Published in final edited form as:
Angew Chem Int Ed Engl. 2007 ; 46(4): 561–564.

Use of Fluorescence Sensors To Determine that 2-Deoxyribonolactone Is the Major Alkali-Labile Deoxyribose Lesion Produced in Oxidatively Damaged DNA**

Liang Xue and **Marc M. Greenberg***
* *Department of Chemistry Johns Hopkins University 3400 N. Charles St., Baltimore, MD 21218 (USA) Fax: (+1) 410-516-7044 E-mail: mgreenberg@jhu.edu*

Keywords

abasic sites; DNA damage; nucleic acids; oxidative stress; sensors

Abasic sites are a family of DNA lesions that do not have a nucleobase. Formal hydrolysis of a nucleotide's glycosidic bond to produce a typical abasic site (**AP**) occurs 10000 times per day per cell.[1] Several oxidized abasic sites (e.g. **L**, **C4-AP**), whose chemical properties and biological effects are distinct from those of **AP**, have also been identified.[2,3] For

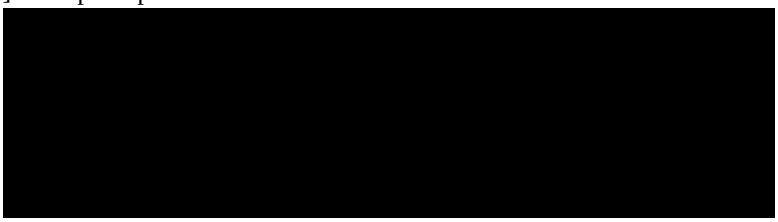
instance, 2-deoxyribonolactone (**L**) forms DNA–protein cross-links with repair enzymes and the outcome of its bypass during replication in *E. coli* is distinct from that of an **AP** site.[4-6] The lactone lesion is often associated with DNA damage induced by antitumor agents, such as neocarzinostatin and the bis-phenanthroline complex of copper, but was believed to form in low yields when diffusible species (e.g. hydroxyl radical) react with DNA due to the inaccessibility of the hydrogen atom at C1′.[7-10] However, 2-deoxyribonolactone was reported to be formed to the exclusion of **AP** sites when DNA was exposed to *tert*-butylperoxy radical.[11] Furthermore, recent reports indicate that ionizing radiation produces this biologically interesting lesion in larger amounts than previously thought.[12-15] Herein we report a new reagent for quantitating the formation of 2-deoxyribonolactone and its use in studies directed towards elucidating how the lesion is produced by the most common means of oxidative damage, γ radiolysis.

We recently exploited the selective reactivity of **L** to develop a biotinylated probe, which covalently tags the lesion with a label that can be quantitated spectroscopically when used in conjunction with avidin and horseradish peroxidase.[16] Building on this basis, we synthesized **1**, which offers the

**We are grateful for support of this research by the National Institute of General Medical Sciences (GM-063028). We thank Professor William Bernhard (University of Rochester) for helpful comments and Dr. Mark Fisher (Varian) for assistance with the fluorimeter.

Supporting information for this article (synthesis and spectral characterization of **1**; experimental procedure for the reaction and analysis of calf-thymus DNA; MALDI-TOF MS analyses) is available on the WWW under http://www.angewandte.org or from the author.

convenience of direct fluorescence detection.[17] The ability of **1** to selectively tag the lactone and not **AP** or **C4-AP** sites was determined using duplexes **3a–c** that contain the independently synthesized lesions, as previously described (Figure 1).[16] DNA containing the abasic sites was cleaved using *N*,*N*′-dimethylethylenediamine (DMEDA), and the butenolide was trapped by **1**. Selective 2-deoxyribonolactone detection was achieved by taking advantage of the heat lability of the adducts formed between **1** and aldehyde-containing lesions (**AP**, **C4-AP**). Adduct formation was confirmed by MALDI-TOF mass spectrometric analysis of the reaction of **1** with **4**.[17] Multiple replications of the



reaction between **1** and **L** in duplex DNA **3a** established the yield for adduct formation to be (17.5 ± 2.2)%.[17] This yield was used in subsequent experiments involving damaged calf-thymus DNA in order to transform the fluorescence signal into a value for the absolute amount of **L**. Similar studies carried out using **2** established that **AP** and **C4-AP,** but not **L,** react with this probe. Commercial reagent **2** (Alexa Fluor 488 hydroxylamine; Molecular Probes) is a fluorescent version of an aldehyde-reactive probe, which was originally designed for **AP** site detection.[18] However, alkoxyamine reagents have been found to be useful for labeling a variety of aldehydes in DNA, including C4-**AP**.[17-20]

Bernhard and co-workers utilized the release of 5-methylene-2-furanone (**5**, Scheme 1) upon heating to quantitate **L** produced in DNA irradiated with X-rays.[14,15] They concluded that 2-deoxyribonolactone accounted for at least 30 % of the sugar damage and postulated that the lesion was formed through an O_2-independent mechanism. The fraction of sugar damage attributable to **L** was based on a comparison of the amounts of **5** and unaltered free base released. We directly compared the yields of respective sugar lesions by using **1** to tag the

lactone and **2** to trap aldehyde-containing lesions. Calf-thymus DNA was pretreated with NaOH (0.1M, 37 °C, 20 min) to remove any abasic sites and rehybridized prior to its exposure to either ^{137}Cs or [Fe^{II}(EDTA)](EDTA = ethylenediaminetetraacetic acid). Individual samples were split into equal parts and treated with **1** and **2**. The yields of **L** and aldehyde-containing lesions varied linearly over the dose range (25–500 Gy) of ^{137}Cs that the DNA was exposed to (Figure 2). The yield of 2-deoxyribonolactone formation was more than threefold greater than for **AP** (and all other lesions that react with **2**). These data indicate that **L** accounts for 76 % of the sugar lesions produced in calf-thymus DNA upon exposure to γ radiolysis that are detectable by these reagents. Irradiating the DNA under anaerobic conditions (Figure 2) resulted in a 3.2-fold decrease in 2-deoxyribonolactone formation and 3.4-fold decrease in **AP** production, indicating that over 75 % of **AP** and **L** lesions form through O_2-dependent mechanisms.

Scheme 1.
The release of 5-methylene-2-furanone (**5**) upon heating **L**.[14,15]

Although these experiments clearly show that 2-deoxyribonolactone is produced in far greater amounts than other abasic lesions (e.g. **AP**, **C4-AP**), how **L** is formed is less certain as a result of the complexities of ionizing radiation. γ Radiolysis damages DNA by two general pathways that produce common products.[21] The "direct" effect of γ radiolysis is the ionization of DNA and the production of cation radicals, including nucleobase holes that are implicated in electron transfer. The cation radicals may react with water and/or deprotonate to form the same radicals generated by hydroxyl radicals, which result from the ionization of water (the "indirect" effect of g radiolysis). Mannitol is often used to probe for the involvement of hydroxyl radicals.[21] Irradiation of calf-thymus DNA containing 1 or 10 mM mannitol (Figure 3) significantly depressed the yields of **L** and **AP**, but did not alter their ratio to one another. The role of the hydroxyl radical in the formation of **L** and **AP** was probed more directly by analyzing calf-thymus DNA that was treated with [Fe^{II}(EDTA)] and H_2O_2 (8.8 mM).[22] [Fe^{II}(EDTA)] generates the hydroxyl radical, which produces a DNA cleavage pattern that is remarkably similar to that of γ radiolysis, indicating that direct strand breaks produced by these agents arise through common reactive species.[23] However, the spectrum of abasic lesions produced by [Fe^{II}-(EDTA)] is unknown, and no **AP** sites were reported in a previous report using *t*BuOOH.[24] Quantitation of 2-deoxyribonolactone and lesions that react with **2** in DNA treated with [Fe^{II}(EDTA)] and H_2O_2 reveals a linear dependence on the concentration of the metal complex (Figure 4). The ratio of products produced (**L:AP** = 3.8) is only slightly larger than when DNA is exposed toγradiolysis, and suggests that **L** accounts for at least 79 % of the detected sugar damage.

The similar distribution of **L** and other sugar oxidation products produced byγradiolysis and [Fe^{II}(EDTA)] suggest that the agents form these lesions by a common pathway. As [Fe^{II}(EDTA)]damages DNA by generating hydroxyl radicals, we propose that the majority of these lesions are produced under these conditions through the indirect effect of γ radiolysis. **AP** sites may arise from scission of a weakened glycosidic bond resulting from radical addition to the nucleobases. However, formation of 2-deoxyribonolactone requires formal hydrogen atom abstraction of a well-concealed carbon–hydrogen bond and further oxidation of the C1′ radical. Consequently, direct C1′ hydrogen atom abstraction by the hydroxyl radical is expected to be a minor contributor to formation of L.[9] In addition, the large reduction in the yield of L upon removal of O_2 suggests that oxidation of a C1′ radical by a proximal purine reactive intermediate is also not a major contributor to the formation of the lesion by γ radiolysis because

this mechanism is independent of O_2.[14] A recently proposed mechanism for the formation of **L** is consistent with the predominant generation of nucleobase radicals by γ radiolysis and the large effect of O_2 on the production of the lesion (Scheme 2).[12,21] Formation of 2-deoxyribonolactone as part of a tandem lesion enables γ radiolysis to utilize the major family of reactive intermediates it produces and overcomes the inaccessibility of the C1′ hydrogen atom. The biological effects of such tandem lesions are unknown. However, these experiments suggest that they warrant investigation.

Scheme 2.
A recently proposed mechanism for the formation of **L**.[12,21]

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

References

1. Lindahl T. Nature 1993;362:709–715. [PubMed: 8469282]
2. Kroeger KM, Jiang YL, Kow YW, Goodman MF, Greenberg MM. Biochemistry 2004;43:6723–6733. [PubMed: 15157106]
3. Kroeger KM, Kim J, Goodman MF, Greenberg MM. Biochemistry 2004;43:13621–13627. [PubMed: 15504024]
4. Hashimoto M, Greenberg MM, Kow YW, Hwang J-T, Cunningham RP. J. Am. Chem. Soc 2001;123:3161–3162. [PubMed: 11457038]
5. Kroeger KM, Hashimoto M, Kow YW, Greenberg MM. Biochemistry 2003;42:2449–2455. [PubMed: 12600212]
6. DeMott MS, Beyret E, Wong D, Bales BC, Hwang J-T, Greenberg MM, Demple B. J. Biol. Chem 2002;277:7637–7640. [PubMed: 11805079]
7. Kappen LS, Goldberg IH. Biochemistry 1989;28:1027–1032. [PubMed: 2523732]
8. Bales BC, PitiK M, Meunier B, Greenberg MM. J. Am. Chem. Soc 2002;124:9062–9063. [PubMed: 12149005]

9. Miaskiewicz K, Osman R. J. Am. Chem. Soc 1994;116:232–238.
10. Dizdaroglu M, Neuwald K, von Sonntag C. Z. Naturforsch. B 1976;31:227–233.
11. Harkin LA, Burcham PC. Biochem. Biophys. Res. Commun 1997;237:1–5. [PubMed: 9266818]
12. Carter KN, Greenberg MM. J. Am. Chem. Soc 2003;125:13376–13378. [PubMed: 14583031]
13. Hong IS, Carter KN, Greenberg MM. J. Org. Chem 2004;69:6974–6978. [PubMed: 15471441]
14. Roginskaya M, Bernhard WA, Marion RT, Razskazovskiy Y. Radiat. Res 2005;163:85–89. [PubMed: 15606311]
15. Roginskaya M, Razskazovskiy Y, Bernhard WA. Angew. Chem 2005;117:6366–6369.Angew. Chem. Int. Ed 2005;44:6210–6213.
16. Sato K, Greenberg MM. J. Am. Chem. Soc 2005;127:2806–2807. [PubMed: 15740088]
17. See Supporting Information
18. Ide H, Akamatsu K, Kimura Y, Michiue K, Makino K, Asaeda A, Takamori Y, Kubo K. Biochemistry 1993;32:8276–8283. [PubMed: 8347625]
19. Byrns MC, Vu CC, Neidigh JW, Abad J-L, Jones RA, Peterson LA. Chem. Res. Toxicol 2006;19:414–420. [PubMed: 16544946]
20. Jeong YC, Sangaiah R, Nakamura J, Pachkowski BF, Ranasinghe A, Gold A, Ball LM, Swenberg JA. Chem. Res. Toxicol 2005;18:51–60. [PubMed: 15651849]
21. Von Sonntag, C. Free-Radical-Induced DNA Damage and Its Repair. Springer; Berlin: 2006.
22. Price MA, Tullius TD. Methods Enzymol 1992;212:194–219. [PubMed: 1325598]
23. Pogozelski WK, McNeese TJ, Tullius TD. J. Am. Chem. Soc 1995;117:6428–6433.
24. Kanazawa A, Sawa T, Akaik T, Maeda H. Cancer Lett 2000;156:51–55. [PubMed: 10840159]

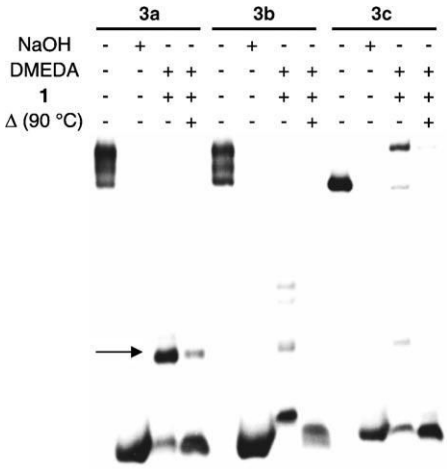


Figure 1.
Autoradiogram demonstrating selective detection of 2-deoxyribonolactone (**L**) in duplex DNA by 1 (50 mM) in the presence of DMEDA (50 mM).

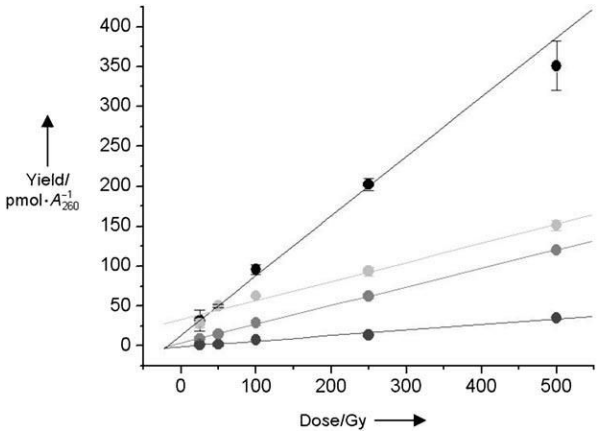


Figure 2.

Yield of 2-deoxyribonolactone and **AP** lesions obtained from g radiolysis of calf-thymus DNA (100 μg/100 μL), normalized for the amount of recovered DNA under aerobic and anaerobic conditions as a function of the dose (Gy = Gray). **L** aerobic (black); **L** anaerobic (pale gray); **AP** aerobic (mid-gray); **AP** anaerobic (dark gray).

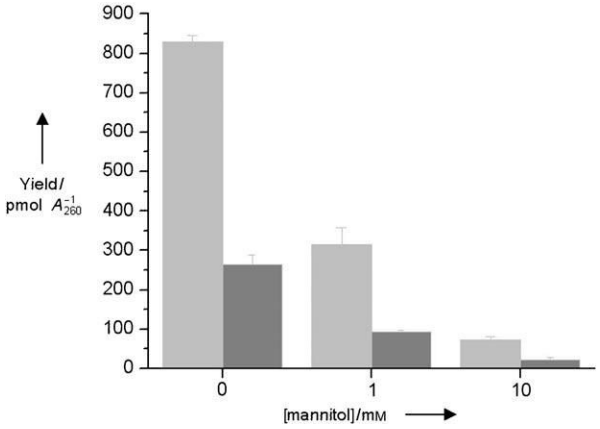


Figure 3.
Effect of mannitol concentration on the yield of 2-deoxyribonolactone (light gray) and **AP** (dark gray) produced in calf-thymus DNA (1.5 μg/10 μL) exposed to γ radiolysis (500 Gy).

Figure 4.
Yield of 2-deoxyribonolactone (●) and **AP** (▲) sites produced in calf-thymus DNA (50 μg/50 μL) exposed to H_2O_2 (8.8 mM) and varying concentrations of [Fe^{II}(EDTA)].