Repair of Oxidative Guanine Damage in Plasmid DNA by Indoles Involves Proton Transfer between Complementary Bases†

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ABSTRACT: We have used the single electron oxidizing agent $(SCN)_2^{\bullet-}$ (generated by γ-irradiation of aqueous thiocyanate) to produce guanyl radicals in plasmid DNA. The stable product(s) formed from these radicals can be detected after conversion with a base excision repair endonuclease to single strand breaks. The yield of enzyme-induced breaks is decreased by the presence during irradiation of indole compounds. Rate constants for the reduction of DNA guanyl radicals by these indoles can be calculated from the concentration dependence of the attenuation in the yield of enzyme sensitive sites. Indoles bearing electron-donating groups (methoxy or methyl) appear to react at the diffusion-controlled rate, but those bearing electron-withdrawing groups (cyano or nitro) are significantly less reactive. At physiological pH values, the reduction of a DNA guanyl radical involves the transfer of a proton as well as an electron. Comparison of the kinetic results with literature thermodynamic data suggests that the source of this proton is the complementary base-paired cytosine.

Electron removal from guanine is involved in the pathology of DNA damage by UV[1] radiation (*1*, *2*), ionizing radiation (*3*), and oxidizing agents (*4*). The resulting species is a DNA guanyl radical (*5*, *6*). This species is a fairly strong oxidizing agent (*7*, *8*) and is expected to be capable of accepting electrons from nearby mild reducing agents if any are available. Examples of mild reducing agents that react in this way are amino acids whose side chains contain imidazole, thioether, thiol, disulfide, phenol, or indole functions (*9*). Derivatives of tyrosine and tryptophan (i.e., phenols and indoles, respectively) are particularly reactive. So for guanyl radicals located in cellular DNA, a possible source of nearby reducing agents may be DNA binding proteins containing tyrosine or tryptophan residues.

This suggestion is supported by the intervention of radicals derived from these amino acids as essential intermediates in some enzyme systems. Examples are ribonucleotide reductase and DNA photolyase (*10*). Tryptophan is a rarer amino acid than tyrosine, and it is also a thermodynamically poorer reducing agent. Reduction potentials at pH 7 for the single electron oxidation products of tyrosine and tryptophan are +0.89 and +1.05 V (*11*, *12*), respectively (a difference equivalent to 15 kJ mol^{-1}). However, tryptophan reacts more rapidly than tyrosine with many single electron oxidizing agents (*13*). The reaction between DNA guanyl radicals and indoles therefore deserves to be studied.

The reaction of phenols and indoles with guanyl radicals involves the transfer of a proton as well as of an electron, because of the acidities of the species involved. In addition to supplying the electron, phenols may also act as the proton donor. This is because their radical cations are very highly acidic [the radical cation of phenol, $PhOH^{\bullet+}$, has $pK_a = -2.0$ (*14*)]. In contrast, indole radical cations are significantly poorer proton donors [the radical cation of indole, $IndNH^{\bullet+}$, has $pK_a = 4.6$ (*15*)], and in some cases this is reflected by mechanistic differences. For example, the reduction of photoexcited 2-benzoylthiophene by phenols takes a proton-coupled electron transfer (PCET) pathway, while in its reduction by indoles electron transfer precedes proton transfer (*16*). In the case of double-stranded DNA, it is possible that the readily available protons residing in the hydrogen bonds between complementary bases may participate in the reduction of guanyl radicals by an indole. Here we have tested this hypothesis by examining the reduction of DNA guanyl radicals by a series of indoles.

EXPERIMENTAL PROCEDURES

Plasmid Substrate. A sample of plasmid pHAZE [10.3 kb (*17*)] was kindly provided by Dr. W. F. Morgan (Department of Radiation Oncology, University of Maryland). It was grown to a large scale, isolated, and purified as described previously (*18*).

Base Excision Repair Endonuclease. The *Escherichia coli* base excision repair endonuclease formamidopyrimidine−DNA *N*-glycosylase (FPG) was obtained commercially (Trevigen).

Irradiation. The plasmid was γ-irradiated in aerobic aqueous solution using an AECL GammaCell-1000 isotopic instrument (^{137}Cs, 662 keV γ-ray photon) at a dose rate of 335 rad min^{-1} (5.6×10^{-2} Gy s^{-1}). The dose rate was quantified using the Fricke system (*19*). The solutions

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[1] Abbreviations: UV, ultraviolet; PCET, proton-coupled electron transfer; FPG, formamidopyrimidine−DNA *N*-glycosylase; AECL, Atomic Energy of Canada Limited; SSB, single strand break; FaPyG, 2,6-diamino-4-hydroxy-5-formamidopyrimidine; 8oxoG, 8-oxo-7,8-dihydroguanine.

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contained plasmid DNA (25 μg mL^{-1}, which is equivalent to 7.7 $\times$ 10^{-5} mol dm^{-3} nucleotide residues or 3.7 $\times$ 10^{-9} mol dm^{-3} plasmid pHAZE), sodium phosphate (5 $\times$ 10^{-3} mol dm^{-3}, pH 7.0), sodium thiocyanate (1 $\times$ 10^{-3} mol dm^{-3}), sodium perchlorate (0.11 mol dm^{-3}), and indole or one of nine derivatives of it (3 $\times$ 10^{-8} to 1 $\times$ 10^{-4} mol dm^{-3}). As such, we sometimes use "indole" in a generic sense. The indole compound was one of the following: 1-methylindole, 2-methylindole, 3-methylindole (skatole), 2,3-dimethylindole, 5-methoxyindole, 5-methylindole, 5-bromoindole, 5-cyanoindole, or 5-nitroindole. Each aliquot was 27 μL in volume.

Enzyme Incubation. After irradiation each aliquot was mixed with 3 mL of a solution containing the enzyme FPG such that the final FPG concentration was either 0 or 3 μg mL^{-1}. This corresponds to a final activity of 30 units/mL (where a unit is defined as the formation of 10^{-12} mol of SSB from abasic sites after incubation at 37 °C for 60 min). The resulting solutions were incubated at 37 °C for 30 min and then assayed for SSB formation.

Determination of Strand Break Yields. Single strand break (SSB) formation in plasmid DNA was detected by agarose gel electrophoresis. The agarose concentration was 0.75%. The procedures for digital video imaging of ethidium fluorescence and for calculating the radiation chemical yield (or *G* value) for SSB formation have been described previously (*18*). SSBs in the plasmid convert the supercoiled conformation to the open circle conformation. The supercoiled conformation contains no breaks, while the open circle conformation contains one or more of them.

If it is assumed that SSBs are introduced according to the Poisson distribution, then the total number of breaks can be estimated from the fraction of the plasmid that is break-free, i.e., the mole fraction of the supercoiled form. The D_0 dose is defined as the radiation dose required to decrease the mole fraction of supercoiled plasmid to 1/*e* of its value in the absence of irradiation. It is numerically equal to the reciprocal of the slope *m* of a straight line fitted to a semilogarithmic yield dose plot. Assuming a Poisson distribution, at the D_0 dose there is a mean of one SSB per plasmid, so that the concentration of the SSB product is equal to the concentration of the plasmid substrate (3.9 $\times$ 10^{-9} mol dm^{-3} for pHAZE). The *G* value for SSB formation (whose units are μmol J^{-1}) is calculated by dividing this concentration by the value of D_0.

RESULTS AND DISCUSSION

Reaction Scheme. We have reported previously on the evidence for the mechanism of DNA damage by γ-irradiation in the presence of thiocyanate ions (*20*). The system is summarized by the reaction scheme in Figure 1. Radiolysis of water produces the hydroxyl radical •OH (reaction 1). Radiolysis also produces reducing species (not shown) which are scavenged by oxygen to form superoxide. The ineffectiveness of superoxide dismutase in this system (*20*) suggests that reactions involving superoxide can be ignored. Some •OH react by hydrogen abstraction with the 2′-deoxyribose groups in DNA to produce carbon-centered radicals (reaction 2). These lead to the formation of strand breaks (reaction 3) (*21*). Some •OH also react by addition to all of the bases in DNA (*22*). The only adduct shown in Figure 1 is to the 8-position of guanine, to produce the

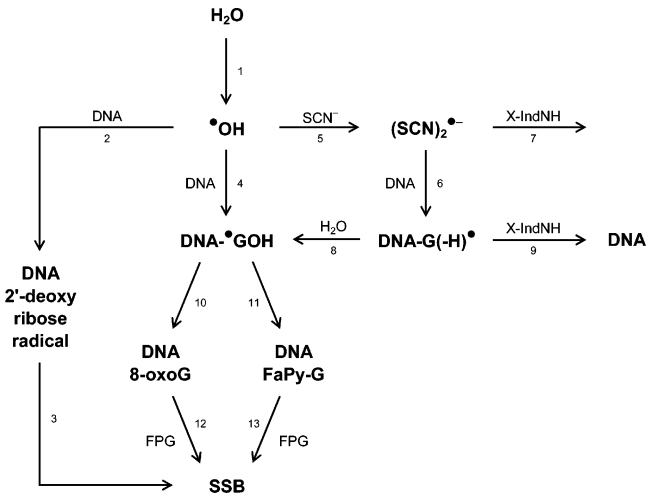

FIGURE 1: Reaction scheme summarizing the mechanism for the formation, repair, and subsequent detection of oxidative damage in plasmid DNA.

8-hydroxy-7,8-dihydroguan-7-yl radical DNA-•GOH (reaction 4).

Under the conditions we have used here (1 $\times$ 10^{-3} mol dm^{-3} thiocyanate and 7.7 $\times$ 10^{-5} mol dm^{-3} nucleotide residues), most •OH are scavenged by thiocyanate to produce the radical anion (SCN)$_2^{\bullet-}$ (reaction 5). The latter behaves as a strong single electron oxidizing agent with $E^\circ = +1.33$ V (*23*). Thus it is capable of removing electrons from guanine bases although probably not from other sites in DNA (*7*, *24*, *25*). The product of single electron removal from guanine is a DNA guanyl radical (reaction 6). Guanyl radicals derived from monomers (*6*) and also from double-stranded oligonucleotides (*26*) are deprotonated at pH 7, so they are symbolized here as DNA-G(−H)•. On the basis of the acidities of the monomers [N-3 protonated cytosine has pK_a = 4.3 (*27*) and G$^{\bullet+}$ has pK_a = 3.9 (*27*)], the complementary cytosine is assumed to be the proton acceptor in double-stranded DNA (*27*). If a reducing agent such a substituted indole X-IndNH is also present in solution, it may compete with the DNA for the oxidizing agent (reaction 7). However, we have previously argued that, under the conditions used here, scavenging of (SCN)$_2^{\bullet-}$ by most mild reducing agents is negligible (*20*).

In the absence of a reducing agent, base-paired guanyl radicals are fairly long-lived species (*28*, *29*). They hydrate to form the 8-hydroxy-7,8-dihydroguan-7-yl radical DNA-•GOH (reaction 8), which is also formed by •OH addition to guanine (see above). Guanyl radicals are fairly strong oxidizing agents [$E_7 = +1.29$ V for the radical derived from guanosine (*7*)], and they can accept electrons from mild reducing agents such as thiols or phenols (*30*−*32*). Here the reducing agent is a substituted indole X-IndNH (reaction 9). Reaction 9 reverses the oxidative damage produced by (SCN)$_2^{\bullet-}$ (or, in general, any of the electron removal processes mentioned in the introduction) by replacing the missing electron.

Stable products derived from the 8-hydroxy-7,8-dihydroguan-7-yl radical DNA-•GOH are 8-oxo-7,8-dihydroguanine DNA-8oxoG (reaction 10) and 2,6-diamino-4-hydroxy-5-formamidopyrimidine DNA-FaPyG (reaction 11). Reaction 10 involves a single electron oxidation and reaction 11 a single electron reduction. Under aerobic conditions but in

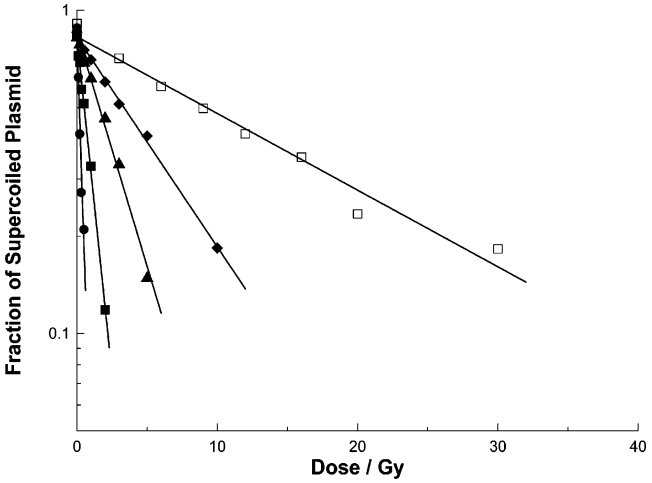

FIGURE 2: Loss of supercoiled plasmid with increasing γ-radiation dose. Aliquots (27 μL) of a solution containing plasmid pHAZE (25 μg mL^{-1}), sodium phosphate (5×10^{-3} mol dm^{-3}, pH 7.0), sodium thiocyanate (1×10^{-3} mol dm^{-3}), sodium perchlorate (1.1×10^{-1} mol dm^{-3}), and 5-methylindole [3×10^{-8} mol dm^{-3} (●), 1×10^{-7} mol dm^{-3} (■, □), 3×10^{-7} mol dm^{-3} (▲), and 1×10^{-6} mol dm^{-3} (◆)] were irradiated under aerobic conditions with cesium-137 γ-rays (662 keV) at a dose rate of 5.6×10^{-2} Gy s^{-1}. After irradiation, the solutions were incubated at 37 °C for 30 min with FPG [0 (□) or 3×10^{-6} g mL^{-1} (closed symbols)]. The fraction of supercoiled plasmid remaining after each radiation dose and subsequent incubation was determined by agarose gel electrophoresis. These five data sets are plotted together, and each is fitted with a least mean square straight line of the form $y = ce^{-mx}$. From the slopes m of these fitted lines, the D_0 doses and SSB yields for the five irradiation and incubation conditions are as follows: (●) 0.338 Gy, 1.10×10^{-2} μmol J^{-1}; (■) 1.05 Gy, 3.56×10^{-3} μmol J^{-1}; (▲) 3.01 Gy, 1.24×10^{-3} μmol J^{-1}; (◆) 6.71 Gy, 5.55×10^{-4} μmol J^{-1}; (□) 18.3 Gy, 2.03×10^{-4} μmol J^{-1}.

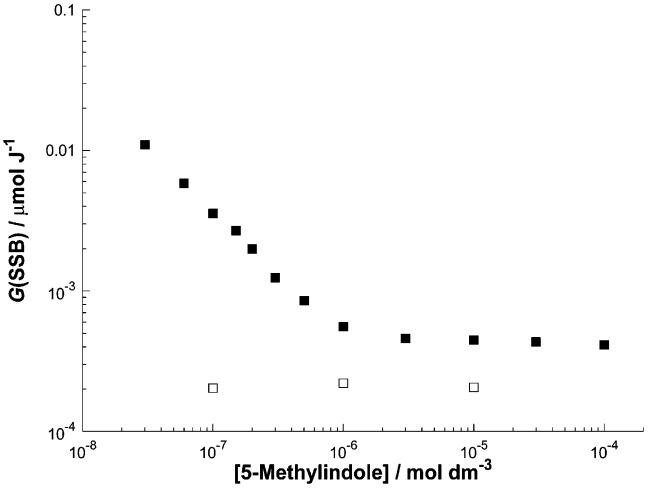

FIGURE 3: Effect of 5-methylindole on the yield of strand breaks after incubation with or without FPG. The SSB yields were determined using the method shown in Figure 1. 5-Methylindole was present during γ-irradiation. After the irradiation was completed, but before assay for breaks by gel electrophoresis, the plasmid was incubated under one of two conditions: (1) in the absence of FPG (open square) or (2) in the presence of 3×10^{-6} g mL^{-1} FPG (closed square).

the absence of a reducing agent, the oxidation (reaction 10) dominates (*33*). Under aerobic conditions and in the presence of a reducing agent such as an indole (the conditions we use here), it is possible that the competition between reactions 10 and 11 may be shifted in favor of the reduced product. Both of these products, DNA-8oxoG and DNA-FaPyG, are stable, but a postirradiation incubation with the base excision repair endonuclease FPG converts both to SSBs via an intermediate abasic site (*34*).

Therefore, SSBs are formed by three distinguishable routes. One (reactions 1−3) produces breaks in the absence of any FPG incubation. A second involves •OH addition to guanine bases in DNA,and produces breaks only after FPG incubation (reactions 1, 4, 10−13). A third is thiocyanate dependent and also produces breaks only after FPG incubation (reactions 1, 5, 6, 8, 10−13). The second and third routes can be distinguished because the second is unaffected by mild reducing agents while the third is. Under the conditions we use here, in the absence of any added reducing agent the SSB yields from these three routes are approximately 1:1:100, respectively. But in the presence of an excess ($>10^{-5}$ mol dm^{-3}) of a substituted indole X-IndNH, the contribution of the third route is attenuated to such an extent that it becomes negligible (see below).

Break Yields. Using a plasmid DNA substrate, the SSB yield can be quantified. Examples of this are shown in Figure 2. Aerobic solutions containing plasmid pHAZE, thiocyanate ions, and 5-methylindole were irradiated with γ-rays and then incubated with or without FPG. Formation of SSBs in the plasmid decreases the mole fraction of the supercoiled form, which can be detected by gel electrophoresis. Postirradiation FPG incubation produces SSBs at a significantly higher frequency, but this effect is attenuated by the presence during irradiation of 5-methylindole. Higher concentrations of 5-methylindole produce a greater attenuation. The radiation chemical yield (referred to as a G value) for SSB formation can be calculated from the slopes of yield dose plots such as the examples reproduced in Figure 2 (details can be found in the Experimental Procedures).

Attenuation of Breaks by Indoles. The three different routes to DNA damage discussed above respond in different ways to the presence of indoles during irradiation. The SSB yield [symbolized by G(SSB)] was quantified over a wide range of 5-methylindole concentrations (from 3×10^{-8} to 1×10^{-4} mol dm^{-3}). The dependence of G(SSB) both with and without FPG on the 5-methylindole concentration is shown in Figure 3.

In the absence of FPG, the value of G(SSB) remains approximately constant at ca. 2×10^{-4} μmol J^{-1}. This yield represents SSBs formed by reactions 2 and 3 and is independent of the concentration of 5-methylindole because any contribution to •OH scavenging by the latter (at concentrations $\leq 1 \times 10^{-4}$ mol dm^{-3}) is insignificant in the presence of 1×10^{-3} mol dm^{-3} thiocyanate.

After incubation in the presence of FPG, G(SSB) is strongly dependent on the concentration of 5-methylindole. The value of G(SSB) in the absence of any indole compound is 4.8×10^{-2} μmol J^{-1} (*35*). The value of G(SSB) decreases from this value to about 5×10^{-4} μmol J^{-1} (about 100-fold decrease) for 5-methylindole concentrations up to about 1×10^{-6} mol dm^{-3}. Further increases in the concentration of 5-methylindole up to 1×10^{-4} mol dm^{-3} have essentially no effect on the SSB yield. This residual SSB yield at high indole concentrations results from •OH addition to guanine bases (reaction 4). Any contribution by the thiocyanate-dependent route (reactions 5, 6, and 8) is diverted by reduction of DNA-G(−H)• (reaction 9). But at 5-methylin-

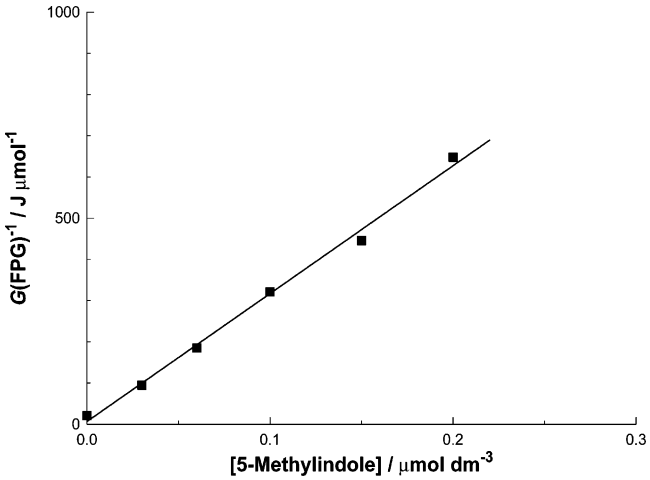

FIGURE 4: Effect of the concentration of 5-methylindole on the yield of enzyme sensitive sites. The reciprocal of the yield of FPG sensitive sites $G(\mathrm{FPG})^{-1}$ is plotted against the 5-methylindole concentration according to competition kinetics (eq 1). The data set is fitted with a least mean square straight line of the form $y = mx + c$. The value of the slope m is 3.10×10^9 MJ dm^3 mol^{-2}.

dole concentrations below about 1×10^{-6} mol dm^{-3}, this reduction reaction becomes too slow to compete with the trapping reaction (reaction 11), and the SSB yield increases significantly.

Repair Kinetics. The yield of FPG sensitive sites produced by the thiocyanate-dependent route [symbolized by G(FPG)] was calculated by subtracting the residual SSB yield (after FPG incubation) of 5×10^{-4} μmol J^{-1} (observed at high concentrations of 5-methylindole) from the SSB yields (after FPG incubation) observed at lower concentrations of 5-methylindole. The reciprocal of G(FPG) is plotted according to competition kinetics against the concentration of 5-methylindole in Figure 4. The competition between the trapping (by reaction 8) and the repair (by reaction 9) of DNA guanyl radicals DNA-G(−H)$^\bullet$ can be interpreted using Figure 4. This competition can be quantified by eq 1, where G(FPG) and G_0(FPG) represent the yields of FPG sensitive sites (produced by the thiocyanate-dependent route) in the presence and absence of 5-methylindole, k_8 and k_9 represent the rate constants of reactions 8 and 9, and [5-MeIndNH] represents the concentration of 5-methylindole.

$$\frac{1}{G(\mathrm{FPG})} = \left\{\frac{1}{G_0(\mathrm{FPG})}\right\}\left\{1 + \frac{k_9[\mathrm{5MeIndNH}]}{k_8}\right\} \quad (1)$$

Equation 1 implies that $1/G$(FPG) should be a linear function of the 5-methylindole concentration. Figure 4 shows that this is the case. The value of k_9 is equal to mk_8/c, where m and c are the slope and intercept of the straight line fitted to Figure 4. The value of m is 3.10×10^9 MJ dm^3 mol^{-2}. In practice, the value of c is estimated as $c = (4.8 \times 10^{-2}$ μmol J$^{-1})^{-1} = 21$ J μmol^{-1}. Assuming that $k_8 = 0.2$ s^{-1} (*28*, *29*), the value of k_9 estimated from Figure 4 is $k_9 = 3.10 \times 10^9 \times 0.2/21 = 3.0 \times 10^7$ dm^3 mol^{-1} s^{-1}.

This procedure was repeated for the other indole compounds listed in the Experimental Procedures. These indoles and the k_9 values obtained for them are listed in Table 1. The value for tryptophan was reported previously (*9*) and is also included in Table 1.

Table 1: Rate Constants for the Reduction of a DNA Guanyl Radical in Plasmid DNA by Substituted Indoles

indole substituent	k_9/dm^3 mol^{-1} s^{-1}	indole substituent	k_9/dm^3 mol^{-1} s^{-1}
2,3-Me$_2$	2.6×10^7	5-Br	8.5×10^6
3-Me (skatole)	5.3×10^7	H (indole)	2.3×10^7
2-Me	2.3×10^7	5-NO$_2$	8.0×10^5
tryptophan	4.4×10^7 [a]	5-CN	1.1×10^6
5-Me	3.0×10^7	1-Me	1.4×10^7
5-MeO	3.5×10^7		

[a] See ref *9*.

Table 2: Thermodynamic Data for Substituted Indoles[a]

indole substituent	pK_a (X-IndNH ⇆ X-IndN$^-$ + H$^+$)[c]	pK_a (X-IndNH$^{\bullet+}$ ⇆ X-IndN$^\bullet$ + H$^+$)[c]	E (X-IndNH$^{\bullet+}$/X-IndNH)/V[c]
2,3-Me$_2$	15.4 (*41*)	6.1 (*44*)	+0.93 (*45*)
3-Me (skatole)	16.6 (*42*)	5.0 (*44*)	+1.07 (*45*)
2-Me	16.6[b]	5.7 (*44*)	+1.10 (*45*)
tryptophan	16.8 (*43*)	4.3 (*15*)	+1.21 (*15*)
5-Me	17.2 (*36*)	5.0 (*15*)	+1.24 (*15*)
5-MeO	17.3 (*36*)	6.1 (*15*)	+1.18 (*15*)
5-Br	16.2 (*36*, *43*)	3.7 (*15*)	+1.32 (*15*)
H (indole)	16.7 (*36*)	4.9 (*44*)	+1.29 (*15*)
	17.0 (*42*)	4.6 (*15*)	
5-NO$_2$	14.8 (*36*, *43*)	2.1 (*15*)	+1.49 (*15*)
5-CN	15.3 (*36*, *43*)	2.8 (*15*)	+1.48 (*15*)
1-Me			+1.22 (*46*)

[a] The abbreviations X-IndNH, X-IndN$^-$, X-IndNH$^{\bullet+}$, and X-IndN$^\bullet$ represent respectively the indole derivative, its conjugate base, its radical cation, and its neutral radical. Ionization constants for 1-methylindole are not listed because it contains no removable N−H proton. [b] Assumed. [c] References are given in parentheses.

Reduction of DNA-G(−H)$^\bullet$. The reduction of a DNA guanyl radical DNA-G(−H)$^\bullet$ (reaction 9) involves the transfer of a proton in addition to an electron. This is because of the acidities of guanine (DNA-G) and of its single electron oxidation product (DNA-G$^{\bullet+}$). The latter is significantly more acidic than the former. pK_a values for monomeric guanine species are 9.5 (guanosine) and 3.9 (radical cation of guanosine) (*27*). These are assumed to be applicable to guanine residues in double-stranded DNA.

It is possible that the indole is the source of the proton as well as of the electron in reaction 9. An alternative source might be the complementary base to which the DNA guanyl radical is hydrogen bonded, the N-3 protonated conjugate acid of cytosine (DNA-CH$^+$). The arguments in favor of the complementary base are (1) DNA-CH$^+$ (pK_a for the monomer is 4.3) is of comparable acidity to the indole radical cations (pK_a values in the range 2.1−6.1; see Table 2), (2) DNA-CH$^+$ is hydrogen bonded to the proton acceptor N-1 atom in guanine (*27*), and (3) the reactivity of 1-methylindole (which is incapable of acting as a proton source) is within a factor of 2 of other indole compounds with similar reduction potentials (see Tables 1 and 2).

Figures 5 and 6 show the reduction reaction resolved into individual proton and electron transfers. In Figure 5 the proton source is the indole (X-IndNH), while in Figure 6 it is the complementary base (DNA-CH$^+$). In both cases, there are three possible mechanisms: (1) electron first and proton second (reactions 9a and 9b or reactions 9f and 9g), (2) both coupled together (reactions 9c or 9h), or (3) proton first and electron second (reactions 9d and 9e or reactions 9i and 9j). Note that reactions 9a and 9f are equivalent.

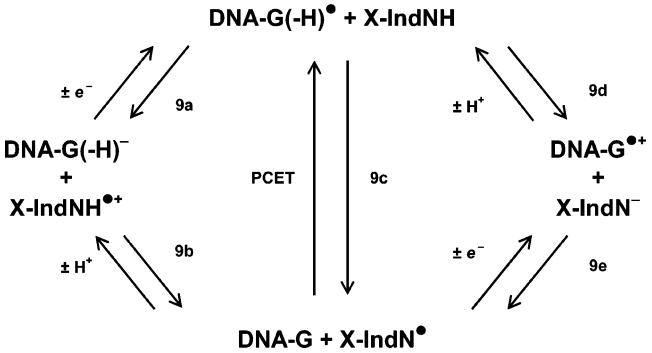

FIGURE 5: Reaction scheme depicting the individual proton and electron transfers for the repair of a DNA guanyl radical by an indole compound (reaction 9), where the indole compound is the source of both the proton and the electron. The three different mechanisms shown here are (1) electron first and proton second (reactions 9a and 9b), (2) both coupled together (PCET, reaction 9c), and (3) proton first and electron second (reactions 9d and 9e).

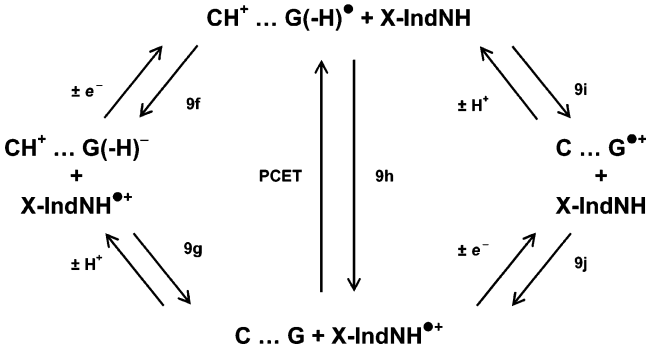

FIGURE 6: Reaction scheme depicting the individual proton and electron transfers for the repair of a DNA guanyl radical by an indole compound (reaction 9), where the indole compound is the source of the electron and the proton is supplied by the complementary base. The three different mechanisms shown here are (1) electron first and proton second (reactions 9f and 9g), (2) both coupled together (PCET, reaction 9h), and (3) proton first and electron second (reactions 9i and 9j).

Repair Energetics. Because the thermodynamic data are available in the literature, it is possible to calculate the driving forces for reactions 9a to 9j for each of the indoles used in this study.

Consider, for example, 5-methylindole. The pK_a value for its weakly acidic N−H proton is 17.2 (*36*). In the radical cation, this proton is about 12 orders of magnitude more acidic with pK_a 5.0 (*15*). From these values, it is possible to calculate the pH dependence of the reduction potential of the couple (5-MeIndN•, H^+/5-MeIndNH), where 5-MeIndNH and 5-MeIndN• represent 5-methylindole and its neutral radical, respectively. Briefly, the reduction potential is constant at pH values lower (more acidic) than the pK_a of the radical cation and constant at pH values greater (more basic) than the pK_a of the indole. Between these values the reduction potential decreases by $RT/F = 0.059$ V per unit increase in pH. Therefore, the reduction potential of the couple at pH 7 is E_7(5-MeIndN•, H^+/5-MeIndNH) = +1.24 − 0.059 × (7.0 − 5.0) = +1.12 V (*15*). At pH values greater than the pK_a of the indole, the reduction potential of the couple is E(5-MeIndN•/5-MeIndN$^-$) = +1.24 − 0.059 × (17.2 − 5.0) = +0.52 V (where 5-MeIndN$^-$ represents the conjugate base of 5-methylindole).

The corresponding values for guanosine are pK_a(guanosine) = 9.5 (*27*), pK_a(guanosine radical cation) = 3.9 (*6*, *27*), and E_7[G(−H)•, H^+/G] = +1.29 V (*7*). We assume that these values apply to guanine residues in plasmid DNA. The reduction potentials of guanine radical species are base sequence dependent (*37*), so this assumption is not strictly valid. The derived values of the reduction potentials are E(G•+/G) = +1.47 V and E[G(−H)•/G(−H)$^-$] = +1.14 V. Here G represents guanosine, G(−H)$^-$ its conjugate base, G•+ its radical cation, and G(−H)• the conjugate base of the radical cation. Again, these values are assumed to be applicable to guanine bases in plasmid DNA.

Table 3: Calculated Driving Forces ΔG for the Individual Proton and Electron Transfers in Figure 5, Where the Indole Acts as the Proton Source

	ΔG/kJ mol^{-1}				
indole substituent	9a e^- first	9b H^+ second	9c PCET	9d H^+ first	9e e^- second
2,3-Me_2	−20	−19	−41	+66	−106
3-Me (skatole)	−7	−26	−33	+72	−105
2-Me	−4	−22	−26	+72	−98
tryptophan	+7	−30	−23	+73	−97
5-Me	+10	−26	−16	+76	−92
5-MeO	+4	−19	−16	+76	−93
5-Br	+17	−33	−16	+70	−86
H (indole)	+14	−28	−14	+73	−86
5-NO_2	+34	−42	−9	+63	−71
5-CN	+33	−38	−6	+65	−71
1-Me	+8				

Using these values, it is possible to calculate the driving forces for the individual proton and electron transfer steps in Figure 5. Driving forces for the proton transfer steps are derived from pK_a differences: $\Delta G = -RT \ln K = 2.3RT(\Delta pK_a)$. For example, the driving force for reaction 9d [in the case of 5-methylindole, this reaction is 5-MeIndNH + DNA-G(−H)• → 5-MeIndN$^-$ + DNA-G•+] is equal to $\Delta G_{9d} = \ln RT(17.2 - 3.9) = +76$ kJ mol^{-1}. Similarly, the driving forces for the electron transfer reactions are derived from the appropriate values of the reduction potentials: $\Delta G = -F\Delta E$. For example, the driving force for reaction 9e (5-MeIndN$^-$ + DNA-G•+ → 5-MeIndN• + DNA-G) is equal to $\Delta G_{9e} = -F(+1.47 - 0.52) = -92$ kJ mol^{-1}. The driving force for the transfer of both a proton and an electron [5-MeIndNH + DNA-G(−H)• → 5-MeIndN• + DNA-G, reaction 9c] is derived from differences in E_7 values: $\Delta G_{9c} = -F(1.29 - 1.12) = -16$ kJ mol^{-1}. This value is also equal to the sum of the driving forces for transferring the proton and electron one at a time, so that $\Delta G_{9a} + \Delta G_{9b} = \Delta G_{9c} = \Delta G_{9d} + \Delta G_{9e}$.

Literature ionization constants and reduction potentials for the other indoles used in this study are reproduced in Table 2 (the pK_a of 2-methylindole was assumed to be identical to that of 3-methylindole). The driving forces calculated as described above for all of the indoles (except for 1-methylindole, which contains no removable amino proton) are summarized in Table 3.

The driving forces for reactions 9f to 9j were calculated in a similar manner and are listed in Table 4. Disagreements of 0.3 unit in the literature pK_a values (Table 2) and base sequence effects on guanine radical reduction potentials of 0.05−0.08 V (*37*) correspond to uncertainties in ΔG of ca. 2 and 5−8 kJ mol^{-1}, respectively. In addition, electrostatic work contributions to ΔG have been ignored. Their contribution is estimated to be about 2 kJ mol^{-1} (*35*).

Table 4: Calculated Driving Forces ΔG for the Individual Proton and Electron Transfers in Figure 6, Where the Complementary Base Acts as the Proton Source[a]

	ΔG/kJ mol^{-1}				
indole substituent	9f e^- first	9g H^+ second	9h PCET	9i H^+ first	9j e^- second
2,3-Me_2	−20	−30	−50	+2	−52
3-Me (skatole)	−7	−30	−36	+2	−39
2-Me	−4	−30	−33	+2	−36
tryptophan	+7	−30	−23	+2	−25
5-Me	+10	−30	−20	+2	−22
5-MeO	+4	−30	−26	+2	−28
5-Br	+17	−30	−12	+2	−14
H (indole)	+14	−30	−15	+2	−17
5-NO_2	+34	−30	+4	+2	+2
5-CN	+33	−30	+3	+2	+1
1-Me	+8	−30	−22	+2	−24

[a] Note that reaction 9f is identical to reaction 9a.

Repair Mechanism. The relationship between the kinetic data in Table 1 and the thermodynamic data in Tables 3 and 4 can be used to eliminate some of the possible pathways for the repair of a DNA guanyl radical by an indole (reaction 9). As shown in Figures 5 and 6, there are three different sequences for the proton and electron transfers (electron first, proton first, or both coupled together) and two sources for the proton (the indole or the complementary base).

Of these six pathways, the proton first from indole route (reaction 9d) can be eliminated because it is energetically so unfavorable. Even the least endoergonic proton first transfer step has $\Delta G = +63$ kJ mol^{-1}. This corresponds to an equilibrium constant of $K_{9d} = 10^{-11}$. This equilibrium constant is equal to the ratio of the forward and reverse reactions, $K_{9d} = k_{9d}/k_{-9d}$. If the reverse reaction is assumed to be diffusion controlled ($k_{-9d} \leq 10^{11}$ dm^3 mol^{-1} s^{-1}), then the forward reaction can be no faster than about 1 dm^3 mol^{-1} s^{-1}. This is slower by 6−7 orders of magnitude than the rate constants observed for the repair reaction (Table 1).

Two other pathways [electron first (reaction 9a, 9f) and PCET proton from indole (reaction 9c)] can be eliminated on the basis of the relationship between the rate constant and the driving force. Marcus theory (*38*) predicts that the rate constant k and driving force ΔG of electron transfer reactions are related by eq 2 (where λ is the reorganization energy).

$$\frac{\partial(RT \ln k)}{\partial(\Delta G)} = \frac{1}{2}\left(1 + \frac{\Delta G}{\lambda}\right) \qquad (2)$$

So irrespective of the value of λ (see below), in principle the slope of a plot of $RT \ln k$ versus ΔG should be steeper than 0.5 for an endoergonic reaction (for $\Delta G > 0$) and less steep than 0.5 for an exoergonic reaction (for $\Delta G < 0$) (*39*, *40*). A plot of $RT \ln k_9$ against ΔG for the electron second route (proton from complementary base) (reaction 9j) is reproduced in Figure 7. It is likely that the faster rate constants ($k_9 > 3 \times 10^7$ dm^3 mol^{-1} s^{-1}) are diffusion limited (*35*), so only the data for the five highest driving forces were used for fitting. The slope of the fitted straight line is −0.38. The corresponding values for reactions 9a and 9c are −0.37 and −0.87 (not shown). Of these three reactions, only a slope of −0.38 for the mildly exoergonic reaction 9j (ΔG values between about −20 and 0 kJ mol^{-1}) is consistent with eq 2.

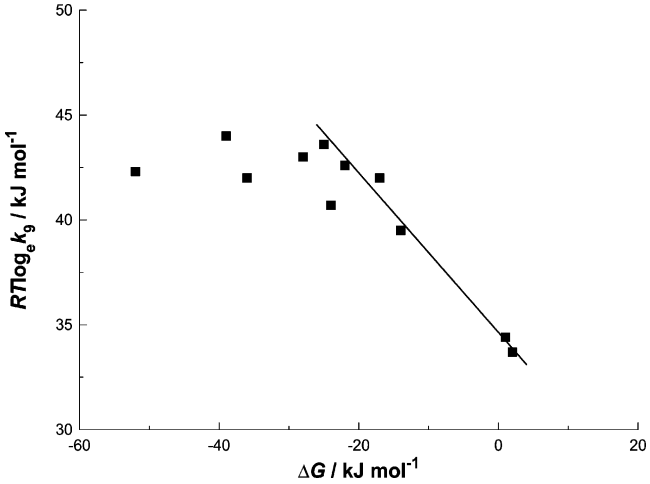

FIGURE 7: Driving force dependence of the rate constant k_9 for the repair of DNA guanyl radicals DNA-G(−H)$^\bullet$ by indole compounds. The value of $RT \ln k_9$ (from Table 1) is plotted against the driving force (from Table 4) of the electron second step where the complementary base acts as the proton donor (reaction 9j) (■). The five highest ΔG values are fitted with a least mean square straight line of the form $y = mx + c$. The value of the slope m of this line is −0.38.

Because the driving forces of reaction 9h are so similar to that of reaction 9j (difference of only 2 kJ mol^{-1}), the data cannot distinguish between these mechanisms.

It is also possible to derive a value for the reorganization energy λ from Figure 7. Equation 2 implies that the value of λ is numerically equal to the ΔG value corresponding to the maximum value of the rate constant [i.e., where $\partial(RT \ln k_9)/\partial(\Delta G) = 0$]. Because of the probable approach of k_9 to a diffusion-limited value, we can only place a lower limit on λ of $>\sim$30 kJ mol^{-1} ($>\sim$0.3 eV).

SUMMARY

Both reactions 9h and 9j involve proton transfer to the guanyl radical from its complementary base. Our observations are in agreement with the emphasis placed by other workers (*27*) on base pair hydrogen bonding in the expected reactivity of guanyl radicals in double-stranded DNA. The differential behavior of phenols (*35*) and indoles as reducing agents for DNA guanyl radicals is presumably related to the acidities of the phenol and indole radical cations. With pK_a values of ca. −2, phenol radical cations easily outcompete any other species as proton donors. The acidity of indole radical cations is significantly lower (see Table 2) and comparable with that of the conjugate acid of the cytosine base to which the DNA guanyl radical is base paired by hydrogen bonding.

REFERENCES

1. Melvin, T., Cunniffe, S., Papworth, D., Roldan-Arjona, T., and O'Neill, P. (1997) Irradiation of DNA with 193 nm light yields formamidopyrimidine DNA glycosylase (Fpg) protein sensitive lesions, *Photochem. Photobiol. 65*, 660−665.
2. Angelov, D., Spassky, A., Berger, M., and Cadet, J. (1997) High intensity UV laser photolysis of DNA and purine 2′-deoxyribonucleosides: formation of 8-oxopurine damage and oligonucleotide strand cleavage as revealed by HPLC and gel electrophoresis studies, *J. Am. Chem. Soc. 119*, 11373−11380.
3. Becker, D., and Sevilla, M. D. (1993) The chemical consequences of radiation damage to DNA, *Adv. Radiat. Biol. 17*, 121−180.

4. Burrows, C. J., and Muller, J. G. (1998) Oxidative nucleobase modifications leading to strand scission, *Chem. Rev. 98*, 1109−1152.
5. Melvin, T., Cunniffe, S. M. T., O'Neill, P. Parker, A. W., and Roldan-Arjona, T. (1998) Guanine is the target for direct ionization damage in DNA as detected using excision enzymes, *Nucleic Acids Res. 26*, 4935−4942.
6. Candieas, L. P., and Stenken, S. (2000) Reaction of HO$^{\bullet}$ with guanine derivatives in aqueous solution: formation of two different redox active OH adduct radicals and their unimolecular transformation reactions. Properties of G(−H)$^{\bullet}$, *Chem. Eur. J. 6*, 475−484.
7. Steenken, S., and Jovanovic, S. V. (1997) How easily oxidizable is DNA? One electron reduction potentials of adenosine and guanosine radicals in aqueous solution, *J. Am. Chem. Soc. 119*, 617−618.
8. Milligan, J. R., Aguilera, J. A., and Ward, J. F. (2001) Redox equilibrium between guanyl radicals and thiocyanate influences base damage yields in gamma irradiated plasmid DNA. Estimation of the reduction potential of guanyl radicals in plasmid DNA in aqueous solution at physiological ionic strength, *Int. J. Radiat. Biol. 77*, 1195−1205.
9. Milligan, J. R., Aguilera, J. A., Ly, A., Tran, N. Q., Hoang, O., and Ward, J. F. (2003) Repair of oxidative DNA damage by amino acids, *Nucleic Acids Res. 31*, 6258−6263.
10. Stubbe, J. A., and van der Donk, W. A. (1998) Protein radicals in enzyme catalysis, *Chem. Rev. 98*, 705−762.
11. Harriman, A. (1987) Further comments on the redox potentials of tryptophan and tyrosine, *J. Phys. Chem. 91*, 6102−6104.
12. DeFilippis, M. R., Murthy, C. P., Faraggi, M., and Klapper, M. H. (1989) Pulse radiolytic measurement of redox potentials: the tyrosine and tryptophan radicals, *Biochemistry 28*, 4847−4853.
13. Neta, P., Huie, R. E., and Ross, A. B. (1988) Rate constants for reactions of inorganic radicals in aqueous solution, *J. Phys. Chem. Ref. Data 17*, 1027−1285.
14. Holton, D. M., and Murphy, D. (1978) Determination of acid dissociation constants of some phenol radical cations. Part 2, *J. Chem. Soc., Faraday Trans. 2 75*, 1637−1642.
15. Jovanovic, S. V., and Steenken, S. (1992) Substituent effects on the spectral, acid base, and redox properties of indolyl radicals: a pulse radiolysis study, *J. Phys. Chem. 96*, 6674−6679.
16. Perez-Prieto, J., Boscá, F., Galian, R. E., Lahoz, A., Domingo, L. R., and Miranda, M. A. (2003) Photoreaction between 2-benzoylthiophene and phenol or indole, *J. Org. Chem. 68*, 5104−5113.
17. Lutze, L. H., Winegar R. A. (1990) pHAZE: a shuttle vector system for the detectin and analysis of ionizing radiation induced mutations, *Mutat. Res. 245*, 305−310.
18. Milligan, J. R., Aguilera, J. A., and Ward, J. F. (1993) Variation of single strand break yield with scavenger concentration for plasmid DNA irradiated in aqueous solution, *Radiat. Res. 133*, 151−157.
19. Spinks, J. W. T., and Woods, R. J. (1976) *An Introduction to Radiation Chemistry*, 2nd ed., Wiley, New York.
20. Milligan, J. R., Aguilera, J. A., Paglinawan, R. A., and Ward, J. F. (2000) Mechanism of DNA damage by thiocyanate radicals, *Int. J. Radiat. Biol. 76*, 1305−1314.
21. von Sonntag, C. (1987) *The Chemical Basis of Radiation Biology*, Taylor and Francis, Philadelphia, PA.
22. Cadet, J., Berger, M., Douki, T., and Ravanat, J.-L. (1997) Oxidative damage to DNA: formation, measurement, and biological significance, *Rev. Physiol. Biochem. Pharmacol. 131*, 1−87.
23. Stanbury, D. M. (1989) Reduction potentials involving inorganic free radicals in aqueous solution, *Adv. Inorg. Chem. 33*, 69−138.
24. Colson, A. O., Besler, B., Close, D. M., and Sevilla, M. D. (1992) Ab initio molecular orbital calculations of DNA bases and their radical ions in various protonation states: evidence for proton transfer in GC base pair radical anions, *J. Phys. Chem. 96*, 661−668.
25. Seidel, C. A. M., Schulz, A., and Sauer, M. H. M. (1996) Nucleobase specific quenching of fluorescent dyes. 1. Nucleobase one electron redox potentials and their correlation with static and dynamic quenching efficiencies, *J. Phys. Chem. 100*, 5541−5553.
26. Kobayashi, K., and Tagawa, S. (2003) Direct observation of guanine radical cation deprotonation in duplex DNA using pulse radiolysis, *J. Am. Chem. Soc. 125*, 10213−10218.
27. Steenken, S. (1992) Electron-transfer induced acidity/basicity and reactivity changes of purine and pyrimidine bases. Consequences of redox processes for DNA base pairs, *Free Radical Res. Commun. 16*, 349−379.
28. Hildenbrand, K., and Schulte-Frohlinde, D. (1990) ESR spectra of radicals of single-stranded and double-stranded DNA in aqueous solution. Implications for $^{\bullet}$OH induced strand breakage, *Free Radical Res. Commun. 11*, 195−206.
29. Shafirovich, V., Dourandin, A., Huang, W., and Geacintov, N. E. (2001) The carbonate radical is a site selective oxidizing agent of guanine in double stranded oligonucleotides, *J. Biol. Chem. 276*, 24621−24626.
30. O'Neill, P., and Chapman, P. W. (1985) Potential repair of free radical adducts of dGMP and dG by a series of reductants, *Int. J. Radiat. Biol. 47*, 71−80.
31. Jovanovic, S. V., and Simic, M. G (1989) The DNA guanyl radical: kinetics and mechanism of generation and repair, *Biochim. Biophys. Acta 1008*, 39−44.
32. Milligan, J. R., Aguilera, J. A., Nguyen, J. V., and Ward, J. F. (2001) Redox reactivity of guanyl radicals in plasmid DNA, *Int. J. Radiat. Biol. 77*, 281−293.
33. Douki, T., Martini, R., Ravanat, J.-L., Turesky, R. J., and Cadet, J. (1997) Measurement of 2,6-diamino-4-hydroxy-5-formamidopyrimidine and 8-oxo-7,8-dihydroguanine in isolated DNA exposed to gamma radiation in aqueous solution, *Carcinogenesis 18*, 2385−2391.
34. Stivers, J. T., and Jiang, Y. L. (2003) A mechanistic perspective on the chemistry of DNA repair glycosylases, *Chem. Rev. 103*, 2729−2759.
35. Milligan, J. R., Aguilera, J. A., Hoang, O., Ly, A., Tran, N. Q., and Ward, J. F. (2004) Repair of guanyl radicals in plasmid DNA by electron transfer is coupled to proton transfer, *J. Am. Chem. Soc. 126*, 1682−1687.
36. Munoz, M. A., Guardado, P., Hidalgo, J., Carmona, C., and Balón, M. (1992) An experimental and theoretical study of the acid base properties of substituted indoles, *Tetrahedron 48*, 5901−5914.
37. Lewis, F. D., Liu, X., Liu, J., Hayes, R. T., and Wasielewski, M. R. (2000) Dynamics and equilibria for oxidation of G, GG, and GGG sequences in DNA hairpins, *J. Am. Chem. Soc. 122*, 12037−12038.
38. Marcus, R. A., and Sutin, N. (1985) Electron transfers in chemistry and biology, *Biochim. Biophys. Acta 811*, 265−322.
39. Graige, M. S., Paddock, M. L., Bruce, J. M., Feher, G., and Okamura, M. Y. (1996) Mechanism of proton coupled electron transfer for quinone (Q_B) reduction in reaction centers of *Rb. Sphaeroides*, *J. Am. Chem. Soc. 118*, 9005−9016.
40. Laranjeira, M. C. M., Marusak, R. A., and Lappin, A. G. (2000) Driving force effects in proton coupled electron transfer, *Inorg. Chim. Acta 300−302*, 186−190.
41. Toledo, J. H., Almeida, M. B., Perez, M. A. M., and Guzman, M. C. C. (1986) Ionization equilibria of rauwolfia alkaloids in concentrated hydroxide solutions, *Tetrahedron 42*, 1497−1500.
42. Yagil, G. (1967) The proton dissocation constant of pyrrole, indole, and related compounds, *Tetrahedron 23*, 2855−2861.
43. Yagil, G. (1967) The effect of ionic hydration on equilibria and rates in concentrated electrolyte solutions. III. The H_- scale in concentrated hydroxide solutions, *J. Phys. Chem. 71*, 1034−1044.
44. Shen, X., Lind, J., and Merenyi, G. (1987) One electron oxidation of indoles and acid base properties of the indolyl radicals, *J. Phys. Chem. 91*, 4403−4406.
45. Merenyi, G., Lind, J., and Shen, X. (1988) Electron transfer from indoles, phenol, and sulfite (SO_3^{2-}) to chlorine dioxide ($ClO_2^{\bullet}$), *J. Phys. Chem. 92*, 134−137.
46. Solar, S., Getoff, N., Surdhar, P. S., Armstrong, D. A., and Singh, A. (1991) Oxidation of tryptophan and *N*-methylindole by $N_3^{\bullet}$, $Br_2^{\bullet-}$, and $(SCN)_2^{\bullet-}$ radicals in light and heavy water solutions: a pulse radiolysis study, *J. Phys. Chem. 95*, 3639−3643.

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