DNA-Bound Peptide Radicals Generated through DNA-Mediated Electron Transport†

Hans-Achim Wagenknecht, Eric D. A. Stemp,‡ and Jacqueline K. Barton*

Division of Chemistry and Chemical Engineering, California Institute of Technology, Pasadena, California 91125

Received December 17, 1999; Revised Manuscript Received February 16, 2000

ABSTRACT: Flash-quench experiments were carried out to explore peptide/DNA electron-transfer reactions. DNA-bound $[Ru(phen)_2(dppz)]^{3+}$ (phen = 1,10-phenanthroline; dppz = dipyridophenazine) and $[Ru(phen)(bpy')(dppz)]^{3+}$ [bpy′ = 4-(4′-methyl-2,2′-bipyridyl)valerate], generated in situ by flash-quench methodology, are powerful ground-state oxidants, capable of oxidizing guanine or tyrosine intercalated in DNA. In flash-quench experiments with mixed-sequence oligonucleotides in the presence of Lys-Tyr-Lys, transient absorption spectroscopy yielded a spectrum with a sharp maximum at 405 nm assigned to the tyrosine radical. Experiments with poly(dG·dC) suggested the intermediacy of the guanine radical, since the rise of the 405 nm signal occurred with the same kinetics as the disappearance of the guanine radical, as monitored at 510 nm. In oligonucleotide duplexes containing $[Ru(phen)(bpy')(dppz)]^{2+}$ tethered at one end, damage to distant guanines was observed by gel electrophoresis, consistent with the mobility of the electron hole through the DNA duplex; the presence of the peptide did not inhibit but instead altered the distribution of guanine damage. Covalent adducts of the DNA and Lys-Tyr-Lys were detected as final irreversible products of this peptide-to-DNA electron-transfer chemistry by mass spectrometric and enzymatic digestive analysis. From these different assays and comparison of reactions of Lys-Trp-Lys and Lys-Tyr-Lys, the reactivity of the DNA-bound tyrosine radical was found to differ considerably from that of the tryptophan radical. These results establish that Lys-Tyr-Lys and Lys-Trp-Lys can participate in long-range electron-transfer reactions through the DNA from a distinct binding site. On that basis, proposals for functional roles for these peptide radicals may be considered.

Electron transfer reactions through DNA have generated tremendous interest, largely because oxidative damage to DNA has been implicated as a major factor in aging and molecular diseases. Furthermore, it is important to consider radical migration through the DNA double helix as a route to mutagenesis and carcinogenesis (*1*). During the past few years, enormous effort has been invested to clarify how charge migrates through the DNA duplex (*2*). In our laboratory, we have systematically probed long-range charge migration through DNA by fluorescence quenching (*3*), transient absorption spectroscopy (*4*, *5*), electrochemical methods (*6*), biochemical methods (*7*), and, most recently, by electron paramagnetic resonance spectroscopy (*8*). It is now well-established that DNA-mediated charge migration is highly dependent on the π-stacking of the electron donor and acceptor, as well as the intervening DNA bases.

Gel electrophoretic analysis of oxidative lesions formed through photoinduced charge migration has shown that guanines can be oxidized from distances of about 200 Å (*9*). This has important implications for the study of DNA damage and repair. Radicals, generated at one location in the DNA duplex, can potentially cause damage at other locations far away in the genome. Importantly, long-range charge transfer is dependent on distance and appears to be modulated by the intervening sequence of the bases (*9*, *10*).

DNA-mediated charge transport can also be modulated by bound proteins. The methylase M.*Hha*I forms a base-flipped complex with each target cytosine in a transient extrahelical conformation and fills the intrahelical space by a glutamine side chain, leaving the overall structure of the DNA free of significant distortions. Due to this significant interruption of the DNA π-stack, M.*Hha*I bound to DNA inhibits long-distance charge transfer (*11*). In contrast, by use of a mutant of M.*Hha*I, containing a substitution of glutamine by tryptophan, long-range oxidation was restored (*11*). Interestingly, DNA interaction with this tryptophan mutant of M.*Hha*I gave rise also to oxidative damage at the central enzyme binding site that was not observed with the wild-type enzyme; the insertion of tryptophan with a lower oxidation potential (1.0 V) (*12*) in comparison to guanine (1.3 V) (*12*) may perturb the overall oxidation potential of the site.

These studies at the macromolecular level provided impetus for an examination of peptide-to-DNA electron transfer reactions. We have recently shown that it is possible to oxidize DNA-bound tryptophan within the tripeptide Lys-Trp-Lys through DNA-mediated charge transfer using the flash-quench technique (*13*). The flash-quench technique (*14*) was originally developed to explore electron-transfer pro-

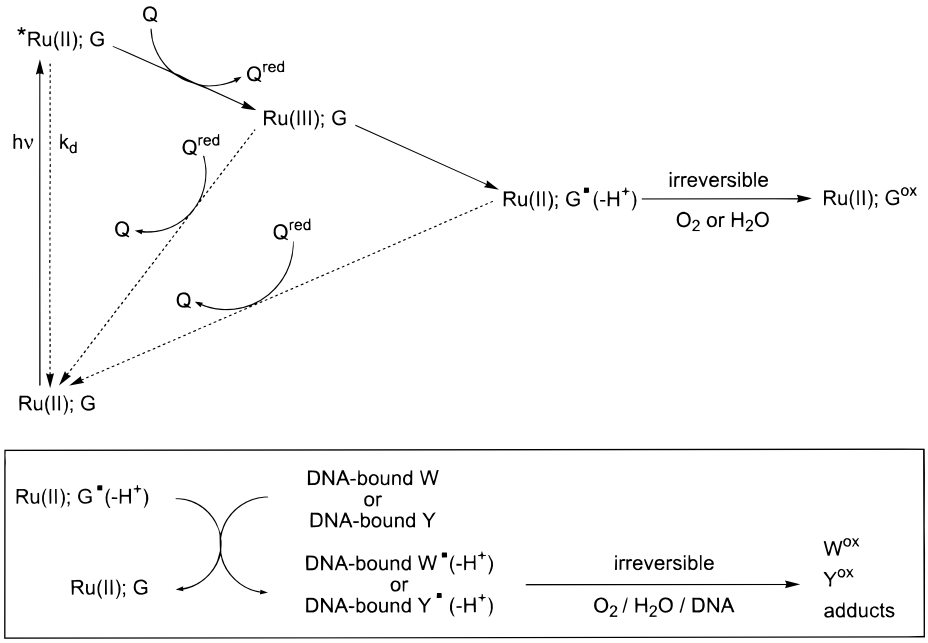
† We are grateful to NIH for financial support (GM49216 to J.K.B.). We thank the Swiss National Science Foundation and the Novartis Foundation for a postdoctoral fellowship to H.A.W., Mount St. Mary's College for a professional development grant, and NSF (MCB981-7338) for funding of E.D.A.S.

* To whom correspondence should be addressed.

‡ Present address: Department of Physical Sciences and Mathematics, Mount St. Mary's College, Los Angeles, CA 90049.

10.1021/bi992897m CCC: $19.00
Published on Web 03/31/2000

Scheme 1: Diagram of the Flash-Quench Technique[a]



[a] G = guanine, G^{ox} = oxidized guanine products, Q = quencher, Q^{red} = reductive state of Q, W = DNA-bound tryptophan, W^{ox} = oxidized tryptophan products, Y = DNA-bound tyrosine, Y^{ox} = oxidized tyrosine products.

cesses in proteins (*15*). Studies in our laboratory have shown that the flash-quench technique is a very useful method to generate guanine radicals in duplex DNA (Scheme 1), detectable by both transient absorption spectroscopy (*5*) and electron paramagnetic resonance spectroscopy (*8*). Excitation of intercalated $[Ru(phen)_2(dppz)]^{2+}$ (phen = 1,10-phenanthroline; dppz = dipyridophenazine) with visible light produces the corresponding excited ruthenium complex, $*[Ru(phen)_2(dppz)]^{2+}$, which subsequently can be quenched via electron transfer to a nonintercalating quencher, like $[Ru(NH_3)_6]^{3+}$, to yield $[Ru(phen)_2(dppz)]^{3+}$ as a ground-state oxidant (1.6 V) (*16*) and the reduced quencher. At this point, $[Ru(phen)_2(dppz)]^{3+}$ can undergo back electron transfer with the reduced quencher or oxidize guanines within the DNA double helix, generating guanine radicals. Due to a low pK_a within the base pair with cytosine (*17*), only the neutral deprotonated guanine radical has been detected spectroscopically (*5*). Subsequently, the guanine radical can either be reduced by the reduced quencher to regenerate the whole redox system or undergo further reactions with oxygen or water yielding oxidative lesions. Piperidine-labile oxidative lesions at guanine sites of the DNA that have been formed during flash-quench experiments have been visualized by gel electrophoretic analysis (*18*).

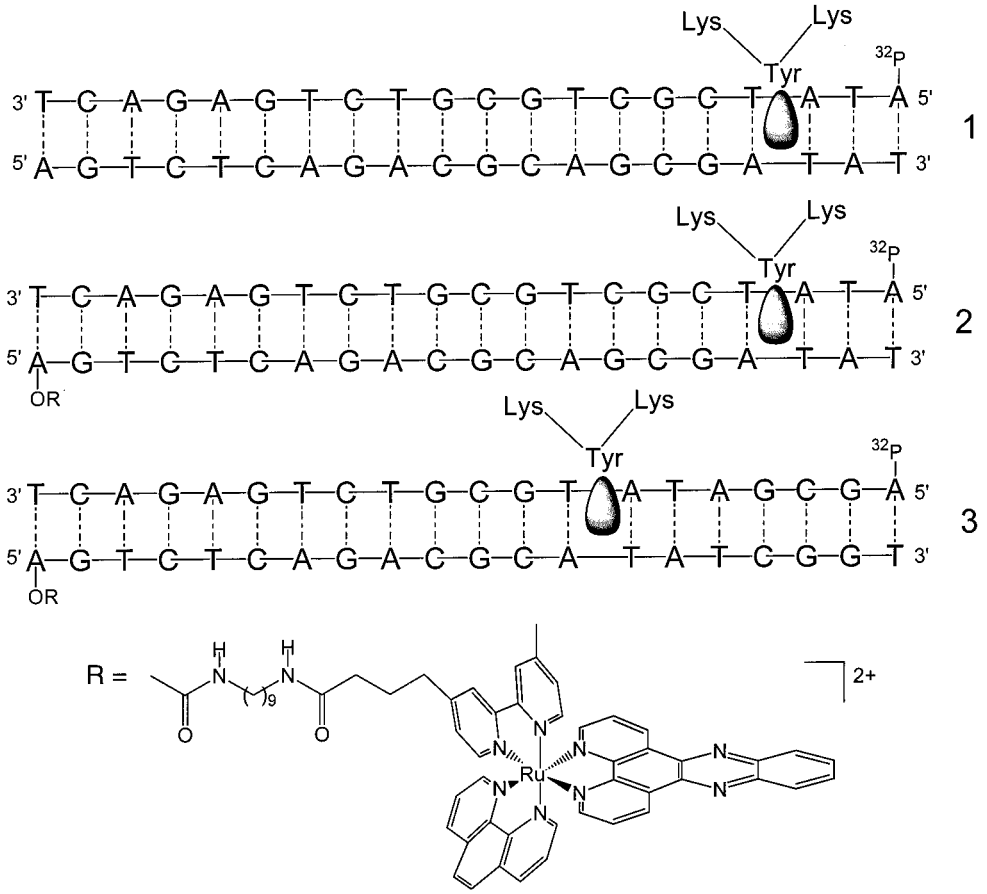
Here we further describe flash-quench experiments carried out to explore electron-transfer reactions between the tripeptide Lys-Tyr-Lys and double-stranded DNA. Tyrosine within DNA-binding proteins, e.g., 3-methyladenine glycosylase (*19*), often intercalates into DNA. The tripeptide Lys-Tyr-Lys exhibits similar electrostatic DNA interactions to those noted for Lys-Trp-Lys (*20*), with the positively charged lysine side chains of each peptide associating with the negatively charged phosphodiester backbone. Additionally, each peptide bears an aromatic moiety capable of stacking within the DNA double helix (*20*). The binding of Lys-Tyr-Lys or Lys-Trp-Lys and stacking of their aromatic side chains (dissociation constant of Lys-Trp-Lys-Gly = 5.9×10^{-4} M for *Escherichia coli* DNA; *21*) is favored with an AT alternating sequence [$K = 3.5 \times 10^{-4}$ M for poly(dA·dT; *21*] or in the presence of an abasic site ($K = 1.9 \times 10^{-4}$ M for apurinic DNA; *22*).

On the basis of thermodynamics, tyrosine should be oxidized by the guanine radical (1.3 V) (*12*), since it has an oxidation potential of 0.9 V (*23*), which is comparable to that of tryptophan (1.0 V) (*12*). We detected the intermediate tyrosine radical by transient absorption spectroscopy and also characterized the final irreversible products of this peptide-to-DNA electron transfer chemistry. The kinetic behavior of the DNA-bound tyrosine radical was compared with that previously observed for the DNA-bound tryptophan radical. We find reactivities of the tyrosine and tryptophan radicals within DNA to differ considerably. On that basis, functional roles for these radicals within DNA may be considered.

EXPERIMENTAL PROCEDURES

Materials. Lys-Tyr-Lys, Lys-Trp-Lys, and hexaammineruthenium(III) chloride were obtained from Sigma or Aldrich and used as received. DNA polymers, poly(dA·dT) and poly(dG·dC), were purchased from Pharmacia and were exchanged via ultrafiltration (Centricon 30, Amicon) into phosphate-buffered saline ($NaHPO_4$, 5 mM, and NaCl, 50 mM), pH = 8.5, prior to use. The oligonucleotides **1**−**3** (Chart 1) were prepared on an Applied Biosystems 394 DNA synthesizer by standard phosphoramidite chemistry (*24*). After preparation, the resin was cleaved and the DNA was deprotected by treatment with NH_4OH at 55 °C for 10 h, dried, and purified by HPLC (Hewlett-Packard HP1050) on a semipreparative reversed-phase C-18 column (Dynamax C18, Rainin, 300 Å) under the following conditions: A = NH_4OAc buffer (50 mM), pH = 6.5; B = MeCN; gradient = 0−15% B over 45 min to elute the DNA. The single strands were lyophilized and quantified by their absorbance at 260 nm. Duplexes were formed by heating a solution

Chart 1: DNA Substrates **1**−**3**



containing equal concentrations of complementary strands to 90 °C, followed by slow cooling. $[Ru(phen)_2(dppz)]Cl_2$ was prepared as described previously (*25*) and used as the enantiomeric mixture. The $[Ru(phen)(bpy')(dppz)]^{2+}$-modified oligonucleotides **2** and **3** were prepared from the racemic metal complex [bpy′ = 4-(4′-methyl-2,2′-bipyridyl)valerate]. This tris(heteroleptic) complex was synthesized according to the general methods of Strouse et al. (*26*) and Anderson et al. (*27*). The two isomers of $[Ru(phen)(bpy')(dppz)]^{2+}$ (with the carboxylate arm axial or equatorial to the dppz ligand) were not separated. Both metal complex isomers were conjugated to the oligonucleotide by solid-phase methodology and purified as described earlier (*18*). The Ru−DNA conjugate was characterized by UV/Vis spectroscopy and matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry. The complement was prepared according to the procedure described for **1**. The single strands were lyophilized and quantified by their absorbance at 260 nm. Duplexes were formed by heating to 90 °C a solution containing equal concentrations of the Ru−DNA strand and its complement, followed by slow cooling.

Laser Spectroscopy. Time-resolved emission and transient absorption measurements used an excimer-pumped dye (Coumarin 480) laser, as described previously (*5*). Laser powers at λ_{exc} = 480 nm ranged from 1 to 1.5 mJ/pulse. The emission of the dppz complexes was monitored at 610 nm, and the emission intensities were obtained by integrating under the decay curve for the luminescence.

Assays of Oxidative Products. Oligonucleotide strands were labeled at the 5′ end with [γ-^{32}P]ATP by standard protocols (*28*), purified by denaturing 5% PAGE, and hybridized to the complementary strands in phosphate buffer (5 mM), pH = 9.0. Samples of 10 μL size containing the radioactively labeled Ru−DNA duplex **2** or **3** (1 μM), $[Ru(NH_3)_6]Cl_3$ (15 μM), and Lys-Tyr-Lys (0−25 μM) in phosphate buffer (5 mM), pH = 8.5, were irradiated at λ = 436 nm (∼6 mW) with a 1000-W Hg/Xe lamp equipped with a monochromator. After irradiation for 10 min, samples were treated with a mixture of piperidine (10 μL) and H_2O (90 μL) at 90 °C for 30 min, lyophilized, resuspended in denaturing gel-loading dye and analyzed by denaturing 20% PAGE. The extent of damage was quantitated by phosphorimagery (Imagequant).

To characterize the oxidation products of Lys-Trp-Lys, samples (60 μL) containing **1** (100 μM), $[Ru(phen)_2(dppz)]Cl_2$ (100 μM), $[Ru(NH_3)_6]Cl_3$ (1.5 mM), and Lys-Trp-Lys (2.0 mM) were irradiated for 30 min at λ = 436 nm and subsequently analyzed by HPLC (Hewlett-Packard HP1050). The irradiated flash-quench samples were analyzed on a semipreparative reversed-phase C-18 column (Dynamax C18, 300 Å, Rainin) under the following conditions: A = NH_4-OAc buffer (50 mM), pH = 6.5; B = MeCN; gradient = 0−6% B in 60 min. After separation, the peptide-containing fraction was lyophilized and dissolved in MeOH. The electrospray ionization (ESI) mass spectrometric analysis of the peptide fraction showed only the correct mass of the original peptide (m/z = 438.2) and no oxidized product. The DNA-containing fraction was analyzed by mass spectrometry (MALDI-TOF) and exhibited the mass of the original DNA single strands (m/z = 6121.6 and 6137.9) and the mass of

the DNA−tripeptide adduct (m/z = 6557.9).

To explore the cross-linking reaction with Lys-Tyr-Lys, the irradiated flash-quench samples were enzymatically digested (*29*). After irradiation the DNA was separated by EtOH precipitation. The resulting pellet was dried and dissolved in NaOAc buffer (50 mM, 80 μL), pH = 5.5, and water (120 μL). The DNA was digested with nuclease P1 (Boehringer Mannheim, 1 unit/μL, 50 μL) for 2 h at 37 °C. Triethanolamine buffer (1 M, 40 μL), pH = 8.5, was added and the sample was subsequently digested with alkaline phosphatase (Boehringer Mannheim, 1 unit/μL, 10 μL) for 2 h at 37 °C. The resulting deoxynucleoside mixture was analyzed by HPLC under the following conditions: A = 12.5 mM citric acid, 25 mM NaOAc, 30 mM NaOH, and 10 mM acetic acid buffer; B = MeCN; isocratic elution with 10% B in 60 min. The different peaks of the HPLC spectrum have been assigned to the corresponding deoxyribonucleosides by co-injections with solutions containing commercially available deoxyribonucleosides. The modified nucleosides T′ and A′ were separated by HPLC on a semipreparative reversed-phase C-18 column (Dynamax C18, 300 Å, Rainin) under the following conditions: A = 12.5 mM citric acid, 25 mM NaOAc, 30 mM NaOH, and 10 mM acetic acid buffer; B = MeCN; gradient = 0−10% B in 45 min. The collected fractions were lyophilized and analyzed by MALDI-TOF mass spectrometry (T′ m/z = 701.1; A′ m/z = 717.7).

RESULTS AND DISCUSSION

Emission Quenching Experiments with [Ru(phen)$_2$(dppz)]$^{2+}$ and Lys-Tyr-Lys Bound Noncovalently to Mixed-Sequence Oligonucleotides. Initial investigations of the oxidation of DNA-bound tyrosine exploited the mixed-sequence oligonucleotide **1** and untethered [Ru(phen)$_2$(dppz)]Cl$_2$. The oligonucleotide **1** contains an alternating AT sequence to ensure that the tyrosine of Lys-Tyr-Lys has a possible intercalation site (Chart 1) (*21*). Upon excitation of [Ru(phen)$_2$(dppz)]$^{2+}$ at 480 nm, the emission decayed biexponentially with τ_1 = 25 ns and τ_2 = 259 ns, as monitored at 610 nm. The fraction quenched (F_q) of this excited state by the quencher [Ru(NH$_3$)$_6$]$^{3+}$ was 87% at 20 equiv of quencher (equivalents relative to the amount of **1**). The quenching efficiency was not diminished in the presence of up to 20 equiv of Lys-Tyr-Lys.

Detection of the Tyrosine Radical by Transient Absorption at 405 nm. Transient absorption spectroscopy was used to monitor the intermediates formed upon oxidative quenching of *[Ru(phen)$_2$(dppz)]$^{2+}$ with [Ru(NH$_3$)$_6$]$^{3+}$. We monitored the absorbance at 405 nm, the absorbance maximum of the tyrosine radical (*30*). Excitation of DNA-bound [Ru(phen)$_2$(dppz)]$^{2+}$ in the presence of [Ru(NH$_3$)$_6$]$^{3+}$ and Lys-Tyr-Lys produces a signal that initially exhibits a negative change in absorbance (ΔA) but then crosses over the baseline to give a positive signal. The initial negative spike at short times can be attributed to absorbance changes of the dppz complex, since the hexaammine quencher exhibits only minimal absorbance in the visible region (*16*). In particular, the negative signal arises from loss of the metal-to-ligand charge transfer (MLCT) band in the 400−500 nm region that is characteristic of ruthenium polypyridyl complexes and is consistent with the presence of *[Ru(phen)$_2$(dppz)]$^{2+}$ and [Ru(phen)$_2$(dppz)]$^{3+}$. This negative ΔA from 400 to 500 nm interferes with observation of the initial rise of the tyrosine radical at 405 nm. However, electron transfer to the intercalated [Ru(phen)$_2$(dppz)]$^{3+}$ occurs approximately over 20 μs, after which the 405 nm signal exhibits a positive ΔA, which then decays nearly completely within 0.2−0.3 ms (Figure 1). The size of the positive signal increased with increasing peptide concentration. It is noteworthy that the signal was not observed upon omission of any one of the ingredients of the flash-quench sample. Exclusion of Lys-Tyr-Lys, [Ru(phen)$_2$(dppz)]Cl$_2$, [Ru(NH$_3$)$_6$]Cl$_3$, or the oligonucleotide **1** abrogated signal formation, thus indicating that the reaction is indeed DNA-mediated. We therefore assigned this 405 nm signal to the DNA-bound tyrosine radical.

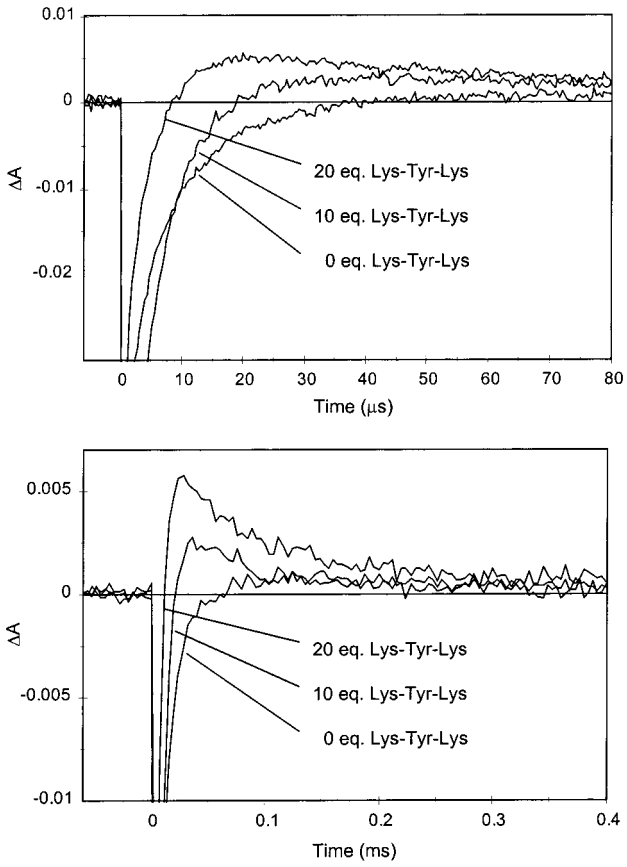


FIGURE 1: Transient absorption at 405 nm for the sample containing oligonucleotide **1**, observed on two different time scales, in the absence (0 equiv) and in the presence of Lys-Tyr-Lys (300 μM = 10 equiv; 600 μM = 20 equiv). Samples contained [Ru(phen)$_2$(dppz)]Cl$_2$ (30 μM), **1** (30 μM), and [Ru(NH$_3$)$_6$]Cl$_3$ (600 μM) in phosphate buffer (5 mM), pH = 9.0, λ_{exc} = 480 nm.

Spectral Characterization of DNA-Bound Radicals of Tyrosine and Tryptophan. To confirm that the flash-quench experiments with Lys-Tyr-Lys and Lys-Trp-Lys generate the expected aromatic radicals, we generated absorbance difference spectra for the long-lived positive transient signals. With the DNA substrate **1**, the spectra of the transients with Lys-Tyr-Lys (Figure 2) and with Lys-Trp-Lys (Figure 3) both show a high degree of similarity to published spectra of the tyrosine and the tryptophan radicals in solution (*30*). These results reveal that under the described conditions of the flash-quench experiments (i) the aromatic peptide radicals are formed bound to DNA and (ii) the absorbance signals at 405 nm (tyrosine radical) or 510 nm (tryptophan radical) can be

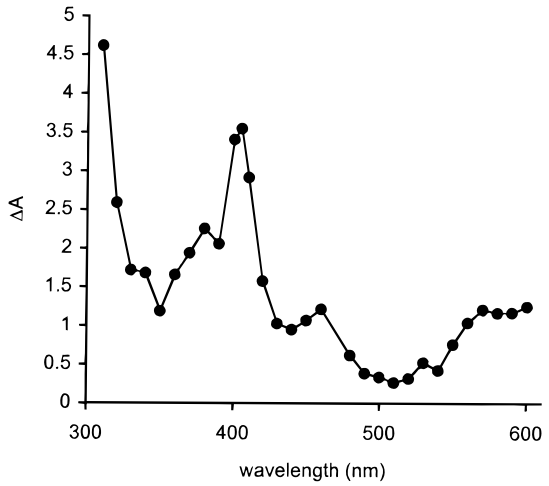


FIGURE 2: Absorbance difference spectrum × 10^3 of the tyrosine radical in its DNA-bound form. The long-lived positive portions [$t > \tau$(*Ru)] of individual signals were fit to the monoexponential function $\Delta A(t) = C + \Delta A(t = 0)[\exp(-kt)]$. $\Delta A(t = 0)$ was plotted against the wavelength λ. Sample contained $[Ru(phen)_2(dppz)]Cl_2$ (30 μM), **1** (30 μM), Lys-Tyr-Lys (600 μM), and $[Ru(NH_3)_6]Cl_3$ (600 μM) in 5 mM phosphate buffer, pH = 9.0, λ_{exc} = 480 nm.

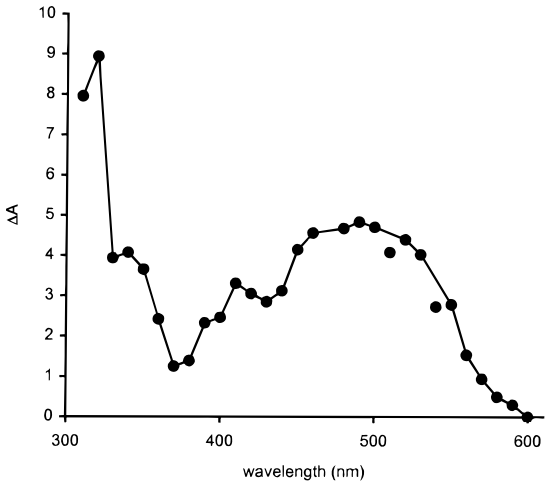


FIGURE 3: Absorbance difference spectrum × 10^3 of the tryptophan radical in its DNA-bound form. The long-lived positive portions [$t > \tau$(*Ru)] of individual signals were fit to the monoexponential function $\Delta A(t) = C + \Delta A(t = 0)[\exp(-kt)]$. $\Delta A(t = 0)$ was plotted against the wavelength λ. Sample contained $[Ru(phen)_2(dppz)]Cl_2$ (30 μM), **1** (30 μM), Lys-Trp-Lys (300 μM), and $[Ru(NH_3)_6]Cl_3$ (600 μM) in 5 mM phosphate buffer, pH = 9.0, λ_{exc} = 480 nm.

used to discern the kinetic behavior of the generated peptide radicals.

Experiments with Covalently Bound Metallointercalator. To define better the intercalation site for the Ru(II) complex, we prepared the DNA substrates **2** and **3**, bearing [Ru(phen)(bpy′)(dppz)]$^{2+}$ covalently tethered to the 5′-end distal to the Lys-Tyr-Lys-binding region (Chart 1). Both DNA substrates **2** and **3** contain a 3′-ATAT binding sequence for Lys-Tyr-Lys but at two different positions. Flash-quench experiments with the duplexes **2** and **3** in the presence of $[Ru(NH_3)_6]^{3+}$ and Lys-Tyr-Lys yielded transient absorption signals (Figure 4) comparable to those seen with duplex **1**. However, the kinetic behavior was somewhat different (Table 1), with a longer rise time and also a longer lifetime (~200 μs) observed for the tethered samples. It is interesting to note that a 4 base-pair shift of the binding site for Lys-Tyr-Lys

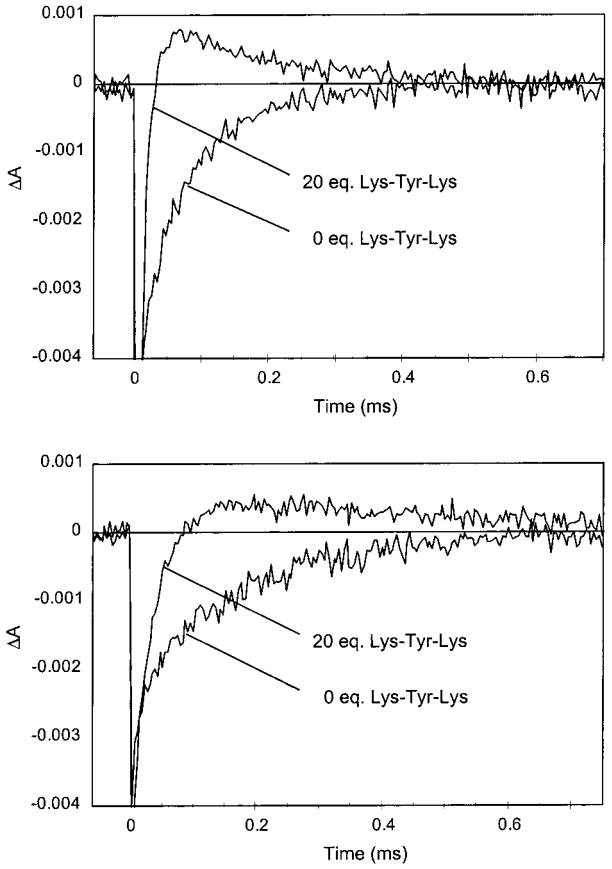


FIGURE 4: Transient absorption at 405 nm for the sample containing oligonucleotide **2** (top) or **3** (bottom), in the absence (0 equiv) and presence of Lys-Tyr-Lys (300 μM = 20 equiv); the sample contained **2** or **3** (each 15 μM), and $[Ru(NH_3)_6]Cl_3$ (300 μM) in phosphate buffer (5 mM), pH = 9.0, λ_{exc} = 480 nm.

of about 13.6 Å further away from the binding region of the covalently tethered [Ru(phen)(bpy′)(dppz)]$^{2+}$ increased the rise time of the DNA-bound tyrosine radical from about 75 μs (for **2**) to about 160 μs (for **3**; Figure 5). Consistently, the rise time observed with the untethered system and DNA **1** is much shorter (~30 μs), presumably because under the latter reaction conditions $[Ru(phen)_2(dppz)]^{2+}$ is randomly bound to the DNA and can assume distances closer to the DNA-bound Lys-Tyr-Lys.

The rise time of the tyrosine radical observed for the DNA substrates **1**−**3** in experiments with Lys-Tyr-Lys (e.g., 160 μs with **2**) is longer than the rise time of the tryptophan radical obtained in similar experiments with Lys-Trp-Lys (e.g., 30 μs with **2**; Table 1). On the basis of oxidation potentials of tyrosine (0.9 V) (*13*) and tryptophan (1.0 V)[12], the driving force for oxidation of tyrosine should be larger than that for tryptophan. Thus, the observed trend in kinetics does not correlate with the driving force. However, assuming that the binding of Lys-Tyr-Lys occurs at similar locations on the DNA as does Lys-Trp-Lys (*20*), the poorer stacking properties of the intercalated phenol-type side chain of tyrosine in comparison to the larger indole-type side chain of tryptophan could be responsible for the differences in the observed rise times of the DNA-bound radicals. A similar sensitivity to stacking of donor and acceptor with the DNA helix has been observed in other systems.

Gel Electrophoretic Analysis. The DNA substrate **3** bearing covalently tethered [Ru(phen)(bpy′)(dppz)]$^{2+}$ and the

Table 1: Quenching Efficiencies and Spectroscopic Results of the Flash-Quench Experiments with Lys-Tyr-Lys (KYK) and Different Oligonucleotides in Comparison with the Results of Experiments with Lys-Trp-Lys (KWK)[a]

		tripeptide			tripeptide radical			
line	oligonucleotide	KWK or KYK	equiv[b]	fraction quenched[c] (%)	rise time (μs)	lifetime (μs)	$conc_{max}$[d] (μM)	guanine radical lifetime (μs)
1	**1**	KYK	10	87	30 ± 6	83 ± 6	0.9 ± 0.1	*e*
2	**1**	KYK	20	83	33 ± 6	83 ± 6	1.8 ± 0.1	*e*
3[g]	**1**	KWK	10	81	7 ± 1	77 ± 10	2.8 ± 0.1	*e*
4	**2**	KYK	20	82	163 ± 30	182 ± 3	0.6 ± 0.1	*e*
5[g]	**2**	KWK	10	76	33 ± 10	238 ± 6	1.9 ± 0.2	*e*
6	**3**	KYK	10	76	76 ± 12	208 ± 4	0.6 ± 0.1	*e*
7	poly(dA·dT)	KYK	20	83	n.o.	n.o.	n.o.	n.o.
8[g]	poly(dA·dT)	KWK	10	87	n.o.	n.o.	n.o.	n.o.
9	poly(dG·dC)	KYK	20	93	15 ± 5	185 ± 3	1.7 ± 0.2	0.5 ± 0.05
10[g]	poly(dG·dC)	KWK	10	93	31 ± 6	100 ± 1	1.0 ± 0.2	8.0 ± 0.6

[a] In experiments with Lys-Tyr-Lys, the tyrosine radical was observed at $\lambda = 405$ nm and the guanine radical at $\lambda = 510$ nm; in experiments with Lys-Trp-Lys (KWK) (*13*), the tryptophan radical was observed at $\lambda = 510$ nm and the guanine radical at $\lambda = 373$ nm. [DNA] = 30 μM (for **1**−**3**), [nucleotide] = 600 μM [for poly(dA·dT) and poly(dG·dC)], $[[Ru(phen)_2(dppz)]Cl_2] = 30$ μM, [Q] = $[[Ru(NH_3)_6]Cl_3] = 600$ μM, [peptide] = 300 μM (for Lys-Tyr-Lys and Lys-Trp-Lys), 5 mM P_i buffer, pH = 9.0, $\lambda_{exc} = 480$ nm; n.o., not observable. [b] Relative to [DNA] or [nucleotide]/20. [c] $1 - I_0/I$, I and I_0 measured by luminescence decay at $\lambda_{em} = 610$ nm. [d] $\epsilon = 3.2 \times 10^3$ M^{-1} cm^{-1} at $\lambda = 405$ nm (tyrosine radical), and $\epsilon = 2.0 \times 10^3$ M^{-1} cm^{-1} at $\lambda = 510$ nm (tryptophan radical) (*30*). [e] Due to overlapping absorbances of Ru(III) and the guanine radical, the decay kinetics could not be determined. [g] Reference *13*.

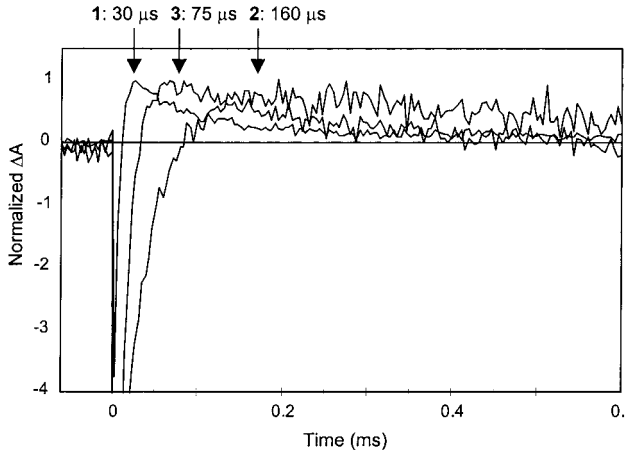


FIGURE 5: Normalized data of the transient absorption at 405 nm of samples containing the oligonucleotides **1**, **2**, or **3** on the same time scale. The conditions are as in Figures 1 and 2.

Lys-Tyr-Lys binding site 12 base pairs from the Ru intercalation site allowed us to investigate damage to the intervening DNA bases between the two redox partners. $[Ru(phen)(bpy')(dppz)]^{3+}$ can oxidize guanines within the DNA from a distance (*18*). Oxidative damage to guanine bases can be visualized by treatment with 1 M piperidine for 30 min at 90 °C and subsequent analysis by denaturing 20% PAGE (*31*). A mixture of oxidative guanine products occurs (*32*), some of which are not piperidine-labile, e.g., 8-oxo-7,8-dihydroguanine (*33*). On the basis of similar experiments in our group, it is known that piperidine-sensitive and piperidine-insensitive guanine damage occur in proportional amounts (S. R. Rajski and J. K. Barton, unpublished results).

Such gel electrophoretic analysis of flash-quench samples containing 0−25 equiv of Lys-Tyr-Lys and irradiated at 436 nm for 10 min showed that the damage to guanine bases in the DNA double helix is modulated by the presence of the peptide (Figure 6). Two observations are noteworthy. First, at higher peptide concentrations (20−25 equiv), Lys-Tyr-Lys competes with the quencher $[Ru(NH_3)_6]^{3+}$ for binding to DNA. Since contact of the quencher with DNA is crucial for the initiation of the DNA-mediated electron-transfer

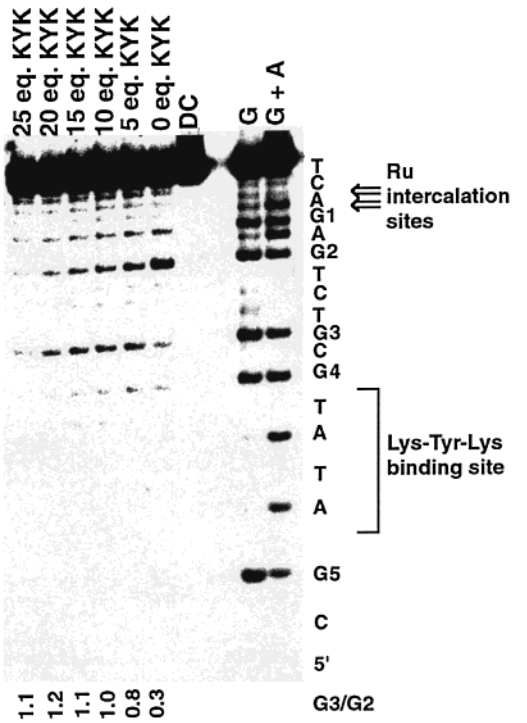


FIGURE 6: Gel electrophoretic analysis of Ru−DNA conjugate **3** after irradiation and treatment with piperidine at elevated temperature in the presence of different concentrations of Lys-Tyr-Lys. Samples contained oligonucleotide **3** (1 μM) and $[Ru(NH_3)_6]Cl_3$ (15 μM) in phosphate buffer (5 mM), pH = 9.0. Samples were irradiated at $\lambda_{exc} = 436$ nm for 10 min. After irradiation, samples were treated with 10% piperidine at 90 ° C for 30 min and analyzed on a denaturing polyacrylamide (20%) DNA sequencing gel.

reaction (*34*), no damage is observed at all at higher peptide concentrations. Second, with increasing peptide concentrations (5−10 equiv), the damage to the guanine nearest to the peptide binding site (G3, Figure 6) increases at the apparent expense of the damage to the guanine proximal to the Ru intercalator (G2, Figure 6). The binding of Lys-Tyr-Lys to DNA clearly alters the distribution of guanine damage throughout the DNA base stack and therefore is able to modulate DNA-mediated charge transfer, as shown previ-

ously with Lys-Trp-Lys (*13*). This observation can be explained by a competition between guanine and tyrosine for the electron hole. Oxidation of guanine (1.3 V) (*12*) is less favorable than that of tyrosine (0.9 V) (*23*); therefore it can be expected that the electron hole primarily resides on the tyrosine, localizing the charge near the binding region of the tripeptide. The guanine radical is expected to be present only in small amounts relative to the concentration of tyrosine radical but is more reactive, yielding irreversible oxidation (G^{ox}, Scheme 1). This charge redistribution leads to an increase of radical concentration in the vicinity of Lys-Tyr-Lys and, if associated with guanine, irreversible DNA damage occurs.

Irreversible Product Analysis. The tyrosine radical is a phenol-type radical (*35*) and therefore would be expected to show different reactivity in comparison to the DNA-bound tryptophan radical (*13*). The flash-quench samples contain several potential reactants, including oxygen, water, and the DNA bases. To investigate the reactivity of the DNA-bound tyrosine radical toward these reactants, we irradiated flash-quench samples at 436 nm containing DNA substrate **1**, $[Ru(phen)_2(dppz)]^{2+}$, $[Ru(NH_3)_6]^{3+}$, and Lys-Tyr-Lys and analyzed the products by HPLC and mass spectrometry. It was possible to separate the peptide fraction from the DNA fraction via reversed-phase HPLC. ESI mass spectrometry of the peptide fraction showed only the mass of the original peptide (m/z = 438.2), thus indicating that the tyrosine radical, once generated in its DNA-bound form, seems to be unreactive toward oxygen or water. Significantly, close examination of MALDI-TOF mass spectrometric analysis of the DNA fraction revealed a peak at m/z = 6557.9, the mass expected if one of the DNA strands were cross-linked to Lys-Tyr-Lys. Formation of this covalent adduct was clearly dependent upon all components of the flash-quench reaction, including Lys-Tyr-Lys. Moreover, analysis of unirradiated samples ruled out that these DNA-peptide adducts are formed under MALDI-TOF measurement conditions. It was not possible to separate reaction constituents by HPLC.

Instead, the DNA fraction of irradiated flash-quench samples was enzymatically digested by use of nuclease P1 and, subsequently, alkaline phosphatase (*29*). The resulting deoxyribonucleosides were then analyzed by HPLC (Figure 7). The different peaks of the HPLC chromatogram have been assigned to the corresponding deoxyribonucleosides by co-injections with solutions containing commercially available deoxyribonucleosides. Two additional product peaks occurred only in irradiated samples that contained Lys-Tyr-Lys. The corresponding HPLC fractions were collected and analyzed by MALDI-TOF mass spectrometry. Both peaks showed the mass of the corresponding nucleoside peptide adduct (dA′ m/z = 717.7 $[M + 23]^+$; dT′ m/z = 701.1 $[M + 23]^+$). It is noteworthy that these adducts occur only with adenosine and thymidine (*36*). This result, together with the different rise time of the tyrosine radical observed with DNA substrates **1**−**3** (Figure 5), establishes that Lys-Tyr-Lys binds preferentially to the AT alternating sequence. Lys-Tyr-Lys therefore undergoes long-range electron-transfer reactions through the DNA from a distinct binding site.

Detection of the Guanine Radical Intermediate in Peptide-to-DNA Electron Transfer. With $[Ru(phen)_2(dppz)]^{2+}$ noncovalently bound to poly(dG·dC) as the DNA substrate in flash-quench experiments, we were able to detect the guanine radical spectroscopically (*5*,*8*). In experiments with poly(dG·dC) and Lys-Tyr-Lys, the transient absorption signal at 405 nm for the tyrosine radical displayed a similar kinetic behavior as for the DNA substrates **1**−**3** (Table 1). However, a transient absorption at 510 nm could be detected on a faster time scale (Figure 8). This signal at 510 nm decayed on the same time scale ($\tau \sim 450$ ns) on which the signal of the tyrosine radical at 405 nm rose. It is important to note that the tyrosine radical has no significant absorbance at 510 nm. On the basis of its kinetic behavior in comparison to previously published flash-quench experiments, we assign

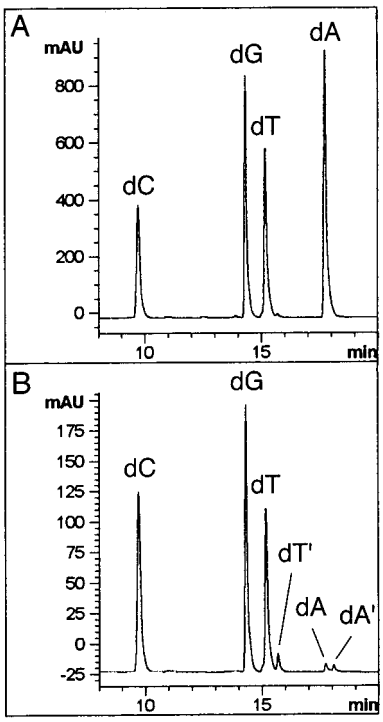


FIGURE 7: HPLC analysis of the mixture of deoxyribonucleosides after enzymatic digestion of the flash-quench sample monitored at 260 nm (A) and 290 nm (B). The sample contained $[Ru(phen)_2(dppz)]Cl_2$ (100 μM), **1** (100 μM), $[Ru(NH_3)_6]Cl_3$ (1.5 mM), and Lys-Tyr-Lys (2.0 mM) in phosphate buffer (5 mM), pH = 9.0. The sample was irradiated at λ_{exc} = 436 nm for 30 min and then digested with nuclease P1 and alkaline phosphatase as described under Experimental Procedures.

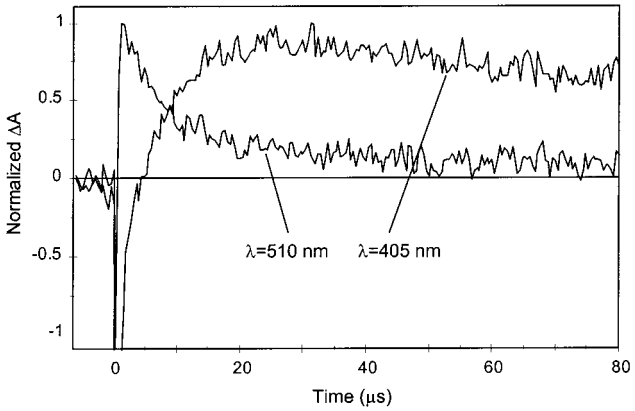
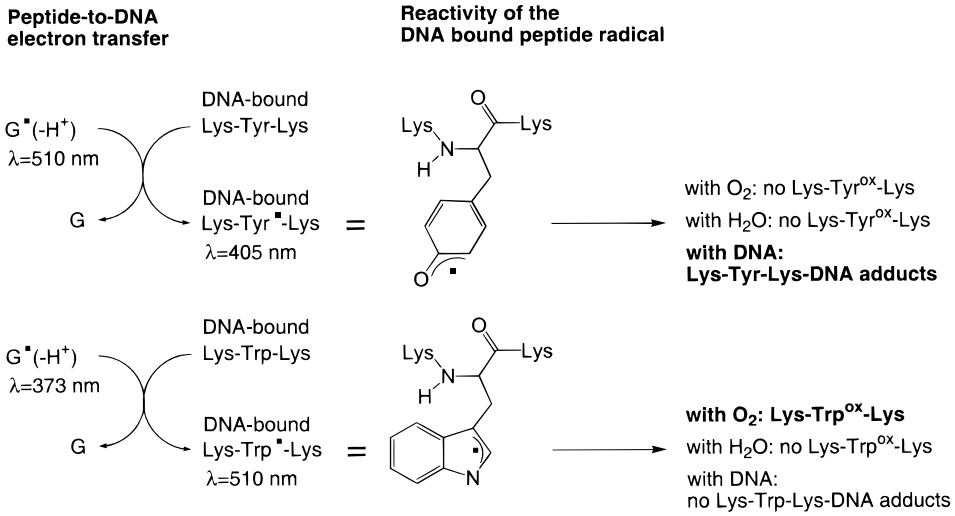


FIGURE 8: Transient absorption at 510 and 405 nm of the solution containing poly(dG·dC) in the presence of Lys-Tyr-Lys (600 μM). Sample contained $[Ru(phen)_2(dppz)]Cl_2$ (30 μM), poly(dG·dC) (600 μM nucleotides), and $[Ru(NH_3)_6]Cl_3$ (600 μM) in 5 mM phosphate buffer, pH = 9.0, λ_{exc} = 480 nm.

Scheme 2: Summary of the Two Observed Peptide-to-DNA Electron-Transfer Reactions and Reactivity of the Generated DNA-Bound Peptide Radicals toward Oxygen, Water, or the DNA Bases



this signal to the guanine radical. This result suggests that the guanine radical is the key intermediate of the observed peptide-to-DNA electron-transfer reactions.

Experiments without DNA do not provide sufficient controls for the intermediacy of the guanine radical since the excited *Ru(II) complex is quenched by water on a very fast time scale (200 ps) in the absence of DNA (*37*). Instead, we repeated the transient absorption experiments with poly(dA·dT) as the DNA substrate. Poly(dA·dT) provides many favorable binding sites for Lys-Tyr-Lys. Although the excited state of $*[Ru(phen)_2(dppz)]^{2+}$ was efficiently quenched by $[Ru(NH_3)_6]^{3+}$ (F_q = 83%, Table 1), we did not detect a transient absorption signal at 405 nm, the absorbance maximum of the tyrosine radical, in the presence of the tripeptide. It is clear, then, that electron transfer from Lys-Tyr-Lys to $[Ru(phen)_2(dppz)]^{3+}$, despite the favorable thermodynamics, is not direct but requires the intermediacy of the guanine radical (vide supra). Perhaps the deprotonation of the guanine radical makes it an effective hole trap within DNA.

By use of the mixed-sequence oligonucleotides **1**−**3**, it was not possible to detect the guanine radical as the intermediate of the DNA-mediated charge migration, because the negative signal from the Ru(III) intercalator persists for tens of microseconds. In the oligonucleotide duplexes, the $[Ru(phen)_2(dppz)]^{3+}$ generated is not completely and rapidly converted to guanine radical, as is the case with poly(dG·dC).

Comparison between DNA-Bound Tyrosine and Tryptophan Radicals and Implications. We have now shown that both the tyrosine radical and the tryptophan radical can be generated in their DNA-bound form by electron transfer through the DNA base stack, and they can be observed by transient absorption spectroscopy. With regard to rise time and decay, both radicals behave remarkably similarly. With poly(dG·dC) as the DNA substrate, the guanine radical was observed as the intermediate in the peptide-to-DNA electron-transfer reactions (Scheme 2).

Nonetheless, we detected a significant difference in the reactivity of the generated DNA-bound peptide radicals. As described earlier (*13*), the tryptophan radical forms an oxidized peptide product in the presence of oxygen or dimerizes in the absence of oxygen at high peptide concentrations in its DNA-bound form. The tryptophan radical exhibits no reactivity toward the DNA bases. In contrast, the tyrosine radical in its DNA-bound form shows no reactivity toward oxygen or water, but cross-links selectively to adenine and thymine at the binding site. This pattern of reactivity of the two peptide radicals is consistent with spin density calculations. In the tryptophan radical, the unpaired electron is located predominantly on the five-membered ring of the indolyl system (*38*). For steric reasons it is very likely, then, that the aromatic part of tryptophan is intercalated mainly with the six-membered ring. As a result, the unpaired electron of the DNA-bound radical is accessible to reactants in solution, mainly oxygen. In contrast, the tyrosine radical spin density is located at or near the phenol oxygen of the aromatic ring (*35*). Assuming significant stacking with the DNA bases, cross-linking reactions with the DNA bases at the binding site are very probable and, in fact, are detected.

It is surprising that small peptides with only three amino acids, such as Lys-Tyr-Lys and Lys-Trp-Lys, are able to participate in DNA-mediated electron-transfer reactions over long distances. Thus, they can serve as models for DNA-binding proteins.The observed reactivity of DNA-bound aromatic peptide radicals is interesting to consider with regard to oxidative damage occurring within the cell. Indeed, given the facility of DNA-mediated charge transfer and of peptide-to-DNA electron transfer, it is tempting to suggest that such redox reactions play some role in DNA repair processes. On the basis of these data, aromatic side chains could serve as electron-transfer relays in protein−DNA redox reactions.

REFERENCES

1. (a) Kelley, S. O., and Barton, J. K. (1999) *Metal Ions Biol. 36*, 211. (b) O'Neill, P., and Frieden, E. M. (1993) *Adv. Radiat. Biol. 17*, 53.
2. (a) Holmlin, R. E., Dandliker, P. J., and Barton, J. K. (1997) *Angew. Chem., Int. Ed. Engl. 36*, 2714. (b) Gasper, S. M., and Schuster, G. B. (1997) *J. Am. Chem. Soc. 119*, 12762. (c) Ly, D., Sanii, L., and Schuster, G. B. (1999) *J. Am. Chem. Soc., 121*, 9400. (d) Lewis, F. D., Wu, T., Zhang, Y., Letsinger, R. L., Greenfield, S. R., and Wasielewski, M. R. (1997) *Science 277*, 673. (e) Meade, T. J., and Kayyem, J. F. (1995) *Angew. Chem., Int. Ed. Engl. 34*, 358. (f) Meggers, E., Kusch,

D., Spichty, U., and Giese, B. (1998) *Angew. Chem., Int. Ed. Engl. 37*, 460. (g) Giese, B., Spormann, M., Lindemann, U., Meggers, E., and Michel-Beyerle, M. E. (1999) *Angew. Chem., Int. Ed. Engl. 38*, 996. (h) Fukui, K., and Tanaka, K. (1998) *Angew. Chem., Int. Ed. Engl. 37*, 158. (i) Beratan, D. N., Priyadarshy, S., and Risser, S. M. (1998) *J. Biol. Chem. 3*, 196. (j) Fink, H.-W., and Schönenberger, C. (1999) *Nature 398*, 407. (k) Harriman, A. (1999) *Angew. Chem., Int. Ed. Engl. 38*, 945.

3. (a) Kelley, S. O., and Barton, J. K. (1999) *Science 283*, 375. (b) Murphy, C. J., Arkin, M. R., Jenkins, Y., Ghatlia, N. D., Bossmann, S. H., Turro, N. J., and Barton, J. K. (1993) *Science 262*, 1025. (c) Kelley, S. O., and Barton, J. K. (1998) *Chem. Biol. 5*, 413.
4. (a) Arkin, M. R., Stemp, E. D. A., Holmlin, R. E., Barton, J. K., Hörmann, A., Olson, E. J. C., and Barbara, P. F. (1996) *Science 273*, 475. (b) Wan, C., Fiebig, T., Kelley, S. O., Treadway, C. R., Barton, J. K., and Zewail. A. H. (1999) *Proc. Natl. Acad. Sci. U.S.A. 96*, 6014.
5. Stemp, E. D. A., Arkin, M. R., and Barton, J. K. (1997) *J. Am. Chem. Soc. 119*, 2921.
6. Kelley, S. O., Jackson, N. M., Hill, M. G., and Barton, J. K. (1999) *Angew. Chem., Int. Ed. Engl. 38*, 941.
7. Hall, D. B., and Barton, J. K. (1997) *J. Am. Chem. Soc. 119*, 5045.
8. Schiemann, O., Turro, N. J., and Barton, J. K. (2000) (submitted for publication).
9. (a) Núñez, M. E., Hall, D. B., and Barton, J. K. (1999) *Chem. Biol. 6*, 85. (b) Henderson, P. T., Jones, D., Hampikian, G., Kan, Y., and Schuster, G. B. (1999) *Proc. Natl. Acad. Sci. U.S.A. 96*, 8353.
10. Kelley, S. O., Holmlin, R. E., Stemp, E. D. A., and Barton, J. K. (1997) *J. Am. Chem. Soc. 119*, 9861.
11. Rajski, S. R., Kumar, S., Roberts, R. J., and Barton, J. K. (1999) *J. Am. Chem. Soc. 121*, 907.
12. Steenken, S., and Jovanovic, S. V. (1997) *J. Am. Chem. Soc. 119*, 617.
13. Wagenknecht, H.-A., Stemp, E. D. A., and Barton, J. K. (2000) *J. Am. Chem. Soc. 122*, 1−7.
14. Dunn, D. A., Lin, V. H., and Kochevar, I. E. (1992) *Biochemistry 31*, 11620.
15. Chang, I. J., Gray, H. B., and Winkler, J. R. (1991) *J. Am. Chem. Soc. 113*, 7056.
16. Juris, A., Balzani, V., Barigelletti, F., Campagna, S., Belser, P., and von Zelewsky, A. (1988) *Coord. Chem. Rev. 84*, 85.
17. Steenken, S. (1992) *Free Radical Res. Commun. 16*, 349.
18. Arkin, M. R., Stemp, E. D. A., Pulver, S. C., and Barton, J. K. (1997) *Chem. Biol. 4*, 389.
19. Lau, A. Y., Schärer, O. D., Samson, L., Verdine, G. L., and Ellenberger, T. (1998) *Cell 95*, 249.
20. Hélène, C., and Maurizot, J. C. (1981) *CRC Crit. Rev. Biochem. 10*, 213.
21. (a) Rajeswari, M. R., Montenay-Garestier, T., and Hélène, C. (1987) *Biochemistry 78*, 926. (b) Rajeswari, M. R. (1996) *J. Biomol. Struct. Dyn. 14*, 25.
22. (a) Behmoras, T., Toulme, J.-J., and Hélène, C. (1981) *Proc. Natl. Acad. Sci. U.S.A. 78*, 926. (b) Behmoras, T., Toulme, J.-J., and Hélène, C. (1981) *Nature 292*, 858.
23. DeFelippis, M. R., Murthy, C. P., Broitman, F., Weinraub, D., Faraggi, M., and Klapper, M. H. (1991) *J. Phys. Chem. 95*, 3416.
24. (a) Beaucage, S. L., and Caruthers, M. H. (1981) *Tetrahedron Lett. 22*, 1859. (b) Goodchild, J. (1990) *Bioconj. Chem. 1*, 165.
25. Amouyal, E., Homsi, A., Chambron, J.-C., and Sauvage, J.-P. (1990) *J. Chem. Soc., Dalton Trans.*, 1841.
26. Strouse, G. F., Anderson, P. A., Schoonover, J. R., Meyer, T. J., and Keene, F. R. (1992) *Inorg. Chem. 31*, 3004.
27. Anderson, P. A., Deacon, G. B., Haarman, K. H., Keene, F. R., Meyer, T. J., Reitsma, D. A., Skelton, B. W., Strouse, G. F., Thomas, N. C., Treadway, J. A., and White, A. H. (1995) *Inorg. Chem. 34*, 6145.
28. Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual*, 2nd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
29. Ito, K., Inoue, S., and Kawanashi, S. (1993) *J. Biol. Chem. 268*, 13221.
30. Prütz, W. A., and Land, E. J. (1979) *Int. J. Radiat. Biol. 36*, 513.
31. Chung, M.-H., Kiyosawa, H., Nishimura, S., and Kasai, H. (1992) *Biochem. Biophys. Res. Commun. 188*, 1.
32. (a) Spassky, A., and Angelov, D. (1997) *Biochemistry 36*, 6571. (b) Kino, K., and Saito, I. (1998) *J. Am. Chem. Soc. 120*, 7373. (c) Gasparetto, D., Ravanat, J.-L., Gérot, O., and Cadet, J. (1998) *J. Am. Chem. Soc. 120*, 10283. (c) Douki, T., and Cadet, J. (1999) *Int. J. Radiat. Biol. 75*, 571.
33. Cullis, P. M., Malone, M. E., and Merson-Davies, L. A. (1996) *J. Am. Chem. Soc. 118*, 2775.
34. Stemp, E. D. A., Holmlin, R. E., and Barton, J. K. (2000) *Inorg. Chim. Acta* (in press).
35. Babcock, G. T., Espe, M., Hoganson, C., Lydakis-Simantiris, N., McCracken, J., Shi, W., Styring, S., Tommos, C., and Warncke, K. (1997) *Acta Chem. Scand. 51*, 533.
36. Connor, D. A., Falick, A. M., and Shetlar, M. D. (1998) *Photochem. Photobiol. 68*, 1.
37. (a) Friedman, A. E., Chambron, J. C., Sauvage, J. P., and Barton, J. K. (1990) *J. Am. Chem. Soc. 112*, 4960. (b) Jenkins, Y., Friedman, A. E., Turro, N. J., and Barton, J. K. (1992) *Biochemistry 31*, 10809. (c) Holmlin, R. E., Stemp, E. D. A., and Barton, J. K. (1998) *Inorg. Chem. 37*, 29. (d) Olson E. J. C., Hu, D., Hormann, A., Jonkman, A. M., Arkin, M. R., Stemp, E. D. A., Barton, J. K., and Barbara, P. F. (1997) *J. Am. Chem. Soc. 119*, 11458.
38. (a) Jensen, G. M., Goodin, D. B., and Bunte, S. W. (1996) *J. Phys. Chem. 100*, 954. (b) Walden, S. E., and Wheeler, R. A. (1996) *J. Phys. Chem. 100*, 1530.

BI992897M