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Oxidation of Guanosine to the Imidazolone Derivative via Proton-coupled Electron Transfer to Hydroperoxy Radical Derived from Superoxide

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Oxidation of guanosine (G) with electrochemically generated superoxide ($O_2^{\bullet-}$) leads to the imidazolone (Iz) derivative as a single-electron oxidation product of G. A crucial step in the mechanism of the oxidation is the proton-coupled electron transfer (PCET) from G to the hydroperoxy radical ($HO_2^{\bullet}$) that is derived from $O_2^{\bullet-}$.

It is well recognized that oxidative damage to DNA bases is deeply involved in mutagenesis and carcinogenesis.[1] Superoxide ($O_2^{\bullet-}$) is an important biological intermediate that forms in living cells and is particularly overproduced in tissues subjected to chronic infection and inflammation.[2] Thus, in living tissues under inflammatory conditions, $O_2^{\bullet-}$ causes oxidative DNA damage.[3–5] A few reports exist on in vivo DNA damage by $O_2^{\bullet-}$, for example, inducing the capability of strain-break damage in intracellular DNA[3] or the plating efficiency of a cell line derived from Chinese hamster ovaries.[4] However, the mechanisms of action are poorly understood. Surprisingly, the electron-transfer reaction of $O_2^{\bullet-}$ with nucleobases has not yet been explored by chemists, even at the nucleoside and nucleotide levels. To the best of our knowledge, only one report exists to date on the reaction of nucleotides with $O_2^{\bullet-}$ resulting in the cleavage of *N*-glycosidic bonds and the release of nucleobases.[6] However, guanosine (G) is the most easily oxidizable nucleoside in DNA and is therefore a primary target of oxidative modification.[7] It is well recognized that one-electron oxidation of guanine residues by hydroxyl radicals or type I photosensitizers first produces the G radical cation, which rapidly deprotonates to yield the G neutral radical G(-H)$^{\bullet}$.[8] Imidazolone (Iz) and oxazolone as its hydrolysis product have currently received considerable attention instead of 8-oxo-G, with the result that they are considered the major stable products of G(-H)$^{\bullet}$ that are ultimately yielded in the presence of molecular oxygen.[7,8] In the present study, we explore the possibility that $O_2^{\bullet-}$ unlike hydroxyl radicals or type I photosensitizers, can rapidly react with the G derivative[9] via a proton- and electron-transfer reaction, which we propose takes the form of a proton-coupled electron-transfer (PCET) pathway for oxidation of G to G(-H)$^{\bullet}$ by $HO_2^{\bullet}$ derived from $O_2^{\bullet-}$.

The presence of G ($pK_a = 9.6$ for N1–H)[10] induces a significant effect upon the $O_2/O_2^{\bullet-}$ electrochemistry.[11] The change in reversibility of the O_2 reduction in the presence of weak acids such as phenols has been well demonstrated.[12,13] In light of these results, it is rationalized that after a primary electron-transfer step associated with proton transfer from G, $O_2^{\bullet-}$ formation leads to the irreversible reduction, driven by the exergonic reduction and the immediate consumption of the resulting $HO_2^{\bullet}$. Controlled potential electrolysis of a DMF solution of G saturated by O_2 was performed on a carbon mesh electrode set at −1.4 V vs. Ag/$AgNO_3$, and an aliquot of the solution was injected into an HPLC. Prominent peaks corresponding to the lesion of G and guanine are observed as it increases over electrolyzed time, as shown in Figure 1a. The cleavage of *N*-glycosidic bonds and the release of the base were previously reported[6] and explained by the SFO mechanism.[14] In fact, H_2O_2 reacts with G to generate the guanine base in DMF containing $(CH_3)_4NOH$. However, the lesion corresponding to Peak A was isolated and subjected to electrospray ionization (ESI)-MS for structural analyses, as shown in Figure 1b. Molecule-related-ion peaks with *m*/*z* 285.2 ($[M + H]^+$) and 307.2 ($[M + Na]^+$), where *M* is the molecular weight of Iz, are observed. High-resolution ion trap (IT)-TOF-MS results (*m*/*z* 285.1202, $[M + H]^+$) provide the chemical formula for the lesion as $C_{11}H_{16}N_4O_5$ (structure shown in Figure 1b).[11] The Iz formation is deduced from the MS/MS spectra for $[M + H]^+$ showing a fragment peak of *m*/*z* 113.0462, corresponding to 2,5-diamino-4*H*-imidazol-4-one + H^+ resulting in the elimination of ribose.[11] The UV spectrum of the lesion is in accordance with the previously published spectrum of Iz.[11,15] These results indicate that G reacts with $O_2^{\bullet-}$ (actually $HO_2^{\bullet}$) via proton and electron transfers to yield G(-H)$^{\bullet}$, leading to the formation of Iz. Note that a direct electron transfer from G to $O_2^{\bullet-}$ is not feasible because of the up-hill electron transfer.[16]

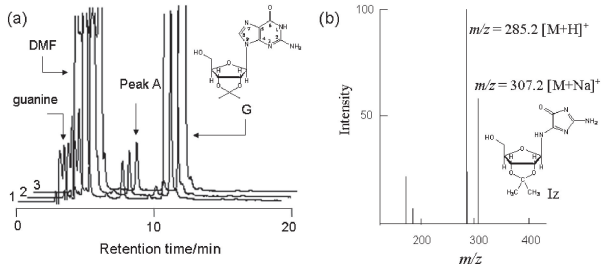

Figure 1. HPLC elution profile for the electrolyzed solution of O_2 in the presence of G with UV detection at 260 nm (a), and ESI-MS spectra of the isolated oxidative lesion corresponding to Peak A (b). HPLC separation was performed on an ODS column with a mobile phase consisting of 0.2 mol dm^{-3} phosphate buffer (pH 7.2) and CH_3CN (15%) at a flow rate of 0.8 mL min^{-1}. The chromatograms of 1–3 were obtained from the sample solutions electrolyzed for 1, 2, and 3 h, respectively.

The key step in the reaction mechanism for G(-H)$^{\bullet}$ generation is the kinetically preferential one-electron transfer conjugated with proton transfer (H transfer) from G to $HO_2^{\bullet}$ generated in the electrochemical process. We explored the energetically favorable H-transfer pathway using UB3LYP/6-31+G(d) calculations, the results being shown in Figure 2. Prior to the H transfer, the pair forms a prereaction hydrogen-bonded complex. The calculation indicates that the complex is nearly planar and lies 18.0 kJ mol^{-1} lower in energy than the separated

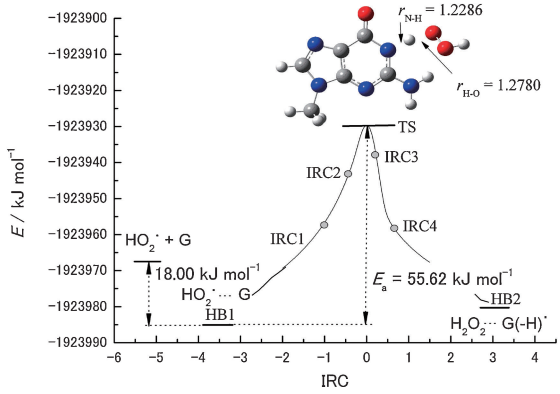

Figure 2. Intrinsic reaction coordinate (IRC) for the H-transfer reaction from G to $HO_2^{\bullet}$, and structure of the transition state, calculated by the DFT (UB3LYP) method with 6-31+G(d) basis sets. The pathway shown here corresponds to a PCET mechanism. See the text for details.

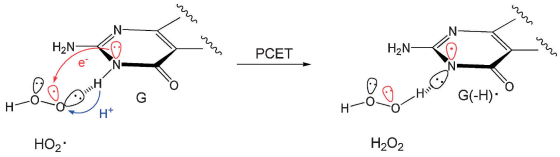

Figure 3. Schematic showing the plausible mechanism for PCET between G and $HO_2^{\bullet}$.

reactants, as shown in Figures 2 and 3. It is found that the H transfer involves three atomic centers consisting of O···H–N and occurs through the migration of the G proton across the hydrogen bond to an O lone pair on $HO_2^{\bullet}$. Any attempt to find a transition state (TS) gives reactant- and product-like geometries in which the H atom lies midway between O and N. The activation energy of 55.62 kJ mol^{-1} is equivalent to a hydrogen-bond energy. This transfer occurs between σ-orbitals that are nearly in the plane of the molecular framework of the TS complex. We find that the proton transfer is accompanied by an electron transfer from a nominally orthogonal lone pair on the G nitrogen to the SOMO on $HO_2^{\bullet}$, with the electron transfer occurring in the same direction as the proton transfer, as shown in Table 1. Therefore, the G(-H)$^{\bullet}$ generation is rationally explained by the PCET mechanism, as shown in Figure 3. Note that the PCET reaction is distinguished from the so-called hydrogen-abstraction reaction of radicals by the fact that the proton and electron are transferred between different molecular orbitals.[17]

In conclusion, we find that, as a typical one-electron oxidation product of G, Iz forms by oxidation of G with $HO_2^{\bullet}$ derived from $O_2^{\bullet-}$. A crucial step in this new mechanism of Iz formation involves the electron-transfer reaction conjugated with a proton transfer from G to $HO_2^{\bullet}$, which we attribute to the PCET mechanism. The PCET reaction currently attracts considerable interest because of its likely involvement in many natural processes.[18] In this connection, the compounds G in which the acidic hydrogen atom is hydrogen-bonded to the oxygen atom of $HO_2^{\bullet}$ are particularly interesting mimics. The present results are important to analyze oxidative DNA damage in living tissues under inflammatory conditions.

Table 1. The number of electrons and spin densities calculated from Mulliken atomic charges with hydrogens summed into heavy atoms and Mulliken atomic spin densities, respectively

IRC[a]	r_{N-H}[b]/Å	Number of electrons	Spin densities
		G/$HO_2^{\bullet}$	G/$HO_2^{\bullet}$
HB1	1.019	86.034/16.966	0.003/0.997
IRC1	1.030	85.889/17.111	0.235/0.765
IRC2	1.071	85.700/17.300	0.472/0.528
TS	1.227	85.584/17.416	0.711/0.289
IRC3	1.336	85.043/17.957	0.898/0.102
IRC4	1.509	85.026/17.974	0.996/0.004
HB2	1.855	85.027/17.975	1.000/0.000

[a]These show the points on the IRC shown in Figure 2. [b]Lengths of the N–H bond in G.

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