DNA Oxidation as a Source of Endogenous Electrophiles: Formation of Ethenoadenine Adducts in γ-Irradiated DNA

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DNA damage resulting from exposure to reactive oxygen species plays a role in mutagenesis and aging.[1] The genotoxicity of reactive oxygen species, which are formed during cellular metabolism, inflammation, and exposure to ionizing radiation,[1b,2] may arise from direct damage to DNA or from reactions with other biomolecules that lead to the formation of DNA-reactive electrophiles.[3] Lipid peroxidation products have been presumed to be an example of the latter indirect pathway.[4] For instance, an epoxide metabolite of 4-hydroxynonenal has been proposed to react with DNA to form the four exocyclic etheno adducts shown in Figure 1A.[4c] Among these adducts, which also arise by reaction of DNA bases with chloroacetaldehyde,[5] 1,N^6-ethenoadenine (ϵA) has been shown to be mutagenic,[6] and it has been detected at levels of 2−2600 adducts per 10^9 nt in mammalian cells.[7] We now report that ϵA adducts also form during oxidation of DNA by γ-radiation.

The premise for these experiments is that oxidation of deoxyribose leads to the formation of electrophilic species capable of forming adducts with nucleobases. As proof-of-concept, we reported that base propenals arising from C4′-oxidation of deoxyribose react with dG to form the mutagenic M_1G adduct, which also arises in a reaction with the structurally analogous malondialdehyde albeit less efficiently.[8]

On the basis of a similar structural analogy, we hypothesized that 3′-phosphoglycoaldehyde residues derived from deoxyribose 3′-oxidation (Figure 1B) would react with nucleobases to form etheno adducts. The phosphoglycoaldehyde residue is produced in DNA treated with γ-radiation[9a] and certain rhodium complexes,[9b] and it is a structural analogue of chloroacetaldehyde, which readily reacts with guanine, cytidine and adenine to form etheno adducts (Figure 1A,B).[5]

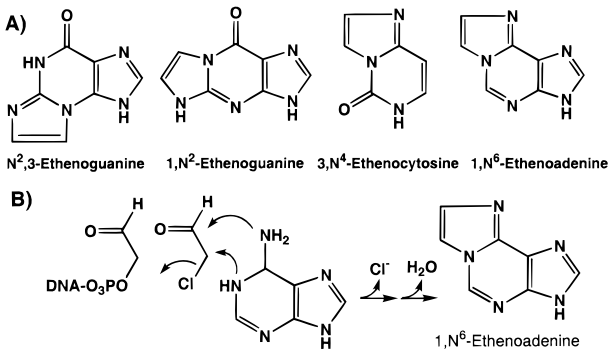

Figure 1. (A) Etheno adducts of DNA bases. (B) Proposed mechanism for the formation of ϵA from a 3′-phosphoglycoaldehyde residue and chloroacetaldehyde.

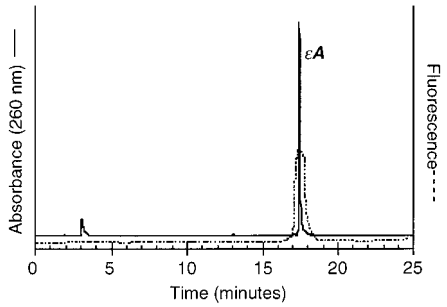

Figure 2. Analysis of ϵA in a reaction of 2-phosphoglycoaldehyde with dA using HPLC with UV (solid line) and fluorescence (dashed line) detection.

To test this hypothesis, we treated dA with synthetic 2-phosphoglycoaldehyde and subjected depurination products to reversed phase HPLC.[10] The reaction resulted in the quantitative formation of ϵA (Figure 2). A UV-absorbing, fluorescent species coeluted with standard ϵA and produced an ESI-MS molecular ion peak $(M+H)^+$ of 160 as expected.[11,12]

We next tested the hypothesis that an oxidizing agent capable of causing formation of 3′-phosphoglycolaldehyde would also produce ϵA. As shown in Figure 3, HPLC analysis of γ-irradiated DNA[13,14] revealed a UV-absorbing and fluorescent species that coeluted at 17−18 min (panel B) with standard ϵA (not shown) and with ϵA generated in chloroacetaldehyde-treated DNA (panel A).[5,7,15b] ESI-MS analysis of the material eluting at 17−18 min

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(10) (a) 2-Phosphoglycoaldehyde was synthesized by treating α-glycerol-phosphate (1 μmol) with 50 mM $NaIO_4$ (1 h, 25 °C). Following Sep-Pak (Waters) purification, product purity was assessed by paper chromatography.[10d,e] (b) dA was treated with 5-fold molar excess of phosphoglycoaldehyde in 100 mM K_2HPO_4 buffer, pH 7.4, for 30 min at ambient temperature. (c) The sample was depurinated (0.1 N HCl, 70 °C, 1 h), and nucleobase products were analyzed by HPLC.[11] (d) Johnson A. W.; Demple, B. *J. Biol. Chem.* **1988**, *263*, 18017−18022. (e) Levin, J. D.; Johnson, A. W.; Demple, B. *J. Biol. Chem.* **1988**, *263*, 8066−8071.

(11) Depurination products neutralized with NH_4OH were analyzed by HPLC using a C-18 reverse-phase column (250 mm × 4.6 mm) and tandem UV diode array and fluorescence (310 nm excitation, 420 nm emission) detectors. The products were eluted with 50 mM ammonium acetate (pH 7.0) with an acetonitrile gradient (0−25%) over 30 min at 25 °C. Peaks were identified by coelution with authentic markers and by ESI-MS.[12a]

(12) (a) Putative ϵA in HPLC fractions was characterized by electrospray ionization mass spectrometry (ESI-MS) using an HP 59987 ESI-MS system with 70 psi nebulizer gas (N_2).[12b] The CID mass spectrum showed fragment ions at 106, 119, and 133 in addition to the molecular ion peak of 160. (b) Yen, T.-Y.; Holt, S.; Sangaiah, R.; Gold, A.; Swenberg, J. A. *Chem. Res. Toxicol.* **1998**, *11*, 810−815.

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(14) DNA (50 μL, 1 μg/μL) in 0.1 M K_2HPO_4 buffer (pH 7.4) was subjected to 0−1000 Gy of γ-radiation in a ^{60}Co source (3 Gy/min). The irradiated DNA was left at ambient temperature for 30 min before depurination.

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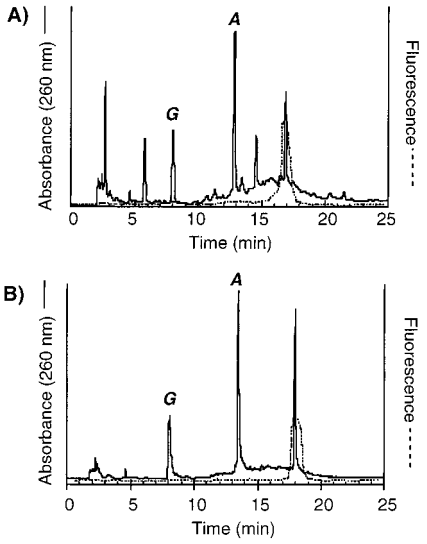

Figure 3. Analysis of ϵA in DNA by HPLC with UV (solid line) and fluorescence (dashed line) detection. **(**A**)** Chloroacetaldehyde-treated and depurinated DNA; the peaks at 8−9, 13−14, and 17−18 min were determined to be guanine, adenine, and ϵA, respectively. **(**B**)** γ-Irradiated (100 Gy) and depurinated DNA.

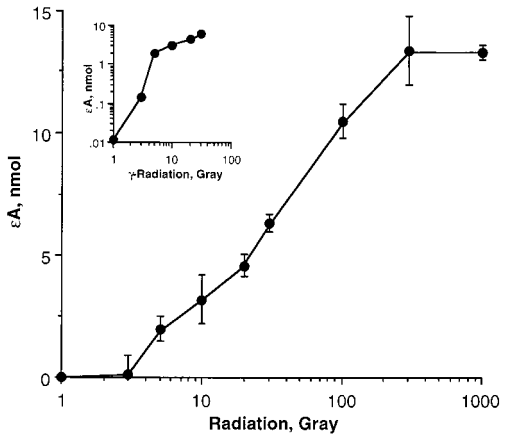

Figure 4. Dose−response for ϵA formation by γ-irradiation (triplicate determinations).

revealed a single molecular ion peak $(M+H)^+$ of 160 and a CID mass spectrum of the known fragmentation ions of ϵA.[12b] The level of ϵA in untreated pUC19 was below the 3−5 picomole detection limit of the fluorescence system employed.[11,15] The radiation dose response for ϵA is shown in Figure 4.[16] At the lowest radiation dose (1 Gy), ϵA was formed at the level of 8 adducts in 10^5 nt.

We have demonstrated that ϵA forms in DNA as a consequence of oxidative DNA damage. Given the precedent for phosphoglycoaldehyde formation by γ-radiation[9a] and our observation that 2-phosphoglycoaldehyde reacts with dA to form ϵdA, it is likely that etheno adduct formation involves a reaction of dA with phosphoglycoaldehyde generated by 3′-oxidation of deoxyribose. The observation of ϵA formation suggests that DNA oxidation will also produce etheno adducts with guanine and cytidine, as observed in reactions of DNA with chloroacetaldehyde and related species.[15b,17]

Several factors suggest that 3′-phosphoglycoaldehyde residues will be a biologically important source of etheno adducts. The first involves proximity to DNA. Although lipids represent a greater target for free radicals than DNA,[3c] the resulting 4-hydroxynonenal must first be metabolized to 2,3-epoxy-4-hydroxynonanal and then diffuse to DNA to react with bases. However, the generation of covalently bound 3′-phosphoglycoaldehyde directly in the DNA target should facilitate reactions with neighboring bases.

The second argument for biological relevance is the highly efficient formation of ϵA in irradiated DNA and in reactions of dA with phosphoglycoaldehyde (Figures 2, 4). This efficiency is highlighted by the observation that 1 Gy of γ-radiation resulted in only 2−3 8-oxo-2′-deoxyguanosine adducts per 10^6 nt, while it produced—as expected for a lesion arising from strand break product—a similar magnitude of strand breaks as ϵA in the present studies.[18]

The radiation dose−response for ϵA formation is notable for its sigmoid shape (Figure 4). The plateau at higher radiation levels probably represents maximal formation of phosphoglycoaldehyde (e.g., 300 Gy yields 1 ϵA in 10 nt). However, the data do not provide an explanation for the more gradual increase in ϵA in the initial portions of the curve. It is possible that the reactivity of 3′-hydrogen atoms varies as a function of sequence context, or that etheno adducts form as a result of other DNA oxidation chemistries that emerge at higher radiation doses.

It remains to be determined how lipid peroxidation and DNA oxidation contribute to the cellular burden of etheno adducts. The level of etheno adducts in human liver ranges from 8 to 26 per 10^9 nt[5,6a] and rises severalfold under conditions of oxidative stress.[7a,c] However, high dietary intake of polyunsaturated fatty acids has also been correlated with an increase in etheno adducts.[7b] Though this problem awaits definitive studies, the relative contributions of 4-hydroxynonenal and 3′-phosphoglycoaldehyde are likely to be tissue- and oxidant-specific.

In conclusion, we have demonstrated that oxidative DNA damage results in the formation etheno adducts. This is consistent with previous observations with base propenal,[8] and it is likely that other electrophilic deoxyribose degradation products will also be genotoxic.[18] Given the continuous production of oxidative DNA damage in cells and the adventitious location of DNA-generated electrophiles, the formation of etheno adducts by phosphoglycoaldehyde may represent a mutagenic burden in vivo.

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(15) (a) Two ϵA standards were prepared: acid depurination of 1,N^6-etheno-2′-deoxyadenosine (Sigma Chemical Co.) and treatment of pUC19 with 2-chloroacetaldehyde in K_2HPO_4 buffer (20 h, ambient temperature) followed by depurination.[15b] (b) Guengerich, F. P. *Chem. Res. Toxicol.* **1992**, *5*, 2−5.

(16) The area integration of the fluorescence peak, relative to a standard curve, was used to generate the dose−response curve for the formation of ϵA by γ-irradiation.

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