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Selective Detection and Quantification of Oxidized Abasic Lesions in DNA

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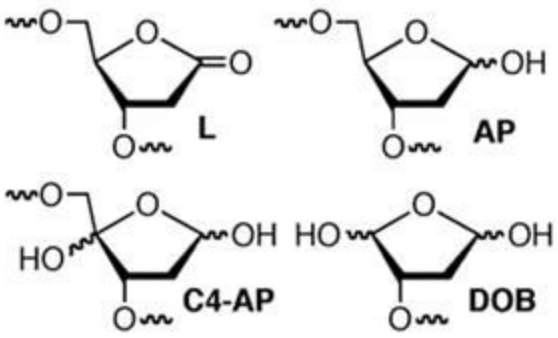
Oxidized abasic lesions are produced via a variety of DNA damaging agents and exert distinctive effects on DNA repair and replication.[1,2] With the exception of 2-deoxyribonolactone (**L**) and **AP**, abasic site (and DNA lesions in general) detection is typically carried out using mass spectrometry, following digestion of the biopolymer, which in some instances is derivatized prior to digestion.[3-6] Selective tagging of DNA lesions offers the possibility of rapid and sensitive lesion detection without the need for completely degrading the biopolymer or expensive instrumentation. We report a reagent, which when used in conjunction with DNA polymerase β and an ELISA like fluorescence assay enables selective quantification of the **C4-AP** and **DOB** oxidized abasic lesions.[7]



C4-AP and **DOB** result from the initial oxidation of the C4′-and C5′-positions of DNA, respectively. Very little is known about the biological effects of **DOB**, although it is a potential source of the DNA alkylating agent, butene dial.[8,9] In contrast, **C4-AP** is known to disrupt replication in *E. coli* and is a substrate for base excision repair.[10,11] **C4-AP** is a major component of DNA damage produced by the antitumor agent bleomycin and is also generated by the enediyne natural products, whereas **DOB** is formed in reactions with neocarzinostatin and enediynes.[12-14] Both lesions are detected following γ-irradiation, presumably because of the accessibility of the respective hydrogen atoms to the freely diffusible hydroxyl radical.[8,15] **C4-AP** can be detected in small DNA fragments via gel electrophoresis following reaction with hydrazine.[16] However, in order to quantify **C4-AP** or **DOB** produced randomly in DNA, one typically stabilizes and/or derivatizes the lesions prior to GC/MS analysis.[8,12]

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Supporting Information Available: Experimental procedures for the synthesis and characterization of **5** and all other experimental procedures; MALDI-TOF MS of DNA adducts and sample fluorescence data. This material is available free of charge via the Internet at http://pubs.acs.org.

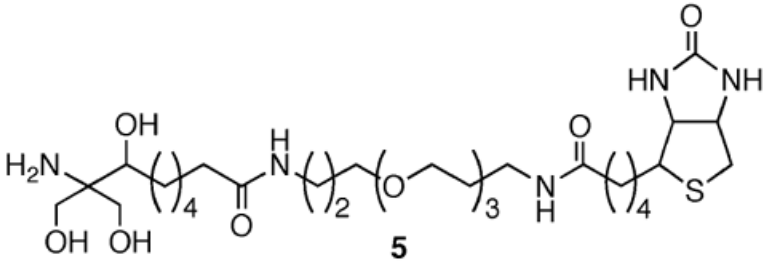
5'-d(CAA GAT TTC AAA TT**X** GAC GAG CTC AAG CAT)
3'-d(GTT CTA AAG TTT AAA CTG CTC GAG TTC GTA)

1 X = **C4-AP**

5'-d(TAA TGG CTA ACG CAG^{OH} **X**CC GTA ATG CAG TCT)
3'-d(ATT ACC GAT TGC GTC——AGG CAT TAC GTC AGA)

2 X = **DOB**

The method described here takes advantage of a common structural feature. The masked 1,4-dicarbonyl group in **C4-AP** and **DOB** is unique to other lesions and offers the opportunity to develop a reagent that would be useful for their selective detection. We previously reported that **DOB** reacted with Tris to form a tricyclic adduct in a reaction that is characteristic of 1,4-dicarbonyl molecules.[17,18] Reaction of two Tris analogues (**3,4**) with **C4-AP** (**1**) indicated that higher adduct yields resulted when a third hydroxyl group was present (Figure 1). Consequently, **5**, which could be used in conjunction with a recently developed fluorescence detection method for quantifying lesions was synthesized.[7] We anticipated that the two lesions could be distinguished by taking advantage of the fact that DNA polymerase β (Pol β) excises **DOB** but not **C4-AP** (Scheme 1, Figure 2).[19]



5

The reactivity of **5** with duplex DNA containing **C4-AP** (**1**) or a ternary complex containing **DOB** (G^{OH} indicates the 3′-hydroxyl terminus of the oligonucleotide that flanks **DOB**, **2**) was examined using previously synthesized oligonucleotides containing the lesions at defined sites.[17,20] Optimum conditions for tagging **C4-AP** (82.3 ± 0.1%) and **DOB** (74.8 ± 0.7%) were found to be 5 mM **5** at pH 8.2 and 55 °C for 4 h (Figure 2). Slightly higher adduct yields did not justify using twice the amount (10 mM) of **5**. No reaction was detected between DNA containing **AP** or **L** lesions and **5** (data not shown). The stereoisomeric mixture of tricyclic adducts were characterized by MALDI-TOF MS.[21] Treatment of **1** with Pol β prior to reaction with **5** had no effect on adduct yield (Figure 2). However, no adduct was detected in **DOB** (**2**) samples pretreated with Pol β, indicating that combined use of the enzyme and **5** will enable one to individually quantify these two lesions.

Quantification was carried out by taking advantage of the biotin covalently linked to the DNA through the lesion of interest (Scheme 1).[7] Lesions were detected by measuring the fluorescence of Resorufin produced from the oxidation of Amplex Red by horseradish peroxidase. Absolute amounts were determined using a calibration curve established side-by-side and by taking into account the adduct yields, per above.[21] Experiments with **1** and **2** indicated that the signal varied linearly over an order of magnitude and one could readily detect 25 fmol of adduct.[21] The method was initially tested by analyzing the reaction of peplomycin with a PCR fragment produced by amplifying a 287 nt region of M13 plasmid (Figure 3). This experiment verified that **C4-AP** formation depended linearly on the concentration of the bleomycin analogue.

Having established the fluorescent assay, **5** was used to measure the levels of **C4-AP** and **DOB** produced in the PCR fragment exposed to γ-radiolysis (Figure 4). The amounts of adduct (s) with and without Pol β treatment were indistinguishable under aerobic conditions, indicating that the amount of **DOB** produced under these conditions was significantly less than that of **C4-AP**. The total amount of lesions formed decreased ~3-fold under anaerobic conditions, consistent with the O_2 enhancement effect in γ-radiolysis.[15] However, under anaerobic conditions **DOB** accounted for ~16% of the lesions detected. Comparing these data to those recently reported reveals that the total amount of 1,4-dicarbonyl containing lesions formed by γ-radiolysis under aerobic conditions account for ~27.5% of the total amount aldehyde reactive DNA damage.[5] Furthermore, these experiments are consistent with the proposal that 2-deoxyribonolactone (**L**) is the major abasic lesion produced by ^{137}Cs irradiation of DNA in aqueous solution, despite the C1′-hydrogen atom's inaccessibility to diffusible species.[5, 22-24] Overall, this study indicates that **5** is a useful research tool for selectively detecting two significant DNA lesions at femtomolar levels. The method will enable one to utilize the **C4-AP** and **DOB** abasic lesions as biomarkers.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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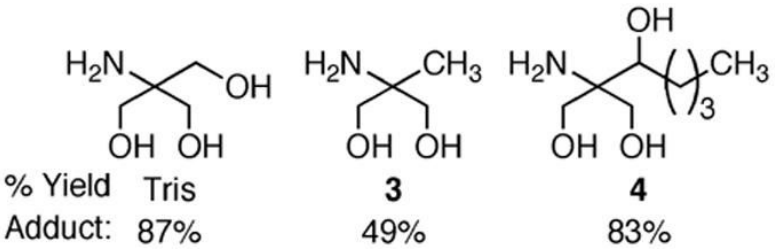


Figure 1.
Yields of adduct with **C4-AP** (**1**).

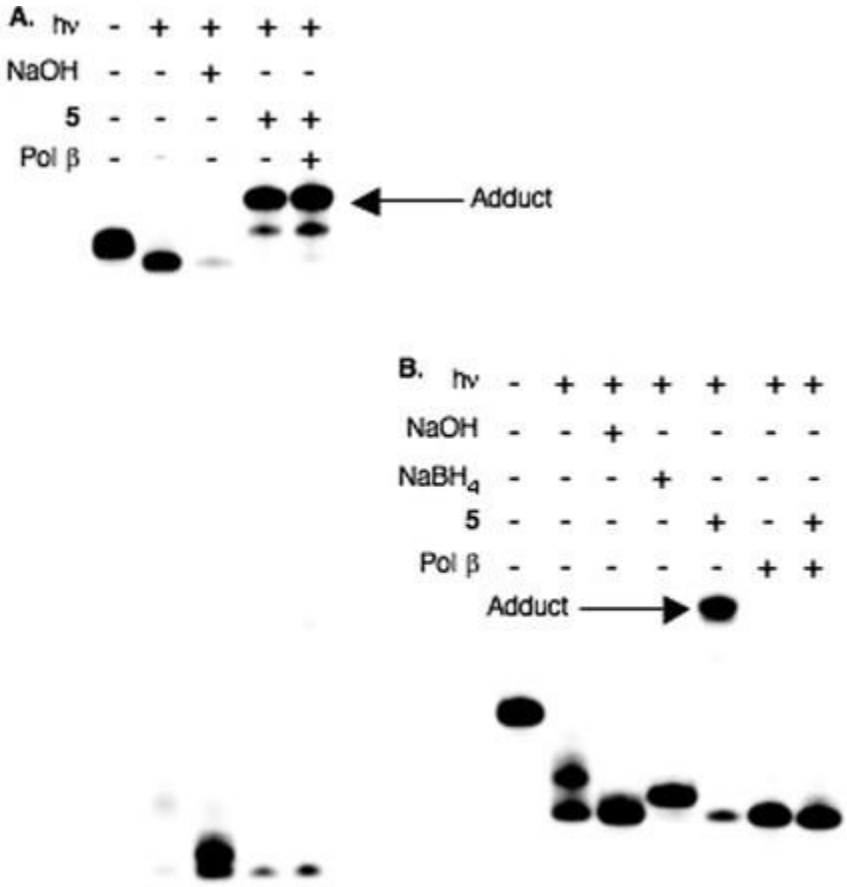


Figure 2.
Reaction of **5** (5 mM, pH 8.2, 55 °C, 4 h) with DNA containing (A) **C4-AP** (**1**) or (B) **DOB** (**2**).

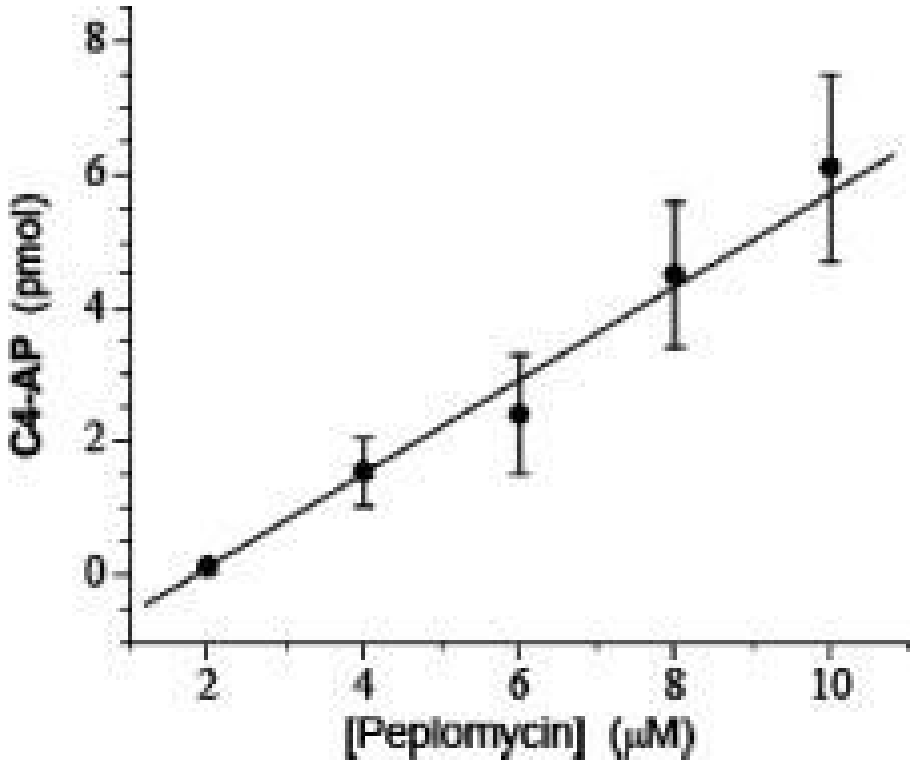


Figure 3.
C4-AP formation as a function of peplomycin concentration.

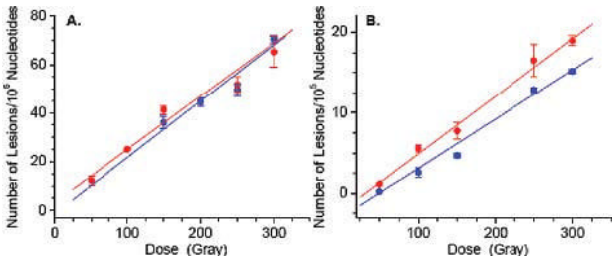
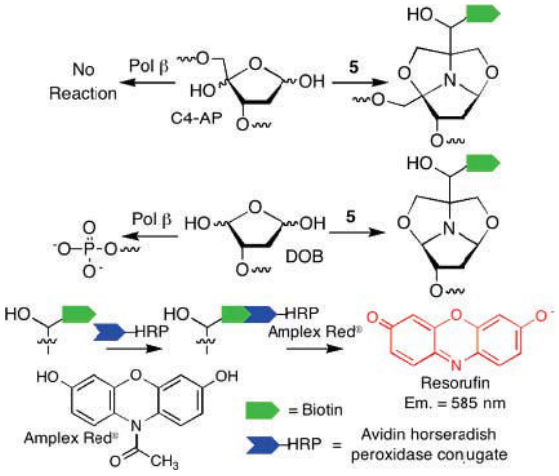


Figure 4.
Quantification of **C4-AP** and **DOB** production by γ-radiolysis of the 287 nt PCR fragment under (A) aerobic (B) anaerobic conditions: with Pol β, blue; without Pol β, red.



Scheme 1.
Fluorescence Detection of **C4-AP** and **DOB** Using **5**