., 2003; Merino and Barton, 2007, 2008, 2009). This may be also related to the base stacking. A recent theoretical study with 5’-X$T_1(T^-)T_2$Y-3’ anion as a model molecule indicated that the influence of the second neighbor (X, Y) is weaker than the effect of adjacent pairs (T_1, T_2). This means the second-neighbor effects are smaller than the influence of electron affinity (EA) at the center T (Voityuk, 2009). Our tetramers are similar to trimers except they have an additional thymine added to the terminal position.

Table 2. Yield of products from LEE-induced DNA damage in tetramers (10 eV).

sample[a]	damage[b]	base release[b]				phosphoester bond cleavage[b]	
		Thy	X	sum[c]	%[d]	sum[e]	%[f]
TTTT	168	23.4		23.4	13.9	17.1	10.2
TTXT X=C	117	13.6	2.1	15.7	13.4	7.9	6.8
TXTT X=C	127	13.8	2.3	16.1	12.7	7.6	6.0
TTXT X=A	77	6.8	0.5	7.3	9.5	7.3	9.5
TXTT X=A	86	6.1	0.8	6.9	8.0	7.9	9.2
TTXT X=G	47	3.0	0.2	3.2	6.8	4.6	9.8
TXTT X=G	41	3.0	0.2	3.2	7.8	3.8	9.3

[a]Each tetramer is written from 5’ to 3’. [b]The results are reported for the number of molecules based on 1000 parent molecules and are the average of 3 independent experiments. The standard deviation is ± 20% (not shown). Thy represents thymine. [c]Sum of base release. [d]Percentage of N–C cleavage based on total damage. [e]Sum of phosphodiester bond cleavage. [f]Percentage of C–O cleavage based on total damage.

Effect of a strong electron captor (5BrU) on total damage, *N*-glycosidic bond (N-C) cleavage, and phosphoester bond (C-O) cleavage in trimers

Recently, Wetmore et al. and Li et al. reported the DFT B3LYP calculations for both gas and solution phase electron affinities of uracil, thymine and a series of 5-halouracils (5XU, X=F, Cl, Br), and the calculated barriers for the dissociation of corresponding radical anions to X^- plus the uracil-5-yl radical (Wetmore et al., 2001; Li et al., 2002b). The sensitivity of the halouracils to LEE is found to be in the order of 5BrU > 5ClU > 5FU > U ≈ T, and the calculated energy barrier for the dissociation of the 5XU anions to X^- and the uracil-5-yl radical decreases in the order of $5FU^-$ > $5ClU^-$ > $5BrU^-$. The high electron affinity and low energy barrier for dissociation of 5 C−X bond of 5BrU support the experimental reports that it may serve as a radiosensitizer in radiation therapy. In our experiments (Chapter IV), substitution of the central thymine of TTT by 5-bromouracil increases the total damage to trimer by 50%, i.e., from 92 to 138 (Table 1, Chapter IV). This change leads to an increase in both C–N bond and C–O bond cleavage. The sum of base release increased from 17 to 32 and the sum of phosphodiester bond cleavage increased from 9.8 to 17.7, which corresponds to a 2-fold increase in the C–N bond and C–O bond cleavage. These findings support the hypothesis that 5-bromouracil is a strong electron captor and it might enhance the sensitivity of DNA bases to ionizing radiation as a potential radiosensitizer and anti-tumor agent.

It has been reported that the replacement of uracil and thymine with 5-halouracil, especially 5-bromouracil, enhances the radiosensitivity of DNA or RNA molecules. The nucleoside of 5-bromouracil has therefore been employed as a sensitizer in radiation therapy by eventually halting DNA or RNA replication in tumor cells (Zamenhof et al., 1958; Kinsella et al., 1984; Coleman et al., 1990; Lawrence et al., 1990; Buchholz et al., 1995). Earlier efforts in model systems showed that halouracils, including 5-bromouracil, capture radiation-produced LEE when incorporated in DNA. 5-Halouracil radical anions formed by radiation dissociate to give halide anions plus the very reactive uracil-5-yl radicals (Zimbrick et al., 1969a, 1969b; Sevilla et al., 1974; Simpson and Zimbrick, 1975; Razskazovskii et al., 1997; Klyachko et al., 1999; Abdoul-Carime et al., 2000a, 2000b, 2001a; Dugal et al., 2000), which produce the ultimate damage in the nucleic acids, including dimerization,

cross-linking, and DNA strand breaking by hydrogen abstraction from the 2-deoxyribose phosphate backbone. In the measurements of dissociative electron attachment (DEA) to gaseous 5-bromouracil (5BrU) for incident electron energies between 0 and 16 eV, Abdoul-Carime et al. reported that the transient negative ion (TNI) or transient molecular anion (TMA) undergoes unimolecular dissociation into a negative ion and a neutral atom or radical via two competing channels, i.e., Br^- + (U-yl)• or Br• + $(U\text{-}yl)^-$, as shown by steps "b1" and "b2", respectively, in Figure 6 (Abdoul-Carime et al., 2000b). Through a theoretical study, Li et al. predicted that the major pathway is Br^- + (U-yl)• (Li et al., 2002b). Our results indicate that the major product is TUT (Table 1, Chapter IV), which may result from the formation of the highly reactive uracil-5-yl radical followed by H-atom abstraction from another molecule (Sugiyama et al., 1996, 1997; Kawai et al., 1999; Oyoshi et al., 2002). This finding supports Li et al.'s prediction and pathway "b1" of Abdoul-Carime et al.'s results. This finding also supports the radiosensitizing mechanisms of 5-halouracils (Zimbrick et al., 1969a, 1969b; Sevilla et al., 1974; Simpson and Zimbrick, 1975; Razskazovskii et al., 1997; Klyachko et al., 1999).

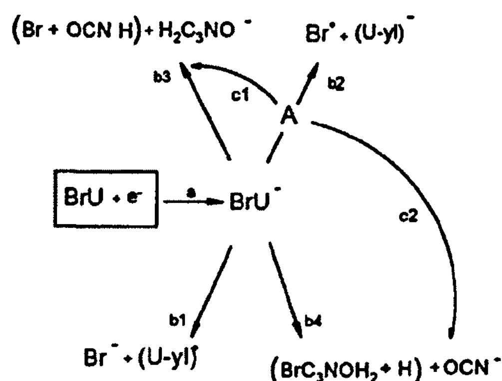


Figure 6 (reprinted from Abdoul-Carime et al., 2000b). In gas phase 5BrU, different dissociation pathways result in different negative ion productions after a LEE is attached to it.

However, Cecchini et al. reported that 5-bromo-2'-deoxyuridine incorporated in oligonucleotides is not found to sensitize DNA to radiation damage in the

double-stranded form but rather it greatly increases damage at non-complementray regions or bubbles within double-stranded DNA (Cecchini et al., 2004; Sevilla, 2004). This is also supported by theoretical studies (Li et al., 2003a) and the proposed mechanism is that double-stranded DNA is not as reactive as single-stranded regions. Cecchini et al. proposed that radiosensitization effects *in vivo* would be maximized during replication and transcription in dividing cells.

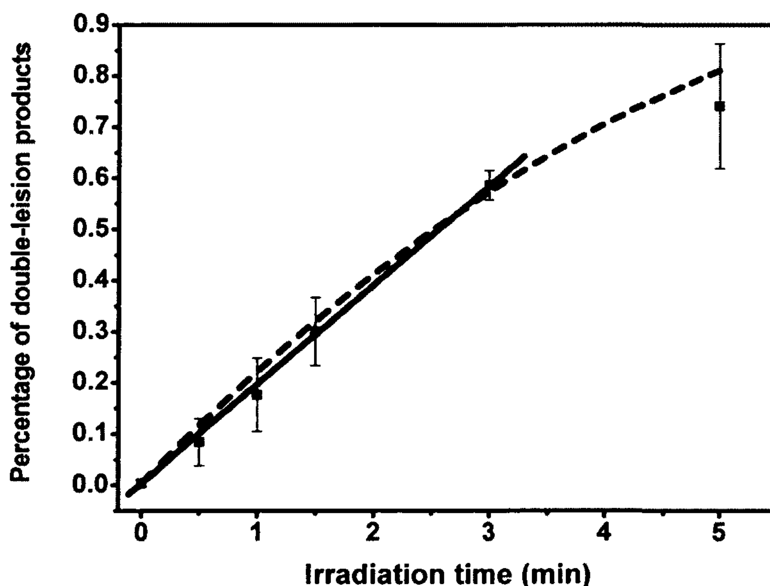
One electron induces double events/lesions

The release of uracil together with 5-bromouracil and fragments containing uracil (pUT and TUp) together with p5BrUT and T5BrUp may suggest that a single 10 eV electron induces double events/lesions in one T5BrUT molecule, i.e., both Br-C and *N*-glycosidic C-N bond cleavage or bromine-carbon Br-C and phosphoester C-O cleavage in the same T5BrUT molecule (Table 1, Chapter IV). The formation of double-lesion products as a function of exposure time was studied at 10 eV (Figure 7). The linear part (< 3 min) of this curve represents the pure interaction of LEE involving single collisions of incident electrons with T5BrUT. Within this regime, the yield of products may be assumed to result from a single electron hit, since damage resulting from multiple successive collisions will necessarily be nonlinear with radiation exposure. In our experiments, the current of LEE and irradiation time were adjusted to give the samples an exposure well within the linear regime of the dose response curve. Thus, one can expect that the majority of products arise from single hits in view of their formation at low exposures (Figure 7). The proposed mechanism is illustrated in Scheme 2, Chapter IV. This case is also referred to as dissociative excitation (DE). It is shown that 5-bromuracil displays resonances at very low energy (0-1 eV), e.g., 5BrU + e^- (~0 eV) → $5BrU^-$ → (U-yl)• + Br^- (Abdoul-Carime et al., 2001a). Different from the well-accepted mechanism that it is uracil-5-yl radical that causes damages to DNA, another possibility is that dissociative excitation of the molecule together with the high cross-section of 5-bromouracil for nearly thermalized electron capture cause more damages adjacent to it.

Biologically, the most severe damage to DNA induced by ionizing radiation is multiply damaged sites (MDS) or clustered damage and it is considered to be crucial

for understanding the biological consequences of ionizing radiation. As for LEE-induced DNA damage, double strand breaks happened in plasmid DNA (Boudaiffa et al., 2000a) and the mechanism of how one electron can induce double strand break remains still unclear. From the present results, one can assume that one electron might induce double lesions in normal DNA, but the amount of the products might be small and below detection. By using 5-bromouracil, a strong electron acceptor or a potential radiosensitizer, we exaggerate the effect that one electron might induce double events/lesions in DNA molecules and this will help us to understand how one electron can induce double strand break in DNA.

Figure 7. Time course of double-lesion products formation by LEE impact. T5BrUT was exposed to 10 eV electrons during 0, 0.5, 1, 1.5, 3, 5 minutes at a constant electron beam flux of 10.6 μA. The amount of double-lesion products was determined by HPLC-UV. The data were fitted to a single exponential (dashed line) and to a straight line at initial times (solid line, R=0.99972). The linear part of this curve represents yields resulting from a single collision of incident electrons with T5BrUT. Each data point corresponds to an average of three independent measurements.



Chapter VI – Conclusions and perspectives

The biological effects of ionizing radiation may be attributed to the reaction of water radiolysis products (hydroxyl radicals, solvated electrons, H-atoms), the reaction of radical ions resulting from the direct ionization of macromolecules, and the reaction of secondary LEE (< 30 eV). In recent years, DNA damage induced by secondary LEE has attracted considerable attention because LEE represent a large percentage of the total energy deposited by ionizing radiation and because they have been shown to induce DNA damage with simple DNA components (bases, nucleosides, nucleotides), oligonucleotides, and plasmid DNA. The majority of studies have examined the desorption of small fragments (<100 a.m.u.) during irradiation of targets in the condensed phase under ultra high vacuum. In addition, a number of studies demonstrate the formation of single and double strand breaks by post-irradiation analysis of damaged plasmids using gel electrophoresis. These results showed that electrons, in particular, LEE (< 30 eV), efficiently induce the formation of stable anions and radical fragments within DNA by a resonance process, i.e., dissociative electron attachment (DEA). Although this process induces DNA damage, there is a paucity of knowledge about the chemistry of the excess LEE in DNA and the chemical steps leading to the modification of DNA by LEE remain to be established. The work described in this thesis is a continuation of studies on measurements of non-volatile damage remaining on the surface of condensed phase irradiations using the newly-developed LEE electron gun irradiating system and using HPLC-UV for analysis. These studies are complementary to electron desorption, combined with strand break analyses, with the goal of identifying the structure of LEE-induced DNA damage products and applying this knowledge to radiotherapy.

The effect of terminal phosphate groups on LEE-induced *N*-glycosidic and phosphodiester bond cleavage was studied in a series of small DNA segments containing thymine. The addition of terminal phosphate group(s) to monomers and dimers resulted in a considerable increase in total damage of DNA; however, it causes a marked decrease in the release of non-modified thymine, and it also affected C–O bond cleavage in dimers, albeit to a smaller extent. These results can therefore be

explained by a simple model in which the electron is initially captured by a base or by a phosphate group with a strong propensity for the terminal positions. The high capture and fragmentation cross section of the phosphate group increases total damage which, in counter balance, decreases rupture of the *N*-glycosidic bond and the 5'-phosphate and 3'-phosphate C–O bonds. Thus, electrons that attach directly to phosphate group do not induce base release and sugar-phosphate C–O σ bond cleavage. In other words, only initial capture by the base directs transfer of the additional electron to the *N*-glycosidic bond or the C–O bond. It appears that, as predicted theoretically and suggested by several experiments, the electron first attaches to the base and either breaks the *N*-glycosidic bond or transfers to the P=O π orbital of the phosphate group and breaks the sugar-phosphate C–O bond.

The effect of base sequence on LEE-induced *N*-glycosidic and phosphodiester bond cleavage was studied in a series of trimeric oligonucleotides. The model compounds included TpXpT where X represents one of the four normal bases in DNA (T, C, A, G). The results show that, when trimers are irradiated with LEE (10 eV), the total damage decreases 2-fold in the series of trimers (T > C > A > G). This study indicates that the initial LEE capture and subsequent bond breaking within the intermediate anion depend on the sequence and electron affinity of the bases, i.e., the base stacking and electron affinity of the bases may play an important role in the LEE-induced fragmentation of DNA. Furthermore, they suggest that LEE may preferentially cause damage to pyrimidine bases and particularly to thymine or clusters of thymine in DNA. Our results with tetramers also support these predictions.

The effect of a strong electron captor, 5-bromouracil, in trimeric oligonucleotide was studied in Tp5BrUpT. The results show that the introduction of 5-bromouracil increased the total damage by at least 50% compared with TpTpT. This change leads to an increase in both C–N bond and C–O bond cleavage. These findings support the hypothesis that 5-bromouracil is a strong electron captor and it might enhance the sensitivity of DNA bases to ionizing radiation as a potential radiosensitizer and anti-tumor agent. Also, these findings support the hypothesis that LEE is initially captured by the base and then transferred from the base to either the C–N bond leading to base release or to the C–O bond leading to phosphodiester bond cleavage. Most importantly, the results of T5BrUT indicated that a single 10 eV

electron induces double events, i.e., Br–C and C–N cleavage or Br–C and C–O cleavage in one T5BrUT molecule. These findings suggest that LEE from ionizing radiation induce multiply damaged sites in DNA especially when it contains modified bases such as 5-bromouracil that have a high cross-section for electron capture.

The limitation of the present LEE gun irradiation system is that it cannot reach very low energies, e.g., <3 eV. So in the present study, all the experiments on DNA molecules with LEE irradiation were performed in the core-excited resonance energy region, i.e., around 10 eV. However, in biological environments or in living cells, the secondary LEE likely thermalize to rapidly become solvated or hydrated electrons by losing their energy to water molecules around them. Thus, the reactions of electrons with energies below 3 eV with DNA, including pre-hydrated electrons or pre-solvated electrons, may be even more interesting. Furthermore, results obtained in this regime may be easier to analyze and interpret due to the limited number of processes available to electrons with such low energies. We recently built and tested a new electron irradiator, which can emit electrons with energies as low as near 0 eV. We are planning to perform experiments with this new electron gun at very low energies, e.g., 1 eV, in the future.

DNA damage is the principle of the radiation carcinogenesis and radiation therapy of cancer. So understanding the fundamental mechanisms of radiation induced DNA damage will benefit the development of new modalities for cancer treatment. Such a comprehension may lead to the development of new drugs and clinical protocols for chemoradiation therapy, a major clinical modality for cancer treatment. For instance, the results in our lab indicated that the gold nanoparticles exposed to radiation produced large quantities of the cell killing low-energy electrons. Therefore, combining the gold nanoparticles in a chemotherapeutic agent and following with radiation therapy may be more effective. In this case, there is a synergistic effect that greatly increases the effectiveness of cancer therapy when both treatments are administerd during the same period of time. Studies of the action of secondary low-energy electrons on DNA allow scientists and clinicians to understand the intimate mechanisms of combined radiation and chemotherapy. In the future, research on the relationship of the mechanism of DNA damage induced by secondary low-energy electrons (LEE) arising from ionizing radiation and its relationship to

radiosensitization and radiotherapy clinically is more significant because this work is extremely promising for the development of improved and new methods for the treatment of a number of diseases including cancer.

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