Reactivity of DNA Guanyl Radicals with Phenolate Anions

Anne Ly, Simona L. Bandong, Nancy Q. Tran, Kathrina J. Sullivan, and Jamie R. Milligan*

Department of Radiology, University of California at San Diego, 9500 Gilman Drive, La Jolla, California 92093-0610

Received: January 28, 2005; In Final Form: May 12, 2005

Guanine bases are the most easily oxidized sites in DNA. Electron-deficient guanine species are major intermediates produced in DNA by the direct effect of ionizing radiation (ionization of the DNA itself) because of preferential hole migration within DNA to guanine bases. By using thiocyanate ions to modify the indirect effect (ionization of the solvent), we are able to produce these single-electron-oxidized guanine radical species in dilute aqueous solutions of plasmid DNA where the direct effect is negligible. The guanyl radical species produce stable modified guanine products. They can be detected in the plasmid by converting them to strand breaks after incubation with a DNA repair enzyme. If a phenol is present during irradiation, the yield of modified guanines is decreased. The mechanism is reduction of the guanine radical species by the phenol. It is possible to derive a rate constant for the reaction of the phenol with the guanyl radical. The pH dependence shows that phenolate anions are more reactive than their conjugate acids, although the difference for guanyl radicals is smaller than with other single-electron-oxidizing agents. At physiological pH values, the reduction of a guanyl radical entails the transfer of a proton in addition to the electron. The relatively small dependence of the rate constant on the driving force implies that the electron cannot be transferred before the proton. These results emphasize the potential importance of acidic tyrosine residues and the intimate involvement of protons in DNA repair.

Introduction

Electron-transfer reactions are common in normal physiology. They also take place in response to pathological events. For example, electron removal is involved in the DNA damage produced by ionizing radiation,[1] photoionization,[2] chemical oxidation,[3] and photosensitization.[4] The most easily oxidized functional group in DNA is guanine.[5,6] The major single-electron-oxidized species is a radical located on guanine.[3,7] Replacing the missing electron represents a means by which this damage may be repaired by reducing agents before it becomes a chemically stable alteration. Diffusible low molecular weight bioreductants such as glutathione and ascorbate are reactive with single-electron-oxidized guanine radical species[8,9] and represent sources of electrons in vivo. However, it is possible that guanine radicals located in regions of chromatin that are tightly bound by proteins are inaccessible to glutathione or ascorbate.[10]

The radical derived from the single-electron oxidation of guanine is a powerful oxidizing agent[11] and is able to accept electrons from other even quite mild reducing agents if any are present. Examples of species that can react in this manner are amino acids with side chains containing a thiol, disulfide, thioether, phenol, or indole. Derivatives of the amino acids tyrosine (a phenol) and tryptophan (an indole) react most rapidly.[12] Thus, a potential source of reducing equivalents for single-electron-oxidized guanine radicals in DNA may be DNA binding proteins that contain tyrosines or tryptophans.

Amino acid residues in proteins can have pK_a values that differ by several units from those of the parent monomers. The phenolic OH group in tyrosine itself has pK_a = 10.1, but a particularly acidic tyrosine residue in a protein would be extensively deprotonated at physiological pH. Examples of acidic tyrosine residues include tyr-9 of human glutathione transferase A4-4 (pK_a = 6.7);[13,14] four tyrosine residues (pK_a's in the range 6.8−8.1) in human serum transferrin apo-hTF/2N;[15] tyr-495 in galactose oxidase from the fungus *Fusarium* (pK_a = 7.0);[16] two tyrosine residues in *N*5-(L-1-carboxyethyl)-L-ornithine synthase from the bacterium *Lactococcus lactis* (pK_a = 8.5);[17] and a tyrosine residue (pK_a between 6 and 7) in UDP-galactose-4-epimerase from the bacterium *Escherichia coli*.[18] The possibility exists that some tyrosine residues in DNA binding proteins may have similarly low pK_a values. The resulting phenolate anions would be expected to react more rapidly in an electron-transfer reaction. See Table 1 for some examples with single-electron-oxidizing agents whose reduction potentials[19,20] are within 0.1 V (in the case of $N_3^{\bullet}$ and $(SCN)_2^{\bullet-}$) of those of single-electron-oxidized guanine radical species.[11,21] Therefore, we sought to determine if a similar increase in reactivity exists in the reaction of these guanine radicals with phenolates relative to phenols.

Because of acidity differences between guanine and its single-electron-oxidized radical, the reduction of the radical at physiological pH values involves the transfer of a proton in addition to the electron.[22,23] In an undissociated phenol, the phenol itself supplies to the guanine radical a proton as well as an electron.[24] This is probably because phenol radical cations are very highly acidic (pK_a values < 0[25]) and excellent proton donors. However, a phenolate anion is unable to act as a proton source. Here, we consider as an alternative source for the proton the complementary cytosine with which the guanine radical is base paired by hydrogen bonding.

* Author to whom correspondence should be addressed. E-mail: jmilligan@ucsd.edu.

10.1021/jp050495k CCC: $30.25
Published on Web 06/16/2005

TABLE 1: Factor by Which Phenoxide Anions React Faster than Their Conjugate Acid Neutral Phenols with Single-Electron-Oxidizing Agents

phenol derivative	oxidizing agent		
	$N_3^{\bullet}$	$(SCN)_2^{\bullet-}$	$Br_2^{\bullet-}$
4-CN	95[26]		
4-H	86[26]	340[27]	84[27]
4-CH_3		55[28]	
4-OCH_3	9[26]		11[27]

Experimental Procedures

Biochemicals. A sample of plasmid pUC18 (2686 base pairs) was obtained commercially (Invitrogen, Carlsbad, CA). It was grown to a large scale, isolated, and purified as previously described.[29] The *E. coli* base excision repair enzyme formamidopyrimidine-DNA *N*-glycosylase (FPG) was obtained commercially (Trevigen, Gaithersburg, MD).

Irradiation. The plasmid was γ-irradiated aerobically in aqueous solution using an AECL GammaCell-1000 isotopic instrument (^{137}Cs, 662 keV). The dose rate of 5.6×10^{-2} Gy s^{-1} was quantified using both the Fricke method[30] and lithium fluoride thermoluminescence dosimetry (Landauer, Glenwood, IL). The solutions contained plasmid pUC18 (50 μg mL^{-1}, equivalent to 1.5×10^{-4} mol dm^{-3} nucleotide residues or to 2.9×10^{-8} mol dm^{-3} plasmid); sodium dihydrogen phosphate (1×10^{-3} to 9×10^{-3} mol dm^{-3}); disodium hydrogen phosphate (1×10^{-3} to 2.9×10^{-2} mol dm^{-3}); sodium perchlorate (2.2×10^{-2} to 9.7×10^{-2} mol dm^{-3}); sodium thiocyanate (1×10^{-3} mol dm^{-3}); and a substituted phenol (10^{-7} to 10^{-4} mol dm^{-3}). To alter the pH, the ratio of the buffer components $H_2PO_4^-$:HPO_4^{2-} was adjusted in the range 12:1−1:30. Sodium perchlorate was used to maintain an ionic strength of 1.1×10^{-1} mol dm^{-3}. The substituted phenol (X-PhOH) was one of the six compounds *p*-cresol, 4-nitrophenol, 4-hydroxybenzaldehde, 4-cyanophenol, 2,5-dichlorophenol, or 2,6-dichlorophenol (X = 4-CH_3, 4-NO_2, 4-CHO, 4-CN, 2,5-Cl_2, or 2,6-Cl_2 respectively). Each aliquot was 16 μL in volume.

Endonuclease Incubation. After irradiation, each aliquot was diluted 2-fold by adding 16 μL of a solution containing the phosphate buffer components and sodium perchlorate so that the final buffer ratio was brought to 1:1 while the ionic strength remained unchanged. The resulting solutions were mixed with 3.5 μL of an FPG solution such that the final concentration was either 0 or 3 μg mL^{-1} (0 or 30 units mL^{-1}) and then were incubated for 30 min at 37 °C. The unit definition is the formation of 10^{-12} mol DNA single-strand breaks (DNA-SSB) from abasic sites after 60 min at 37 °C. We have shown previously that these conditions represent an excess of FPG.[31]

Strand Break Yields. After enzyme incubation, the plasmid was assayed using agarose gel electrophoresis. We have reported previously on the procedure for digital video imaging of ethidium fluorescence and for calculating the radiation chemical yield (conventionally referred to as a *G*-value, having units of mol J^{-1}) for DNA-SSB formation.[29] Briefly, the D_0 dose (the radiation dose required to reduce the fraction of SSB free plasmid by a factor of *e*, equivalent to the introduction of a mean of one SSB per plasmid) is equal to the reciprocal of the slope *m* of a straight line fitted to a semilogarithmic yield dose plot. Therefore, at the D_0 dose, the concentration of the DNA-SSB product is equal to the concentration of the plasmid (2.9×10^{-8} mol dm^{-3}). The *G*-value for DNA-SSB formation is calculated by dividing this concentration by the value of D_0.

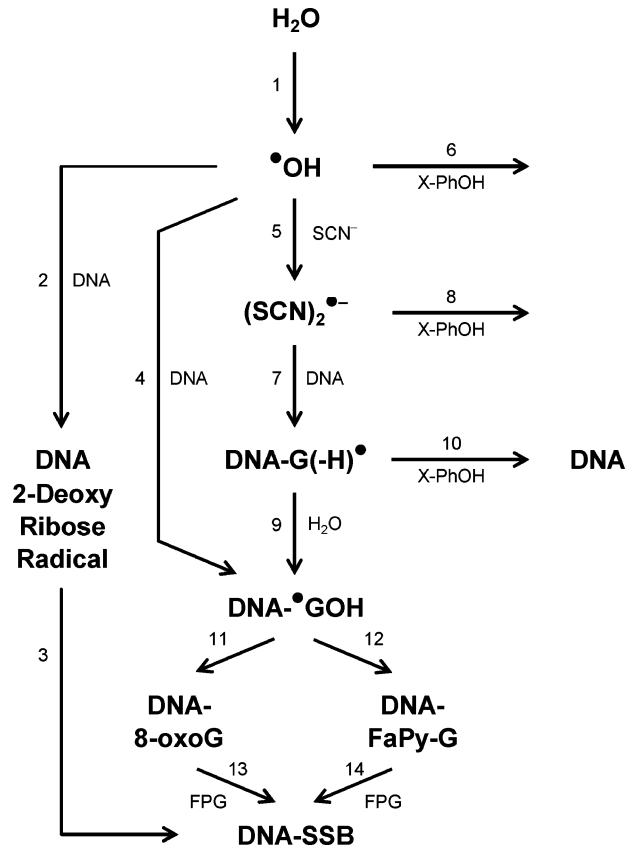

Figure 1. Reaction scheme depicting the mechanism for the formation and repair of oxidative DNA damage by γ-irradiation in the presence of thiocyanate ions.

Results and Discussion

Reaction Scheme. The system we use here produces DNA damage as a result of γ-irradiation in the presence of thiocyanate ions. We have already reported on the reactions involved.[31] They are summarized by the reaction scheme in Figure 1. Water radiolysis produces hydroxyl radical •OH (reaction 1) and reducing species which are scavenged by oxygen (not shown). A small fraction of •OH (about 1%) reacts with the 2-deoxyribose residues in DNA to produce carbon-centered radicals (reaction 2). These produce DNA strand breaks DNA-SSB (reaction 3).[29] Some •OH (again about 1%) reacts with the bases in DNA to form adducts.[32,33] Although several are formed in significant amounts, the only adduct shown in Figure 1 is at the 8-position of guanine to form the 8-hydroxy-7,8-dihydroguan-7-yl radical DNA-•GOH (reaction 4).

Under the conditions we have used here (1.5×10^{-4} mol dm^{-3} nucleotide residues and 10^{-3} mol dm^{-3} thiocyanate), the majority of •OH react with thiocyanate. The product of this reaction is $(SCN)_2^{\bullet-}$ (reaction 5).[34] This radical anion is a single-electron-oxidizing agent.[19] It is strong enough to oxidize the guanines in DNA but not the other bases or the 2-deoxyribose residues.[11,19,20] The product is a single-electron-oxidized DNA guanine radical species (reaction 7). Guanyl radical cations $G^{\bullet+}$ in monomers[35] have $pK_a = 3.9$ and deprotonate at physiological pH values. The DNA species are therefore symbolized as DNA-G(−H)• and are commonly referred to as DNA guanyl radicals. This deprotonation has also been observed for base-paired guanine radicals in double-stranded oligonucleotides.[36] In the absence of reducing agents, guanyl radicals are trapped by addition of water at the 8-position (reaction 9) to form the 8-hydroxy-7,8-dihydroguan-7-yl radical DNA-•GOH (also formed by the addition of •OH to guanine, see above).[37] Hydration of

the guanine radical cation is too slow to compete with deprotonation.[36]

If a reducing agent is also present during irradiation (in this case, a phenolic compound X-PhOH or its conjugate base X-PhO$^-$), it may react with one or more of the oxidizing species in the scheme. These are •OH, $(SCN)_2^{\bullet-}$, and DNA-G(−H)• (reactions 6, 8, and 10, respectively). The reaction conditions (maximum phenol concentration of 1×10^{-4} mol dm^{-3}) were chosen such that reactions 6 and 8 do not compete with reactions 5 and 7, respectively. The major effect of the phenol additive results from reduction of the guanyl radical DNA-G(−H)• (reaction 10). Many other mild reducing agents react in a similar manner.[8,9,38−40] This reduction reaction is too slow to compete with deprotonation of the guanine radical cation.[36,39]

The stable products formed from the 8-hydroxy-7,8-dihydroguan-7-yl radical DNA-•GOH are 8-oxo-7,8-dihydroguanine DNA-8-oxoG (reaction 11, by a single-electron oxidation) and 2,6-diamino-4-hydroxy-5-formamidopyrimidine DNA-FaPy-G (reaction 12, by a single-electron reduction). Under aerobic conditions and absent a reducing agent, the oxidized product is the major product.[33] The conditions we use here are aerobic but also include a reducing agent (the substituted phenol X-PhOH), so the distribution between reactions 11 and 12 might be altered in favor of the reduced product. Although both products DNA-8-oxoG and DNA-FaPy-G are stable under the irradiation conditions, their *N*-glycosidic bond is susceptible to hydrolysis upon subsequent incubation with the base excision repair enzyme FPG. The activity of FPG converts both of these guanine derivatives to a single-strand break (DNA-SSB).[41,42]

The strand break assay is able to detect stable guanine oxidation products at the level of about one per plasmid. In the case of the substrate pUC18, this is equivalent to a yield of guanine oxidation of slightly less than 1×10^{-3}. We assume that further oxidation[43,44] of these low levels of DNA-8-oxoG to ring-opened products is unable to compete with oxidation of the large excess of unmodified guanines.

Thus, the three routes to DNA-SSB formation shown in Figure 1 are (1) 2-deoxyribose oxidation (reactions 1−3); (2) •OH addition to guanine (reactions 1, 4, 11−14); and (3) $(SCN)_2^{\bullet-}$ oxidation of guanine (reactions 1, 5, 7, 9, 11−14). These three routes can be distinguished by the presence of a reducing agent during irradiation and a post-irradiation incubation with FPG. The first route produces breaks in the absence of FPG. The second and third routes produce breaks only after FPG incubation.[45] The second is unaffected by mild reducing agents while the third is.

Strand Break Yields. By using a plasmid as the DNA target, it is possible to quantify the DNA-SSB yield. Examples are shown in Figure 2. Buffered aerobic solutions containing pUC18, thiocyanate, and 4-nitrophenol were subjected to γ-irradiation and then were incubated either with or without FPG. The formation of DNA-SSB in the plasmid converts the supercoiled conformation into the open circle conformation. The decrease in the mole fraction of the supercoiled form is plotted against the radiation dose in Figure 2. Incubation after irradiation with FPG produces DNA-SSB in large numbers, but the presence of 4-nitrophenol during irradiation decreases this effect. The radiation chemical yield (called a *G*-value) for DNA-SSB formation can be estimated from the slopes of yield dose plots such as Figure 2 (see Experimental Procedures). This yield is symbolized as *G*(SSB).

Protection by Phenols. The three routes to DNA-SSB formation (Figure 1) are affected in different ways by the presence of 4-nitrophenol during irradiation. The value of

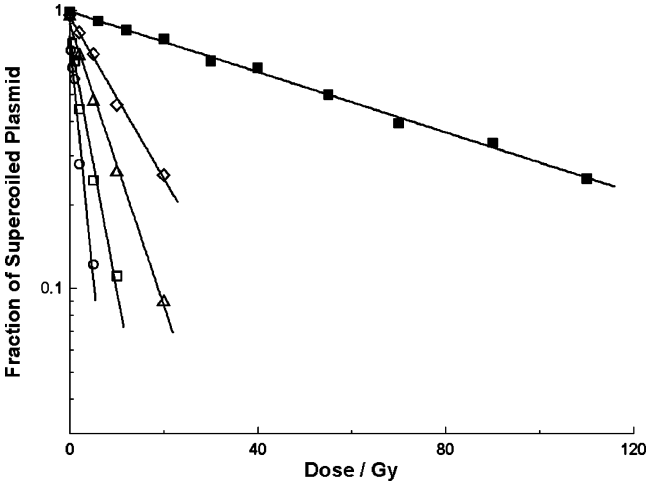

Figure 2. Loss of supercoiled plasmid with increasing dose of γ-radiation. Aliquots (16 μL) of a solution containing plasmid pUC18 (50 μg mL^{-1}), sodium dihydrogen phosphate (NaH_2PO_4, 1×10^{-3} mol dm^{-3}), disodium hydrogen phosphate (Na_2HPO_4, 9×10^{-3} mol dm^{-3}), sodium perchlorate (8.1×10^{-2} mol dm^{-3}), sodium thiocyanate (1×10^{-3} mol dm^{-3}), and 4-nitrophenol (3×10^{-7} mol dm^{-3} (open circle ○), 1×10^{-6} mol dm^{-3} (open square □, closed square ■), 2×10^{-6} mol dm^{-3} (open triangle △), or 5×10^{-6} mol dm^{-3} (open rhombus ◇)) were subjected to cesium-137 γ-irradiation (662 keV) under aerobic conditions. After irradiation, each aliquot was treated with 16 μL of a solution containing sodium dihydrogen phosphate (NaH_2PO_4, 9×10^{-3} mol dm^{-3}), disodium hydrogen phosphate (Na_2HPO_4, 1×10^{-3} mol dm^{-3}), and sodium perchlorate (9.7×10^{-2} mol dm^{-3}). The resulting solutions were incubated for 30 min at 37 °C with FPG (zero (closed square ■) or 3 (open symbols ○□△◇) μg mL^{-1}). The fraction of supercoiled plasmid remaining after these treatments was determined using agarose gel electrophoresis. All five data sets are plotted on the same scale and each is fitted with a least mean square straight line of the form $y = ce^{-mx}$. From the slopes m of the fitted lines, the D_0 doses and SSB yields for the five irradiation and incubation conditions are (open circle ○) 2.52 Gy, 1.14×10^{-2} μmol J^{-1}; (open square □) 4.76 Gy, 6.01×10^{-3} μmol J^{-1}; (open triangle △) 8.62 Gy, 3.32×10^{-3} μmol J^{-1}; (open rhombus ◇) 14.9 Gy, 1.92×10^{-3} μmol J^{-1}; and (closed square ■) 80.0 Gy, 3.58×10^{-4} μmol J^{-1}.

G(SSB) was calculated for 12 different 4-nitrophenol concentrations over almost 3 orders of magnitude up to a maximum of 1×10^{-4} mol dm^{-3}. Most of these have been omitted from Figure 2 for clarity. In Figure 3, the values of *G*(SSB) after incubation in the presence and absence of FPG are plotted against the concentration of 4-nitrophenol. For incubation in the absence of FPG, the effect of 4-nitrophenol is negligible and the value of *G*(SSB) remains constant at about 4×10^{-4} μmol J^{-1}. The SSB yield under these conditions is controlled by the competition for •OH between DNA (reaction 2) and the dominant •OH scavenger present (thiocyanate, reaction 5). Since the thiocyanate concentration exceeds that of 4-nitrophenol by at least 10-fold, the contribution of the latter to •OH scavenging (reaction 6) can be ignored.[46]

For incubation with FPG, *G*(SSB) decreases from ca. 5×10^{-2} to ca. 1×10^{-3} μmol J^{-1} (factor of 50) as the concentration of 4-nitrophenol increases from zero to 1×10^{-4} mol dm^{-3}. The residual FPG sensitive sites at the highest concentrations of 4-nitrophenol represent the contribution of •OH addition to guanine (reaction 4). We have previously reported that the yield of this pathway is comparable to that of the FPG-independent 2-deoxyribose damage pathway (reactions 2 and 3). At lower concentrations of 4-nitrophenol, the value of *G*(SSB) is significantly larger. This represents a significant contribution to SSB formation by the thiocyanate dependent route (reactions 5, 7, and 9). The lower concentrations of 4-nitrophenol are

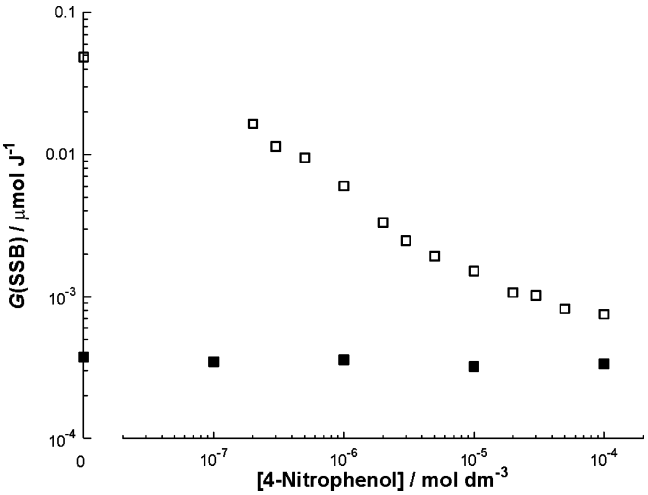

Figure 3. Effect of 4-nitrophenol on the SSB yield after incubation in the presence or absence of FPG. The SSB yields were determined using the method shown in Figure 2. 4-Nitrophenol was present during γ-irradiation. After irradiation but before SSB determination (by gel electrophoresis), the plasmid was incubated either in the absence (closed square ■) or presence (open square □) of FPG.

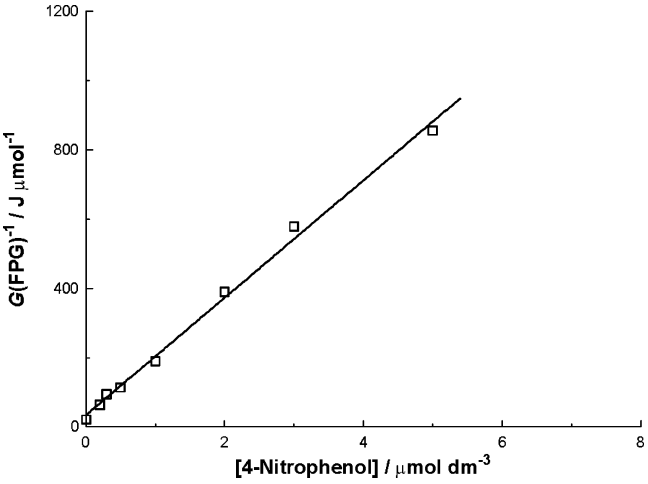

Figure 4. Effect of 4-nitrophenol on the yield of FPG sensitive sites. The reciprocal of the yield of FPG sensitive sites (see text) is plotted against the concentration of 4-nitrophenol present during irradiation. The data set is fitted with a least mean square straight line of the form $y = mx + c$. The value of the slope m is 170 J dm^3 mol^{-2}. See the text for the evaluation of the intercept c.

unable to attenuate this pathway by competing (via reaction 10) with the trapping (reaction 9) of the intermediate deprotonated guanine radical cation DNA-G(−H)•.

Quantifying Phenol Repair. It is possible to interpret this competition between the trapping and repair of the guanyl radical quantitatively by using eq 1. Here, G(FPG) represents the yield of FPG sensitive sites (the value of G(SSB) after incubation with FPG minus the value after incubation in its absence) in the presence of 4-nitrophenol, G_0(FPG) represents the yield of FPG sites in the absence of any 4-nitrophenol, and k_9 and k_{10} represent the rate constants of reactions 9 (trapping of the guanyl radical by water) and 10 (repair of the guanyl radical by the phenol), respectively. Equation 1 implies that $1/G$(FPG) should be a linear function of the concentration of 4-nitrophenol and that the ratio of the rate constants is $k_{10}/k_9 = m/c$, where m and c are the slope and intercept, respectively, of the fitted straight line. The data set from Figure 3 (for the lower 4-nitrophenol concentrations) are replotted according to eq 1 in Figure 4 and agree with this linear competition model. The value of the slope is $m = 170$ J dm^3 μmol^{-2}. The value of the intercept of the straight line in Figure 4 is calculated from the data on the Y-axis of Figure 3 as $c = (4.8 \times 10^{-2}$ to $3.8 \times 10^{-4})^{-1} = 21$ J μmol^{-1}. Therefore, the value of the ratio k_{10}/k_9 derived from Figure 4 is $m/c = 8.1 \times 10^6$ dm^3 mol^{-1}.

TABLE 2: Experimentally Determined Values of the Ratio k_{10}/k_9 for the Reduction of a Deprotonated DNA Guanine Radical Cation DNA-G(−H)• by Selected Phenols at Selected pH Values

phenol derivative	pH	k_{10}/k_9 /dm^3 mol^{-1}
4-CH_3	6.0	1.2×10^7
4-CH_3	7.0	1.3×10^7, 1.8×10^7
4-CH_3	8.0	1.2×10^7
4-NO_2	6.0	9.7×10^5, 1.7×10^6
4-NO_2	6.5	2.3×10^6, 2.7×10^6
4-NO_2	7.0	6.0×10^6
4-NO_2	7.5	5.9×10^6, 6.1×10^6
4-NO_2	8.0	6.2×10^6, 8.7×10^6
4-CN	8.5	2.1×10^7
4-CHO	8.0	2.3×10^7
2,5-Cl_2	8.0	3.8×10^7
2,6-Cl_2	8.0	7.6×10^7

This method was used to estimate the ratio k_{10}/k_9 for 4-nitrophenol after γ-irradiation at five other pH values and then was repeated with *p*-cresol at three pH values. Four other acidic phenols were also examined under conditions where they were extensively deprotonated. The observed values of the ratio k_{10}/k_9 are listed in Table 2.

$$\frac{1}{G(\mathrm{FPG})} = \left\{\frac{1}{G_0(\mathrm{FPG})}\right\} \times \left\{1 + \frac{k_{10}[4-\mathrm{NO_2}-\mathrm{PhOH}]}{k_9}\right\} \quad (1)$$

Dependence of Repair on pH. One aim here is to examine the effect of pH on the rate constant for the repair of a DNA guanyl radical by a phenol (k_{10}). However, the competition kinetics approach described above only provides an estimate of the ratio k_{10}/k_9. Quantifying k_{10} itself requires an independent determination of k_9. Imprecise values for k_9 are available in the literature. The lifetime (i.e., a close approximation to the value of k_9^{-1}) lies in the range 1−10 s,[47,48] but there are no reports of its pH dependence. As an alternative, we have compared over the pH range 6−8 the value of the ratio k_{10}/k_9 for 4-nitrophenol (p$K_a = 7.1$[49]) with that for *p*-cresol (p$K_a = 10.1$[49]). Since the phenolate anion of *p*-cresol is formed to a negligible extent over this pH range, we argue that it represents a suitable control compound.

The pH dependence of k_{10}/k_9 for both 4-nitrophenol and *p*-cresol is plotted in Figure 5. The former is pH dependent, increasing 7-fold as the pH increases from 6 to 8. In contrast, the latter is independent of pH in this range. The implication is that the reduction of the DNA guanyl radical by the 4-nitrophenolate anion is faster than by its conjugate acid, the neutral phenol. A similar difference might reasonably be expected for acidic tyrosine residues in DNA binding proteins. The difference in reactivity between phenolate and phenol is, however, significantly smaller than for the reduction of the species listed in Table 1. At the lowest pH value of 6.0, 4-nitrophenol (mostly in its protonated form) is significantly less reactive than *p*-cresol. This observation is consistent with the expected poorer ability of the former to act as a reducing agent, since the nitro group is a strongly electron-withdrawing substituent.

Energetics of DNA Repair. It is possible to quantify the reactivity of phenolate anions with DNA guanyl radicals only for phenols which are extensively deprotonated over at least

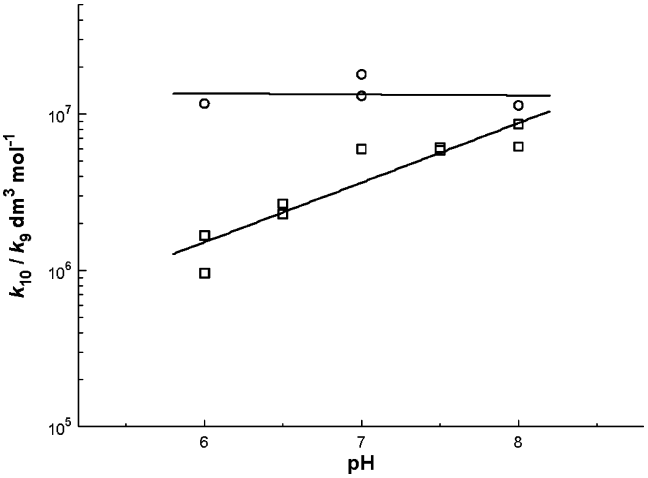

Figure 5. pH dependence of DNA repair. The value of the ratio k_{10}/k_9 is plotted against pH for the phenolic compounds 4-nitrophenol (open square □) and *p*-cresol (open circle ○). The data sets are fitted with least mean square straight lines of the form $y = ce^{-mx}$.

TABLE 3: Acid Dissociation Constants and Reduction Potentials of Selected Phenols

phenol derivative	pK_a (X-PhOH ⇆ X-PhO$^-$ + H$^+$)	$E°$ (X-PhO•/ X-PhO$^-$)/V
4-NO_2	7.14[52]	+1.22[50]
	7.15[53]	+1.28[51]
	7.15[54]	
4-CHO	7.60[53]	+1.14[51]
	7.62[54]	
	7.66[55]	
4-CN	7.95[51]	+1.12[50]
	7.74[56]	+1.14[51]
	7.80[57]	
2,5-Cl_2	7.51[51]	+0.94[51]
2,6-Cl_2	6.80[51]	+0.90[51]
	6.78[55]	

TABLE 4: Acid Dissociation Constants and Reduction Potentials for Guanines and Cytosines

reaction	pK_a	couple	E_7/V
$CH^+ \leftrightarrows C + H^+$	4.3[23]	G(−H)•, H$^+$/G	+1.29 V[11]
$G \leftrightarrows G(-H)^- + H^+$	9.5[23]		
$G^{\bullet+} \leftrightarrows G(-H)\bullet + H^+$	3.9[23,35]		

part of the relatively narrow pH range (about pH 6.0−8.5) over which plasmid DNA is stable on the time scale of several hours that we require for data collection. Depurination becomes significant under more acidic conditions (FPG sensitive sites form in the absence of irradiation because guanine bases are lost from the DNA substrate in an acid-catalyzed reaction), and guanine deprotonation cannot be ignored under more basic conditions. Reduction potential data is available for a limited number of such fairly acidic (pK_a < 8) phenols.[50,51] Examples are listed in Table 3. The corresponding data for guanine and cytosine are listed in Table 4.[11,23,35] From these data, the derived value of the reduction potential for the couple G(−H)•/G(−H)$^-$ is $E = +1.29 - (\log_e(10) \times RT/F) \times (9.5-7.0) = +1.14$ V (where R, T, and F represent the gas constant, the absolute temperature, and the Faraday constant, respectively).

Because of the acidity differences (see Table 4), guanine and its single-electron-oxidized radical species are both largely uncharged over a pH range of about 4−9. So, the reduction of this guanyl radical at physiological pH values involves the transfer of a proton as well as of an electron. While a phenolate anion is able to supply an electron more rapidly than a neutral phenol, it is not able to act as a proton source. It has been argued

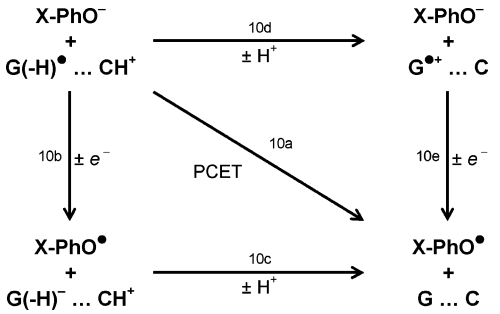

Figure 6. Reaction scheme showing the individual electron and proton transfers involved in the reduction of a deprotonated guanine radical cation G(−H)• by a phenolate anion X-PhO$^-$ (reaction 10 from Figure 1). The proton is assumed to be supplied by the conjugate acid CH$^+$ of the cytosine to which the guanine base is hydrogen bonded. The three pathways shown here are (1) electron and proton transfers coupled together (reaction 10a); (2) electron before proton (reaction 10b and 10c); and (3) proton before electron (reaction 10d and 10e).

TABLE 5: Driving Forces for Individual Proton and Electron Transfers in the Reaction of a Phenolate Anion with a Deprotonated DNA Guanine Radical Cation DNA-G(−H)• (reactions 10a to 10e in Figure 6) Where the Proton Source Is Assumed to Be the Conjugate Acid of the Complementary Cytosine Base

	ΔG/kJ mol^{-1}				
phenolate derivative	10a	10b	10c	10d	10e
4-NO_2	−16	+14	−30	+2	−18
4-CHO	−30	0	−30	+2	−32
4-CN	−30	0	−30	+2	−32
2,5-Cl_2	−49	−19	−30	+2	−51
2,6-Cl_2	−53	−23	−30	+2	−55

that protons present in the hydrogen bonds between complementary bases in DNA are intimately involved in proton-transfer reactions of base radical species.[23] In Figure 6, the reaction between a phenolate anion and the deprotonated guanine radical cation (reaction 10 in Figure 1) is shown in greater detail with the individual proton and the electron transfers resolved into three different pathways: (1) both reactions coupled together (reaction 10a); (2) electron before proton (reaction 10b and 10c); or (3) proton before electron (reaction 10d and 10e). The proton source is the conjugate acid of the cytosine base to which the guanyl radical is hydrogen-bonded.

We can calculate the driving force of the repair reaction (reaction 10) and of the individual electron- and proton-transfer reactions. The driving force for reaction 10b (electron transferred first) can be estimated from the difference in reduction potentials. For 4-nitrophenoxide, the driving force is $\Delta G_{10b} = -F\Delta E = -96.5 \times (+1.14 - 1.28) = +14$ kJ mol^{-1}. The driving force of reaction 10c (proton transfer after the electron transfer) can be estimated from the pK_a difference as $\Delta G_{10c} = -RT \log_e K = \log_e(10) \times RT \times \Delta(pK_a) = 2.3 \times 8.3 \times 298 \times (4.3-9.5) = -30$ kJ mol^{-1}. The driving force for the overall reaction (transfer of both an electron and a proton) is simply the sum of the two individual steps: $\Delta G_{10a} = \Delta G_{10c} + \Delta G_{10b}$. The driving forces for reaction 10d and 10e were estimated in the same way. These values and the corresponding values for the other acidic phenols are listed in Table 5.

There are some uncertainties in these driving force estimates. Disagreements in phenol reduction potential and acid dissociation constant values (Table 3) of up to 0.06 V and 0.2 units correspond to 6 and 1 kJ mol^{-1}, respectively. The values in Table 4 are not strictly applicable to all of the guanine and cytosine residues in DNA. The effects of base sequence can alter reduction potential and acid dissociation constant values

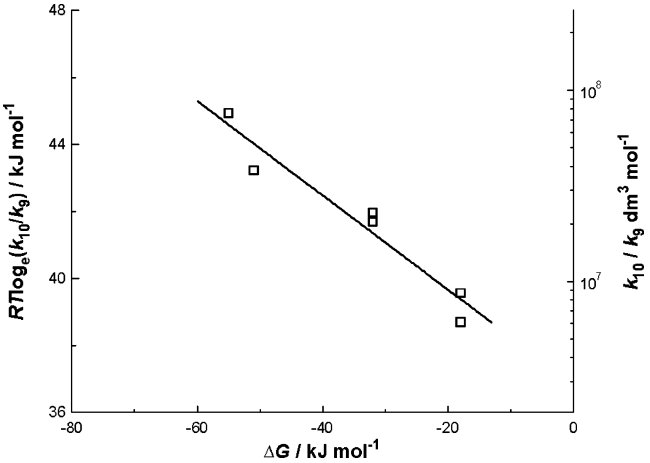

Figure 7. Driving force dependence of the ratio k_{10}/k_9 for the repair of a deprotonated DNA guanine radical cation by phenols. The value of $RT\ \log_e(k_{10}/k_9)$ is plotted against the driving force of reaction 10e (see Figure 6). The data are fitted with a least mean square straight line of the form $y = mx + c$. The value of the slope m is 0.12.

by up to 0.08 V[59] and 1.6 units[60] (equivalent to 8 and 9 kJ mol^{-1}, respectively).

Driving Force Dependence of DNA Repair. It is possible to make use of the relationship between the driving force of an electron-transfer reaction and its rate constant to examine the feasibility of the three steps in Figure 6 which involve the transfer of an electron. These are reaction 10a, in which the electron transfer is coupled to the proton transfer; 10b, in which the electron is transferred before the proton; and 10e, in which the electron is transferred after the proton. The Marcus treatment argues that the relationship can be described in differential form as $\partial(RT\ \log_e k)/\partial\Delta G = {}^1/_2 + (\Delta G/2\lambda)$, where k is the rate constant, ΔG the driving force, and λ is the reorganization energy.[61] Even if λ is unknown, the slope of a plot of $RT\ \log_e k$ against ΔG should still have a slope less steep than $m = {}^1/_2$ for an exoergonic step but steeper than $m = {}^1/_2$ for an endoergonic step.[62,63] According to this relationship, Figure 7 shows the value of $RT\ \log_e(k_{10}/k_9)$ plotted against the driving force for reaction 10e. Only k_{10}/k_9 ratios measured at pH 8.0 (in one case, pH 8.5 for the weakest acid 4-cyanophenol) are plotted in this figure. We assume that the k_{10}/k_9 ratios apply to the phenolate form, which is a reasonable assumption for the most acidic phenols (X = 2,6-Cl_2 and 4-NO_2) but only an approximation for the others. Although we do not have absolute values for k_{10} (only for the ratio k_{10}/k_9), we argue from the *p*-cresol data (Figure 5) that the value of k_9 does not appear to be not strongly dependent on pH over the range we have used.

The value of the slope of the fitted straight line in Figure 7 is $m = 0.12$. The same slope is applicable to reaction 10a, which has a very similar driving force to reaction 10e (difference of only 2 kJ mol^{-1}). This slope is consistent with a reaction that is exoergonic by at least 16 kJ mol^{-1}. It is, however, not nearly steep enough to be consistent with reaction 10b, whose driving force covers the range $\Delta G = -23$ to $+14$ kJ mol^{-1}. Therefore, the possible pathways would appear to be a proton coupled electron transfer or a proton transfer before the electron transfer.

Summary

The deprotonated DNA guanine radical cation is a major intermediate produced by the direct effect of ionizing radiation and other electron-removal processes such as photoionization and photosensitization. The oxidative damage can be repaired by mild reducing agents. An understanding of the mechanisms of DNA damage and its repair involves characterizing this reaction. Acidic phenols that form phenolate anions to a significant extent are particularly reactive with guanyl radicals located in a plasmid DNA substrate. This electron-transfer reaction also requires the transfer of a proton. Our data suggest that the electron transfer is unable to take place without a preceding or coupled proton transfer. Other workers have also drawn attention to this intimate involvement of a proton transfer in the redox reactivity of DNA radical species.[23,48,64]

Acknowledgment. Supported by NIH grant CA46295.

References and Notes

(1) Becker, D.; Sevilla, M. D. *Adv. Radiat. Biol.* **1993**, *17*, 121−180.
(2) Angelov, D.; Spassky, A.; Berger, M.; Cadet, J. *J. Am. Chem. Soc.* **1997**, *119*, 11373−11380.
(3) Burrows, C. J.; Muller, J. G. *Chem. Rev.* **1998**, *98*, 1109−1152.
(4) Melvin, T.; Botchway, S. W.; Parker, A. W.; O'Neill, P. *J. Am. Chem. Soc.* **1996**, *118*, 10031−10036.
(5) Sevilla, M. D.; Besler, B.; Colson, A.-O. *J. Phys. Chem.* **1995**, *99*, 1060−1063.
(6) Seidel, C. A. M.; Schulz, A.; Sauer, M. H. M. *J. Phys. Chem.* **1996**, *100*, 5541−5553.
(7) Melvin, T.; Cuniffe, S. M. T.; O'Neill, P.; Parker, A. W.; Roldan-Arjona, T. *Nucleic Acids Res.* **1998**, *26*, 3935−4942.
(8) O'Neill, P.; Chapman, P. W. *Int. J. Radiat. Biol.* **1985**, *47*, 71−80.
(9) Jovanovic, S. V.; Simic, M. G. *Biochim. Biophys. Acta* **1989**, *1008*, 39−44.
(10) Allard, S.; Masson, J. Y.; Cote, J. *Biochim. Biophys. Acta* **2004**, *1677*, 158−164.
(11) Steenken, S.; Jovanovic, S. V. *J. Am. Chem. Soc.* **1997**, *119*, 617−618.
(12) Milligan, J. R.; Tran, N. Q.; Ly, A.; Ward, J. F. *Biochemistry* **2004**, *43*, 5102−5108.
(13) Hubatsch, I.; Mannervik, B. *Biochem. Biophys. Res. Commun.* **2001**, *280*, 878−882.
(14) Ibarra, C. A.; Chowdhury, P.; Petrich, J. W.; Atkins, W. M. *J. Biol. Chem.* **2003**, *278*, 19257−19265.
(15) Sun, X.; Sun, H.; Ge, R.; Richter, M.; Woodworth, R. C.; Mason, A. B.; He, Q. Y. *FEBS Lett.* **2004**, *573*, 181−185.
(16) Wright, C.; Im, S.-C.; Twitchett, M. B.; Saysell, C. G.; Sokolwski, A.; Sykes, A. G. *Inorg. Chem.* **2001**, *40*, 294−300.
(17) Sackett, D. L.; Rubinov, S. B.; Thompson, J. *Protein Sci.* **1999**, *8*, 2121−2129.
(18) Liu, Y.; Thoden, J. B.; Kim, J.; Berger, E.; Gulick, A. M.; Ruzicka, F. J.; Holden, H. M.; Frey, P. A. *Biochemistry* **1997**, *36*, 10675−10684.
(19) Stanbury, D. M. *Adv. Inorg. Chem.* **1989**, *33*, 69−138.
(20) Wardman, P. *J. Phys. Chem. Ref. Data* **1989**, *18*, 1637−1755.
(21) Milligan, J. R.; Aguilera, J. A.; Ward, J. F. *Int. J. Radiat. Biol.* **2001**, *77*, 1195−1205.
(22) Steenken, S. *Chem. Rev.* **1989**, *89*, 503−520.
(23) Steenken, S. *Free Radical Res. Commun.* **1992**, *16*, 349−379.
(24) Milligan, J. R.; Aguilera, J. A.; Hoang, O.; Ly, A.; Tran, N. Q.; Ward J. F. *J. Am. Chem. Soc.* **2004**, *126*, 1682−1687.
(25) Holton, D. M.; Murphy, D. *J. Chem. Soc., Faraday Trans. 2* **1978**, *75*, 1637−1642.
(26) Alfassi, Z. B.; Schuler, R. H. *J. Phys. Chem.* **1985**, *89*, 3359−3363.
(27) Alfassi, Z. B.; Huie, R. E.; Neta, P.; Shoute, L. C. T. *J. Phys. Chem.* **1990**, *94*, 8800−8805.
(28) Kemsley, K.; Moore, J. S.; Phillips, G. O.; Sosnowski, A. *Acta Vitaminol. Enzymol.* **1974**, *28*, 263−267.
(29) Milligan, J. R.; Aguilera, J. A.; Ward, J. F. *Radiat. Res.* **1993**, *133*, 151−157.
(30) Spinks, J. W. T.; Woods, R. J. *An Introduction to Radiation Chemistry*, 2nd ed.; Wiley: New York, 1976.
(31) Milligan, J. R.; Aguilera, J. A.; Paglinawan, R. A.; Ward, J. F. *Int. J. Radiat. Biol.* **2000**, *76*, 1305−1314.
(32) Fuciarelli, A. F.; Wegher, B. J.; Blakely, W. F.; Dizdaroglu, M. *Int. J. Radiat. Biol.* **1990**, *58*, 397−415.
(33) Douki, T.; Martini, R.; Ravanat, J.-L.; Turesky, R. J.; Cadet, J. *Carcinogenesis* **1997**, *18*, 2385−2391.
(34) Neta, P.; Huie, R. E.; Ross, A. B. *J. Phys. Chem. Ref. Data* **1988**, *17*, 1027−1284.
(35) Candeias, L. P.; Steenken, S. *Chem. Eur. J.* **2000**, *6*, 475−484.
(36) Kobayashi, K.; Tagawa, S. *J. Am. Chem. Soc.* **2003**, *125*, 10213−10218.
(37) Giese, B. *Acc. Chem. Res.* **2000**, *33*, 631−636.

(38) Willson, R. L.; Wardman, P.; Asmus, K.-D. *Nature* **1974**, *252*, 323−324.
(39) Milligan, J. R.; Aguilera, J. A.; Mares, E. J.; Paglinawan, R. A.; Ward, J. F. *Int. J. Radiat. Biol.* **2001**, *77*, 1095−1108.
(40) Kavnah, S.; Schuster, G. B. *J. Am. Chem. Soc.* **2002**, *124*, 11286−11287.
(41) Krokan, H. E.; Standal, R.; Slupphaug, G. *Biochem. J.* **1997**, *325*, 1−16.
(42) Stivers, J. T.; Jiang, Y. L. *Chem. Rev.* **2003**, *103*, 2729−2759.
(43) Tretyakova, N. Y.; Wishnok, J. S.; Tannenbaum, S. R. *Chem. Res. Toxicol.* **2000**, *13*, 658−664.
(44) Duarte, V.; Gasparutto, D.; Jaquinod, M.; Ravanat, J. L.; Cadet, J. *Chem. Res. Toxicol.* **2001**, *14*, 46−53.
(45) Cullis, P. M.; Malone, M. E.; Merson-Davies, L. A. *J. Am. Chem. Soc.* **1996**, *118*, 2775−2781.
(46) Buxton, G. V.; Greenstock, C. L.; Helman, W. P.; Ross, A. B. *J. Phys. Chem. Ref. Data* **1988**, *17*, 513−887.
(47) Hildenbrand, K.; Schulte-Frohlinde, D. *Free Radical Res. Commun.* **1990**, *11*, 195−206.
(48) Shafirovich, V., Dourandin, A., Huang, W., Geacintov, N. E. *J. Biol. Chem.* **2001**, *276*, 24621−24626.
(49) Liptak, M. D.; Gross, K. C.; Seybold, P. G.; Feldgus, S.; Shields, G. C. *J. Am. Chem. Soc.* **2002**, *124*, 6421−6427.
(50) Lind, J.; Shen, X.; Eriksen, T. E.; Merenyi, G. *J. Am. Chem. Soc.* **1990**, *112*, 479−482.
(51) Li, C.; Hoffman, M. Z. *J. Phys. Chem. B* **1999**, *103*, 6653−6656.
(52) Ba−Saif, S. A.; Davis, A. M.; Williams, A. J. *Org. Chem.* **1989**, *54*, 5483−5486.
(53) Stradins, J.; Hasanli, B. J. *Electroanal. Chem.* **1993**, *353*, 57−69.
(54) *Dean's Handbook of Organic Chemistry*, 2nd ed.; Gokel, G. W., Ed.; McGraw-Hill: New York, 2004.
(55) Hunter, A.; Renfrew, M.; Rettura, D.; Taylor, J. A.; Whitmore, J. M. J.; Williams, A. *J. Am. Chem. Soc.* **1995**, *117*, 5484−5491.
(56) Schulman, S. G.; Vincent, W. R.; Underberg, W. J. M. *J. Phys. Chem.* **1981**, *85*, 4068−4071.
(57) Stefanidis, D.; Cho, S.; Dhe-Paganon, S.; Jencks, W. P. *J. Am. Chem. Soc.* **1993**, *115*, 1650−1656.
(58) Ly, A.; Tran, N. Q.; Ward, J. F.; Milligan, J. R. *Biochemistry* **2004**, *43*, 9098−9104.
(59) Lewis, F. D.; Liu, X.; Liu, J.; Hayes, R. T.; Wasielewski, M. R. *J. Am. Chem. Soc.* **2000**, *122*, 12037−12038.
(60) Acharya, S.; Barman, J.; Cheruku, P.; Chatterjee, S.; Acharya, P.; Isaksson, J.; Chattopadhyaya, J. *J. Am. Chem. Soc.* **2004**, *126*, 8674−8681.
(61) Marcus, R. A.; Sutin, N. *Biochim. Biophys. Acta* **1985**, *811*, 265−322.
(62) Graige, M. S.; Paddock, M. L.; Bruce. J. M.; Feher, G.; Okamura, M. Y. *J. Am. Chem. Soc.* **1996**, *118*, 9005−9016.
(63) Laranjeira, M. C. M.; Marusak, R. A.; Lappin, A. G. *Inorg. Chem.* **2000**, *300−302*, 186−190.
(64) Debije, M. G.; Bernhard, W. A. *J. Phys. Chem. B* **2000**, *104*, 7845−7851.