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# Glycosidic Bond Cleavage of Thymidine by Low-Energy Electrons

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It is well-known that high-energy ionizing particles induce DNA strand breaks, which can be toxic, mutagenic, and recombinogenic. However, recent experiments and theoretical studies have demonstrated that at very low energies (5−20 eV), even below the ionization threshold, electrons induce single- and double-strand breaks in DNA via dissociative electron attachment.[1−3] Electrons of 0−20 eV are generated in large amounts as secondary particles in irradiated cells (∼40 000 are produced by a 1 MeV electron), with a most probable energy lying below 10 eV.[4] Hence, because they carry a large portion of the energy of primary radiation, they are expected to induce a substantial amount of chemical damage. A detailed understanding of the mechanisms responsible for DNA damage via low-energy electron (LEE) attack requires knowledge of their interaction with individual basic compounds.[5] Damage to such compounds has been obtained by the analysis of species undergoing desorption from the condensed films during electron bombardment under high vacuum.[6−8] However, the majority of biological compounds are nonvolatile and, thus, do not desorb for detection using conventional systems. To solve this problem, we have developed a novel high vacuum system in which relatively large amounts of substrate can be bombarded (milligrams), and chemical modifications on the surface can be determined by HPLC and/or GC/MS analysis.[9] Here, we report our initial results with this apparatus, showing that LEE efficiently breaks the *N*-glycosidic bond of thymidine (dThd).

Thymidine was deposited on the inside surface of tantalum cylinders (3.2 cm × 2.5 cm diameter) by the addition of dThd in methanol to cylinders rotating at a speed of 1500 rpm under a vacuum of 400 m Torr. The resulting thin solid film of dThd consisted of four to five monolayers (2.5 nm). The distribution of molecules was homogeneous as inferred by spin-coating, using radioactive substrates and autoradiography. The sample was then irradiated with monoenergetic LEE in the range of (3−100) ± 0.5 eV under high vacuum of $10^{-8}$ Torr and ambient temperature. After irradiation, the cylinders were transferred into a glovebox, which was constantly purged with an atmosphere of pure nitrogen or oxygen (humidity 20%). To recover dThd and its radiation products, the surface of the cylinder was washed repeatedly with methanol in the glovebox, sonicated in the same solution, and evaporated to dryness under vacuum.

The mixture of dThd radiation products was examined by HPLC/UV and GC/MS (Figure 1). HPLC/UV analysis revealed the formation of several products. The appearance of products in nonirradiated samples could be attributed to physical contact of dThd with the cylinder surface as well as to sonication required for optimal recovery. The major product was identified as thymine by comparison of the HPLC retention time and UV spectrum to authentic standards (Figure 1a) and by comparison of GC/MS features (Supporting Information). The yield of thymine was determined by GC/MS (Figure 1b,c). For these analyses, the initial solution of dThd was spiked with isotopically labeled thymine

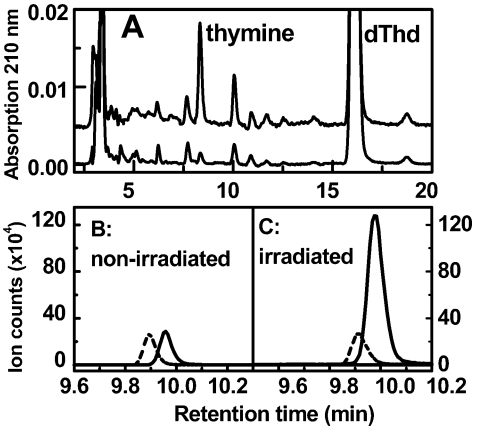


***Figure 1.*** (A) Analysis of dThd radiation products by HPLC/UV. The upper and lower traces correspond to irradiated and nonirradiated samples of dThd respectively (irradiated samples were exposed to $1.9 \times 10^{16}$ electrons of 15 eV). The separation of products was carried out using an analytical ODS-AQ column with 10% methanol in water as the mobile phase with detection at 210 nm. (B and C) Analysis of thymine by GC/MS with isotopic dilution. The separation of thymine was achieved by derivatization with BSFTA and GC/MS analysis with selective monitoring at 270 *m*/*z* (solid line) and 274 *m*/*z* (dashed line), which correspond to the molecular ions for nonlabeled and $d_4$-labeled thymine, respectively (see Supporting Information for details).

(α,α,α,H6-$d_4$-thymine) before spin-coating and LEE irradiation to correct for any losses of thymine during sample preparation. Thymine, including both labeled and natural isotopes, was then purified from the mixture of radiation products by HPLC. This step excluded the possibility that dThd decomposed into thymine during sample preparation for GC/MS analysis. To estimate the percent decomposition of dThd into thymine, dThd was bombarded with LEE (15 eV) for relatively long exposure times (10 min) to overcome variability in the recovery of samples from the surface. Accordingly, the average decomposition of dThd was 30% on the basis of HPLC/UV analysis. From the same samples, the yield of thymine was 10% (2.7 nmol) of the initial amount of dThd (26.5 nmol). Although the yield of thymine may have been affected by secondary processes at these exposures, one expected that the majority of thymine arose from single hits in view of its formation at low exposures (Figure 2). Thus, we estimated that thymine constituted approximately one-third (10% of 30%) of LEE-induced decomposition of dThd.

The formation of thymine as a function of electron energy (3−100 eV) exhibited a broad maximum around 15 eV, indicating that at this energy, thymine was produced essentially via the formation of a transient anion. At energies greater than 15 eV, the formation of thymine decreased and then increased gradually starting at 30 eV. From these preliminary results, the formation of thymine as a function of exposure time was studied in more detail near the resonance maximum of thymine formation at 15 eV (Figure 2). The linear part of this curve represents the pure interaction of LEE


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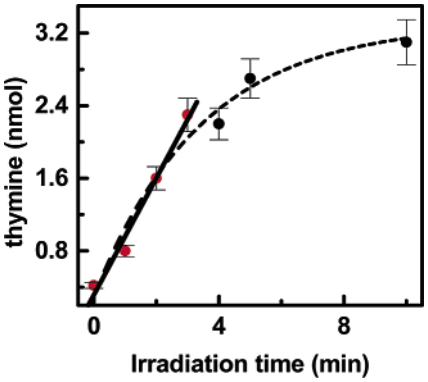


***Figure 2.*** Time course of thymine formation by LEE. dThd was exposed to 15 eV electrons at a constant electron beam flux of 10.6 $\mu$A = 6.6 $\times$ $10^{13}$ electrons. The amount of thymine was determined by HPLC/UV and GC/MS analysis. The data were fit to a single exponential (dashed line) and to a line at initial times (solid line). Each data point corresponds to an average of three independent experiments.

involving single collisions of incident electrons with dThd. At longer exposure times (>3 min), the yield of thymine reached a plateau because of changes in the film as a result of dThd decomposition and continuous trapping of electrons. Trapping of electrons by the film during exposure modified the surface potential and thus changed the energy of incident electrons. Both of these changes likely lowered the yield of thymine, explaining the deviation from linearity for long exposure times (>3 min). Nevertheless, for exposure times of less than 3 min, the formation of thymine from dThd could be attributed to the interaction of electrons with dThd and not to other factors. A linear fit for data at the early times gave a quantum yield of $3.2 \times 10^{-2}$ per incident electron or 32 thymine molecules per 1000 incident electrons.

For conversion of dThd to thymine, electrons with low kinetic energy probably localized in the antibonding orbitals of the N1-glycosidic bond of dThd. This can lead to either homolytic or hetereolytic cleavage. It is doubtful that thymine N1-centered radicals were involved because these species gave diagnostic dimeric products that were not detected in our analysis.[11] In addition, no change in the yield of thymine was observed when oxygen, in place of nitrogen, was introduced into the irradiation chamber after irradiation. Thus, we propose that hetereolytic cleavage takes place with the formation of thymin-N1-yl anions and neutral 2-deoxy-D-ribose-C1(H)-yl radicals. The latter radicals may undergo reduction into 1,2-dideoxy-D-ribose or oxidation into 2-deoxy-D-ribose. Of these two products, 2-deoxy-D-ribose appears to be the main product as inferred by GC/MS analysis (see Supporting Information). Alternatively, 2-deoxy-D-ribose-C1(H)-yl radicals may give rise to other sugar radical species and final sugar decomposition products. It is important to note, however, that these radicals are not identical to those resulting from H-atom abstraction or deprotonation of pyrimidine nucleosides at C1′ of the sugar moiety.

The release of thymine and other nonmodified bases is an important pathway of damage upon exposure of DNA to ionizing radiation in the solid state.[12,13] This damage has been attributed to the oxidation of DNA involving either the deprotonation of base radical cations or fragmentation of initial sugar phosphate radical cations.[13−15] Alternatively, the ability of LEE to release thymine from dThd implies that LEE is involved in the release of this base from irradiated DNA. The higher electron affinity of pyrimidines compared to purines may explain the bias of base release at pyrimidine residues in irradiated DNA.[14,15] Moreover, the involvement of LEE is consistent with the independence of base release on the extent of hydration (<15 water molecules per DNA base).[13,14] If oxidative processes are involved in base release, then this process should increase with hydration due to transfer of water radical cations to the sugar moiety. Thus, novel reactions induced by LEE may explain the bias of base release at pyrimidines and the lack of an effect of hydration on base release during exposure of DNA to ionizing radiation.

In summary, our studies indicate that LEE efficiently breaks the N1-glycosidic bond of dThd. The mechanism of base release is likely different from other well-studied pathways of DNA damage, involving base ionization, OH radicals, and solvated electrons. This work provides quantitative information of LEE-induced damage in complex biological systems that will be necessary to compare the chemical damage produced by LEE to that produced by other particles (e.g., $\alpha$, $\beta$, UV, X-rays).

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**Supporting Information Available:** Experimental procedures as well as GC/MS analyses of selected compounds (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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