Peptide Repair of Oxidative DNA Damage†

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ABSTRACT: Guanyl radical species are produced in DNA by electron removal caused by ionizing radiation, photoionization, oxidation, or photosensitization. DNA guanyl radicals can be reduced by electron donation from mild reducing agents. Important biologically relevant examples are the redox active amino acids cysteine, cystine, methionine, tryptophan, and tyrosine. We have quantified the reactivity of derivatives of these amino acids with guanyl radicals located in plasmid DNA. The radicals were produced by electron removal using the single electron oxidizing agent $(SCN)_2^{\bullet-}$. Disulfides (cystine) are unreactive. Thioethers (methionine), thiols (cysteine), and phenols (tyrosine) react with rate constants in the range 10^4-10^6, 10^5-10^6, and 10^5-10^6 dm^3 mol^{-1} s^{-1}, respectively. Indoles (tryptophan) are the most reactive with rate constants of 10^7-10^8 dm^3 mol^{-1} s^{-1}. Selenium analogues of amino acids are over an order of magnitude more reactive than their sulfur equivalents. Increasing positive charge is associated with a ca. 10-fold increase in reactivity. The results suggest that amino acid residues located close to DNA (for example, in DNA binding proteins such as histones) might participate in the repair of oxidative DNA damage.

The DNA damage produced by the direct effect of ionizing radiation (ionization of the DNA itself (*1*, *2*)), photoionization (*3*), chemical oxidation (*4*), and photosensitization (oxidation by a species generated by photoexcitation (*5*)) involves the transfer of an electron away from the DNA. The location of the missing electron (often referred to as a hole) shows a strong tendency to migrate from the site of ionization in DNA to a guanine base (*6*, *7*). This is presumably because the single electron oxidation products of guanine bases (i.e., guanyl radicals) have the lowest reduction potentials of the functional groups present in DNA (*8*, *9*). They are therefore the most stable location available for the hole within the macromolecule. Guanyl radicals are still fairly strong oxidizing agents ($E_7 = +1.29$ V for guanosine (*10*)), and are capable of accepting electrons from even quite mild reducing agents. For example, guanyl radicals are repairable by antioxidants such as ascorbate (*11*, *12*).

However, these compounds may be hindered in their access to nucleosomal DNA. The tight association with histone proteins limits access to DNA by at least some small diffusible species (*13*, *14*). But it is possible that DNA binding proteins such as histones possess the capacity to donate electrons to guanyl radicals. The side chains of some amino acids are able to behave as mild reducing agents. The six most easily oxidized amino acids (the six best reducing agents) are cysteine, cystine, histidine, methionine, tryptophan, and tyrosine (*15*). The reduction potentials of these amino acids, dipeptides containing them, and structurally very similar compounds are available in the literature. Values at pH 7 are $E_7 = +0.9$ (cysteine (*16*)), +1.1 (cystine (*17*)), +1.2 (histidine (*18*)), +1.5 (methionine (*19*)), +1.0 (tryptophan (*20*)), and +0.9 V (tyrosine (*20*)). Comparison of these data with the corresponding value for guanosine (see above) suggests that electron transfer from amino acid to guanyl radical is in many cases thermodynamically favorable. This reaction has been observed in several model systems usually employing amino acids or small peptides as the electron donors (*21*−*24*).

The product of the reaction is a repaired guanine base and an amino acid radical. Such radicals have an undeserved reputation as highly unstable intermediates produced only under artificial conditions. It is clear that they are essential intermediates in the normal activity of some redox enzyme systems (*25*). Examples include tyrosine radicals in ribonucleotide reductase (*26*) and prostaglandin H synthase (*27*), and tryptophan radicals in DNA photolyase (*28*) and cytochrome *c* peroxidase (*29*). It is therefore possible that amino acid residues in DNA binding proteins such as histones may back-donate an electron to DNA in response to the pathological stimulus of prior electron removal from DNA by ionizing radiation, or by any of the other processes mentioned above. To examine this possibility, we have measured the rate constants for the repair of guanyl radicals in plasmid DNA by the five amino acids cysteine, cystine, methionine, tryptophan, and tyrosine, structural analogues of them, and di- and tripeptides containing them.

MATERIALS AND METHODS

Plasmid Substrate. A sample of plasmid pHAZE (10.3 kb (*30*)) 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 (*31*).

Base Excision Repair Endonuclease. An expression vector containing *Escherichia coli* formamido-pyrimidine-DNA

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Table 1: Rate Constants for Repair of DNA Guanyl Radicals by Amino Acids,[a] Simple Amino Acids Derivatives, and Selected Structurally Similar Compounds

parent amino acid (structure)	derivative	net charge	k_9/dm^3 mol^{-1} s^{-1}
cysteine (thiol)	coenzyme A (co-A)	−3	1.7×10^5
	cysteine	0	8.8×10^5
	cysteamine	+1	5.7×10^5
cystine (disulfide)	cystine	0	$< 2.0 \times 10^4$
	homocystine	0	8.3×10^4
	4,5-dihydroxy-1,2-dithiane (DHD)	0	$< 2.0 \times 10^4$
	selenocystine	0	1.3×10^6
	cystamine	+2	$< 2.0 \times 10^4$
methionine (thioether)	biotin	−1	$< 2.0 \times 10^4$
	methionine	0	5.0×10^4
	ethyl 2-hydroxyethyl sulfide	0	$< 2.0 \times 10^4$
	selenomethionine	0	1.2×10^7
	methioninamide	+1	1.1×10^6
tryptophan (indole)	tryptophan	0	1.0×10^7
	Trp−Gly	0	7.7×10^6
	tryptophanamide	+1	4.5×10^7
	Lys−Trp−Lys	+2	9.2×10^7
tyrosine (phenol)	4-hydroxyphenylacetic acid	−1	3.0×10^5
	3-(4′-hydroxyphenyl)propionic acid	−1	2.8×10^5
	tyrosine	0	4.0×10^5
	N-acetyltyrosinamide	0	8.3×10^5
	N-acetyltyrosine ethyl ester	0	3.2×10^5
	Tyr−Ala	0	3.4×10^5
	Ala−Tyr	0	5.2×10^5
	tyramine	+1	4.4×10^6
	tyrosinamide	+1	1.0×10^6
	Lys−Tyr−Lys	+2	3.6×10^6

[a] Rate constants for the parent amino acids have been reported previously (*51*).

N-glycosylase (abbreviated as FPG)[1] was kindly provided by Dr. Y. W. Kow, Department of Radiation Oncology, Emory University. The enzyme was expressed, isolated, and purified as described previously (*32*, *33*).

Irradiation. The plasmid substrate was γ-irradiated at 25 °C in aerobic aqueous solution using an AECL GammaCell-1000 isotopic device (cesium-137, 662 keV γ-ray photon). These solutions contained pHAZE (25 μg mL^{-1}, which assuming a mean of 325 g mol^{-1} per nucleotide is equivalent to 7.7×10^{-5} mol dm^{-3} nucleotide residues), sodium phosphate (pH 7.0, 5×10^{-3} mol dm^{-3}), sodium thiocyanate (1×10^{-3} mol dm^{-3}), sodium perchlorate (1.1×10^{-1} mol dm^{-3}), and an amino acid derivative, peptide, biochemical, or drug (5×10^{-8} to 1×10^{-4} mol dm^{-3}, see Table 1 for a list of these compounds). Each aliquot was 27 μL in volume. The dose rate of 5.6×10^{-2} Gy s^{-1} was determined by the Fricke method (*34*).

Enzyme Incubation. After irradiation, each 27 μL aliquot was mixed with 3 μL of a solution containing the enzyme FPG, such that the final FPG concentration was either zero or 5 μg mL^{-1}. This corresponds to a final activity of 30 units/mL, where a unit is defined as the formation of 1 pmol of single strand breaks (SSBs) 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 electrophoretically.

Determination of Strand Break Yields. After incubation, the yield of SSBs 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 production have been described previously (*31*). Briefly, the D_0 value is the radiation dose required to reduce the fraction of SSB-free plasmid to 1/*e* of its original value. At this dose, the plasmid population contains a mean of one SSB per plasmid. Numerically, the D_0 dose is equal to the reciprocal of the slope *m* of a straight line fitted to a semilogarithmic yield dose plot. At the D_0 dose, the concentration of the SSB product is equal to the concentration of the plasmid substrate, and the *G* value for SSB formation is calculated by dividing this concentration by the value of D_0.

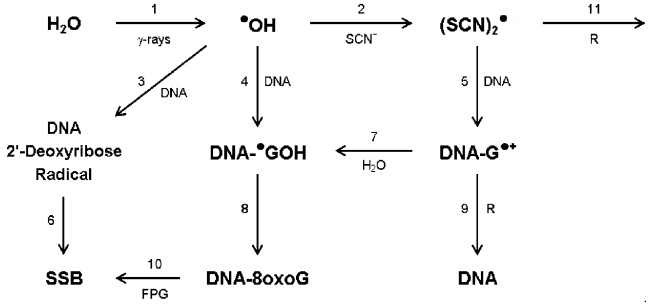

FIGURE 1: Reaction scheme summarizing the mechanism for the formation, repair, and subsequent detection of oxidative DNA damage in plasmid DNA.

RESULTS AND DISCUSSION

Reaction Scheme. We have discussed the mechanism of DNA damage by γ-irradiation in the presence of thiocyanate ions (*35*). The system is summarized by the reaction scheme in Figure 1. Radiolysis of water produces the hydroxyl radical •OH (reaction 1) and reducing species which are scavenged by oxygen to form superoxide. The ineffectiveness of superoxide dismutase in this system (*35*) suggests that reactions involving superoxide can be ignored. Some •OH

[1] Abbreviations: FPG, formamidopyrimidine-DNA *N*-glycosylase; SSB, single-strand break; *G*(SSB), radiation chemical yield of single-strand breaks.

react with the 2′-deoxyribose groups in DNA by hydrogen abstraction to produce C-centered radicals (reaction 3) and also by addition to the bases to produce adducts such as the 8-hydroxy-7,8-dihydroguanyl radical DNA-•GOH (reaction 4). Adducts with the other bases are also formed (*36*), but are not shown. However, under the conditions used here (1×10^{-3} mol dm^{-3} thiocyanate and 7.7×10^{-5} mol dm^{-3} nucleotide residues), the majority of •OH react with thiocyanate to produce the radical anion $(SCN)_2^{\bullet-}$ (reaction 2). The species $(SCN)_2^{\bullet-}$ is a single electron oxidizing agent, $E^\circ = +1.33$ V (*37*). It is sufficiently reactive to remove electrons from guanine, although not from other sites in DNA (reaction 5 (*8*, *9*)). The resulting intermediate is a DNA guanyl radical cation, DNA-G$^{\bullet+}$.

There are differences in the products formed from monomeric guanyl radicals and from those located in double-stranded substrates and which are therefore base paired. The major product formed from monomeric guanyl radicals is a 2,2-diamino-5(2*H*)-oxazolone, while base paired guanyl radicals produce mostly 8-oxo-7,8-dihydroguanine (*38*−*40*). This difference has been rationalized by partial protonation of base paired guanyl radicals by their cytosine partner. This argument (*41*) is based on the similar pK_a values derived from the radical cation of deoxyguanosine (p$K_a = 3.9$ (*42*)) and the *N*3-protonated conjugate acid of cytosine (pK_a = 4.5 (*43*)). However, recent evidence suggests that at pH 7 guanyl radicals in double stranded oligonucleoties are also deprotonated (*44*). The deprotonated neutral radical is symbolized as DNA-G(-H)•.

In the presence of a reducing agent *R*, such as a redox active amino acid, the DNA guanyl radical cation DNA-G$^{\bullet+}$ or its conjugate base DNA-G(-H)• may be reduced back to the original guanine base (reaction 9). The stable product 8-oxo-7,8-dihydroguanine (DNA-8oxoG) is formed by hydration of the guanyl radical cation DNA-G$^{\bullet+}$ via the intermediate 8-hydroxy-7,8-dihydroguanyl radical DNA-•GOH (reactions 7 and 8). This hydroxylated adduct can also be produced by •OH addition (reaction 4) even in the absence of thiocyanate. The yield of 8-oxo-7,8-dihydroguanine derived from •OH by reactions 4 and 8 is about 2-fold greater than the SSB yield derived from •OH by H-atom abstraction from the 2′-deoxyribose groups (reactions 3 and 6). It is highly doubtful that mild reducing agents can protect against the formation of 8-oxo-7,8-dihydroguanine bases from •OH, because reducible DNA guanyl radicals are not intermediates. Minor products other than 8-oxo-7,8-dihydroguanine can also be formed from DNA-•GOH (see below). Finally, 8-oxo-7,8-dihydroguanine products are detected by converting them to SSB using the *E. coli* base excision repair enzyme FPG (reaction 10 (*45*)). Note that FPG is not present during irradiation. It is added after irradiation is complete.

It is unlikely that the additive *R* attenuates the yield of FPG-sensitive sites by scavenging of $(SCN)_2^{\bullet-}$ (reaction 11). This is because of (1) the lack of correlation of the attenuation produced by different reducing agents with their reactivities with $(SCN)_2^{\bullet-}$ (*46*), (2) the equal attenuations observed for a single reducing agent with different single electron oxidants ($(SCN)_2^{\bullet-}$, $Br_2^{\bullet-}$, $SeO_3^{\bullet-}$, and $Tl^{II}OH^+$) which is suggestive of a common intermediate (*12*, *46*, *47*), 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 (*12*), such as the polyanionic plasmid.

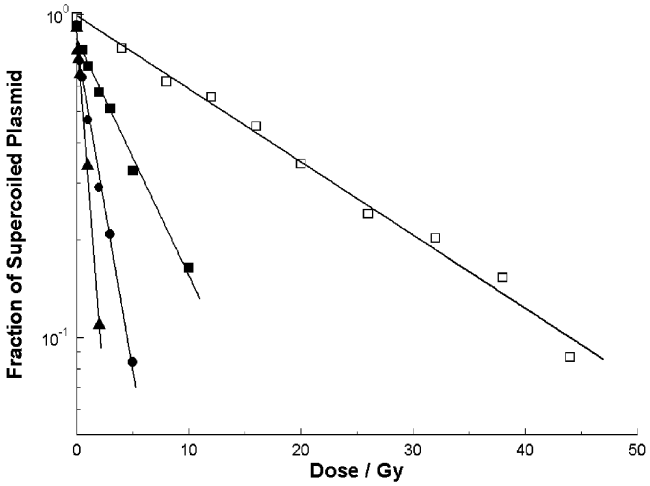

FIGURE 2: Loss of supercoiled plasmid with increasing dose of γ-radiation. Aliquots of a solution containing pHAZE (25 μg mL^{-1}), sodium phosphate (pH 7.0, 5×10^{-3} mol dm^{-3}), sodium thiocyanate (1×10^{-3} mol dm^{-3}), sodium perchlorate (1.1×10^{-1} mol dm^{-3}), and the tripeptide Lys−Tyr−Lys (6×10^{-7} (▲), 2×10^{-6} (●), 6×10^{-6} (■), or 1×10^{-6} (□) mol dm^{-3}) were irradiated at 25 °C under aerobic conditions with cesium-137 γ-rays (662 keV photon) 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 at a concentration of either zero (□) or 5 μg mL^{-1} (▲, ●, ■). The fraction of the plasmid remaining in the supercoiled conformation after each radiation dose and incubation was determined using agarose gel electrophoresis. These four data sets are plotted together, and each is fitted with a least mean square straightline 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 and incubation conditions are (▲) 0.962 Gy, 3.87×10^{-3} μmol J^{-1}; (●) 2.16 Gy, 1.73×10^{-3} μmol J^{-1}; (■) 5.92 Gy, 6.29×10^{-4} μmol J^{-1}; (□) 19.2 Gy, 1.94×10^{-4} μmol J^{-1}.

Strand Break Yields. Using a plasmid DNA substrate, it is possible to detect SSBs and to quantify their yields. SSB formation leads to the conversion of the supercoiled conformation of the plasmid into the relaxed (or open circle) conformation. These two forms are easily separated by gel electrophoresis, and their mole fractions can be estimated from fluorescence of bound ethidium.

Figure 2 shows examples of typical yield dose plots in which an aerobic solution of plasmid pHAZE (25 μg mL^{-1}) was irradiated at 25 °C with cesium-137 γ-rays in the presence of sodium phosphate (pH 7.0, 5×10^{-3} mol dm^{-3}), sodium thiocyanate (1×10^{-3} mol dm^{-3}), sodium perchlorate (1.1×10^{-1} mol dm^{-3}), and the tripeptide Lys−Tyr−Lys (6×10^{-7} to 6×10^{-6} mol dm^{-3}). After irradiation, the plasmid solutions were incubated for 30 min at 37 °C under one of two conditions. Either with or without the *E. coli* base excision repair endonuclease FPG. For each radiation dose and incubation, the fraction of the plasmid remaining in the supercoiled form was determined using agarose gel electrophoresis. Increasing radiation doses convert an increasing fraction of the supercoiled form of the plasmid into the open circle form. Therefore, the loss of the supercoiled form results from the introduction of SSB. In the absence of FPG, a radiation dose of about 40 Gy is required to reduce the fraction of the supercoiled form to 0.1. After incubation with FPG, the plasmid is significantly more (5−20-fold) sensitive to irradiation, and increasing concentrations of the tripeptide decrease this sensitivity.

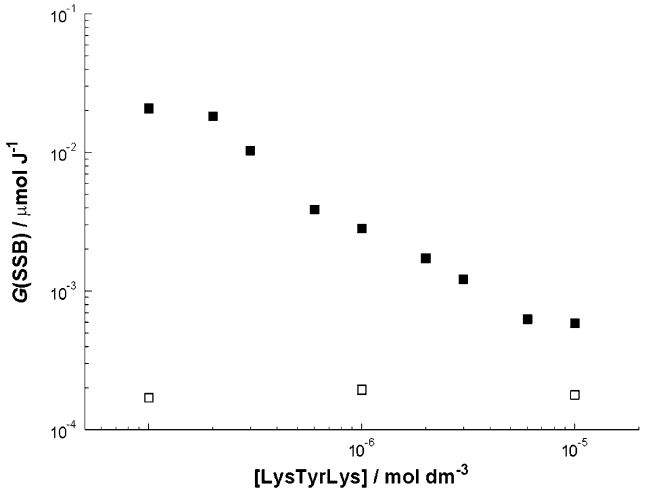

FIGURE 3: Effect of the concentration of the tripeptide Lys−Tyr−Lys during irradiation on the SSB yield after incubation with or without FPG. Plasmid pHAZE was γ-irradiated in the presence of various concentrations of Lys−Tyr−Lys, and the resulting SSB yield was determined (see Figure 2) after incubation in the presence (■) or absence (□) of FPG.

The radiation chemical yield (or *G* value) for SSB formation can be calculated from the slopes of the straight lines fitted to Figure 2 (see Materials and Methods). The SSB yield (symbolized by *G*(SSB)) was determined for additional concentrations of Lys−Tyr−Lys not shown in Figure 2.

Attenuation by Peptides. Figure 2 reveals that the presence of Lys−Tyr−Lys results in a protection against the formation of SSB in the plasmid, as detected by a post-irradiation FPG incubation. The concentration dependence of the SSB yield on the Lys−Tyr−Lys concentration is shown in Figure 3. In the absence of FPG, the value of *G*(SSB) remains essentially constant at about 1.5×10^{-4} μmol J^{-1}. In the presence of FPG, the SSB yield is strongly dependent on the concentration of Lys−Tyr−Lys. The value of *G*(SSB) decreases from 2×10^{-2} to 5×10^{-4} μmol J^{-1} (factor of 40) as the Lys−Tyr−Lys concentration increases from 1×10^{-7} to 1×10^{-5} mol dm^{-3} (factor of 100). We have previously reported that under the conditions used here the values of *G*(SSB) observed with and without FPG incubation after irradiation in the absence of any additive (i.e., a tripeptide concentration of zero) are respectively 4.8×10^{-2} and 1.5×10^{-4} μmol J^{-1} (*12*). In the presence of increasing concentrations (up to 1×10^{-4} mol dm^{-3}) of many additives such as tyrosine (*12*), the value of *G*(SSB) without FPG incubation remains constant at about 1.5×10^{-4} μmol J^{-1}, and the value of *G*(SSB) with FPG incubation reaches an asymptotic value about 2-fold greater than this (3×10^{-4} to 4×10^{-4} μmol J^{-1}). Note that the concentration of the additive must remain significantly smaller than the thiocyanate concentration (which is 1×10^{-3} mol dm^{-3}), so that $^{\bullet}$OH scavenging by the former can be ignored.

Therefore, the additive Lys−Tyr−Lys is unable to protect against SSB formation from 2′-deoxyribose radicals (reactions 3 and 6). However, it is able to protect against about 99% of the SSB produced by FPG incubation after irradiation in the absence of the additive, while a residual 1% remain unaffected. In terms of the reaction scheme in Figure 1, the preventable FPG-sensitive sites are formed via reactions 7 and 8, and are prevented by a competing reduction of the guanyl radical DNA-G$^{\bullet+}$ by the additive *R* (reaction 9). This reduction or repair reaction becomes increasingly rapid as the concentration of the additive is increased. The residual unpreventable FPG-sensitive sites are formed via reactions 4 and 8. They cannot be prevented by the presence of a mild reducing agent because the species DNA-G$^{\bullet+}$ (or its conjugate base) is not an intermediate in their formation. The yield of FPG-sensitive sites produced by reactions 4 and 8 is about 2-fold greater than the SSB yield produced by reactions 3 and 6 (*48*).

Nature of FPG-Sensitive Sites. In the absence of a reducing agent, the major product formed from the 8-hydroxy-7,8-dihydroguanyl radical (DNA-$^{\bullet}$GOH) is 8-oxo-7,8-dihydroguanine (after a one electron oxidation (*36*, *48*)). A smaller amount of 2,6-diamino-4-hydroxy-5-formamido-pyrimidine (DNA-FaPyG) is also formed from DNA-$^{\bullet}$GOH (by a one-electron reduction reaction (*36*, *48*)). Because both of these stable products are substrates for FPG, it is not possible to distinguish between them with the assay we use here. In addition to the repair of DNA-G$^{\bullet+}$, the presence of a mild reducing agent (even in the presence of oxygen) may also alter the product distribution from DNA-$^{\bullet}$GOH away from the oxidized product (DNA-8oxoG) and in favor of the reduced product (DNA-FaPyG). Therefore, the modified bases that are the precursors of the breaks produced by FPG incubation will be referred to functionally as FPG-sensitive sites.

Rate Constant for Repair. The competition between the trapping and the reductive repair of DNA-G$^{\bullet+}$ (between reactions 7 and 9, respectively) can be analyzed quantitatively by competition kinetics to derive a value for the rate constant of reaction 9 (symbolized by k_9) using eq 1.

$$\frac{1}{G(\mathrm{FPG})} = \frac{1}{G_0(\mathrm{FPG})} \times \left\{1 + \frac{k_9[R]}{k_7}\right\} \qquad (1)$$

In eq 1, *G*(FPG) represents the yield of FPG-sensitive sites in the presence of a reducing agent *R*, G_0(FPG) is the yield of FPG-sensitive sites in the absence of any added reducing agent (this value is 4.8×10^{-2} μmol J^{-1}, see above), and k_7 and k_9 represent the rate constants of reactions 7 and 9. The residual yield of unpreventable FPG-sensitive sites derived from reaction 4 has been subtracted from the value plotted in Figure 3 (this represents a small correction of less than 10% of *G*(FPG) for Lys−Tyr−Lys concentrations below 2×10^{-6} mol dm^{-3}).

Equation 1 predicts that 1/*G*(FPG) should be linearly dependent on the concentration of the reducing agent *R*. Figure 4 shows that this is true for *R* = Lys−Tyr−Lys. The slope *m* of the straight line fitted to this plot is $m = 3.75 \times 10^8$ MJ dm^3 mol^{-2}. The intercept *c* is assumed to be equal to the reciprocal of G_0(FPG) (i.e., $c = (4.8 \times 10^{-2})^{-1} = 20.8$ J μmol^{-1}). Comparison of eq 1 with that of a straight line ($y = mx + c$) suggests that the value of k_9 can be calculated as $k_9 = mk_7/c$. The value of k_7 has not been determined precisely, but may be assumed to be 0.2 s^{-1} (*49*, *50*). Therefore, the value of k_9 derived from Figure 4 is $k_9 = 3.75 \times 10^8 \times 0.2/20.8 = 3.6 \times 10^6$ dm^3 mol^{-1} s^{-1}. The largest source of error is the uncertainty in the value of k_7, which may be as large as an order of magnitude (*49*, *50*).

Using this method, we have calculated rate constants for a variety of different reducing agents. These include: (1)

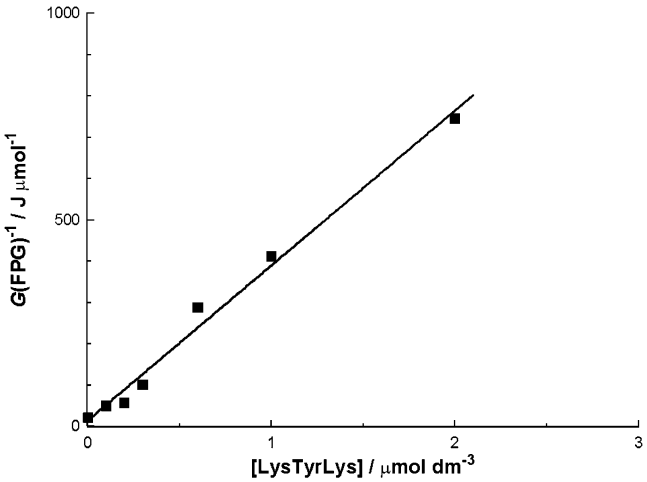

FIGURE 4: Reciprocal plot of the attenuation of the yield of FPG sensitive sites, G(FPG), against the concentration of the tripeptide Lys−Tyr−Lys according to competition kinetics (see text). The data set is fitted with a least mean square straight line of the form $y = mx + c$. The slope m of the line is 3.75×10^8 MJ dm^3 mol^{-2}. See the text for evaluation of the intercept c.

simple derivatives of amino acids such as amides, di- and tripeptides; (2) structurally similar naturally occurring compounds such as biotin (vitamin H), coenzyme A, and selenium analogues of sulfur containing amino acids; and (3) structurally related drugs such as phenol derivatives. The rate constants are listed in Table 1 along with the net charges carried by the reducing agents under the irradiation conditions (pH 7). Previously reported values for the parent amino acids (*51*) are included for comparison.

From the data in Table 1 it is possible to draw conclusions about the relationship between the structure of the reducing agents and their reactivity with DNA guanyl radicals.

Effect of Amino Acid Type. The data in Table 1 reveal that disulfides (e.g., cystine and its derivatives) are generally unreactive (rate constants $< 10^5$ dm^3 mol^{-1} s^{-1}), thiols and thioethers (e.g., derivatives of cysteine and methionine) are mildly reactive (rate constants in the range 10^4-10^6 dm^3 mol^{-1} s^{-1}), phenols (e.g., derivatives of tyrosine) are more reactive (rate constants in the range 10^5 to 10^7 dm^3 mol^{-1} s^{-1}), and indoles (e.g., tryptophan compounds) are highly reactive (rate constants $> 10^7$ dm^3 mol^{-1} s^{-1}). The selenium derivatives of cystine and methionine are far more reactive (about 100-fold) reducing agents than the parent sulfur compounds, in agreement with previous reports of their chemical properties (*52*, *53*) and their biochemical role in redox enzymes (*54*).

Effect of Charge. An effect of charge on reactivity can be discerned for the relatively large number of phenolic compounds and tyrosine derivatives that we examined. The geometric mean for uncharged tyrosine derivatives is $k_9 = 4.5 \times 10^5$ dm^3 mol^{-1} s^{-1}. With respect to this value, a single negative charge produces a small decrease of about 1.5-fold (hydroxyphenyl-acetic and propionic acids). One or two positive charges produce a significant increase of up to 10-fold (e.g., the tripeptide Lys−Tyr−Lys). Effects of this magnitude are consistent with a previous report of the reactivity of charged sulfur compounds with plasmid DNA (*55*), also at approximately physiological ionic strength. The increase in the rate constant derives from counterion condensation (*56*), an increase in the concentration of the positively charged reducing agent close to the polyanionic plasmid.

Expected Reactivity of Amino Acid Residues in Proteins. The general similarity of the rate constants for compounds with common chemical structures suggests that the behavior of the model compounds we have used here forms a basis to estimate their reactivity in vivo. But because in vivo the vast majority of amino acids are incorporated into proteins, any such estimate entails large uncertainties. The main issues involved are the reduction potentials of amino acid residues in proteins, the distance between the reactants, and comparison of the intermolecular rate constants we have quantified here with those in cells which are far better described as intramolecular.

Intramolecular Rate Constants. The rate constants reported here refer to intermolecular reactions. In contrast, the reaction of a DNA guanyl radical with an amino acid residue located in a DNA binding protein (for example, a histone protein) is intramolecular. Values for the latter could be estimated from the former by comparison with literature data for electron transfer from tyrosine to tryptophan radicals. Bimolecular rate constants between dipeptides lie in the range 5×10^5 to 2×10^6 dm^3 mol^{-1} s^{-1} (*57*, *58*). Unimolecular rate constants within di- and tripeptides (such as Tyr−Gly−Trp or Trp−Gly−Tyr) lie in the range 2×10^4 to 8×10^4 s^{-1} (*58*). The uncertainties involved in such an extrapolation therefore exceed an order of magnitude.

Reduction Potential of Amino Acid Residues in Proteins. Values of the reduction potentials of monomeric amino acids are available in the literature (see introduction). The values are close to those of chemically similar model compounds. At pH values lower than the pK_a of the amino acid, but greater than the pK_a of its radical cation, these reduction potentials should be pH dependent according to the Nernst equation, increasing by $RT/F = 0.059$ V per unit decrease in pH. This pH range is large enough to include realistic physiological values, since the pK_a values of the most acidic redox active amino acids are 8.3 (cysteine) and 10.1 (tyrosine), while that of the most weakly acidic radical cation is 4.3 (tryptophan (*20*)). In many cases, this pH dependence has been verified experimentally (*20*, *59*). It is well-known that the ionization constants of amino acid residues in proteins may differ from the monomers by up to 2 or even 3 units. For example in chicken lysozyme, the observed tyrosine pK_a values are 9.8, 10.3, and 12.1 (*60*). A difference of 2 or 3 pK_a units corresponds to a reduction potential difference of 2×0.059 or $3 \times 0.059 = 0.12$ or 0.18 V. Unlike ionization constants experimental determinations of amino acid reduction potentials in proteins are rare. In agreement with this estimate, a difference of 0.2 V has been reported for a tyrosine residue located in an artificial protein (*59*) relative to tyrosine itself. According to Marcus theory (*61*), a difference of 0.2 eV (i.e., 19 kJ mol^{-1}) in the driving force of an electron transfer reaction which is not strongly endo- or exoergonic (i.e., with $|\Delta G| < 19$ kJ mol^{-1}) can change its rate constant by ca. 50-fold.

Distance between Donor and Acceptor. It is well established that the rate constant of an electron-transfer reaction decreases with increasing separation between the electron donor and acceptor. Again, taking literature data for the tyrosine to tryptophan radical reaction as an example, the intramolecular rate constant decreases by 3-fold with each

additional proline residue in the six peptide series Trp−$(Pro)_n$−Tyr ($n = 0-5$ (*58*, *62*, *63*)). Typical distances between guanyl radicals and redox active amino acid residues in the nucleosome (*64*, *65*) may therefore change the rate constant by at least an order of magnitude.

Conclusions. We have quantified intermolecular rate constants for electron transfer from redox active amino acid derivatives (and structurally related compounds) to guanyl radicals in plasmid DNA. The purpose is to model the behavior expected from these compounds in their most common cellular form (amino acid residues in proteins) and most reactive location (close proximity to DNA). Principal examples for eukaryotes are presumably the histone proteins. The most reactive amino acids are the selenium analogues and tryptophan. Occurrence of the selenium containing amino acids is restricted to a small number of mostly redox enzymes (*66*, *67*), which have limited relevance for nuclear DNA, and even the regular amino acid tryptophan is relatively rare. No tryptophan residues are present in the histone octamer (*68*). The combination of nucleosomal abundance with the reactivity data in Table 1 suggests that tyrosine residues may dominate the reduction of guanyl radicals in vivo. Further work using a nucleosomal model system will be required to address this issue.

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