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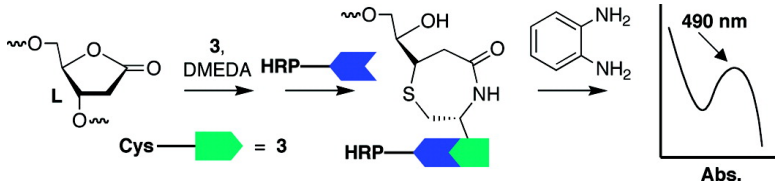

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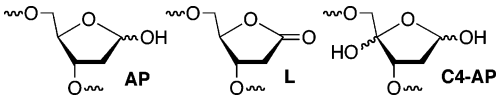
Selective Detection of 2-Deoxyribonolactone in DNA

Kousuke Sato and Marc M. Greenberg*

Department of Chemistry, Johns Hopkins University, 3400 North Charles Street, Baltimore, Maryland 21218

Received December 8, 2004; E-mail: mgreenberg@jhu.edu

DNA lesions give rise to mutations and can be a primary chemical transformation en route to cancer. Elegant methods exist for detecting specific sequences in DNA, which are useful for sensing mutations and single nucleotide polymorphisms.[1−4] However, sensitive detection of DNA lesions is mostly limited to mass spectrometric analysis of individual lesions (or their nucleobases) following complete chemical or enzyme degradation of the nucleic acids.[5,6] Appropriately tagged alkoxy amines (e.g., aldehyde reactive probe, **ARP**), which selectively react with abasic sites (**AP**), are one of the few reagents available for detecting specific lesions in DNA.[7,8] We now report a sensitive method for selectively detecting the oxidized abasic lesion 2-deoxyribonolactone (**L**) in DNA.



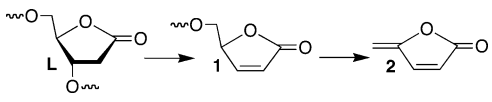
2-Deoxyribonolactone is produced in DNA by the neocarzinostatin chromophore, and other damaging agents, including γ-radiolysis.[9,10] Recent experiments using independently generated reactive intermediates suggest that **L** is produced in greater quantities than predicted by the accessibility of the C1′-hydrogen atom to a diffusible species.[11−13] The formation and detection of **L** is important because the lesion possesses interesting properties. For example, 2-deoxyribonolactone is one of only two lesions known to form DNA-protein cross-links.[14−16] Furthermore, **L** exhibits a mutation spectrum in *Escherichia coli* that is clearly distinct from that of an **AP** site.[17]

5′-d(GTC ACG TGC TGC A**X**A CGA CGT GCT GAG CCT)
3′-d(CAG TGC ACG ACG TAT GCT GCA CGA CTC GGA)
4a X = L; 4b X = AP; 4c X = C4-AP

5′-d(GAA GAC CT**L** GCC GTC C)
5

2-Deoxyrbionolactone has been detected using GC/MS and/or HPLC following alkaline digestion of oxidatively damaged DNA as its persilylated ether and by direct analysis of its elimination product (**2**).[18,19] These methods underestimate the amount of **L** present in DNA because the conditions used to release the lactone prior to silylation may destroy some of the lesion, and **2** is unstable (Scheme 1). We designed a strategy that takes advantage of the unique reactivity of **L** and incorporates an amplification mechanism to increase sensitivity. The butenolide (**1**) has previously been generated using *N*,*N*′-dimethylethylenediamine (DMEDA) and trapped in situ by nucleophiles.[20] The reaction products have been used as a fingerprint to detect **L** in oligonucleotides.[12,21] These studies suggested that an appropriately substituted cysteine could serve as a sensor for 2-deoxyribonolactone by trapping **1**. The utility of biotinylated cysteine (**3**) for detecting **L** was examined using a duplex (**4a**) containing the lesion at a defined site.[22]A cleavage product (**6a**) with considerably retarded migratory ability relative to those consisting of 3′-phosphate or amine adducts was observed when **4a** was treated with **3** and DMEDA (Figure 1). This product was stable to heating at 90 °C, which is consistent with the expectations for cyclic amide **6a**. Furthermore, MALDI-TOF MS analysis of reaction between **5** and **3** under comparable conditions detected a product that corresponds to the expected molecular weight of **6b**.[23]

Scheme 1



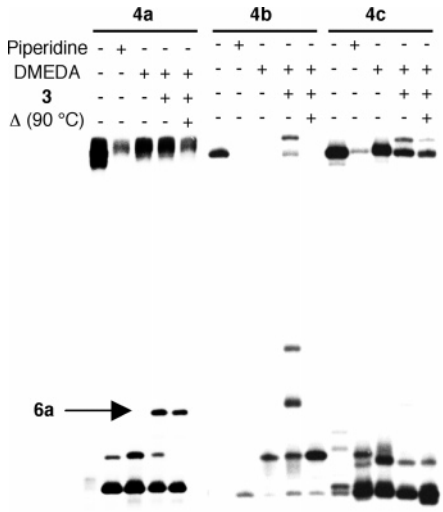
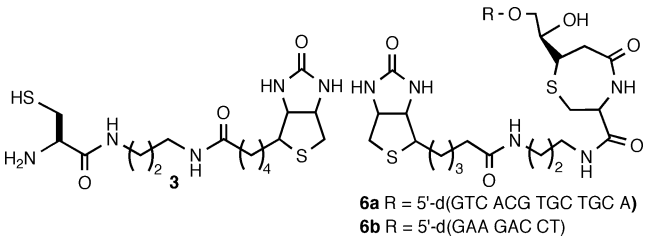


Figure 1. Autoradiogram demonstrating selective detection of **L** by **3** (50 mM) in the presence of DMEDA (50 mM).



Optimized reaction conditions that produce **6a** in 20% yield were used to determine the selectivity of **1** for the butenolide derived from **L** over **AP** and **C4-AP**, which were independently generated in otherwise identical duplexes (**4b**, **4c**).[24,25] The latter is formed in significant amounts by agents such as bleomycin and the enediyne antitumor antibiotics, whereas endogenous conditions produce approximately 10,000 **AP** sites per cell per day.[9,26,27] Cysteine derivative **3** did not trap the **C4-AP** lesion in **4c** (Figure 1). In contrast, two adducts were detected when the AP site (**4b**) was subjected to the conditions used for trapping **L**, but these were

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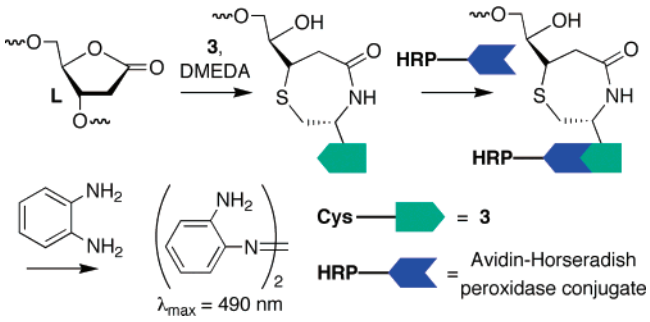
unstable at 90 °C. These data show that **3** selectively detects **L** over **AP** or **C4-AP**. The biotinylated cysteine also does not react with thymidine glycol, a prototypical dihydropyrimidine lesion.[23] Finally, it is worth noting that **L** (**4a**) does not react with commercially available **ARP** (data not shown), suggesting that simultaneous detection of **AP** and 2-deoxyribonolactone lesions will be possible using these two reagents.

The sensitivity for 2-deoxyribonolactone (**L**) detection was enhanced by taking advantage of biotin−avidin binding in conjunction with horseradish peroxidase (HRP) catalysis (Scheme 2). After

Scheme 2



treating **4a** with DMEDA and **3** (followed by heating at 90 °C) and removing excess reagents, the DNA was adsorbed on the surface of a well in a microtiter plate. The plate was washed with avidin−HRP conjugate, followed by addition of 1,2-diaminobenzene. Oxidation of the diamine was monitored at 490 nm after 15 min (Figure 2). A linear correlation with respect to the quantity of **L** was observed, and absolute amounts of 2-deoxyribonolactone present were determined via phosphorimage analysis of radiolabeled **4a**.

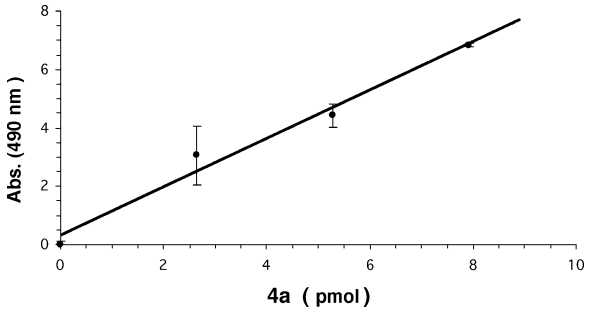


Figure 2. Detection of 2-deoxyribonolactone (**L**) in **4a** by **3** and avidin−HRP conjugate.

Finally, the horseradish peroxidase assay was used to measure **L** in DNA treated with the neocarzinostatin chromophore (NCS). A PCR fragment (287 nt) produced from amplification of pM13 was reacted with varying concentrations of NCS. The oxidatively damaged DNA was then subjected to reaction with **3**, followed by amplification of the signal (Figure 3). The absorbance at 490 nm was corrected for the yield of biotinylated adduct (20%) and calibrated using an identical duplex in which one of the primers contained a 5′-biotin. The lowest concentration of NCS (5 μM) employed produced 390 fmol of **L**. No signal above background was observed when the PCR fragment was treated with acid or with bleomycin, conditions that result in **AP** and **C4-AP** lesions, respectively.[23] However, abasic sites were detected followng treatment with **ARP**.[23]

In conclusion, we have developed the first method to selectively detect the oxidized abasic lesion, 2-deoxyribonolactone (**L**), by

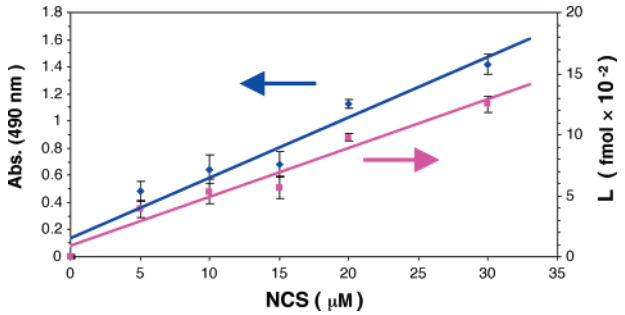


Figure 3. Detection of 2-deoxyribonolactone (**L**) by **3** and avidin−HRP conjugate in duplex DNA (287 nt) treated with varying amounts of NCS.

taking advantage of its distinctive chemical reactivity. The cysteine biotin probe (**3**) will be useful for measuring the amounts of 2-deoxyribonolactone formed in DNA exposed to various forms of oxidative stress.

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Supporting Information Available: Experimental procedures for carrying out lactone detection using **3** and control experiments; MALDI-TOF analysis of reaction of **5**. This material is available free of charge via the Internet at http://pubs.acs.org.

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