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Repair of Guanyl Radicals in Plasmid DNA by Electron Transfer Is Coupled to Proton Transfer

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Abstract: By using γ-irradiation in the presence of thiocyanate ions, we have generated guanyl radicals in plasmid DNA. These can be detected by using an *Escherichia coli* base excision repair endonuclease to convert their stable end products to strand breaks. The yield of enzyme-sensitive sites is strongly attenuated by the presence of micromolar concentrations of one of a series of singly substituted phenols, and it is possible to derive bimolecular rate constants for the reduction of DNA guanyl radicals by these phenols. More strongly reducing phenols were found to react more rapidly. This electron-transfer reaction also involves a proton transfer. By comparing the expected energetics of the reaction with the observed rate constants, the electron transfer is found to be mechanistically coupled with the proton transfer.

Introduction

One-electron oxidation of compounds tends to increase their acidity significantly. Proton loss from the product, therefore, makes the driving force for the reaction more favorable. But individual electron and proton transfers frequently involve an unfavorable first step. Initial electron transfer produces a strongly acidic intermediate, and initial proton transfer produces a strongly reducing species. The resulting kinetic barriers can be large (see Results and Discussion section). This often results in the fastest available route for the reaction being the concerted transfer of both an electron and a proton, also referred to as a proton-coupled electron transfer (PCET). Most analyses of PCET employ inorganic reagents,[1,2] but one important organic substrate is the nucleic acid base guanine.

Electron-transfer reactions are common in normal biology. They are also involved in pathological processes. For example, electron removal results in the DNA damage produced by ionizing radiation,[3] photoionization,[4,5] chemical oxidation,[6] and photosensitization.[7,8] Refilling the vacancy offers a simple and effective route to repairing or preventing this DNA damage. Characterizing the mechanism of this reaction is essential before rational efforts can be made to influence the rate or extent to which it takes place.

Guanine is the most easily oxidized component in DNA.[9,10] Even at cryogenic temperatures, the sites of electron loss from DNA are located mostly on guanine bases.[3] At more biologically relevant temperatures in liquid aqueous solutions, the principal one-electron-oxidized intermediate is a guanyl radical.[6,7] If a guanine base is involved in a base pair with cytosine, then the radical species derived from it tends to be significantly less reactive. The main product derived from an unpaired guanyl radical is 2,2,4-triamino-5-(2*H*)-oxazolone,[11] but 8-oxo-7,8-dihydroguanine is the major product from base-paired guanine.[12] These differences are probably a result of partial protonation of the base-paired radical.[13] Therefore, the issue of coupling proton transfer to electron transfer is particularly important for DNA.

Electron removal from guanine proceeds by a PCET mechanism.[14,15] This also applies when guanine is base-paired in a double-stranded oligonucleotide.[16−18] Here we examine the reduction of guanyl radicals located in a biologically active plasmid DNA substrate. We have examined the energetics and mechanism of electron transfer in the repair of guanyl radicals in plasmid DNA by using a series of phenols. The values of the bimolecular rate constants and their dependence on the

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phenol reduction potential imply that the electron transfer is coupled to the transfer of a proton.

Experimental Section

Plasmid Substrate. A sample of plasmid pHAZE (10 327 base pairs)[19] was generously supplied 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.[20]

Base Excision Repair Endonuclease. An expression vector containing formamidopyrimidine−DNA *N*-glycosylase from *Escherichia coli* (FPG) was generously provided by Dr. Y. W. Kow, Department of Radiation Oncology, Emory University. The enzyme was overexpressed, isolated, and purified as described previously.[21]

Irradiation. The plasmid substrate was γ-irradiated in aerobic aqueous solution with an AECL GammaCell-1000 isotopic device (cesium-137, 662 keV γ-ray photon). These solutions contained pHAZE (25 μg mL^{-1}, equivalent to 7.7×10^{-5} mol dm^{-3} nucleotide residues or 3.7×10^{-9} mol dm^{-3} plasmid), sodium phosphate (5×10^{-3} mol dm^{-3}, pH 7.0), sodium thiocyanate (10^{-3} mol dm^{-3}), sodium perchlorate (1.1×10^{-1} mol dm^{-3}), and a phenolic compound (10^{-7} to 10^{-4} mol dm^{-3}). The phenol was one of the following: 4-aminophenol, catechol, *p*-cresol, 4-cyanophenol, hydroquinone, 4′-hydroxyacetophenone, 4-methoxyphenol, or resorcinol. Each aliquot was 27 μL. The dose rate of 335 rad min^{-1} (5.58×10^{-2} Gy s^{-1}) was quantified by means of the Fricke method.

Enzyme Incubation. After irradiation, each 27 μL aliquot was mixed with 3 μL of a solution containing FPG, such that the final FPG concentration was either 0 or 3 μg mL^{-1}. This corresponds to a final activity of 30 units per mL, where a unit is defined as the formation of 1 pmol single strand breaks (SSB) from abasic sites after 60 min incubation at 37 °C. The resulting solutions were incubated at 37 °C for 30 min and then assayed electrophoretically.

Determination of Strand Break Yields. After incubation, the yield of SSB was quantified after agarose gel electrophoresis. The procedures for digital video imaging of ethidium fluorescence and for calculating the radiation chemical yield (or *G* value, with units of mol J^{-1}) for SSB formation have been described previously.[20] Briefly, the D_0 dose (dose required to reduce the fraction of SSB free plasmid by a factor of *e*, which is equivalent to introducing 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 SSB product is equal to the concentration of the plasmid substrate (3.7×10^{-9} mol dm^{-3} plasmid), and the *G* value for SSB formation (whose units are mol J^{-1}) is calculated by dividing this concentration by the value of D_0.

Results and Discussion

Reaction Scheme. We have previously reported on the mechanism of DNA damage by γ-irradiation of solutions containing thiocyanate ions.[22] This is summarized by the reaction scheme in Figure 1. Radiolysis of aqueous thiocyanate produces the species $(SCN)_2^{\bullet-}$ (reactions 1 and 2). This strong single-electron-oxidizing agent is capable of removing electrons from guanine bases in plasmid DNA to produce guanyl radicals DNA-G$^{\bullet+}$ (reaction 3). Trapping of this intermediate by water produces mainly 8-oxo-7,8-dihydroguanine residues DNA-8oxoG (reactions 5 and 7) via an intermediate 8-hydroxy-7,8-dihydroguanyl radical DNA-$^{\bullet}$GOH. The stable 8-oxo-7,8-dihydroguanine bases are detected after conversion to SSB by a subsequent incubation with FPG (reaction 9). Back-donation of an electron to DNA-G$^{\bullet+}$ and/or its conjugate base DNA-G(-H)$^{\bullet}$ by a reducing agent *R* leads to the repair of this oxidative damage (reaction 4). It is possible that the presence of a reducing agent might also alter the product distribution from DNA-$^{\bullet}$GOH in favor of 2,6-diamino-4-hydroxy-5-formamido-pyrimidine residues DNA-FaPyG, which are also recognized by FPG (reaction 10). Some additional breaks (reactions 11 and 12) and additional DNA-8oxoG residues (reactions 6 and 7) are also produced by a thiocyanate-independent route involving the reaction of $^{\bullet}$OH with the plasmid. Superoxide is also produced by aerobic γ-irradiation of aqueous solutions, but the ineffectiveness of superoxide dismutase in this system suggests that reactions involving superoxide can safely be omitted from the scheme.[22]

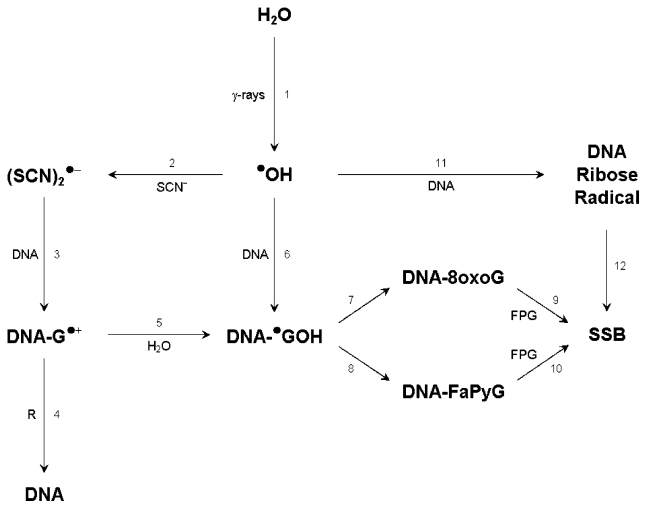

Figure 1. Reaction scheme summarizing the mechanism for the introduction and repair of oxidative DNA damage in the experimental system.

Break Yields. The formation of strand breaks can be quantified. Four examples of strand break yield measurements are depicted in Figure 2. Aerobic solutions containing plasmid pHAZE, thiocyanate ions, and *p*-cresol were subjected to γ-irradiation. Introduction of SSB into the plasmid is detected by the decrease of the fraction of its supercoiled (SSB free) form. Post irradiation incubation of the plasmid with the base excision repair endonuclease FPG introduces SSB with approximately 10-fold greater frequency. Lower *p*-cresol concentrations result in higher frequencies. The radiation chemical yield (or *G* value) of the SSB can be calculated from the slopes of the straight lines fitted to yield dose plots such as that in Figure 2 (see Experimental Section). The SSB yield (hereafter *G*(SSB)) was determined over a wide range of *p*-cresol concentrations.

Attenuation by Phenols. Figure 3 shows the dependence of *G*(SSB) as a function of the *p*-cresol concentration after incubation in both the presence and absence of FPG. In the absence of FPG, *G*(SSB) remains approximately constant at 2×10^{-4} μmol J^{-1}. After FPG treatment, *G*(SSB) values are strongly dependent on the *p*-cresol concentration. The value of *G*(SSB) observed for FPG incubation after irradiation in the absence of *p*-cresol is 4.83×10^{-2} μmol J^{-1}. This value is significantly smaller than the yield of the oxidizing agent $(SCN)_2^{\bullet-}$, which is about 0.3 μmol J^{-1} for 10^{-3} mol dm^{-3}

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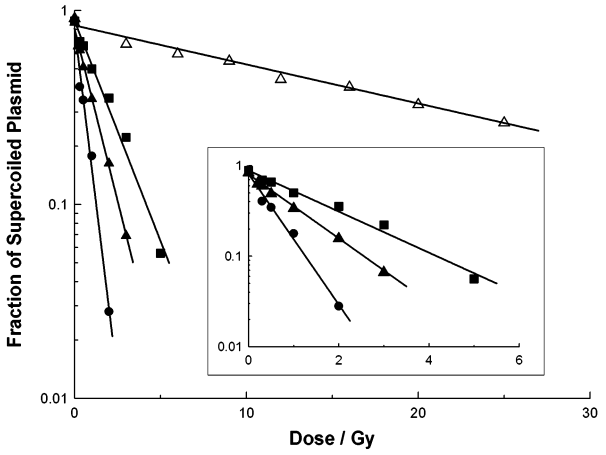

Figure 2. Loss of supercoiled plasmid with increasing dose of γ-radiation (see Experimental Section). Aliquots of a solution containing the phenol *p*-cresol at a concentration of 0.5 μmol dm^{-3} (●), 1 μmol dm^{-3} (△, ▲), or 1.5 μmol dm^{-3} (■) were γ-irradiated. After irradiation, the solutions were incubated with (●, ▲, ■) or without (△) FPG. The mole fraction of supercoiled plasmid remaining was plotted against the radiation dose (in the insert, the *x*-axis was expanded by 5-fold). The data sets were fitted with least mean square straight lines of the form $y = ce^{-mx}$. From the slopes *m* of these fitted lines, the D_0 doses and SSB yields for the four irradiation conditions were: (△) 21.6 Gy, 1.72×10^{-4} μmol J^{-1}; (●) 0.625 Gy, 5.96×10^{-3} μmol J^{-1}; (▲) 1.23 Gy, 3.04×10^{-3} μmol J^{-1}; and (■) 1.91 Gy, 1.95×10^{-3} μmol J^{-1}.

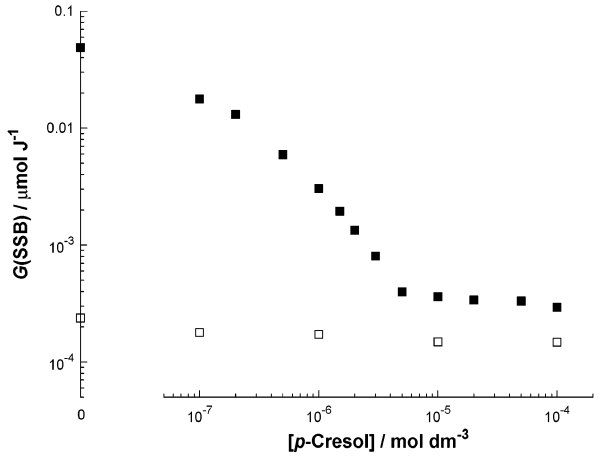

Figure 3. Effect of *p*-cresol concentration on the SSB yield. The SSB yield was determined by the method shown in Figure 2. After irradiation, but before electrophoresis, the plasmid was incubated under one of two conditions: in the absence of FPG (□) or in the presence of 3 μg mL^{-1} FPG (■).

thiocyanate. We have previously argued that under these conditions, the majority of $(SCN)_2^{\bullet-}$ reacts by disproportionation.[22]

Nature of FPG-Sensitive Sites. Under the conditions we employ here, two different types of DNA damage can be detected and their yield quantified. After incubation in the absence of FPG, the SSBs derive from the reaction of the plasmid with hydroxyl radicals (•OH, reactions 11 and 12).[20] The yields of these breaks are represented by the open symbols in Figure 3. The yield of 2×10^{-4} μmol J^{-1} is unaffected by *p*-cresol (at concentrations up to 10^{-4} mol dm^{-3}) because the latter makes no significant contribution to •OH scavenging in the presence of 10^{-3} mol dm^{-3} thiocyanate.[23]

After FPG incubation, the SSB yield increases by a factor of 2−200 (closed symbols in Figure 3). In the absence of *p*-cresol, these breaks are formed by reaction of FPG mainly with 8-oxo-7,8-dihydroguanine residues (DNA-8oxoG) in the plasmid (reaction 9). This is because 8-oxo-7,8-dihydroguanine is the major product derived from single-electron oxidation of guanine in double-stranded DNA,[5,12,24,25] and it is a substrate for FPG.[26−28] The action of FPG is to both remove 8-oxo-7,8-dihydroguanine and subsequently create a SSB (by a β,δ-elimination reaction) at the resulting abasic site.[29,30] These 8-oxo-7,8-dihydroguanine residues are produced via the 8-hydroxy-7,8-dihydroguan-7-yl radical (DNA-•GOH) by the action of the oxidizing agent $(SCN)_2^{\bullet-}$, itself derived from thiocyanate scavenging of •OH (reaction 2)[31] produced by water radiolysis (reaction 1). Strand breaks are not produced by $(SCN)_2^{\bullet-}$, but the reaction of •OH with plasmid DNA does produce the 8-hydroxy-7,8-dihydroguan-7-yl radical (and 8-oxo-7,8-dihydroguanine, the major product derived form it) (reactions 6−8) with a yield of the same order as the SSB yield (reactions 11 and 12).

An additional product formed from the DNA-•GOH is 2,6-diamino-4-hydroxy-5-formamidopyrimidine (DNA-FaPyG, in which the five-membered ring of the purine is opened). Because this reaction involves a one-electron reduction, it is possible that it might dominate (even under aerobic conditions) in the presence of a reducing agent such as *p*-cresol. Since the 2,6-diamino-4-hydroxy-5-formamidopyrimidine residue is also a substrate for FPG,[28] we are unable to distinguish between DNA-8oxoG and DNA-FaPyG, both of which are derived from the same intermediate, DNA-•GOH.[32]

The extensive attenuation of the yield of FPG-sensitive sites that is caused by *p*-cresol (Figure 3) cannot be explained by a change in the product distribution between DNA-8oxoG and DNA-FaPyG. The *p*-cresol (or in general the reducing agent *R*) must act at an earlier stage. We have previously argued that the attenuation does not result from scavenging of the oxidant $(SCN)_2^{\bullet-}$. This is because of (1) the lack of correlation of the attenuation produced by different reducing agents with their reactivities with $(SCN)_2^{\bullet-}$,[33] (2) the equal attenuations observed for a single reducing agent with different single-electron oxidants ($Br_2^{\bullet-}$, $SeO_3^{\bullet-}$, and $Tl^{II}OH^+$) which is suggestive of a common intermediate,[33−35] and (3) in the case of the cationic oxidant $Tl^{II}OH^+$, ionic strength effects suggest that the reducing agent reacts with a negatively charged species[35] such as the polyanionic plasmid.

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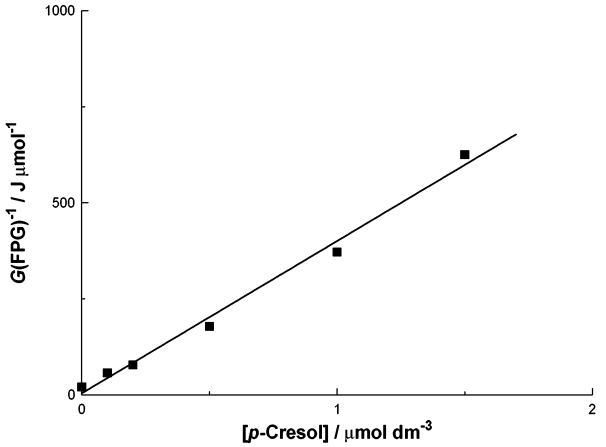

Figure 4. Effect of *p*-cresol concentration on the SSB yield. The reciprocal of the yield of FPG-sensitive sites, *G*(FPG), was plotted against the concentration of *p*-cresol according to competition kinetics. The yield of FPG-sensitive sites did not include the contribution made by •OH (see text). The data set was fitted with a least mean square straight line of the form $y = mx + c$. The slope m of this line was 3.97×10^{14} J dm^3 mol^{-2}.

The precursor of the DNA-•GOH is the guanine radical cation DNA-G•$^+$, but the conjugate base of the latter, DNA-G(-H)•, gives rise to quite different products.[5,6,12,25] The pK_a of the radical cation of deoxyguanosine is 3.9,[6] but in double-stranded DNA its deprotonation may be inhibited by the base-paired cytosine (pK_a of the *N*-3 protonated conjugate acid of cytosine is 4.3).[6] Recent evidence suggests that guanyl radicals are at least partially deprotonated in double-stranded oligonucleotides.[36] Therefore, we assume that the attenuation in the yield of FPG-sensitive sites produced by *p*-cresol results from the reduction of the DNA guanyl radical DNA-G•$^+$ or of its conjugate base DNA-G(-H)•. Many other mild reducing agents have the same effect on guanyl radicals.[37,38] This reaction is of some interest, because it represents the chemical repair of DNA damage produced by oxidizing agents or by ionizing radiation.

Rate Constant for Phenol Repair. The data from Figure 3 are re-plotted in Figure 4 according to competition kinetics. Here, the yield of FPG-sensitive sites (hereafter *G*(FPG)) has been estimated by subtracting the contribution made by •OH. From the slope and intercept of the straight line fitted to the data in Figure 4, it is possible to quantify the competition between the repair (by *p*-cresol) of a guanyl radical located in plasmid DNA (reaction 4) and its irreversible trapping (reaction 5). Because estimates for the rate constant k_5 of the trapping reaction are available in the literature, we can arrive at an estimate for the rate constant k_4 of the repair reaction for a given reducing agent *R*.

The competition can be quantified by eq 1:

$$\frac{1}{G(\mathrm{FPG})} = \left(\frac{1}{G_0(\mathrm{FPG})}\right)\left(1 + \frac{k_4[R]}{k_5}\right) \qquad (1)$$

In eq 1, *G*(FPG) and G_0(FPG) are, respectively, the yield of FPG-sensitive sites in the presence and absence of *R*. The contribution of •OH to the yield of FPG-sensitive sites was removed by subtracting the value of *G*(SSB) observed in the presence of excess *p*-cresol (4×10^{-4} μmol J^{-1}). Equation 1 implies that *G*(FPG)$^{-1}$ should be linearly dependent on the concentration of *R*. Figure 4 shows that this is the case when *R* = *p*-cresol. The value of k_4 is equal to mk_5/c, where m and c are, respectively, the slope and intercept of the fitted straight line in Figure 4. In practice, the intercept was estimated from Figure 3 as $c = 1/(4.83 \times 10^{-2}) = 20.7$ J μmol^{-1}. Assuming that $k_5 = 0.2$ s^{-1},[18,39] the value of k_4 obtained from Figure 3 is $3.97 \times 10^8 \times (0.2/20.7) = 3.8 \times 10^6$ dm^3 mol^{-1} s^{-1}.

This procedure was repeated for seven other phenols. These phenols and their k_4 values are listed in Table 1. Values of the single-electron reduction potentials at pH 7 (E_7) are also included in Table 1.[40−42]

Table 1. Reduction Potentials[40−42] and Rate Constants (this work) for the Reduction of a Guanyl Radical in Plasmid DNA by Selected Phenols

phenol	E_7/V	k_4/dm^3 mol^{-1} s^{-1}
4-NH_2	+0.41	4.7×10^7
4-OH (hydroquinone)	+0.46	2.1×10^7
2-OH (catechol)	+0.53	1.4×10^7
4-OCH_3	+0.72	1.8×10^7
3-OH (resorcinol)	+0.81	7.8×10^6
4-CH_3 (*p*-cresol)	+0.87	3.8×10^6
4-$COCH_3$	+1.06	1.6×10^6
4-CN	+1.17	7.3×10^5

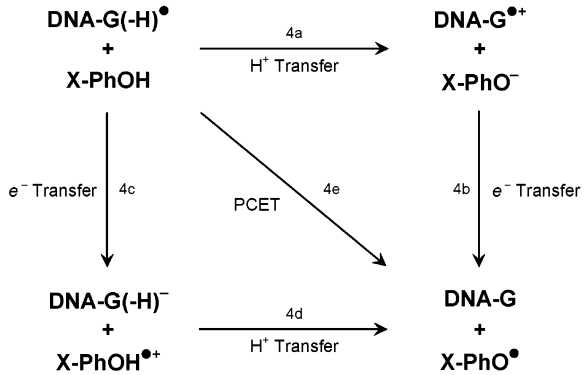

Figure 5. The individual proton and electron transfers involved in reaction 4 were depicted here in the form of a thermodynamic cycle for the case where the reducing agent *R* was a phenolic compound. The horizontal reactions were proton transfers, the vertical reactions electron transfers, and the diagonal reaction represented a PCET. Three possible mechanisms for reaction 4 were proton transfer followed by electron transfer (reactions 4a and 4b), electron transfer followed by proton transfer (reactions 4c and 4d), or both coupled together (reaction 4e).

Energetics of Repair by Phenols. The reduction of the DNA guanyl radical by a phenolic compound (reaction 4) is shown in greater mechanistic detail in Figure 5. At pH 7, the overall reaction 4e involves the transfer of a proton as well as an electron.

$$\text{DNA-G(-H)}^\bullet + p\text{-MeC}_6\text{H}_4\text{OH} \rightarrow \text{DNA-G} + p\text{-MeC}_6\text{H}_4\text{O}^\bullet \qquad (4e)$$

A proton is also transferred because of the acidities of the reactants and products. Single-electron oxidation products are,

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Table 2. Ionization Constants and Reduction Potentials for Guanosine, Plasmid DNA, and *p*-Cresol

reaction			pK_a	reference
G	⇆	$G(-H)^- + H^+$	9.5	43
$G^{\bullet+}$	⇆	$G(-H)^{\bullet} + H^+$	3.9	43
p-MeC_6H_4OH	⇆	p-$MeC_6H_4O^- + H^+$	10.19	50
p-$MeC_6H_4OH^{\bullet+}$	⇆	p-$MeC_6H_4O^{\bullet} + H^+$	−1.6	47

couple			E_7/V	reference
$G(-H)^{\bullet}$, H^+	/	G	+1.29	49
DNA-$G(-H)^{\bullet}$, H^+	/	DNA-G	+1.39	56
p-$MeC_6H_4O^{\bullet}$, H^+	/	p-MeC_6H_4OH	+0.87	40

Table 3. Driving Forces for the Individual Reactions in Figure 6 for the Eight Phenols Examined in This Study

	ΔG/kJ mol^{-1}				
phenol	4a	4b	4c	4d	4e
4-NH_2	37	−121	−22	−62	−84
4-OH (hydroquinone)	35	−115	−18	−62	−80
2-OH (catechol)	32	−105	−10	−63	−73
4-OCH_3	36	−91	7	−62	−55
3-OH (resorcinol)	32	−78	20	−67	−46
4-CH_3 (*p*-cresol)	36	−76	23	−63	−41
4-$COCH_3$	24	−46	42	−65	−22
4-CN	23	−35	53	−64	−12

in general, significantly more acidic than the parent compounds.[13,43] Literature ionization constants and reduction potentials for guanosine and *p*-cresol (and radicals derived from them) are listed in Table 2. From these values, it is possible to calculate the driving forces for each of the five reactions (4a−4e) in Figure 5. These calculations assume that the pK_a and E_7 values for the monomer guanosine are applicable to 2′-deoxyguanosine residues in plasmid DNA. This assumption is not strictly valid because there is evidence that multiple neighboring guanines in DNA are particularly easily oxidized.[6,44]

Driving forces for the proton-transfer steps are derived from differences in pK_a values. For example, the driving force for reaction 4a is equal to $\Delta G = \log_e 10 \times RT \times (10.19 - 3.9) = +36$ kJ mol^{-1} (where R represents the gas constant, and T represents the absolute temperature). The driving force for the overall reaction 4e is derived from differences in E_7 values: $\Delta G = -F(1.29 - 0.87) = -41$ kJ mol^{-1} (where F represents the Faraday constant). Driving forces for the electron-transfer steps are also derived from differences in reduction potentials, after allowing for their pH dependence.[45,46] For example, the reduction potentials applicable to reaction 4b are E(DNA-$G^{\bullet+}$/DNA-G) $= +1.29 + (7 - 3.9) \times RT/F = +1.47$ V and E(p-$MeC_6H_4O^{\bullet}$/p-$MeC_6H_4O^-$) $= +0.87 + (7 - 10.19) \times RT/F = +0.68$ V. Using these values, we found that $\Delta G = -F(1.47 - 0.68) = -76$ kJ mol^{-1}. Table 3 contains ΔG values calculated in this manner for all of the eight phenols used in this study.[40,42,47−50] Electrostatic work contributions[51] to ΔG have been ignored. We estimate that they are on the order of 2 kJ mol^{-1}. This effect is small because of (1) the high static dielectric constant of water, (2) the relatively high physiological ionic strength, and (3) the relatively large diffuse π-systems of the species involved.

Driving Force Dependence of Repair. Conclusions can be drawn about the detailed mechanism of reaction 4 by comparing the energetics summarized in Table 3 with the experimental data in Table 1.

For example, the proton transfer step (reaction 4a) of the proton first route is strongly endoergonic, and its contribution to the experimentally determined value of k_4 can be shown to be negligibly slow. A ΔG value of +36 kJ mol^{-1} corresponds to an equilibrium constant of $K_{4a} = 10^{-6.3}$. This equilibrium constant is equal to the ratio of the rate constants of the forward and reverse reactions, $K_{4a} = k_{4a}/k_{-4a}$. If the reverse reaction is assumed to be diffusion-controlled, ($k_{-4a} \leq 10^{11}$ dm^3 mol^{-1} s^{-1}), then the upper limit for k_{4a} is $10^{-6.3} \times 10^{11} = 10^{4.7} = 5 \times 10^4$ dm^3 mol^{-1} s^{-1}. This estimate is significantly lower than the observed value of $k_4 = 3.6 \times 10^6$ dm^3 mol^{-1} s^{-1} for *p*-cresol (Table 1) and argues strongly against a major contribution by the proton first route. This argument also applies to the other phenols.

The electron-transfer step (reaction 4c) of the electron first route is endoergonic, except for phenols bearing strongly electron-donating substituents such as $-NH_2$ or $-OH$. In contrast, the PCET route (reaction 4e) is in all cases exoergonic. The driving force dependence of the rate constant provides a means to distinguish between these two mechanisms. The Marcus theory[51] predicts that for an electron-transfer reaction, the variation of the rate constant k with the driving force ΔG is $\partial(RT \log_e k)/\partial(\Delta G) = 1/2(1 + \Delta G/\lambda)$. Therefore, irrespective of the value of the reorganization energy λ, the slope of a plot of $RT \log_e k$ against ΔG should be steeper than 0.5 for endoergonic reactions (positive ΔG) and less steep than 0.5 for exoergonic reactions (negative ΔG).[52,53]

Figure 6 shows the experimentally derived values of k_4 plotted in this fashion (as $RT \log_e k_4$) against the driving force ΔG of the PCET reaction (reaction 4e). The magnitude of the slope of the straight line fitted to the data is $m = 0.17$ (because the driving force of reaction 4d is very weakly dependent on the phenol substituent, the same value of the slope also applies when $RT \log_e k_4$ is plotted against the driving force of reaction 4c, the electron-transfer step of the electron first route). It is possible that the fastest rate constants are close to the diffusion-controlled limit, which may be significantly smaller than the normal bimolecular value of 10^{10} to 10^{11} dm^3 mol^{-1} s^{-1} when one of the reactants is a macromolecule such as a plasmid (10 327 base pairs is equivalent to a molecular weight of 6.7×10^6 g mol^{-1}). A representative example of a difference in reactivity of 2 orders of magnitude would be the slower reaction of mercaptoethanol with carbon-centered radicals in plasmid DNA[54] compared with its reaction with radicals derived from simple alcohols.[55] Since

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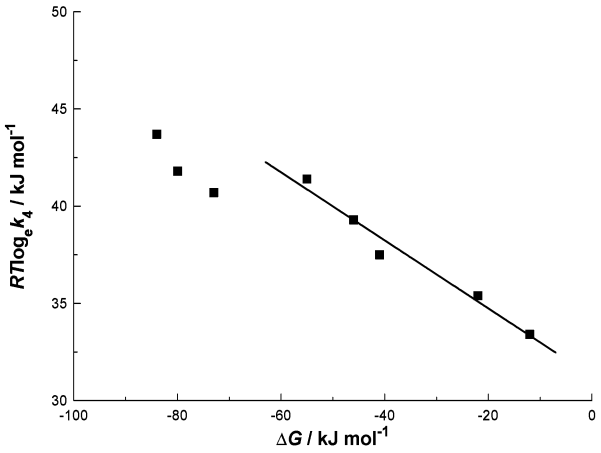

Figure 6. Driving force dependence of the rate constant k_4 for repair of the DNA guanyl radical by phenols. The value of $RT \log_e k_4$ (from Table 1) was plotted against the driving force of the PCET (reaction 4e). The data for $\Delta G > -60$ kJ mol^{-1} were fitted with a least mean square straight line of the form $y = mx + c$. The value of the slope m of this line was -0.17.

these values might artifactually decrease the slope, they were excluded. The relatively mild driving force dependence (slope of only 0.17) argues against the endoergonic electron transfer of the electron first route and in favor of the exoergonic PCET mechanism. It is possible that a PCET mechanism is favorable for double-stranded DNA since available protons exist in the hydrogen bonds between complementary strands.

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Comparison with Other Studies. The issue of the coupling of proton and electron transfer for guanine in mono- and oligonucleotide environments has been addressed by other workers using different experimental approaches. A solvent isotope effect has been observed for the electron transfer between guanosine monophosphate and 2-aminopurine monomers and between these bases in double-stranded oligonucleotide using time-resolved spectrophotometry.[15,18] Driving force effects and solvent isotope effects have also been examined by electrochemical methods in monomers, oligonucleotides, and genomic DNA.[16,17] All of these studies suggest that electron-transfer reactions involving guanine are coupled to proton transfer. Here, we provided evidence that the same is true of a biologically active plasmid substrate.

Summary

We have argued that the repair of guanyl radicals in plasmid DNA by phenols takes place by a proton-coupled electron-transfer reaction. The extensive thermodynamic data available for phenols permit calculation of the energetics of the individual electron- and proton-transfer steps. The data in Table 3 clearly show that transfer of a proton on the same time scale as the electron transfer offers a significant improvement in the driving force for the redox reaction.

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