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Guanine Oxidation

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Oxidation of Guanine and 8-oxo-7,8-Dihydroguanine by Carbonate Radical Anions: Insight from Oxygen-18 Labeling Experiments**

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The carbonate radical anion ($CO_3^{\bullet-}$) is of significant current interest, as it plays an important oxidative role in biological systems.[1] These radical anions arise during the inflammatory response in tissues[2] by the one-electron oxidation of bicarbonate anions in the active site of copper–zinc superoxide dismutase,[3] and the reaction of peroxynitrite with carbon dioxide.[4,5] The $CO_3^{\bullet-}$ radical is a strong one-electron oxidant ($E^\circ = 1.59$ V versus normal hydrogen electrode)[6] which gives rise to the post-translational modification of proteins[7,8] and causes oxidative DNA damage.[2] Guanine is the most susceptible base in DNA toward oxidative damage,[9] and is oxidized by $CO_3^{\bullet-}$ radicals[10,11] predominantly to oxidative end-products such as spiroiminodihydantoin (Sp) lesions,[12] which are highly mutagenic.[13,14] We have shown earlier that 8-oxo-7,8-dihydroguanine, itself the product of a two-electron oxidation of guanine, is an intermediate in the

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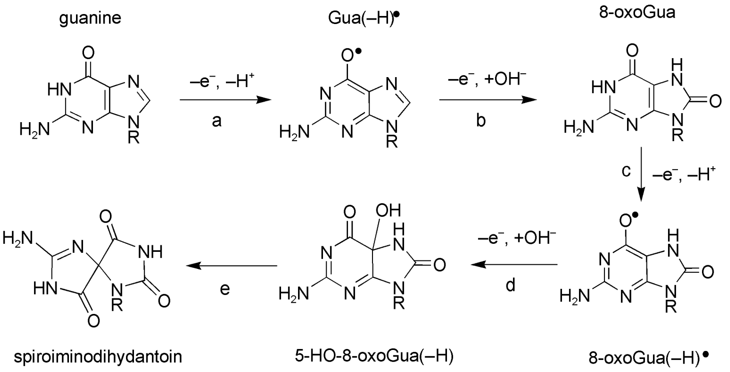
complex series of reactions that ultimately results in the formation of Sp.[11] The generation of Sp formally requires four electron-transfer steps[12] (Scheme 1) and the transfer of oxygen atoms from either the $CO_3^{\bullet -}$ radical species or water.



Scheme 1. Formation of spiroiminodihydantoin by the consecutive abstraction of four electrons from guanine (a–d) followed by an acyl shift (e) in the 5-HO-8-oxoGua(-H) intermediate.[12]

However, the critical mechanistic aspects of the consecutive four-electron oxidation of G by $CO_3^{\bullet -}$ radicals that culminate in the formation of Sp are still obscure.

Herein, we report the use of a combination of ^{18}O labeling and mass spectrometry to address these questions with 2′,3′,5′-tri-*O*-acetylguanosine (tri-*O*-Ac-Guo) or 2′,3′,5′-tri-*O*-acetyl-8-oxo-7,8-dihydroguanosine (tri-*O*-Ac-8-oxoGuo) derivatives as standard model systems.[12,15] We show that the oxygen atoms incorporated into the Sp end-products formed by the oxidation of either tri-*O*-Ac-Guo or tri-*O*-Ac-8-oxoGuo originate from $CO_3^{\bullet -}$ radicals and not from H_2O. These observations indicate that the intermediate steps occur by the radical–radical combination of $CO_3^{\bullet -}$ with Gua(-H)• and 8-oxoGua(-H)•, and that these reactions can be formally viewed as O^--transfer mechanisms.

The $CO_3^{\bullet -}$ radicals were generated by the laser photolysis method used in our previously reported experiments.[10,11] This method involves the photodissociation of persulfate to sulfate radical anions followed by one-electron oxidation of HCO_3^- anions by $SO_4^{\bullet -}$ radicals. Solutions of tri-*O*-Ac-Guo or tri-*O*-Ac-8-oxoGuo (30 μM), $NaHCO_3$ (0.3 M), and $Na_2S_2O_8$ (0.01M) containing ≈43% $H_2{}^{18}O$ were equilibrated in air, adjusted to pH 7.5 by NaH_2PO_4 (1M), and excited by excimer laser pulse train ($\lambda = 308$ nm, 15 mJ pulse^{-1} cm^{-2}, 10 pulse s^{-1}) for ≈10 s. After irradiation, the $Na_2S_2O_8$ was reduced by the addition of a solution of $Na_2S_2O_4$ to quench any further potential oxidation reactions. The Sp nucleoside products, in agreement with our previous experiments,[11] were formed in high yield (40–60%). The Sp products were isolated by reversed-phase HPLC and subjected to ESI MS–MS analysis. The major signals from the Sp molecular ions $[M{+}H]^+$ were observed at $m/z = 442$; the two additional O atoms in Sp (relative to the parent guanine) are ^{16}O (Figure 1a and c, black traces). The presence of lower-intensity peaks at $m/z = 444$ in these spectra suggests that some ^{18}O atoms are incorporated into Sp nucleosides. These incorporation pathways could include hydration[12,16–21] of the Gua and 8-oxoGua radicals or isotope exchange[22] of $HC^{16}O_3^-$ with $H_2{}^{18}O$ that occurs slowly in the absence of the carbonic anhydrase catalyst[23]. Hence, the O atoms in the Sp nucleosides could have been transferred either from molecular oxygen ($^{16}O_2$) or from $C^{16}O_3^{\bullet -}$ radicals, but not from $H_2{}^{18}O$. In contrast, generation of Sp by the oxidation of tri-*O*-Ac-8-oxoGuo by $IrCl_6^{2-}$ in $H_2{}^{18}O$ solution results in the incorporation of an ^{18}O atom from the heavy water molecules.[12] As Sp can be formed in the absence of oxygen,[11] O_2 can be ruled out as the source of the additional oxygen atoms in Sp, and the $C^{16}O_3^{\bullet -}$ radical can be concluded as the source of ^{16}O.

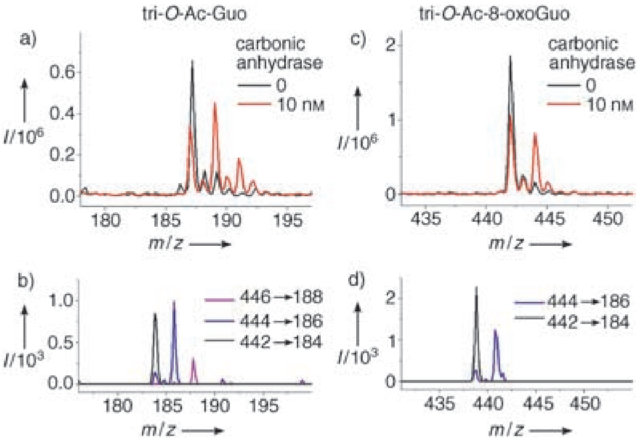
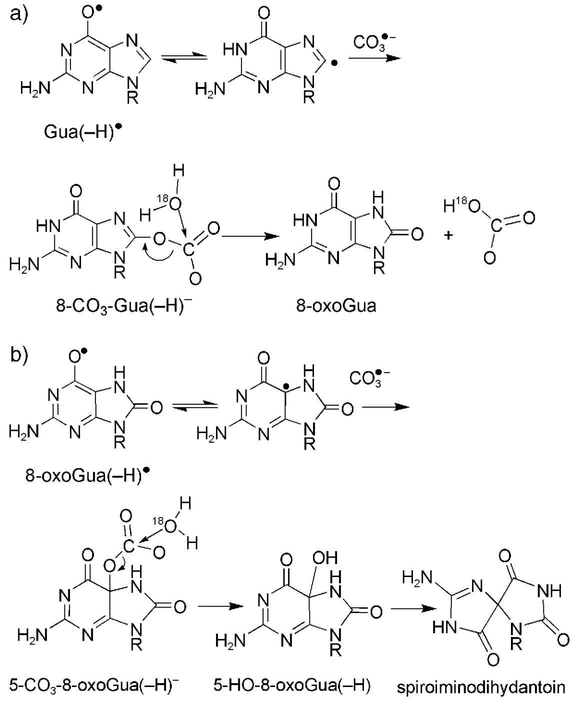


Figure 1. Positive ion spectra of the spiroiminodihydantoin nucleosides prepared by the oxidation of a) tri-*O*-Ac-Guo and c) tri-*O*-Ac-8-oxoGuo by $CO_3^{\bullet -}$ radicals in buffer solutions (pH 7.5) containing $H_2{}^{18}O$ (fraction of ^{18}O atoms $\alpha = 0.43 \pm 0.03$). The reaction mixtures contained either no carbonic anhydrase (black) or carbonic anhydrase at 10 nM to provide a complete exchange of ^{18}O between $HC^{16}O_3^-$ and $H_2{}^{18}O$ (red); Mass spectra obtained by fragmentation of the molecular ion ($[M{+}H]^+$) at $m/z = 442$ (black), 444 (blue), and 446 (magenta) from b) Sp nucleosides prepared by the oxidation of tri-*O*-Ac-Guo and the oxidation of d) tri-*O*-Ac-8-oxoGuo. In the presence of carbonic anhydrase, the experimentally observed fractions of isotope-labeled Sp nucleosides are 0.37 ± 0.04 for the m/z 442→184 ions, 0.46 ± 0.02 in the case of the m/z 444→186 ions, and 0.17 ± 0.03 for the m/z 446→188 ions. These fractions are close to the expected values of $(1\text{-}\alpha)^2 = 0.33 \pm 0.04$ ($^{16}O^{16}O$), $2(1\text{-}\alpha)\alpha = 0.49 \pm 0.01$ ($^{16}O^{18}O$), and $\alpha^2 = 0.19 \pm 0.03$ ($^{18}O^{18}O$) for a random transfer of two oxygen atoms from the carbonate radical anions to the Sp products.

Normally, isotope exchange between $HC^{16}O_3^-$ and $H_2{}^{18}O$ is slow because it occurs only through the hydration of carbon dioxide in equilibrium with bicarbonate.[22] Therefore, even if the reactions are carried out in heavy water, the isotopic composition of the $CO_3^{\bullet -}$ radicals will be close to that of the parent $HC^{16}O_3^-$ anions, and Sp will contain only ^{16}O. To confirm that the source of the O atoms in Sp are transferred from the carbonate radical anions, the oxidation reactions were conducted in the presence of carbonic anhydrase (Figure 1). This enzyme catalyzes the oxygen isotope exchange between $HC^{16}O_3^-$ and $H_2{}^{18}O$[23] and thus the ^{18}O isotope content in the carbonate radical anions is expected to be the same as that of the water at equilibrium. Indeed, the MS data of the Sp samples prepared by the oxidation of tri-*O*-Ac-Guo in $H_2{}^{18}O/H_2{}^{16}O$ solutions incubated with carbonic anhydrase (≈10 nM) for ≈2 h, are characterized by molecular ions at $m/z = 442$, 444 and 446 (Figure 1a, red trace). The assignment of these molecular ions as the Sp nucleosides was confirmed by monitoring the 442→184, 444→186, and 446→188 transitions (Figure 1 b), which correspond to the abstraction of the sugar residues (258 au) from the $^{16}O^{16}O$-, $^{16}O^{18}O$-, and $^{18}O^{18}O$-containing Sp molecular ions.[12] Therefore, these results confirm that both O atoms in the Sp nucleoside derived from the oxidation of tri-*O*-Ac-Guo originate from the $CO_3^{\bullet-}$ radicals.

The MS data of the Sp samples prepared by the oxidation of tri-*O*-Ac-8-oxoGuo in $H_2{}^{18}O/H_2{}^{16}O$ solutions incubated with carbonic anhydrase are characterized by molecular ions at $m/z = 442$ and 444 (Figure 1 c, red trace) and their fragmentation patterns at $m/z = 184$ and 186 (Figure 1 d). In these spectra, the signals at $m/z = 446$ and 188 were not detected because the first O atom in the Sp nucleoside is derived from a tri-*O*-Ac-8-oxoGuo molecule, and only the second originates from $CO_3^{\bullet-}$ labeled with ^{18}O.

The results of this work suggest that the reaction mechanisms leading to Sp oxidation products involve bimolecular combinations of the $CO_3^{\bullet-}$ radicals with Gua(-H)$^\bullet$ or 8-oxoGua(-H)$^\bullet$ radicals (Scheme 2). The primary products of radical addition can be considered as monoesters of carbonic acid (H_2CO_3). The seminal works of Polanyi,[24] Ingold[25] and co-workers showed that, in the acid–base hydrolysis of esters, the ^{18}O atoms from water are transferred to the acid component and not to the alcohol product. In agreement with this mechanism of ester hydrolysis, the O atoms in Sp originate from the $CO_3^{\bullet-}$ radicals and not from H_2O (Figure 1). Therefore, the combination reactions of $CO_3^{\bullet-}$ radicals with Gua(-H)$^\bullet$ and 8-oxoGua(-H)$^\bullet$ radicals can be formally considered as a transfer of O^- from $CO_3^{\bullet-}$ to these radicals. The O^--transfer mechanism has been proposed earlier on the basis of the results of pulse radiolysis experiments for the reactions of $CO_3^{\bullet-}$ radicals with inorganic radicals $^\bullet NO_2$, $SO_3^{\bullet-}$, and $^\bullet NO$, resulting in the formation of CO_2 and the anions NO_3^-, SO_4^{2-}, and NO_2^-.[26,27] In contrast, metal complexes such as $IrCl_6^{2-}$ induce consecutive abstraction of two electrons from 8-oxoGua followed by the addition of water to the carbocation intermediate, and the O atom in the Sp residue originates from water.[12]



Scheme 2. The O^- transfer mechanism of the combination of $CO_3^{\bullet-}$ radicals with a) Gua(-H)$^\bullet$ and b) 8-oxoGua(-H)$^\bullet$ radicals.

These results constitute the first experimental evidence that the combination of carbonate radical anions with organic radicals occurs through the transfer of oxygen atoms from $CO_3^{\bullet-}$ to the target nucleobase radicals, formally corresponding to the transfer of an O^- anion.

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Keywords: DNA damage · isotopic labeling · oxidation · radical reactions · reaction mechanisms

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