The Trap Depth (in DNA) of 8-Oxo-7,8-dihydro-2′deoxyguanosine as Derived from Electron-Transfer Equilibria in Aqueous Solution

Steen Steenken,* Slobodan V. Jovanovic,[1] Massimo Bietti,[2] and Klaus Bernhard

Max-Planck-Institut für Strahlenchemie D-45413 Mülheim, Germany

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8-Oxo-7,8-dihydro-2′deoxyguanosine (pK_{a1} = 8.6, pK_{a2} = 11.7),[3] frequently called 8-hydroxy-2′deoxyguanosine (≡ 8-OHdG), is probably the most important and best-documented product of "oxidative stress"[4−7] in biological systems. Its concentration in the cellular DNA is, in fact, a quantitative measure of the degree of damage that an organism has undergone.[8−12] Unless 8-OHdG is built by nature into DNA on purpose, it is the product of oxidative *decomposition* of 2′deoxyguanosine (dG). A large number of oxidants/oxidizing environments leading from dG to its 8-hydroxy derivative have been identified, some of the more important ones being singlet oxygen,[13,14] the OH radical (produced by ionizing radiation or transition metal catalyzed decomposition of hydroperoxides),[15,16] and *photo*-oxidation[17] (which is believed[18−21] to proceed via the guanosine radical cation).

8-OHdG is a mutagenic lesion involved in carcinogenesis and aging,[8,22] but *as such* it does *not* lead to DNA strand breakage. However, it is much more easily oxidized than its natural "parent", dG,[14,21,23−26] and its oxidation product *is* a candidate for (piperidine-induced) strand breakage.[27−29] In view of this pronounced sensitivity, it is necessary to fully understand the radical chemistry of 8-OHdG and particularly the one-electron redox properties of this physiologically important molecule. With this aim, the pulse radiolysis method with optical and conductance detection was applied. 8-OHdG[30] in 0.01 to 1 mM aqueous solutions was one-electron-oxidized with the radiation-chemically produced *inorganic* radicals $Br_2^{\bullet-}$, $N_3^{\bullet}$, Tl^{2+}, or $SO_4^{\bullet-}$ (Table 1). From the pH-dependent absorption spectra[31] (Figure 1, insets d, e) are derived the (de)protonation equilibria (Scheme 1)[32] with pK_{a1} = 6.6 and pK_{a2} = 12.3. These values are higher than those for dGuo (3.9 and 10.8)[33] which reflects the increase in electron density due to the oxygen at C8.

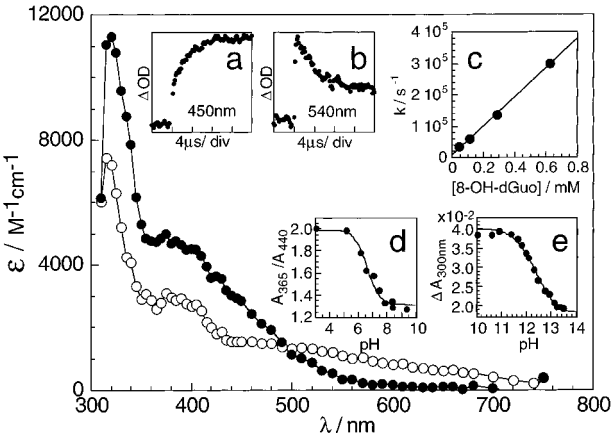

Figure 1. Oxidation of 8-OHdG by G(−H)• at pH 7. The pulse (200 ns 3 MeV electrons)-irradiated solution contained 2 mM guanosine, 0.6 mM 8-OHdG, 20 mM $K_2S_2O_8$ and 0.5 M *tert*-butyl alcohol. Absorption spectra (G(radical) ≡ 3.3) of G(−H)•, observed at 1.5 μs: ○, and of 8-OHdG-(−H)•, observed at 15 μs after the pulse: ●. In insets d and e are shown the determinations of the pK_a-values of 8-OHdG(−H)• using, however, $N_3^{\bullet}$ to oxidize 8-OHdG.

The same conclusion can be drawn from the reduction potential (0.74 V/NHE, see later) of 8-OHdG(−H)• as compared to the 1.29 V/NHE[34] of G(−H)•.

8-OHdG can also be oxidized with various *organic* radicals (Table 1), such as tyrosyl or tryptophyl or enolether radical cations,[35] peroxyls, and even with the deprotonated radical cation of 2′deoxyguanosine-5′-monophosphate or that of guanosine, G(−H)•. The protocol for this reaction is shown in Figure 1.

The initial spectrum (Figure 1, ○) is that of G(−H)• (produced via $SO_4^{\bullet-}$ from the excess guanosine present). Due to the presence of 8-OHdG, G(−H)• disappears (inset b) to give rise to 8-OHdG-(−H)• (see ● and inset a). The rate constant for the oxidation of 8-OHdG by G(−H)• (obtained via inset c) is 4.6×10^8 M^{-1} s^{-1} at pH 7.

(1) Helix International, 381 Viewmount Dr., Nepean, ON, Canada K2E 7R9.
(2) Chemistry Department, Universitá "Tor Vergata", I-00133 Roma, Italy.
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(31) The spectra of oxidized 8-OHdGuo at pH 3, 7.7, and 13.3 are available in Supporting Information.
(32) With the time-resolved AC-conductance method (using $SO_4^{\bullet-}$ at pH 4.5 to one-electron-oxidize 8-OHdG and, for calibration, anisole radical cation (O'Neill, P.; Steenken, S.; Schulte-Frohlinde, D. *J. Phys. Chem.* **1975**, *79*, 2773)) under the same conditions, it was established (the conductance signals had the same shape and amplitude within ±4%) that the species existing at pH < 6 is the radical *cation* of 8-OHdG.
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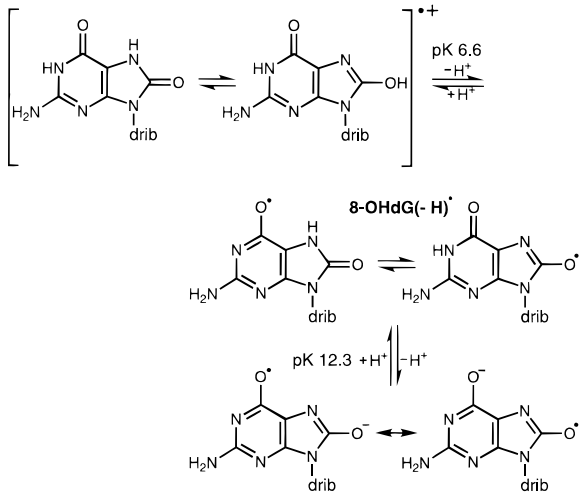
Table 1. Rate and Equilibrium Constants[a] for Oxidation of 8-OHdG in Aqueous Solution at 20−25 °C

oxidizing radical// $\lambda_{observation}$/nm	pH	k_{ox}/ 10^9 M^{-1} s^{-1}	redox standard S[b] $S^{\bullet}+8\text{-OHdG} \underset{k_r}{\overset{k_f}{\rightleftharpoons}} S+8\text{-OHdG}^{\bullet}$	pH	E_S/V_{NHE}	k_f[b]	k_r	K_{eq}[c]	E_{pH}/V_{NHE} 8-OHdG
$Br_2^{\bullet-}$/325	6.9	1.1	tryptophan	3.0	1.19[d]	1.2×10^9		3025	0.99
$N_3^{\bullet}$/330	7.6	5.6							
Tl^{2+}/325	3.2	1.6	3,5-dihydroxyanisole	7.5	0.84[e]	1.2×10^7	2×10^5	58	0.74
$SO_4^{\bullet-}$/330, 390	7.0	4.3	3,5-dihydroxyanisole	13.2	0.46[e]	4.4×10^8	3.5×10^6	164	0.33
$CH_3O_2^{\bullet}$/320	7.0	0.83	4-methyl-2-methoxyphenol	7.0	0.68[f]		1.3×10^6	0.1	0.74
$CCl_3O_2^{\bullet}$/370	*g*	0.38							
TyrO$^{\bullet}$/480	7.0	≈0.02	1,2,4-trimethoxybenzene	3.0	1.14[h]	2.6×10^9	2.6×10^7	112	1.02
$Trp^{\bullet+}$/420, 565	3.0	1.2							
Trp(−H)$^{\bullet}$/400	7.0	0.017	1,2,4,5-tetramethoxybenzene	6.0	0.889[i]	1.8×10^7	5.4×10^5	25.9	0.81
dG(−H)$^{\bullet}$/450, 540	7.0	0.46							
G5′-MP(−H)$^{\bullet}$/330, 550	5.0	0.84							
cis-MeOCH=CHOMe$^{\bullet+}$/330	7.7	0.83							
2,3-Me_2-4,5−$DHF^{\bullet+}$[j]/400	7.5	0.56							

[a] Error limits ±10%. [b] The forward reaction (k_f) is between the one-electron-oxidized redox standard and 8-OHdG. The redox standards were oxidized with $Br_2^{\bullet-}$. [c] The constant is from the *concentrations* at equilibrium and, where possible, from the *kinetically* measured values (from k_f and k_r). [d] From ref 36. [e] From ref 37. [f] From ref 38. [g] Solvent 65% (v/v) water, 25% 2-propanol, 10% acetone, saturated with CCl_4. [h] Electrochemically determined. From ref 39. [i] From ref 40. [j] 2,3-Dimethyl-4,5-dihydrofurane.

Scheme 1



To better understand the role in DNA of 8-OHdG, it is necessary to know its redox potential. From the fact that the G radical is able to oxidize 8-OHdG (Figure 1, Table 1) and also from the literature[14,23,24,26] it is evident that 8-OHdG is more easily oxidized than G. It was found that the compounds tryptophan (E_3 = 1.2 V/NHE),[36,41] 3,5-dihydroxyanisole (E_7 = 0.84 V),[37] 4-methyl-2-methoxyphenol (E_7 = 0.68 V),[38] 1,2,4-trimethoxybenzene (E = 1.14 V),[39] or 1,2,4,5-tetramethoxybenzene (0.889 V)[40] are able to establish an electron transfer *equilibrium* with 8-OHdG radical. From the measured equilibrium constants K_{eq} for electron transfer between the systems and applying the Nernst equation to convert K_{eq} into ΔE,[42] the reduction potential E of the one-electron-oxidized 8-OHdG radical was determined at different pH values (Table 1). The pH-dependence of E is shown in Figure 2, the (averaged) value at pH 7 being 0.74 V/NHE.[43]

The difference in reduction potential at pH 7 between G(−H)$^{\bullet}$ (1.29 V) and 8-OHdG(−H)$^{\bullet}$ (0.74 V) of 0.55 V[44,45] corresponds to a driving force for oxidation of 8-OHdG by G(−H)$^{\bullet}$ of 13 kcal/mol. It is thus clear that not the guanine but the 8-OH-guanine moiety is the *ultimate* sink of oxidizing equivalents in DNA.[46,47]

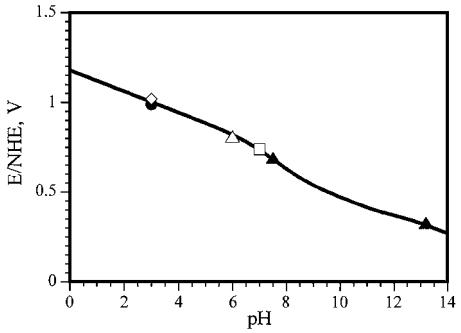


Figure 2. Dependence of the reduction potential E/NHE of one-electron oxidized 8-OHdG on pH. The solid line is a computer fit to the formula given in ref 43. Redox standards used: ● tryptophan, ◇ 1,2,4-trimethoxybenzene, △ 1,2,4,5-tetramethoxybenzene, □ 4-methyl-2-methoxyphenol, ▲ 3,5-dihydroxyanisole.

The presence of 8-OHdG in DNA makes this macromolecule vulnerable to oxidation from "environmental" radicals such as peroxyl (E_7 = 1.05 V),[48,49] and even amino acid radicals such as tyrosyl (0.93 V),[50] tryptophyl (1.01−1.08 V)[36,41] or to thiyl radicals (0.75 V),[51] as they may exist in the histone proteins covering the DNA. By functioning as the ultimate "positive hole" sink, the oxidation of DNA is funneled into this (desired?) direction. By serving this function, 8OH-dG *protects the other bases* from oxidation.[46]

Supporting Information Available: The spectra of one-electron-oxidized 8-OHdG at pH 3, 7.7, and 13.3 (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.
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(44) The difference in the (irreversible) "half peak" potentials *in DMF* has been measured to be 0.43 V (ref 14).
(45) As shown in ref 26, *stacking* of 8-OHdG with G does not change the relative potential of G and 8-OHdG moieties.
(46) Although the redox potentials of the bases *in the DNA polymer* are not known, it is likely that the change from aqueous to "DNA phase" or stacking interactions (ref 26) do not change the *relative* potentials.
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