analytical response was observed. The analytical response in all of these studies was an upfield or downfield shift of a $^1H$ NMR resonance peak; specifically, the upfield shift of the imide NH proton was observed on dilution. If a minimal change in chemical shift (<0.5 ppm) was observed through a large range of concentrations ($0.25-10^{-5}$ M) it was concluded that the self-association was negligible. For a reference study the cis-trans methyl ester derivative **8c** was concentrated to 1.25 M (268 mg/mL) and cooled to 230 K, at which temperature the chemical shift of the imide NH proton ceased to change (9.8 ppm). This value was subsequently used as the intrinsic chemical shift of the hydrogen-bonded dimer, and 7.6 ppm was used as the chemical shift of the free imide NH. By using these end points, self-association constants of Table II were calculated for these receptors (**5a-e**, **8c**).

**(b) Stoichiometry.** The complexes with adenine derivatives (**10**, **11**, **13**) were determined to be of 1:1 stoichiometry by using Job's method[3] (Figure 2). For a specific case, the Job plot of **5k** and **10** is described here in detail. Solutions of **5k** and **10** (0.01 M each) were made in separate volumetric flasks. In nine separate 5-mm-o.d. NMR tubes mixtures of each solution were added such that the stoichiometry of each component varied but the total volume was 500 μL. For example, the first tube contained only the receptor; the second tube contained 5 μL of the solution of **5k** and 495 μL of the solution of **10**. Likewise, the last tube contained only the ligand, and the tube before that contained 495 μL of the solution of **5k** and 5 μL of the solution of **10**. The $^1H$ NMR spectra were obtained for each tube, and the chemical shift of the imide NH proton was used to calculate the complex concentration. This value was plotted against the mole fraction of the **5k**. The resulting Job plot showed a maximum at 0.5 mole fraction (see Figure 2b).

**(c) Titrations.** For a specific example, the titration of **5g** with **10** will be described here. A 0.01 M solution of **5g** (4.77 mg in 1 mL of $CDCl_3$) and a 0.1 M solution of **10** (32.6 mg in 1 mL of $CDCl_3$) were prepared in separate 1-mL volumetric flasks. A 500-μL portion of the solution of **5g** was added to a 5-mm-o.d. NMR tube. An initial NMR spectrum was obtained, and the initial chemical shift of the imide NH proton was 7.78 ppm at this concentration. The guest was initially added in 10-μL portions, and the chemical shift of the imide proton was recorded at each increment. (Care was taken to recover the stock solution of guest to prevent evaporation, which causes significant deviations in the resulting Eadie plots.) After 60 μL (~1 equiv) of guest had been added, the aliquot size was increased to 20 μL. After 200 μL had been added the aliquot size was increased to 50 μL until 500 μL was added, then 100-μL aliquots were added until 1000 μL of guest had been added, and finally 250-μL portions were added until 1750 μL (35 equiv) of the guest had been added. The chemical shift of the imide NH proton at this concentration of guest was 12.8 ppm. The experiment was repeated three additional times to give $K_a = 125 \pm 8$ $M^{-1}$. Typically, 20-30 equiv of guest was needed for the chemical shift of the imide NH proton to reach saturation. The value 13.2 ppm was experimentally determined to be the maximal chemical shift by cooling a 2:1 solution of **10** and the chelating molecule **5h** below the coalescence temperature (~210 K), at which temperature the chemical shift of the imide NH proton was 13.2 ppm. This value was used in all subsequent titrations, especially those for which it was not experimentally possible to reach the limiting chemical shift of the imide NH proton.

**(d) Thermodynamic Studies.** An equimolar solution of **5k** and **10** were made up in a volumetric flask by using 6.30 mg of **5k** and 1.63 mg of **10** in 2 mL of $CDCl_3$. A 500-μL portion of this solution was added to a 5-mm-o.d. NMR tube and cooled to 273 K. The chemical shift of the imide NH proton was recorded at this temperature and at every 5 K temperature increment as the sample was warmed to 323 K (equilibrium time at each temperature was 5 min.). The van't Hoff plots of the $K_a$'s calculated at each temperature gave $-\Delta H$ and $-\Delta S$. In order to ensure that a reasonable range of the saturation plot was covered, the initial solution concentration was adjusted such that, over the range of temperatures studied, $0.2 \leq$ fraction saturation $\leq 0.8$ was maintained.

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**Registry No. 4c**, 109216-51-5; **4d**, 117873-87-7; **5a**, 109216-52-6; **5b**, 109216-53-7; **5c**, 109216-54-8; **5d**, 109216-55-9; **5e**, 117873-88-8; **5f**, 111689-17-9; **5g**, 111635-64-4; **5h**, 117873-89-9; **5l**, 117873-90-2; **5j**, 117873-91-3; **5k**, 117873-92-4; **5l**, 117895-86-0; **5m**, 117873-93-5; **8c**, 117873-94-6; **9a**, 117873-95-7; **9b**, 117873-96-8; **9c**, 117873-97-9; **10**, 2715-68-6; **11**, 15888-38-7; **13**, 21031-78-7; $CDCl_3$, 865-49-6; $CD_3CN$, 2206-26-0; $CD_3OD$, 811-98-3.

# Structure and Acid-Base Properties of One-Electron-Oxidized Deoxyguanosine, Guanosine, and 1-Methylguanosine

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**Abstract:** Deoxyguanosine, guanosine, and 1-methylguanosine react in aqueous solution with $SO_4^{\bullet-}$ with nearly diffusion-controlled rates and with $Br_2^{\bullet-}$ with rate constants close to $\approx 5 \times 10^7$ $M^{-1}$ $s^{-1}$. The resulting radical cations have $pK_a$ values of 3.9 and 4.7 for the nonmethylated and methylated systems, respectively, so that at pH 7 the products of one-electron oxidation are neutral radicals, formed by deprotonation from N(1) in the case of guanosine and deoxyguanosine and from the exocyclic $N^2$ in the case of 1-methylguanosine. The radicals of deoxyguanosine and guanosine, but not that from 1-methylguanosine, further deprotonate to give radical anions with $pK_a$ values of 10.8 and 10.7, respectively. An implication of these results to the radiation chemistry of DNA is that the radical cation formed upon ionization of a guanine moiety shifts a proton (and thereby a positive charge) to its complementary base cytosine, i.e., that separation of charge from spin occurs by proton transfer.

Guanine has been known for a long time to be the most easily oxidized of the nucleic acid bases.[2] This property is in line with (gas-phase) ionization potential[3] as well as aqueous solution redox potential data,[4] and it is in accord with the results of MO calculations.[5] In agreement with this concept, the guanine moiety appears to be the ultimate trap of oxidative damage to DNA, as concluded from ESR data on "dry" DNA, irradiated frozen solutions,[6] and oriented DNA fibers.[7] These data have so far been

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**Table I.** Rate of Reaction of Sulfate Radical $k(SO_4^{\bullet-})$ and Dibromine Radical $k(Br_2^{\bullet-})$ with Guanosine and Derivatives (pH 7 and Room Temperature)

| | $k(SO_4^{\bullet-})$,[a] $10^9$ $M^{-1}$ $s^{-1}$ | $k(Br_2^{\bullet-})$,[b] $10^7$ $M^{-1}$ $s^{-1}$ |
|---|---|---|
| guanosine | 4.1 | 3.9 |
| guanosine-$H^+$ | 3.2[c] | |
| deoxyguanosine | 4.1 | 4.6 |
| 1-methylguanosine | 4.6 | 5.6 |

[a] From 248-nm photolysis of 0.1 M aqueous solutions of $S_2O_8^{2-}$ in the presence of 0.1–1 mM of the substrates, monitoring the decay of $SO_4^{\bullet-}$ at 450 nm and the formation of radicals at 310–350 nm and with 1-methylguanosine at 630 nm. [b] From pulse radiolysis of $N_2O$-saturated aqueous solutions containing 0.1 M $Br^-$ and 0.25–1.5 mM of the substrates, monitoring the decay of $Br_2^{\bullet-}$ at 360 nm. [c] Determined at pH 0.

interpreted in terms of formation of a radical cation, following an early suggestion based on pulse radiolysis results.[8] The radical cation has been proposed to be able to initiate strand breaks.[9] However, there is evidence from recent single-crystal ESR work[10,11] and less recent pulse radiolysis data[12] that the guanine radical is not a cationic but a neutral species. In view of the widely accepted importance of the guanine radical in the radiation chemistry of DNA, it was considered necessary to establish its nature unambiguously. This is most easily done in aqueous solution by studying the oxidation of deoxyguanosine and its derivatives by one-electron oxidants such as sulfate and dibromine radical anions by use of pulse radiolysis with both optical and conductometric detection. From the results it is evident that in neutral aqueous solution the one-electron-oxidized guanosine or deoxyguanosine systems are neutral, i.e., uncharged radicals. These radicals are, however, relatively strong Brønsted bases, such that protonation to yield radical cations takes place at relatively low (≈0.1 mM) $H^+$ concentrations.

## Results

**a. $SO_4^{\bullet-}$ as an Oxidant.** The sulfate radical anion $SO_4^{\bullet-}$ was produced by reacting peroxydisulfate with hydrated electrons formed on irradiation of aqueous solutions containing *tert*-butyl alcohol as OH radical scavenger:

$$e_{aq}^- + S_2O_8^{2-} \rightarrow SO_4^{2-} + SO_4^{\bullet-} \quad (1)$$

$SO_4^{\bullet-}$ reacts with the nucleosides studied with rate constants not far from the diffusion-controlled limit (see Table I)[13] to give transients that absorb in the range ≈300 to >700 nm. The spectra are pH dependent. In Figure 1 are presented the spectra recorded after completion of the reaction of $SO_4^{\bullet-}$ with deoxyguanosine at pH 3, 7, and 12. At pH 3 and 7, small differences between the spectra are visible, particularly at $\lambda \geq 610$ nm. The dependence of optical density (OD) on pH, determined at 700 nm (see inset a of Figure 1), is a sigmoidal curve with an inflection point at pH 3.9. In basic medium, a more pronounced change in the spectrum occurs. The OD changes also follow a p$K$ profile with an inflection point at pH 10.8, determined by monitoring the optical density at 720 nm (see inset b of Figure 1) or at 375 nm (not shown).

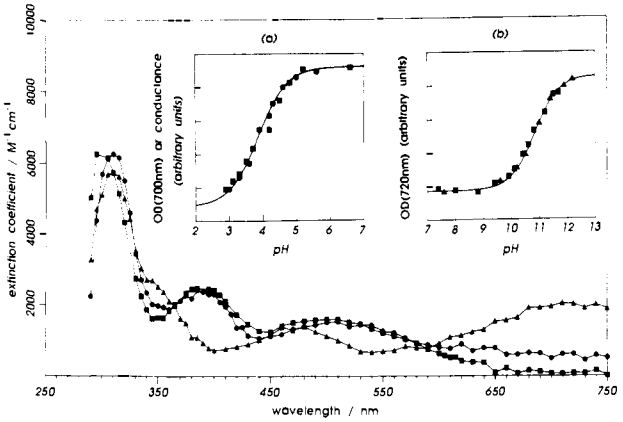


**Figure 1.** Absorption spectra recorded after reaction of $SO_4^{\bullet-}$ with 0.1 mM deoxyguanosine ($[S_2O_8^{2-}]$ = 10 mM; [*tert*-butyl alcohol] = 50 mM) at pH 3.1 (squares) and 6.6 (circles) 10 μs after the pulse and with 1 mM deoxyguanosine ($[S_2O_8^{2-}]$ = 20 mM; [*tert*-butyl alcohol] = 400 mM) at pH 11.7 (triangles) 5 μs after the pulse. Inset a: pH dependence of conductance change (circles) and optical density at 700 nm (squares) after reaction of deoxyguanosine with $SO_4^{\bullet-}$. Inset b: pH dependence of optical density at 720 nm after reaction of deoxyguanosine with $SO_4^{\bullet-}$ (squares) and $Br_2^{\bullet-}$ (triangles).

The same system was studied by AC–conductometric detection in order to identify the protonation states of the radicals. In the acid range a conductance increase was observed that follows the same pH dependence as the optical density (see inset a of Figure 1). The lower plateau corresponds, after correction for the buffering effect[14] of deoxyguanosine (p$K_a$ = 2.4), to conversion of $SO_4^{\bullet-}$ into sulfate dianion and an organic cation:

$$SO_4^{\bullet-} + G \rightarrow SO_4^{2-} + G^{\bullet+} \quad (2)$$

The upper plateau results from the further replacement of the cation by the more conducting proton, thus indicating that reaction 2 is followed by deprotonation. The p$K_a$ of $G^{\bullet+}$, the radical cation of deoxyguanosine, is 3.9, as determined by both optical and conductance detection (see inset a of Figure 1):

$$G^{\bullet+} \rightleftarrows G(-H)^{\bullet} + H^+ \quad (3)$$

This means that one-electron oxidized deoxyguanosine exists as a neutral radical at pH 7.

**b. $Br_2^{\bullet-}$ as an Oxidant.** As a second oxidant, the dibromine radical anion $Br_2^{\bullet-}$ was produced by irradiating $N_2O$-saturated aqueous solutions containing 0.1 M potassium bromide:

$$OH^{\bullet} + 2Br^- \rightarrow OH^- + Br_2^{\bullet-} \quad (4)$$

$Br_2^{\bullet-}$ reacts with guanosine and derivatives with rate constants close to $\approx 5 \times 10^7$ $M^{-1}$ $s^{-1}$ (see Table I).[15] The absorption spectra and inflection points in the OD vs pH dependencies obtained on reaction with deoxyguanosine were found to be the same as those obtained with sulfate radical (for the high-pH p$K$ curve, see inset b of Figure 1). This demonstrates that the two oxidants lead to the same radical(s). Conductance experiments on guanosine showed the production of $H^+$, if performed at pH 5–6, whereas at pH ≈ 3 the conductance change corresponded to formation of an organic cation. The optical and conductometric results are

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(13) A similar value ($2.3 \times 10^9$ $M^{-1}$ $s^{-1}$) for the rate of reaction of deoxyguanosine with $SO_4^{\bullet-}$ is given in ref 23.

(14) The correction for the buffering effect was made by measuring the $OH^{\bullet}$-induced yields of conductance in (a) an $N_2O$-saturated aqueous solution of 10 mM dimethyl sulfoxide and (b) then adding the nucleoside in the same concentration (0.1 mM) as in the real experiment. The concentration of dimethyl sulfoxide being such that in both conditions it reacts with all the OH radicals, the difference in the conductance is due only to buffering. The observed conductance yield in (b) was ≈90% of that in (a); the corresponding factor (1.11) was used to correct the value measured in the experiment with $SO_4^{\bullet-}$.

(15) A similar value ($4 \times 10^7$ $M^{-1}$ $s^{-1}$) for reaction with 2′-deoxyguanosine 5′-monophosphate is reported in ref 8.

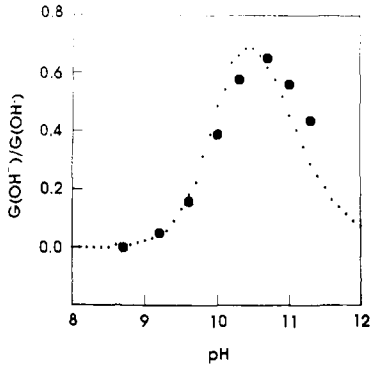


**Figure 2.** pH dependence of the yield of $OH^-$ produced by the indirect reaction of $OH^•$ with deoxyguanosine (see text); dotted line: calculated curve considering $pK_a(G) = 9.4$ and $pK_a[G(-H)^•] = 10.8$; ([deoxyguanosine] = 0.5 mM; $[Br^-] = 0.1$ M).

explained in terms of one-electron oxidation of deoxyguanosine to yield, at pH ≤ 3.9, the radical cation, eq 5, followed by its deprotonation, eq 3.

$$Br_2^{•-} + G \rightarrow 2Br^- + G^{•+} \quad (5)$$

With respect to the optical properties, guanosine behaves in a way analogous to deoxyguanosine. When reacted with $Br_2^{•-}$ or $SO_4^{•-}$, nearly identical pH-dependent absorption spectra were measured and the OD vs pH profiles were found to have inflection points at pH 3.9 and 10.7, the first value identical with and the second one very similar to the one in the case of the *deoxy*-nucleoside.

Using $Br_2^{•-}$, conductance experiments were performed on guanosine and deoxyguanosine also in the pH range 8–12, where the parent molecules undergo deprotonation [$pK_a$(deoxy)guanosine = (9.4) 9.25]. The pH dependence of the conductance change, using 1-methylguanosine for dosimetry,[16] is shown in Figure 2. At pH 8 the conductance change is essentially zero, which is due to the occurrence of reaction 4 followed by 5 and 3, which, if combined, yield eq 6, which demonstrates that $Br^-$ functions as

$$OH^• + G \rightarrow G(-H)^• + H_2O \quad (6)$$

an electron carrier between G and $OH^•$. At pH ≈ 9 an $OH^•$-induced conductance *in*crease, which reaches a maximum at pH ≈ 10.5, was observed (see Figure 2). This increase is due to formation of $OH^-$ resulting from the (indirect) reaction of $OH^•$ with the deoxyguanosine *anion*, $G(-H)^-$ (cf. eq 7):

$$OH^• + G(-H)^- \xrightarrow{[Br^-]} OH^- + G(-H)^• \quad (7)$$

At pH > 10.5 the conductance change was found to become smaller again, and this shows that the neutral radical $G(-H)^•$ produced in eq 7 undergoes deprotonation to yield the radical anion $G(-2H)^{•-}$:

$$G(-H)^• \rightleftarrows G(-2H)^{•-} + H^+ \quad (8)$$

The $pK_a$ of $G(-H)^•$ is (10.8) 10.7 for (deoxy)guanosine, as deduced from the optical experiments (see Figure 1b). In Figure 2, the experimentally observed conductance change is presented as a function of pH and this dependence is compared with that calculated for deoxyguanosine on the basis of eq 6–8, the neutralization of $H^+$ and $OH^-$, and $pK_a(G) = 9.4$ and $pK_a[G(-H)^•] = 10.8$. The agreement between experiment and expectation is considered very satisfactory in view of the difficult measurements at high ionic strength and pH under conditions of buffering by the parent compound.

**c. 1-Methylguanosine.** A third nucleoside, 1-methylguanosine, was studied. The absorption spectra of the transients obtained on reaction with $Br_2^{•-}$ at different pH values are presented in Figure 3. At low pH, the absorption spectrum is very similar to the ones of the nonmethylated nucleosides, but the spectrum recorded in neutral and basic solution is quite different; in particular, there is a broad absorption band centered at ≈630 nm that is absent in the case of the nonmethylated systems. The inflection point in the optical density vs pH dependence is 4.7, as determined by monitoring the optical density at 630 nm (see inset of Figure 3) or at 295 and 400 nm (not shown). No further spectral changes were observed up to pH 13.

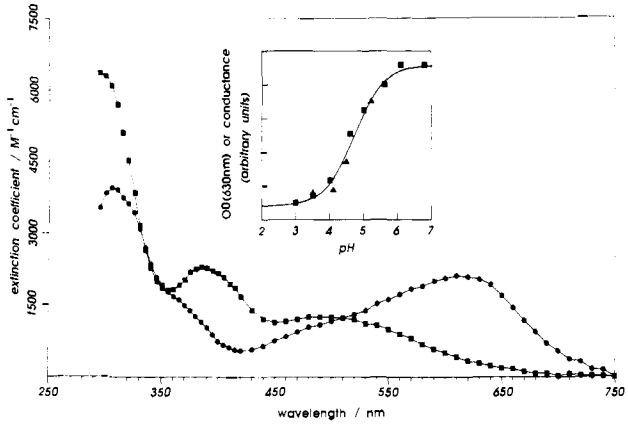


**Figure 3.** Absorption spectra recorded after reaction of $Br_2^{•-}$ with 1 mM 1-methylguanosine at pH 3.0 (squares) and 7.3 (circles) 100 μs after the pulse. Inset: pH dependence of optical density at 630 nm after reaction of 1-methylguanosine with $Br_2^{•-}$ (squares) and of conductance change after reaction with $SO_4^{•-}$ (triangles).

The reaction of this nucleoside with $SO_4^{•-}$ was studied by conductometric detection. The same $pK_a$ value (4.7) as that observed with optical detection was found (see inset of Figure 3). At pH 3–4, the conductance change indicates the formation of an organic cation; at pH ≈ 6 production of $H^+$ was seen. In basic medium, a conductance decrease was observed, with an amplitude that corresponds to the conversion of $SO_4^{•-}$ into $SO_4^{2-}$ and the depletion of one $OH^-$ per $SO_4^{•-}$ reacted, due to neutralization of the proton formed in eq 3. The conductance signal remained unchanged up to pH 11.5.[17]

The results thus show that the reaction of $SO_4^{•-}$ with 1-methylguanosine at pH 6–11.5 leads to the production of 1 equiv of $H^+$, which means that the radical is a neutral species in this pH range.

## Discussion

The results presented are summarized in Scheme I. At neutral pH, reaction of guanosine and derivatives with $SO_4^{•-}$ or $Br_2^{•-}$ is accompanied, or followed, by fast deprotonation, i.e., the resulting radicals are uncharged. This is in agreement with earlier conclusions of O'Neill[18] which were based on kinetic salt effect studies. In the acid range, the conductance signals show a pH dependence, which reflects the existence of a protonation equilibrium, so that at pH < 3.9 [in the case of (deoxy)guanosine] and at pH < 4.7 (in the case of 1-methylguanosine) the prevailing species are the radical cations.

The tendency of the one-electron-oxidized guanine system to undergo deprotonation at pH 7 is also exhibited by 9-methylguanine [oxidized by Tl(II)].[19]

Inspection of the absorption spectra of the radical cations of (deoxy)guanosine and 1-methylguanosine shows that these are very similar, which indicates that the π systems are essentially the same, independent of whether N(1) is substituted by H or by $CH_3$. In contrast, the spectra of the neutral radicals, formed by

(16) 1-Methylguanosine, which does not undergo deprotonation below pH 12 but whose radical cation does ($pK_a = 4.7$, see text), has the same reactivity with $Br_2^{•-}$ as (deoxy)guanosine (see Table I). At pH > 6 it leads to the pH-independent production of 1 equiv of $H^+$ against which the (deoxy)guanosine system was calibrated.

(17) At pH > 11.5 the competing reaction of $SO_4^{•-}$ with $OH^-$ becomes noticeable.

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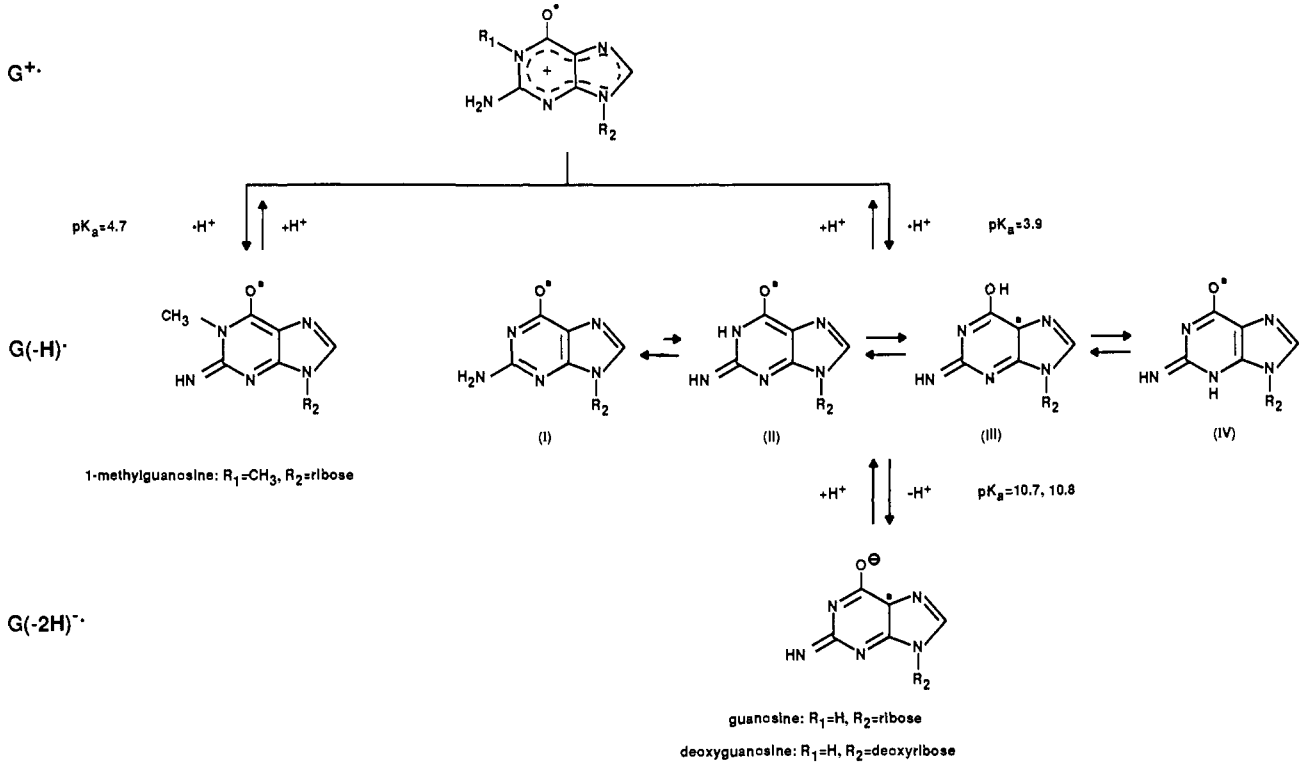
**Scheme I**




deprotonation, are very different (see Figures 1 and 3). The radical cation of 1-methylguanosine can only deprotonate from (the exocyclic) $N^2$, to yield an imino-substituted radical (see Scheme I). The $pK_a$ for this process is 4.7, almost 1 unit higher than that (3.9) for (deoxy)guanosine. The difference between the absorption spectra of the methylated and the nonmethylated neutral radicals means that with the latter deprotonation takes place from a different site, evidently from N(1), to give the "$O^6$-centered"[20] radical I (see Scheme I). This structure should be favored over the tautomeric imino-substituted ones (II, III, and IV) because it has the highest degree of aromaticity. Furthermore, on the basis of this structure (a) the oxidizing properties[18] of the radical and (b) the relatively small $\Delta pK_a$ between the parent molecule [$pK_a$ (deoxy)guanosine = (9.4) 9.25] and the radical cation ($pK_a$ = 3.9, vide supra) are easily understandable. The $\Delta pK_a$, which reflects the increase in acidity on one-electron oxidation, corresponds to only $\approx$5.5 orders of magnitude as compared with the 12 orders of magnitude observed for, e.g., the "classical" phenol ($pK_a$ = 10.0)/phenol radical cation ($pK_a = -2$)[21] system. The smaller $\Delta pK_a$ for the (deoxy)guanosine case may be taken as evidence for an extensive reorganization of the $\pi$-electronic systems in going from the amido-type structure of the parent compound to the oxyl-substituted pyrimidine structure of the radical. The $\Delta pK_a$ of 5.5 for the deoxyguanosine system corresponds to a differential free energy of deprotonation of the radical cation as compared to the parent of 7.5 kcal·mol$^{-1}$ at 298 K.

The deprotonation reaction of the radical cations of guanine nucleosides to give a neutral radical is in line with the results of recent ESR experiments on $\gamma$-irradiated single crystals of 2′-deoxyguanosine 5′-phosphate.[10,11] There is agreement that the radical cation deprotonates between 10 and 70 K; however, different conclusions have been reached with respect to the site of deprotonation: Rakvin et al.[10] favor N(1); Close et al.[11] prefer the exocyclic $NH_2$ group as the proton donor, in which case the imino-substituted oxyl radical II would be formed. In aqueous solution, the various tautomeric forms I–IV (plus other possible but unlikely forms) are in principle able to coexist. However, as pointed out above, tautomer I is probably the dominant one.

At high pH the (neutral) (deoxy)guanosine radical undergoes deprotonation from $N^2$ to yield an imino-substituted radical anion (eq 8). This type of reaction is not possible in the case of 1-methylguanosine, whose neutral radical is already an imino-substituted radical. This system therefore has only one $pK$ (=4.7) compared to the two $pK$ equilibria in the (deoxy)guanosine case, and this is further evidence in support of radical anion formation in the (deoxy)guanosine case.

The conclusions presented here can be extended and applied to the reaction of the OH• radical with guanosine and derivatives. This reaction leads to an "oxidizing guanosine radical" with a yield of $\approx$50%,[22] and it has been unambiguously demonstrated that this radical is the same as that formed by reaction with $Br_2^{\bullet-}$[18] or $SO_4^{\bullet-}$.[23] The identity was established on the basis of chemical reactivity, in particular with respect to reductants, and the loss of oxidative power at high pH.[18] This decrease in oxidizing strength (with an inflection point at $\approx$10.9)[18] can now be attributed to the formation of the radical anion, which is expected to be a much weaker oxidant than its neutral conjugate acid (see Scheme I). The identity of the (deoxy)guanosine radical from the reaction with OH• with that from $Br_2^{\bullet-}$ and $SO_4^{\bullet-}$ can also be demonstrated via the acid–base equilibria of the radicals: Scholes et al.[12] showed that the OH•-generated guanosine radical cation proposed by Willson et al.[8] has a $pK_a$ of 3.9, i.e., is not a radical cation at neutral pH. That this $pK_a$ is identical with that found with $Br_2^{\bullet-}$ or $SO_4^{\bullet-}$ as oxidants makes it likely that all three oxidants produce the same radical. The upper $pK_a$ (10.8) has also been observed: O'Neill reports[22] a value of 10.9 for the radical from the reaction of OH• with deoxyguanosine. Our results thus confirm formation of the one-electron-oxidized (deoxy)guanosine systems by the OH• radical. This statement implies that the "reducing radical(s)"[22] do not contribute to the OD changes at 720 nm (at pH 10–12) or to the conductance change (at pH 3–5). Another consequence is that in order for OH• to give a one-electron-oxidized guanosine, elimination of $OH^-$ (or of $H_2O$) from the primarily formed OH adduct must occur. This type of mechanism is in agreement with

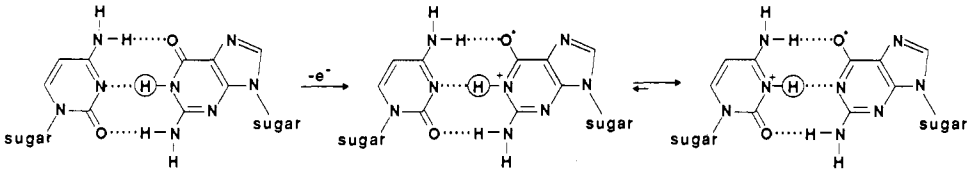
(20) This radical has, however, considerable unpaired electron density at the endo- and exocyclic nitrogens (cf. ref 10 and 11); we thank W. A. Bernhard for pointing this out.

(21) Dixon, W. T.; Murphy, D. *J. Chem. Soc., Faraday Trans. 2* **1976**, *72*, 1221.

(22) O'Neill, P. *Radiat. Res.* **1983**, *96*, 198.

(23) O'Neill, P.; Davies, S. E. *Int. J. Radiat. Biol.* **1987**, *52*, 577.

Scheme II



the known reactivity of OH•, which usually does *not* react by outer-sphere electron transfer but by addition/elimination.[24]

It has been suggested that the neutral radical existing in neutral solution may be the OH adduct to the 4-position formed from the (deoxy)guanosine radical cation by hydration[25] (followed by deprotonation of the adduct) or the equivalent reaction with $OH^-$.[18,23] However, we consider this hypothesis very unlikely. With adenines and hypoxanthine, the opposite to hydration, namely *de*hydration was observed.[26] On the basis of the lower ionization potentials[3] (IP) and one-electron redox potentials[4] ($E^2$) of guanines as compared to adenines, dehydration (of the OH adducts) should be even more favored and hydration (of the radical cations) even less in the case of the guanines, since the ease of dehydration of an OH adduct *in*creases with *in*creasing electron density of the system.[24,27] Conversely, the activity of an aromatic radical cation with respect to electrophilic reaction with water to yield hydroxycyclohexadienyl-type radicals (OH adducts) *in*creases with *de*creasing electron density. An example of this relation is benzene (IP = 9.24 eV) and anisole (IP = 8.20 eV): the radical cation of benzene hydrates even in strongly acidic aqueous solution completely and irreversibly in ≤20 ns,[24] whereas the OH adduct of anisole is irreversibly *de*hydrated in acid solution to give the radical cation, which has a lifetime with respect to hydration of ≥10 ms.[28,29]

## Summary and Conclusions

Deoxyguanosine, guanosine, and 1-methylguanosine react with one-electron oxidants to give radicals that are uncharged in neutral solution, identified as the products of electron loss followed by (or accompanied by) deprotonation with a p$K$ of 3.9 [presumably from position N(1)] in the case of guanosine and deoxyguanosine) and 4.7 (from position $N^2$) in the case of 1-methylguanosine. Further deprotonation to give radical anions takes place with a p$K$ of 10.8 and 10.7 for deoxyguanosine and guanosine radicals, respectively, but does not occur for the radical of 1-methylguanosine.

The acid–base properties of the one-electron-oxidized guanine moiety as determined in aqueous solution do not allow a simple decision to be made regarding the protonation state of that unit in DNA. Due to the *pairwise* existence of the bases in DNA, changes in the proton affinity of one base, due to ionization (electron loss), or, conversely, to electron pickup, will affect the *complementary base* as the "natural" proton acceptor (or donor in the case of electron capture), whereas in aqueous solution the proton is transferred from the solvated acid to a (solvated) water molecule to yield the (solvated) hydronium ion. It is, however, reasonable to assume that in the case of an oxidized guanine moiety (p$K_a$ = 3.9) in DNA, the equilibrium positions of one or all of the three protons involved in the hydrogen bonds with the cytosine moiety (p$K_a$ = 4.45) will be shifted toward the cytosine, resulting in an at least partial transfer of positive charge to that base, as shown in Scheme II.[30] This proton transfer leads to a separation of charge from spin. In so far as the reaction is in principle reversible, the question of the protonation state of one-electron-oxidized guanine in DNA may be a matter of degree rather than of kind.

It is likely that the proton is finally picked up by a water molecule; if this happens, the base pair will be held together by only two hydrogen bonds. The resulting increased flexibility in the DNA chain[31] could enable the radical to abstract a hydrogen atom from a deoxyribose unit above or below its own plane.[32] Such a mechanism could in principle explain the observation[9] that one-electron-oxidized guanine in DNA may induce strand breaks.[33] One-electron-oxidized guanine is expected to have the ability to abstract H atoms, due to the oxyl-type structure of the neutral or of the cation radical.

## Experimental Section

The nucleosides were obtained from Sigma and were used as received. The solutions were prepared with water purified with a Millipore Milli-Q system. In experiments with sulfate radical, they contained 0.1–1 mM nucleoside, 10–20 mM potassium peroxydisulfate (Merck), and 50–500 mM *tert*-butyl alcohol (Merck) and were saturated with Ar. $G(SO_4^{\bullet-})$ = 3.15[34] was assumed. In experiments with $Br_2^{\bullet-}$, the solutions contained 0.1 M potassium bromide (Merck) and 0.5–1 mM nucleoside and were saturated with $N_2O$. $G(Br_2^{\bullet-})$ = 6.0 was assumed. The pH was adjusted with NaOH or $HClO_4$, and when close to neutrality, the solutions were buffered with ≈1 mM phosphate. For the optical experiments, dosimetry was performed with $N_2O$-saturated 10 mM KSCN aqueous solutions for which $G[(SCN)_2^{\bullet-}]$ = 6.0 and $\epsilon[(SCN)_2^{\bullet-}]$ = 7600 $M^{-1}$ $cm^{-1}$ at 480 nm.[35] For the conductance experiments, dosimetry was performed by using Ar-saturated aqueous solutions containing 10–20 mM $K_2S_2O_8$ and 0.1 M methanol[36] or 10 mM $K_2S_2O_8$, 0.4 mM anisole,[37] and 50 mM *tert*-butyl alcohol. The measurements were performed at room temperature (20 ± 1 °C). The computer-controlled 3-MeV pulse radiolysis apparatus is described elsewhere.[38] Pulses of 0.4 μs were used that supplied doses such that 1–2 μM radicals were produced.

**Acknowledgment.** L.P.C. thanks the Max-Planck-Gesellschaft for a stipend.

(24) Steenken, S. *J. Chem. Soc., Faraday Trans. 1* **1987**, *83*, 113. Steenken, S. In *Free Radicals: Chemistry, Pathology and Medicine*; Rice-Evans, C., Dormandy, T., Eds.; Richelieu: London, 1988; p 51.

(25) We use the terms hydration (rather than hydroxylation) and dehydration (rather than dehydroxylation) to emphasize that the essential feature of this reaction is the nucleophilic attack of a water molecule to a radical cation or the reverse, the elimination of $H_2O$ or $OH^-$ from the OH adduct.

(26) Vieira, A. J. S. C.; Steenken, S. *J. Phys. Chem.* **1987**, *91*, 4138.

(27) Holcman, J.; Sehested, K. *Nukleonika* **1979**, *24*, 887.

(28) O'Neill, P.; Steenken, S.; Schulte-Frohlinde, D. *J. Phys. Chem.* **1975**, *79*, 2773.

(29) Holcman, J.; Sehested, K. *J. Chem. Phys.* **1976**, *80*, 1642.

(30) (Gas-phase) proton affinities rather than (aqueous-phase) p$K_a$ values are possibly more appropriate to characterize the equilibrium position of the proton, since the species involved are not hydrated in DNA; such data are not available for the base radicals.

(31) Lifetimes of undamaged base pairs with respect to unpairing have recently been determined to be of the order of milliseconds to minutes, depending on type and location in the DNA chain; see: Leroy, J. L.; Kochoyan, M.; Huyn-Duih; Gueron, M. J. *Mol. Biol.* **1988**, *200*, 223. Gueron, M.; Kochoyan, M.; Leroy, J. L. *Nature* **1987**, *328*, 89. Kochoyan, M.; Leroy, J. L.; Gueron, M. *Mol. Biol.* **1987**, *196*, 599.

(32) This mechanism is implied by the results of: Lemaire, D. G. E.; Bothe, E.; Schulte-Frohlinde, D. *Int. J. Radiat. Biol.* **1984**, *45*, 351. Deeble, D. J.; von Sonntag, C. *Ibid.* **1984**, *46*, 247.

(33) Strand breaks have been suggested to occur via the C-4′ mechanism, cf.: Schulte-Frohlinde, D. In *Radioprotectors and Anticarcinogens*; Nygaard, O. F., Simic, M. G., Eds.; Academic Press: New York, 1983.

(34) This $G$ value takes into account the reaction of $S_2O_8^{2-}$ with the electrons in the spurs: Balkas, T. I.; Fendler, J. H.; Schuler, R. H. *J. Phys. Chem.* **1970**, *74*, 4497.

(35) Schuler, R. H.; Hartzell, A. L.; Behar, B. *J. Phys. Chem.* **1981**, *85*, 192.

(36) This produces a neutral radical in 100% yield: Bansal, K. M.; Fessenden, R. W. *Radiat. Res.* **1978**, *75*, 497.

(37) This produces a radical cation in 100% yield (cf. ref 28).

(38) Jagannadham, V.; Steenken, S. *J. Am. Chem. Soc.* **1984**, *106*, 6542.

**Note Added in Proof.** Since Tl(II) has been suggested to one-electron oxidize 9-methylguanine,[19] this reagent was used (as a third oxidant) with guanosine, and the reaction was monitored with conductance in the pH range 3-6. At pH 3 (where the oxidizing species is $Tl^{2+}$),[39] the reaction led to removal of 1 equiv of $H^+$, whereas at pH 6 (where the oxidizing species is $TlOH^+$),[39] the net change of $[H^+]$ was zero.[40] The inflection point of the conductance vs pH plot was 3.9, in perfect agreement with the $pK_a$ value of $G^{\bullet+}$ as determined by using $Br_2^{\bullet-}$ or $SO_4^{\bullet-}$ as oxidants. The observations are described by, at pH 6,

$$OH^\bullet + Tl^+ \rightarrow TlOH^+$$

$$TlOH^+ + G \rightarrow Tl^+ + H_2O + G(-H)^\bullet$$

at pH 3,

$$OH^\bullet + Tl^+ + H^+ \rightarrow Tl^{2+} + H_2O$$

$$Tl^{2+} + G \rightarrow Tl^+ + G^{\bullet+}$$

and eq 3.

(39) O'Neill, P.; Schulte-Frohlinde, D. *J. Chem. Soc., Chem. Commun.* **1975**, 387.

(40) The reaction of $Tl^{2+}$ with anisole (≡100%)[28] was used for calibration. On this basis, Tl(II) reacts with guanosine quantitatively by electron transfer.

**Registry No.** $SO_4^{\bullet-}$, 12143-45-2; $Br_2^{\bullet-}$, 12595-70-9; deoxyguanosine, 961-07-9; guanosine, 118-00-3; 1-methylguanosine, 2140-65-0.

# Chemically Induced Release of Charge from a Rectifying Polymer Based on Viologen and Quinone Subunits

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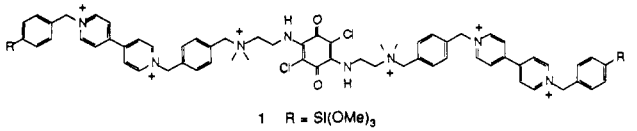
**Abstract:** Charge associated with the reduction of quinone, $2e^- + 2H^+ + Q \rightarrow QH_2$, can be trapped at low pH in the electrode-confined siloxane polymer, $(BV\text{-}Q\text{-}BV^{6+})_n$, which is derived from a monomer that consists of a benzoquinone unit flanked by two benzyl viologen units. $2ne^-$'s can be released from the polymer by raising the solution pH to neutral or basic pH where the viologen can reoxidize the $QH_2$ to Q, ultimately delivering the charge to the electrode. Chemical redox reagents, added to the polymer film, can also be used to release the charge and deliver it to the electrode. The use of $I_3^-/I^-$ and $Fe(CN)_6^{3-/4-}$ as charge-release mediators is demonstrated. Oxidation of $QH_2$ centers occurs when the potential of an electrode modified with $(BV\text{-}Q\text{-}BV^{6+})_n$ is brought close to $E^{\circ\prime}(I_3^-/I^-)$ or $E^{\circ\prime}(Fe(CN)_6^{3-/4-})$. Because $Fe(CN)_6^{3-/4-}$ is concentrated by the polycationic $(BV\text{-}Q\text{-}BV^{6+})_n$ polymer, only very small solution concentrations, approximately 1 $\mu$M, are required to effectively mediate the oxidation of $QH_2$.

In this article we describe chemical mechanisms that can be used to release charge trapped in an electrode-confined, rectifying, redox polymer. In particular, we show that a variation in pH from low to high results in delivery of charge to the electrode. In addition, we show that certain solution redox additives can serve as mediators to deliver charge to the electrode. The significance of this work is 2-fold: (1) we demonstrate a system where a pH change brings about a thermodynamically allowed electrochemical process converting the chemical change to an electric current, and (2) we demonstrate that the rectifying properties of a polymer film can be rationally altered by judicious choice of charge-release agents added to the rectifying film.

This work continues the characterization of the electrode-confined polymer, $(BV\text{-}Q\text{-}BV^{6+})_n$, derived from base hydrolysis of the pendant trimethoxysilyl units on **1**.[1] Electrode surfaces



**1** R = Si(OMe)$_3$

modified with $(BV\text{-}Q\text{-}BV^{6+})_n$ undergo a $4ne^-/2nH^+$ reduction between pH 9 and 1 upon moving the potential from +0.2 to −0.7 V vs SCE. This corresponds to $2n$ 1e reductions of the viologen units, $BV^{2+}$, to the radical cationic form, $BV^+$, and $n$ $2e^-/2H^+$ reductions of the quinone centers, Q, to the hydroquinone form, $QH_2$ (eq 1). The interesting observation is that while the reduction is completely reversible above pH 6, only the $2ne^-$ in the viologen system can be removed electrochemically below pH 6, even when the electrode is held substantially positive of the $Q/QH_2$

$$(BV\text{-}Q\text{-}BV^{6+})_n \underset{\text{pH 6-9, } -4ne^-, -2nH^+}{\overset{+0.2 \rightarrow -0.7 \text{ V vs SCE}, \text{ pH 1-9, } +4ne^-, +2nH^+}{\rightleftharpoons}} (BV\text{-}QH_2\text{-}BV^{4+})_n \quad (1)$$

$$-0.7 \rightarrow +0.2 \text{ V vs SCE}$$

$$\text{pH 1-5, } -2ne^- \quad -0.7 \rightleftharpoons +0.2 \text{ V}$$

$$(BV\text{-}QH_2\text{-}BV^{6+})_n$$

(1) (a) Smith, D. K.; Lane, G. A.; Wrighton, M. S. *J. Am. Chem. Soc.* **1986**, *108*, 3522. (b) Smith, D. K.; Lane, G. A.; Wrighton, M. S. *J. Phys. Chem.* **1988**, *92*, 2616.

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