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Hole Migration is the Major Pathway Involved in Alkali-Labile Lesion Formation in DNA by the Direct Effect of Ionizing Radiation

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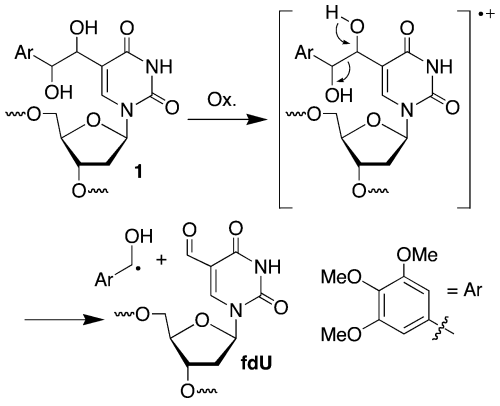
Ionizing radiation is the most commonly used nonsurgical method for treating cancer, and DNA damage is the source of its cytotoxic effects. DNA is damaged directly (the "direct effect") and via reactive species (e.g., hydroxyl radical, OH•) generated from water (the "indirect effect") by ionizing radiation.[1] The direct and indirect effects are believed to contribute approximately equally to DNA damage and to produce a common spectrum of products from overlapping sets of initially formed reactive intermediates. Nucleobase addition is the predominant pathway for OH•. In contrast, the direct effect is sufficiently energetic to ionize the sugar, phosphate, and nucleobase groups.[1−3] The latter produces nucleobase holes, whose migration in DNA has been the focus of extensive investigation.[4−7] Our goal was to determine how large a contribution hole migration makes in the distribution of DNA damage resulting from the direct effect of ionizing radiation.

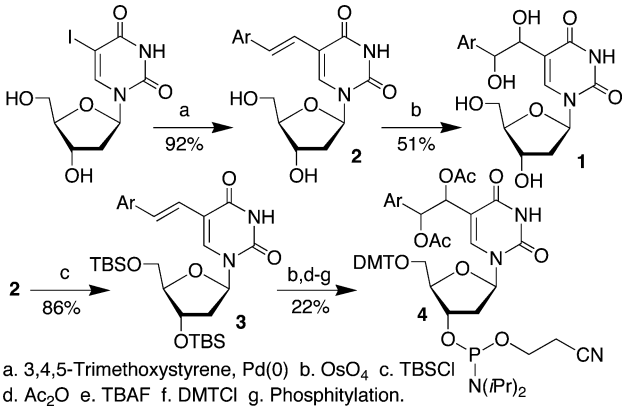
Molecular probes that facilitate detecting excess electron transfer or short circuit hole migration in DNA have been reported.[8,9] We designed a molecular probe (**1**, Scheme 1) to selectively detect hole migration using gel electrophoresis. The trimethoxyphenyl group was included in **1** to serve as a sink in which to localize a cation radical (hole). Using established chemistry as a precedent, the corresponding cation radical was expected to fragment to 5-formyl-2′-deoxyuridine (**fdU**), an alkali-labile lesion.[10−12] Monomeric **1** was readily synthesized via Pd(0) coupling of the corresponding styrene to 5-iodo-2′-deoxyuridine (Scheme 2). The vicinal diol was introduced as a mixture of diastereomers via OsO_4. The nucleoside's hydroxyl groups were silylated (**3**) prior to carrying out the osmylation reaction when preparing phosphoramidite **4**, in order to distinguish them from the vicinal hydroxyl groups. Oligonucleotides containing **1** were synthesized via automated solid-phase synthesis using commercially available reagents with minor modifications.[13]

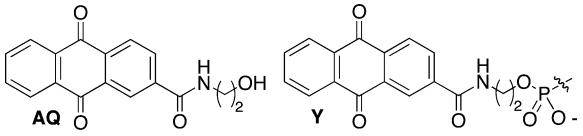
Cyclic voltammetry measurements on **1** confirmed that the trimethoxyphenyl group provided a suitable driving force (E^0(**1**) = 1.36 V versus E^0(dG) = 1.58 V) for hole localization, consistent with that measured for other trimethoxyphenyl containing molecules.[13−15] In addition, monomeric **1** met the requirements for utilization as a probe for photoinduced electron transfer. The nucleoside was unaffected by direct photolysis (350 nm), but HPLC and ESI-MS analysis revealed that it was rapidly converted to **fdU** when irradiated in the presence of the substituted anthraquinone (**AQ**) previously used to inject holes in DNA.[7,13]

Scheme 1. Alkali-Labile Lesion Formation via Hole Trapping by **1**



Scheme 2. Synthesis of Hole Migration Probes





5′-d(GGC ACG **X**GT TTT)
3′-d(CCG TGC ACA AAA)
5a **X** = T
5b **X** = **1**

5′-d(GGC ACG **1**GT TTT)
3′-d(CCG TGC ACA AAA **Y**)
6

5′-d(**X** AAA TTG ACA ATG CAA CGT TAA TCA GTT)
3′-d(TTT AAC TGT TAC G**1**T GCA ATT AGT CAA)
7a **X** = H
7b **X** = **Y**

Having established the integrity of the oxidative fragmentation process in **1**, we sought to determine if this molecule functioned as an efficient hole trap in DNA without disrupting duplex structure. The T_M of **5b** (52.2 ± 0.7 °C) was only 1.8 °C lower than that of **5a** (54.0 ± 1.2 °C), indicating that the substituent in **1** oriented toward the major groove did not significantly perturb duplex structure. The functionality of **1** as a hole trap in DNA was examined qualitatively using **6** in which anthraquinone was used as a hole injector.[16] Evidence for the formation of **fdU** was gleaned by the appearance of a gel shift product upon reaction of the photolysate with a commercially available aldehyde reactive probe.[13] Quantitative studies were carried out using **7a**,**b** in which the sequence was chosen to maximize the efficiency of hole injection and migration (Figure 1a).[16] Treatment of **7a** with $NaIO_4$ produced an alkali-labile lesion at the original site of **1**, consistent with the formation of **fdU** via oxidation of the vicinal diol. However,

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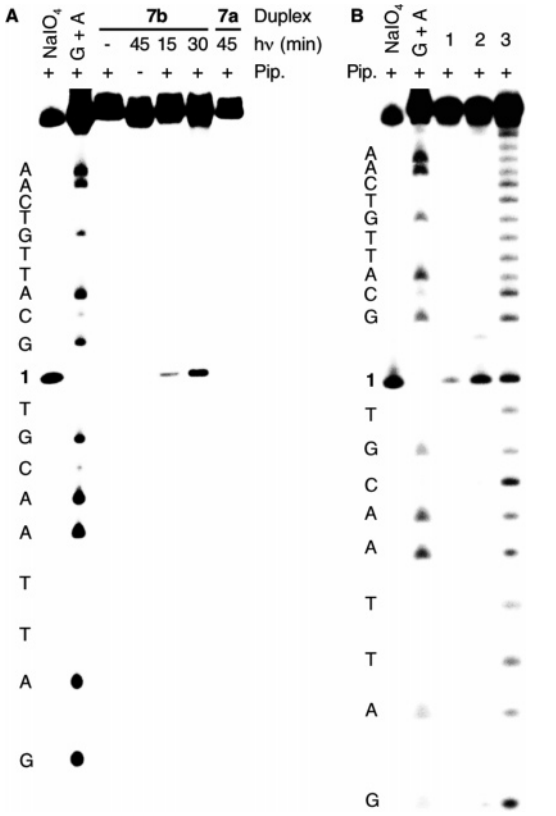

Figure 1. Formation of alkali-labile lesions via hole trapping by **1**. (A) Photolysis (350 nm) of duplexes containing **1** with or without covalently linked anthraquinone hole injector. (B) Use of **1** to detect hole migration in **7a** exposed to ^{137}Cs. Lane 1, 150 Gy; Lane 2, 300 Gy; Lane 3, Fe•EDTA. Lanes 1−3 were treated with piperidine.

Table 1. Alkali-Labile Lesion Formation in **7a** upon Exposure to ^{137}Cs

trial	% **1** (#)[a]	% other (#)[a,b]	ratio **1**:other
1	9.1 ± 1.3 (9)	4.1 ± 1.3 (9)	2.2 ± 0.8
2	9.5 ± 0.8 (10)	4.1 ± 0.8 (10)	2.3 ± 0.5
3	7.9 ± 0.9 (10)	3.8 ± 1.2 (10)	2.1 ± 0.7

[a] # = Number of replicates. [b] Other = nucleotides other than **1**.

piperidine labile sites were not detected following direct photolysis (350 nm) of **7a**. In contrast, photolysis of **7b** produced an alkali-labile lesion solely at the site of **1** in amounts proportional to the irradiation time. Additional cleavage sites were not detected upon treatment with base excision repair enzymes (Fpg, Endo III) that excise damaged purines and pyrimidines.[13,17] Any concern that **1** perturbed the duplex structure in such a way so as to favor its own cleavage was dispelled by analyzing piperidine treatment of **7a** following exposure to OH• generated by Fe•EDTA (Figure 1b). Overall, these data demonstrated that **1** was the sole repository of damage following hole injection in **7b**. Furthermore, the probe provides a convenient readout for hole migration but does not bias the reactivity of the primary DNA damaging reactive species (OH•) produced by the indirect effect.

Hole formation by ionizing radiation (^{137}Cs) was investigated in **7a**. Irradiation of **7a** generated an alkali-labile lesion at the site of **1** whose quantity varied linearly with dose.[13] Although **1** was the major piperidine labile site, alkali-labile lesions were formed in small amounts at all other nucleotides in **7a**.[13] The ratio of alkali-labile lesions produced at **1** relative to all other nucleotides in **7a** was determined in three separate experiments (Table 1). Multiple replicates were carried out in each experiment in order to accurately

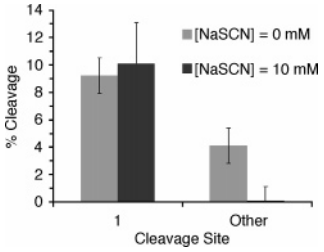

Figure 2. Effect of sodium thiocyanate (NaSCN) on yield of alkali-labile lesions in **7a** upon exposure to ^{137}Cs.

measure the small amounts of (background corrected) cleavage at individual nucleotides other than **1**.

Assuming that cleavage at **1** was attributable to direct ionization, and that the indirect effect was responsible for alkali-labile lesions at all other nucleotides, we estimated that the direct effect accounted for at least two-thirds of the alkali-labile lesions produced in **7a** upon exposure to ^{137}Cs. Support for mechanistically partitioning the cleavage products in this manner was obtained by irradiating **7a** in the presence of the known OH• scavenger, sodium thiocyanate (Figure 2).[18] Cleavage at **1** was at most modestly reduced due to competition for the energy emitted by ^{137}Cs. More importantly, cleavage at nucleotides other than **1** was reduced to background levels. This indicated that the indirect effect of γ-radiolysis was responsible for the detected damage at these positions and not direct ionization of the deoxyribose backbone or phosphate groups. From these data, we conclude that alkali-labile lesions produced by the direct effect of ionizing radiation on DNA are funneled to **1**. Moreover, hole formation/migration is the dominant component of the direct effect of ^{137}Cs that gives rise to alkali-labile lesions in solution.

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Supporting Information Available: Experimental procedures for the synthesis/characterization of all molecules, and all other experiments. HPLC, ESI-MS, phosphorimages from experiments described herein. This material is available free of charge via the Internet at http://pubs.acs.org.

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