How Easily Oxidizable Is DNA? One-Electron Reduction Potentials of Adenosine and Guanosine Radicals in Aqueous Solution

Steen Steenken*,† and Slobodan V. Jovanovic‡

Max-Planck-Institut für Strahlenchemie
D-45413 Mülheim, Germany
Department of Chemistry, University of Ottawa
10 Marie Curie, Ottawa, Canada K1N 6N5

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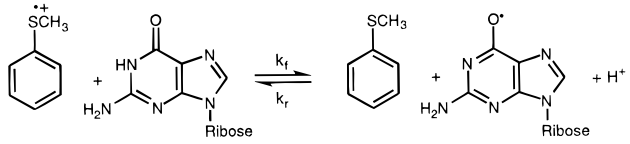
DNA interacts with fluorescent dyes,[1,2] intercalating agents,[2] and transition metal complexes,[3] often through charge transfer complexes involving the DNA bases. These interactions, which are also of interest in photodynamic therapy, depend on the electron-donating abilities of the bases.[4] Furthermore, the direct and indirect effects of ionizing radiation on DNA, particularly those relating to base alterations and single-strand breaks, are mediated by (the one-electron deficient) DNA base radicals.[5−12] In order to better understand these effects, it is necessary to know the reduction potentials of the one-electron deficient bases (radicals), which are a measure of the susceptibility of DNA to damage by endogenous oxidizing radicals (e.g., peroxyl radicals from lipids or amino acids, superoxide radical, singlet oxygen) and exogenous oxidants (UV light, ionizing radiation).

The ionization of DNA, induced either by ionizing radiation,[5,7,9] 193 nm photolysis,[8,12] or chemi-ionization by strong transient oxidants such as $SO_4^{\bullet-}$ or Tl(II)[6,13−17] results in the formation of a positive "hole". ESR experiments show that this positive "hole" is transmitted to a guanine moiety.[6,14] The driving force for such intramolecular electron "hops" is the difference in the reduction potentials of the DNA base radicals. The guanine radical apparently has the lowest reduction potential. Values of the reduction potentials of the DNA base radicals in aqueous media do exist.[15,16] However, even if corrected on the basis of improved values for the reduction potentials of the reference redox couples, these values[15,16] do not appear to be sufficiently accurate or reliable.[1] The main reason for this is that reference redox couples with sufficiently high potentials (higher than 1.2 V vs NHE) were previously not available. The situation has now changed, since recently the reduction potentials of substituted anisole and thioanisole radical cations became available,[18,19] whose values are in the range of 1−1.6 V. Using these compounds as references, we report here the determination of the reduction potentials of the adenosine and guanosine radicals in neutral and acidic aqueous solutions and the evaluation of the pH dependence of the reduction potentials ranging from 0 to 14.

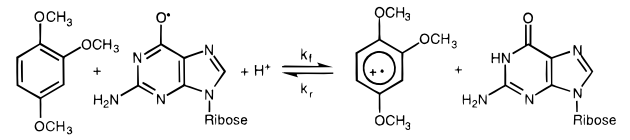
The purine and reference radicals were generated by pulse radiolysis (using a 3 MeV van de Graaff accelerator and optical detection)[20] in Ar-purged aqueous solutions of 25 mM $K_2S_2O_8$, 1 M 2-methyl-2-propanol, and millimolar parent compounds at 20 °C. The composition of the aqueous solution ensures the selective generation of strongly oxidizing $SO_4^{\bullet-}$ radicals ($G \approx 3.0$) by the $e_{aq}^- + S_2O_8^{2-}$ reaction. The OH radicals ($G \approx 2.8$) are scavenged by 2-methyl-2-propanol to the inert $^{\bullet}CH_2(CH_3)_2COH$. The $SO_4^{\bullet-}$ single electron oxidizes the purine nucleosides and reference compounds at diffusion-controlled rates ($k > 10^9\ M^{-1}\ s^{-1}$).[13] In order to minimize radical−radical decay rates, low-dose rates (0.8−2 Gy/pulse, corresponding to 0.5−1.2 μM radicals) were used. Computer averaging of multiple traces (50−200 pulses/trace) improved the accuracy of the data.

The reduction potential of the neutral guanosine radical was determined at pH 7 using thioanisole, with $E_7 = 1.44$ V,[19] and 1,2,4-trimethoxybenzene, with $E_7 = 1.13$ V vs NHE,[18] as the reference redox couples. In the presence of guanosine (Guo, from 0.018 to 0.175 mM), the absorbance of thioanisole radical cation (generated by the $SO_4^{\bullet-}$ oxidation of 2.8−3.4 mM thioanisole) at 540 nm[19] decayed exponentially. Both the rate of the reaction and the absorbance upon completion of the first-order decay depended on the ratio of the concentrations of thioanisole and Guo, which indicates the following electron transfer equilibrium:



$k_f = (1.4\pm0.1)\times10^9\ M^{-1}\ s^{-1}$; $k_r = (7\pm2)\times10^6\ M^{-1}\ s^{-1}$
$K_{abs} = 185\pm18$, $\Delta E = 0.14$ V, $E(Guo^{\bullet}/Guo)_7 = 1.31$ V

The reduction potential of the guanosine radical at pH 7 was also determined via the electron transfer equilibrium with 1,2,4-trimethoxybenzene (monitored as a buildup of 1,2,4-trimethoxybenzene radical cation at 450 nm[21] in an aqueous solution of 2.2 mM Guo and from 0.012 to 0.05 mM 1,2,4-trimethoxybenzene, see Figure 1):



$k_f = (6.3\pm0.6)\times10^8\ M^{-1}\ s^{-1}$
$K_{abs} = 140\pm10$, $\Delta E = 0.13$ V, $E(Guo^{\bullet}/Guo)_7 = 1.26$ V

The absorbance equilibrium constants were determined from the plot of absorbances of radicals at equilibrium vs the ratio of concentrations of the parent compounds,[19] as illustrated in Figure 2.

† Max-Planck-Institut für Strahlenchemie.
‡ University of Ottawa.

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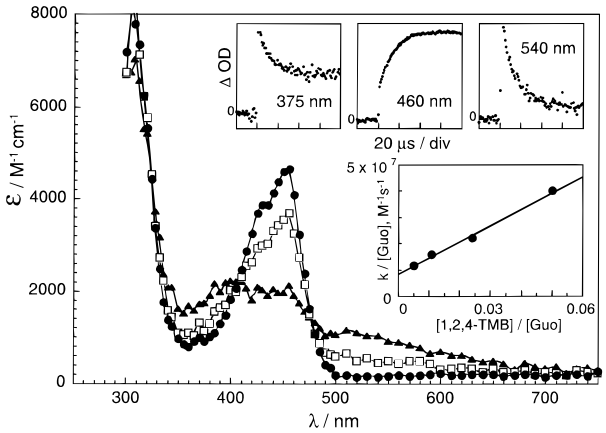


Figure 1. Time-resolved radical spectra at various stages of the reaction of the guanosine radical with 1,2,4-trimethoxybenzene obtained by pulse radiolysis in Ar-saturated aqueous solution of 2 mM Guo, 0.1 mM 1,2,4-trimethoxybenzene, 25 mM $K_2S_2O_8$, and 1 M 2-methyl-2-propanol. Spectra: ▲, at 3 μS; □, at 8 μS; ●, at 95 μS (after the pulse). The kinetic traces in the inset show the progress of the reaction at 460 nm (maximum of the 1,2,4-trimethoxybenzene radical cation) and at 375 and 540 nm (maxima of the Guo radical cation). Lower inset is the kinetic analysis of the electron transfer equilibrium.

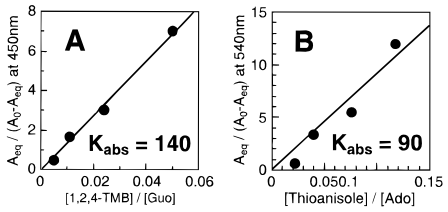


Figure 2. Representative plots used to determine the equilibrium constants from the absorbances of radicals at equilibrium (A_{eq}, equilibrium absorbance of one radical in the presence of a redox substrate; A_0 in the absence of the substrate). (A) Guo + 1,2,4-trimethoxybenzene and (B) Ado + thioanisole (see text for details).

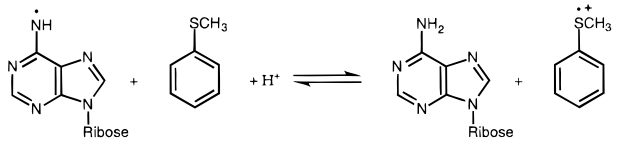
The mean of the two measured values of the reduction potential of the neutral guanosine radical at pH 7 obtained from the electron transfer equilibria in both directions, $E_7 = 1.29 \pm 0.03$ V, is used to evaluate the standard reduction potential of the guanosine radical (assuming that the activity coefficients of guanosine and corresponding radical are ~1 at pH = 0). Taking p$K_{a1} = 1.9$, p$K_{a2} = 9.25$, p$K_{a3} = 12.33$ for Guo and p$K_{r1} = 3.9$, p$K_{r2} = 10.9$ for the corresponding radicals[6,13,16] and using the following equation,[22]

$$E_{pH} = E^{0\prime} + 0.059 \log \times \frac{(K_{a1}K_{a2}K_{a3} + K_{a1}K_{a2}10^{-pH} + K_{a1}10^{-2pH} + 10^{-3pH})}{(K_{r1}K_{r2} + K_{r1}10^{-pH} + 10^{-2pH})}$$

we obtain $E^0 = 1.58$ V. This value is 0.26 V higher than the 1.33 V calculated from literature data,[15] using corrected reduction potentials of the reference compounds (E^0(Trp•,H$^+$/Trp) = 1.19 V).[23−25] The reason for this difference is most likely the incomplete equilibrium in the previous study,[15] as a consequence of the unsuitability of the redox reference used (redox potential too low) and of the relatively high dose. Such high doses (~6 Gy/pulse and higher, corresponding to more than 4 μM radicals) favor second-order radical termination processes and thus affect the measurements of the first-order rates of the equilibrium reactions and of the equilibrium concentrations of the radicals.

The value $E^0 = 1.58$ V is even higher than the recently published $E^0 = 1.49$ V[1] obtained by cyclic voltammetry of guanine in acetonitrile. Other than the solvent effect, the reason for the difference may be the irreversibility of the electrode processes in the electrochemical measurement,[1] due to subsequent rapid chemical reactions of the guanine radical cations.

The adenosine (Ado) radical[26] was found to oxidize thioanisole to its radical cation at pH 3 and 5 in a reversible manner, which indicates that the following reaction occurs:



At pH 3, $k_f = (5.0 \pm 0.5) \times 10^8$ M^{-1} s^{-1} and the equilibrium constant from the absorbances, $K_{abs} = 1870 \pm 200$, and at pH 5, $k_f = 1.9 \times 10^7$ M^{-1} s^{-1} and $K_{abs} = 90 \pm 10$ (Figure 2). The increase in K with decreasing pH reflects the involvement of H$^+$ in the electron exchange. The pH dependence of the reduction potential of the adenosine radical can be evaluated from the values determined at pH 5 and 3, $E_5 = 1.56 \pm 0.02$ V and $E_3 = 1.64 \pm 0.04$ V and p$K_{a1} = 3.3$ and p$K_{a2} = 12.5$, using the equation[27]

$$E_{pH} = E^0 + 0.059 \log(K_1K_2 + K_1 10^{-pH} + 10^{-2pH})$$

The value of $E^0 = 2.03 \pm 0.02$ V (giving more statistical weight to the more accurate $E_5 = 1.56$ V) is again considerably higher than the corrected literature value of $E^0 = 1.73$ V, the explanation being the same as for the guanosine radical.

The reduction potentials of the neutral purine radicals in neutral solution, E_7(Guo•/Guo) = 1.29 V and E_7(Ado•/Ado) = 1.42 V, are higher than the previously[1,15,16] estimated ones. Even higher are the reduction potentials of pyrimidine radicals for which we now estimate (using the reduction potential difference previously reported[15] at pH 13, $\Delta E = 0.6$ V) E_7(thymidine) ≅ 1.7 V and E_7(deoxycytidine) ≅ 1.6 V. These numbers explain the observed[6,14] positive hole transfer from an oxidized base to the guanine moiety.

The reduction potential of the guanosine radical, $E_7 = 1.29$ V, is higher than that of alkyl peroxyl radicals ($E_7 = 1.05$ V),[28] which means that (the electrophilic) lipid peroxyl radicals cannot oxidize DNA bases by one-electron transfer. On the other hand, the reduction potential of guanosine radical is considerably higher than those of aromatic and sulfur amino acids (e.g., $E_7 = 1.01$ V (tryptophan)[23−25] and E^0(RS•/RS$^-$) = 0.75 V[29]), which means that the repair of a positive hole (electron deficiency) in DNA by a histone protein is thermodynamically feasible.

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