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Factors Affecting the Yields of C1' and C5' Oxidation Products in Radiation-Damaged DNA: The Indirect Effect

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Abstract

This study reports the effects of denaturation and deoxygenation on radiation-induced formation of 2-deoxyribonolactone (2-dL) and 5'-aldehyde (5'-Ald) lesions in highly polymerized DNA. The radiation-chemical yields of 2-dL were determined through quantification of its dephosphorylation product 5-methylenefuranone (5MF). The formation of 5'-Ald was monitored qualitatively through the release of furfural (Fur) under the same conditions. The yields of 2-dL were found to be $7.3 \pm 0.3 \text{ nmol J}^{-1}$, or about 18% of the yield of free base release measured in the same samples. Denaturation increased the efficiency of 2-dL formation approximately twofold while deoxygenation resulted in a fourfold decrease. The release of Fur is about twofold lower than that of 5MF in aerated native DNA samples and is further reduced by denaturation of the DNA. Unlike 5MF, the formation of Fur requires the presence of molecular oxygen, which is consistent with peroxy radical-mediated oxidation of C5' radicals into 5'-Ald. In contrast, the existence of an oxygen-independent pathway of 2-dL formation suggests that C1' sugar radicals can also be oxidized by radiation-produced oxidizing intermediates such as electron-loss centers on guanines.

INTRODUCTION

The specificity of hydrogen abstraction from the deoxyribose moiety of DNA by hydroxyl radicals has been a subject of discussion for over a decade. Early theoretical simulations gave preference to the C4' and C5' hydrogen abstractions based on the accessibility of these positions to hydroxyl radicals generated in the bulk (1,2). The lack of effect of deuteration at the C1' position on DNA strand scission was also interpreted as meaning that C1' plays a negligible role in oxidative damage of the deoxyribose moiety (3). More recent studies (4–6) have shown, however, that the actual yields of the C1'-oxidation end product, 2-deoxyribonolactone (2-dL, Fig. 1), are much greater than predicted by this model. The published values, however, vary from 76% (7) to 7% (8) of all deoxyribose damages. The goal of this study is to determine whether this broad range of estimates results from the 2-dL yields being highly sensitive to DNA quality and irradiation conditions or from differences in interpretation of the experimental data. The factors investigated in this study are partial denaturation of the DNA, the role of molecular oxygen, and the presence of common ionic species such as magnesium and chloride ions. Magnesium affects the secondary DNA structure (9) while oxidation of chloride with hydroxyl radicals gives rise to new hydrogen-

abstracting species such as ClOH^- , chlorine atoms and Cl_2^- (10). Reactions of these intermediates with DNA may follow different selectivity rules and alter the relative efficiency of end-product formation.

Our approach is based on direct HPLC quantification of 5MF released from 2-dL upon treatment of irradiated DNA with spermine in neutral solutions. This process is quantitative, the amount of 5MF being a direct measure of the amount of 2-dL. As shown below, the sensitivity of this technique is sufficient to quantify the formation of 2-dL at doses as low as a few Gy. Oxidative degradation of the deoxyribose moiety in most cases also results in unaltered base release (11). This makes base release a convenient measure of the overall oxidative damage to the sugar-phosphate backbone. In the present study, it was used to determine what fraction of all hydroxyl radical attacks on deoxyribose led to the formation of 2-dL.

Other well known products of deoxyribose damage are 5'-aldehydes (5'-Ald, Fig. 1) originating from oxidation at the C5' position. These lesions are known to release furfural (Fur) (12,13). In a previous study (5) we found that this process, like 5MF release, is catalyzed by polyamines such as spermine and polylysine and, that with minor modifications it can be used for simultaneous quantification of both 5MF and Fur in the same sample by HPLC. In the present study this technique was used to compare the efficiencies of 5MF and Fur release from radiation-damaged DNA. Although the yields of Fur cannot be converted directly into the 5'-Ald yields within this approach since the chemical efficiency of Fur release remains undetermined, the technique is still useful for comparing the sensitivity of C1' and C5' oxidations to factors like DNA strandedness and oxygenation levels.

MATERIALS AND METHODS

Reagents

A solution of calf thymus DNA from Sigma (5 mM in base pairs) in 10 mM phosphate (pH 6.9) was treated with Chelex[®] and passed through a solid-phase extraction cartridge (Waters C18, 10 mm × 10 mm) to remove non-ionic organic contaminants. This stock solution incubated at 90°C for 15 min and cooled down slowly to room temperature (1 h) is referred to here as “denatured” DNA. The denatured DNA had a 50% greater absorbance at 260 nm than the unheated DNA, indicating that in spite of the attempted “rehybridization” procedure the DNA in this preparation was mostly single-stranded. These solutions were used shortly after preparation. All other reagents were used as received from Sigma except 5MF, which was synthesized as described elsewhere (14).

Sample Preparation and Irradiation

The DNA solutions (200 µl) were irradiated at room temperature in standard 2-ml ampoules (Wheaton). When needed, the samples were degassed by three standard freeze-pump-thaw cycles (15) and sealed. X rays were generated by a Philips tube operated at 40 kV and 20 mA. The tube output was attenuated by passing through either 0.32 mm or 0.16 mm of lead and through the glass bottom of the ampoules before they reached the solution. The dose rates measured inside the ampoules by Fricke dosimetry were 0.12 Gy/s and 0.50 Gy/s, respectively.

Product Analysis

Irradiated samples were mixed with 20 µl of 0.1 M spermine tetrahydrochloride containing 10 µM uracil (used as an internal standard for HPLC measurements), incubated for 40 min at 70°C, and centrifuged to separate the precipitated DNA. The free bases, 5-MF and Fur were quantified in the supernatant by reverse-phase HPLC with UV detection (254 nm) using

authentic compounds as references. The choice of conditions for postirradiation heat treatment was based on the kinetics of end-product formation studied in a separate set of experiments (Fig. 2). One of the criteria was the stability of 5MF and Fur under the conditions of their release. The radiation-chemical yields of 5MF, Fur and free bases, further denoted as $G(5MF)$, $G(Fur)$ and $G(B)$, respectively, were determined from the corresponding dose–response plots and are reported in Table 1 in nmole/J.

RESULTS AND DISCUSSION

Two representative HPLC chromatograms illustrating pronounced differences in the formation of 5MF and Fur in native and denatured DNA samples are shown in Fig. 3. Representative dose–response curves are shown in Fig. 4. The radiation-chemical yields of product formation obtained in these two systems and in other systems are summarized in Table 1.

Under the conditions of this study, most of the DNA oxidations are initiated by hydroxyl radicals. These species produce oxidative damage in the sugar-phosphate backbone either directly through hydrogen abstraction or, according to Xue *et al.* (7), indirectly, through addition to the bases followed by formation of base peroxy radicals and “intramolecular” hydrogen atom transfer from the C1' position of the neighboring nucleotide (Fig. 5). The carbon-centered deoxyribose radicals thus formed must be further one-electron oxidized to form stable end products such as 2-dL and 5'-Ald. In the presence of oxygen the second oxidation step involves the formation of peroxy radicals, as shown in Fig. 5. There is growing evidence, however, that oxidizing intermediates generated in DNA by ionizing radiation, such as electron-loss centers on guanines (aka holes), can also act as oxidants of sugar radicals. This so-called carbocation mechanism (16), or the “double-hit” event, also shown in Fig. 5, allows the same end products to form under anoxic conditions. This mechanism has been suggested, in particular, to describe product formation in directly ionized DNA (17). Our primary interest is whether it can also contribute to the formation of 2-dL and 5'-Ald through the indirect effect, when all DNA damages are induced by water radiolysis products. The feasibility of this process follows from the ability of hydroxyl radicals to generate holes through one-electron oxidation of the guanine bases (18).

5MF Release and the Relative Efficiency of 2-dL Formation

The $G(5MF)/G(B)$ ratios from Table 1 show that approximately 18% of deoxyribose oxidations led to 2-dL formation in aerated solutions of native DNA. The absolute yield of 2-dL reported recently by Chan *et al.* is greater than ours (13 compared to 7.3 nmol J⁻¹), but the relative yield is smaller (7% of all deoxyribose damages). The latter value, however, is likely an underestimate. To produce 2-dL with a 13 nmol J⁻¹ absolute yield and 7% relative efficiency, the total yield of deoxyribose damage must be 186 nmol J⁻¹. This number is unreasonably high. It is commonly accepted that only about 20–25% of hydroxyl radical attacks on DNA result in sugar damages (19). Given that the radiation-chemical yield of hydroxyl radicals in water is about 250 nmol J⁻¹, the yield of total deoxyribose damage cannot substantially exceed 63 nmol J⁻¹. A large block of data on radiation-induced DNA cleavage and unaltered base release (19) as well as from Table 1 are fully consistent with this limit.

Our data show that denaturation increases both the relative and absolute yields of 2-dL formation by a factor of two. This effect is not unexpected since C1'-hydrogens in single-stranded DNA are more accessible to reactive species formed in the bulk water. The greater 2-dL yields reported by Xue *et al.* (7) can be partially attributed to this effect since DNA in that study was pretreated with 0.1 M NaOH alkali at 37°C and likely remained substantially single-stranded after subsequent neutralization and annealing.

In contrast, deoxygenation drastically (by a factor of 4) decreases the absolute (but not the relative) yields of 2-dL. The removal of oxygen does not affect the initial hydroxyl radical production and their reactions with DNA, but the following free radical reactions are likely to be affected. It is not surprising that 2-dL yields drop under anoxic conditions since the major pathways of 2-dL formation from the C1' radicals are believed to be dependent on oxygen. The fact that $G(B)$ drops to the same extent, keeping the $G(5MF)/G(B)$ ratio approximately constant, may be a coincidence. Unfortunately, a lack of knowledge regarding anoxic transformations of sugar radicals makes it difficult to assess the mechanistic implications of the latter observation.

The more interesting finding is that the production of 2-dL does not drop to zero even under strictly anoxic conditions. As discussed earlier in this section, this means that something else besides oxygen must be able to perform one-electron oxidation of the C1' radical. The most likely candidates for that role are one-electron oxidized guanines (G^+) due to a unique combination of oxidizing properties [$E(G^+/G) = +1.29$ V compared to NHE at pH 7 (20)] and mobility in DNA through site-to-site hopping (21,22). It cannot be excluded that other species like hydroxyl radical adducts to DNA bases may also oxidize C1' radicals, but this possibility seems less likely since neutral carbon-based radicals are known primarily as reducing agents and not as oxidizers (23).

The presence of Mg^{2+} has no visible effect on the 2-dL relative yield, and small concentrations of Cl^- reduce the relative efficiency of 2-dL formation marginally or, based on a 95% confidence level, not at all. In fact, the existing data on the kinetics of chloride oxidation by OH give us no reason to expect large effects under our experimental conditions. Although the equilibrium $Cl^- + OH \leftrightarrow ClOH^-$ establishes within nanoseconds, the small equilibrium constant ($1.4 M^{-1}$) allows less than 1% of all OH to be present in the $ClOH^-$ form at millimolar concentrations of chloride (10). Second, the pseudo-first-order protonation of $ClOH^-$ by H_3O^+ leading to the production of chlorine atoms should be too slow at pH 7 ($k_{eff} = 2.1 \times 10^3 s^{-1}$) to make this pathway competitive with direct hydroxyl radical attack on DNA. Chlorine atoms and Cl_2^- , however, could play a greater role if formed through protonation of $ClOH^-$ by HPO_4^{2-} and $H_2PO_4^-$ anions, whose concentrations in phosphate-buffered solutions are several orders of magnitude greater than that of H_3O^+ .

Furfural Release

The data from Table 1 show that denaturation reduces the relative yield of 5'-Ald by more than a factor of two while deoxygenation completely suppresses the formation of this product. The drop in going from double- to single-stranded DNA likely results from the more efficient competition of other deoxyribose positions, which in single-stranded DNA become more accessible to hydroxyl radical attack. The parallel sharp increase in 2-dL production observed in the same system is fully consistent with this model. The suppression of 5'-Ald formation under anoxic conditions strongly suggests that oxidation of C5' radicals proceeds exclusively through the intermediate formation of peroxy radicals. The alternative "carbocation" mechanism that would involve oxidation of C5' radicals by electron-loss centers in DNA appears to be far less efficient in this case. This is consistent with the facts that (a) C5' and C1' radicals are rated the least and the most oxidizable sugar radicals in DNA (24) and (b) the distance of electron transfer to the holes in the base stack is much greater for C5' radicals than for C1' radicals.

Although the effects of Mg^{2+} and Cl^- on the 5'-Ald production are questionable at the 95% confidence level, they appear to be more pronounced than the effect on the yields of 2-dL. Both agents tend to increase the relative yields of Fur release (up to 1.5-fold in chloride-containing solutions). The effect of magnesium can be associated with the narrowing of the

minor groove through Mg^{2+} binding to the phosphate (25) that would make the inner hydrogens less accessible to reactive species from the bulk. A similar effect of DNA conformation on its radiation sensitivity was described by Isabelle *et al.* (26). The effect of Cl^- , as in the case of 2-dL formation, could again be attributed to the involvement of reactive chlorine species. In particular, Cl and Cl_2^- , being bulkier than OH , could react more selectively with the most exposed C5'-hydrogens.

CONCLUSION

Our results show that the relative yield of 2-dL in aerated phosphate-buffered solutions of highly polymerized DNA is around 18% of all radiation-induced sugar oxidations resulting in unaltered base release. This number is significantly increased only by denaturation of the DNA. The existence of an oxygen-independent pathway of 2-dL formation provides additional evidence that the so-called double oxidations contribute to C1' damage even under the conditions of the indirect effect. Unlike 2-dL, the production of 5'-Ald drops upon DNA denaturation and is 100% dependent on oxygen. The latter indicates that, in this case, double oxidations play an insignificant role. Magnesium and chloride ions, whose presence in different preparations may vary significantly, may also alter the relative efficiencies of the C1'- and C5'-oxidations, but this effect is relatively small.

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FIG. 1.
Chemical structures of 2-dL and 5'-Ald shown with their respective low-molecular-weight decomposition products, 5MF and Fur.

FIG. 2.
Kinetics at 70°C for the release of 5MF and Fur from irradiated native DNA in 10 mM phosphate buffer (pH 6.9) containing 10 mM spermine tetrahydrochloride.

FIG. 3. Representative chromatograms of irradiated (200 Gy) solutions of native and denatured DNA in 10 mM phosphate showing free base (C, G, T, A), Fur and 5MF release. Uracil (U, 0.88 μ M) was used as an internal standard.

FIG. 4.
Dose-response curves for the Fur, 5MF and total unaltered base release from native DNA in 10 mM phosphate.

FIG. 5.
Oxygen-dependent and -independent pathways of hydroxyl radical-induced formation of 2-dL.

Radiation-Chemical Yields (in nmole J⁻¹) of Free Base, 5MF and Fur Release in Different Preparations of DNA (5 mM in bp) in 10 mM Phosphate (pH 6.9)

TABLE 1

DNA preparation	Gassing	Additives (concentration, mM)	G(B)	G(5MF)	G(Fur)	G(5MF)/G(B)	G(Fur)/G(B)
native	air	none	41.3 (1.2)	7.3 (0.3)	3.8 (0.6)	0.18	0.093
native	air	Mg ²⁺ (1)	38.0 (5.0)	7.0 (1.1)	4.8 (0.3)	0.18	0.13
native	air	Cl ⁻ (10)	58.0 (8.0)	8.2 (0.6)	9.0 (1.0)	0.14	0.15
native	anoxic	none	12.7 (0.5)	2.5 (0.5)	n.d. ^a	0.20	n.d. ^a
denatured	air	none	55.3 (4.5)	26.0 (2.1)	2.2 (0.4)	0.47	0.040

Note. The numbers in parentheses are standard deviations.

^aNot detected.