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Evidence of Electron Transfer from Peptides to DNA: Oxidation of DNA-Bound Tryptophan Using the Flash-Quench Technique

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Abstract: A flash-quench method has been employed to probe electron-transfer reactions from peptides to DNA. The photoexcited intercalators $[Ru(phen)_2(dppz)]^{2+}$ (phen = 1,10-phenanthroline; dppz = dipyridophenazine) and $[Ru(phen)(bpy')(dppz)]^{2+}$ (bpy′ = 4-(4′-methyl-2,2′-bipyridyl)valerate) are quenched by a nonintercalating and weakly bound electron-transfer quencher to generate the corresponding DNA-bound Ru(III) complexes *in situ*. Both Ru(III) complexes are powerful ground-state oxidants, capable of oxidizing guanine in DNA or DNA-bound tryptophan of the intercalating peptide, Lys-Trp-Lys. In mixed-sequence oligonucleotide duplexes containing $[Ru(phen)(bpy')(dppz)]^{2+}$ tethered at one end, damage to distant guanines is observed by gel electrophoresis, consistent with the mobility of the electron through the DNA duplex. This damage at guanines is observed in both the presence and absence of Lys-Trp-Lys, but the presence of the peptide affects the distribution. In flash-quench experiments using mixed-sequence oligonucleotides or poly(dG·dC) in the presence of Lys-Trp-Lys, transient absorption spectroscopy reveals a signal at $\lambda = 510$ nm assigned to the tryptophan radical; it decays on the time scale of 60−250 μs. The final peptide product of this electron-transfer reaction has been described by UV/vis spectroscopy and mass spectrometry. No DNA−peptide adducts were detected. Significantly, the tryptophan radical is not observed in reactions with Ru(III) bound to poly(dA·dT), an observation that suggests the intermediacy of the guanine radical cation in generating the tryptophan radical. These results indicate that charge migration from tryptophan to $[Ru(phen)(bpy')(dppz)]^{3+}$ occurs to produce the tryptophan radical and that this process is DNA mediated. This work establishes methodology to probe tryptophan intercalation in DNA by protein or peptides. Moreover, this methodology demonstrates an electron-transfer event between peptides and DNA and suggests the consideration of such events within the cell.

Introduction

An understanding of DNA-mediated electron-transfer chemistry is critical in characterizing oxidative damage to the DNA double helix as a route to mutagenesis and carcinogenesis.[1] Recently, photoinduced long-range electron-transfer reactions with DNA as a bridge between bound donors and acceptors have been probed in our laboratory by various methods, including fluorescence quenching,[2] transient absorption spec-

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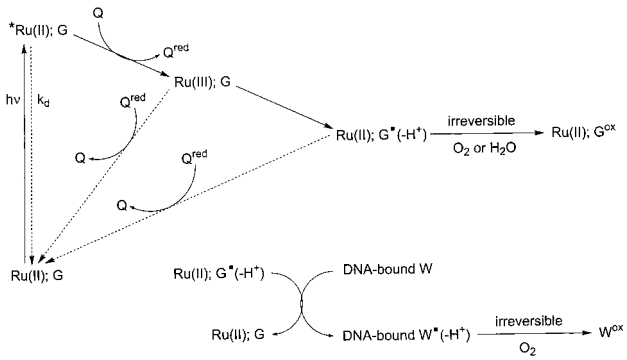
troscopy,[3,4] electrochemistry,[5] and gel electrophoretic analysis of oxidative lesions.[6] These experiments have all shown that DNA-mediated electron transfer is extremely sensitive to the π-stacking of the intervening DNA bases as well as the stacking of both donor and acceptor. DNA-mediated electron transfer can occur on an ultrafast time scale with well-stacked reactants, and with tethered intercalators, can result in reactions over long distances. Indeed, electron-transfer chemistry mediated by the base pair stack can yield both the damage and repair of DNA from a distance.[7−9]

The interactions of proteins with nucleic acids provide a molecular basis for gene expression and DNA repair. Aromatic amino acid residues in particular have been shown to engage in stacking interactions with nucleic acid bases[10] and could represent a possible target for oxidative damage through radical migration. It was also reported that electron transfer from histones to DNA could be observed by EPR upon γ-irradiation in the presence of chromatin.[11] Certainly stacking interactions between proteins and DNA play an important role in protein−nucleic acid binding, recognition, and conformation.[12] The exquisite sensitivity of electron transfer through the DNA base stack may provide a useful tool to explore these noncovalent interactions as well as the structural perturbations in DNA that arise with bound proteins. We have therefore become interested in how proteins and peptides modulate DNA-mediated electron-transfer chemistry.[13]

It seemed reasonable that a detailed investigation of protein−DNA electron transfer processes might begin with the study of model systems containing only one DNA-interactive aromatic amino acid.[14] The tripeptide Lys-Trp-Lys has been extensively studied as a model compound for DNA-binding proteins, because it exhibits stacking properties with both single-stranded and double-stranded nucleic acids coupled with a high degree of favorable electrostatic interactions between the cationic lysine amines and the phosphodiester DNA backbone.[15] The stacking of the tryptophan residue with base pairs in DNA is similar to that observed with classical intercalators and is favored with AT alternating sequences[16] or in the presence of an abasic site.[17] NMR studies with different di- and tripeptides reveal that both lysines are required for effective stacking within double-stranded DNA, resulting in a binding constant of 1.25×10^4 M^{-1} for poly(dA·dT).[16]

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. The tryptophan radical W˙ can undergo back reactions to the unoxidized tryptophan in the presence of guanine radicals G˙ or reduced quencher Q^{Red}. These reactions were omitted from the scheme for clarity.

Complexes of Ru(II) bearing a dipyridophenazine (dppz) ligand, such as $[Ru(phen)_2(dppz)]^{2+}$ (phen = 1,10-phenanthroline) or $[Ru(phen)(bpy')(dppz)]^{2+}$ (bpy′ = 4-(4′-methyl-2,2′-bipyridyl)valerate), have been used as luminescent probes of DNA due to the large (10^3) enhancements in emission observed upon DNA intercalation.[19,20] Ruthenium(III) intercalators, generated *in situ* from the dppz complexes of Ru(II) in a flash-quench methodology, have also been utilized to explore oxidative reactions of the DNA bases.[4] Flash-quench reactions are initiated by visible light which generates an excited *Ru(II) complex (Scheme 1). Upon irradiation, nonintercalating oxidants such as $[Ru(NH_3)_6]^{3+}$ and methyl viologen (MV^{2+}) efficiently quench the emission of the excited state of intercalated Ru(II) complexes on the nanosecond time scale. The resulting intercalated Ru(III) complexes are powerful ground-state oxidants with 3+/2+ reduction potentials of 1.5 V,[21] indicating that these complexes

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should oxidize guanine nucleotides (1.3 V).[22] In fact, the subsequent generation of guanine radicals within the DNA double helix is easily observable by transient absorption spectroscopy at $\lambda = 373$ nm,[4] an isosbestic point in the *Ru(II)−Ru(II) difference spectrum.[23] The spectrum that is obtained corresponds to that assigned previously as the neutral, deprotonated guanine radical,[24] which has an absorption maximum near $\lambda = 373$ nm, rather than the guanine radical cation. Within poly(dG·dC), the oxidation of guanine by intercalated and *in situ* generated $[Ru(phen)_2(dppz)]^{3+}$ occurs in less than 200 ns using $[Ru(NH_3)_6]^{3+}$ as the quencher.[23] Once formed, nearly all of the guanine radical decays within 100 μs. Irreversible reactions of the guanine radical with oxygen or water yield oxidative lesions which could then be analyzed by gel electrophoresis.[25]

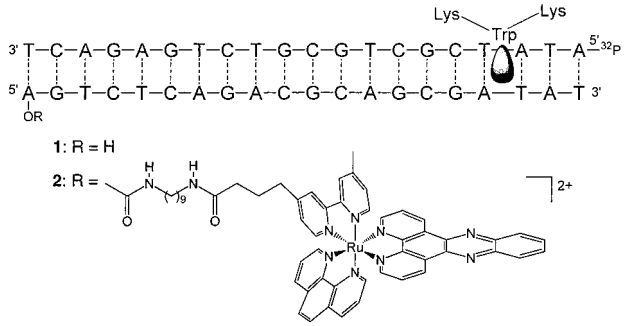
Based on thermodynamics, the guanine radical cation should be able also to oxidize tryptophan residues (~1 V)[22,26] within peptides or proteins. Indeed, in pulse radiolysis experiments, it was demonstrated that the guanine radical cation, whether present as the nucleoside or in single-stranded DNA, does oxidize both tryptophan and tyrosine in solution.[27] The redox potential of the tryptophan radical exhibits a strong pH dependence, ranging from 0.9 V at pH 3 to 0.6 V at pH 13.[28]

Herein we present the application of the flash-quench technique,[29] originally developed to explore electron-transfer processes in proteins,[30] in probing the oxidation of the intercalated indole residue of tryptophan through DNA-mediated electron transfer. Through transient absorption spectroscopy and gel electrophoretic analysis, we have examined the formation and decay of the tryptophan radical in experiments with both covalently bound $[Ru(phen)(bpy')(dppz)]^{2+}$ and noncovalently associated $[Ru(phen)_2(dppz)]^{2+}$ using a variety of quenchers and DNA duplexes.

Experimental Methods

Materials. Lys-Trp-Lys, methyl viologen (MV^{2+}), and hexaammineruthenium(III) chloride were obtained from Sigma-Aldrich and used as received. DNA polymers, poly(dA·dT) and poly(dG·dC), were purchased from Pharmacia and were dialyzed against a buffer of phosphate (5 mM) and NaCl (50 mM), pH 8.5, prior to use. The oligonucleotides **1** and **2** (Scheme 2) were prepared on an Applied Biosystems 394 DNA synthesizer, using standard phosphoramidite chemistry.[31] After preparation, the resin was cleaved and the DNA was deprotected by treatment with NH_4OH at 55 °C for 10 h, dried, and

Scheme 2. DNA Substrates **1** and **2**



purified by HPLC (Hewlett-Packard HP1050) on a semipreparative reversed-phase C-18 column (Dynamax C18, Rainin, 300 Å) using 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 $\lambda =$ 260 nm. Duplexes were formed by slow cooling of equal concentrations of complementary strands.

$[Ru(phen)_2(dppz)]Cl_2$ was prepared as described previously[32] and used as the enantiomeric mixture. The $[Ru(phen)(bpy')(dppz)]^{2+}$-modified oligonucleotide **2** was prepared from the racemic metal complex. This tris(heteroleptic) complex was synthesized according to the general methods of Strouse et al.[33] and Anderson et al.[34] 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 a solid-phase methodology and purified as described earlier.[25] The Ru−DNA conjugate was characterized by UV/vis spectroscopy and 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 $\lambda = 260$ nm. Duplexes were formed by slow cooling of equal concentrations of the Ru−DNA strand with its complement.

Laser Spectroscopy. Time-resolved emission and transient absorption measurements used an excimer-pumped dye laser (Coumarin 480) as described previously.[35] Laser powers at $\lambda_{exc} = 480$ 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 [^{32}P]-γ-ATP by standard protocols[36] and hybridized to the complementary strands in a buffer of triethanolamine (75 mM), pH 8.5. Samples of 10 μL size containing the radioactively labeled Ru−DNA−duplex **2** (1 μM), MV^{2+} (15 μM), and Lys-Trp-Lys (0−100 μM) in triethanolamine buffer (75 mM), pH 8.5, were irradiated at $\lambda = 436$ 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, dried, and analyzed through a 20% denaturing polyacrylamide gel. 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 (0.5 mM) were irradiated for 30 min at $\lambda = 436$ nm and subsequently analyzed by HPLC (Hewlett-Packard HP1050). The original peptide and the oxidized peptide product could

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be separated using a semipreparative reversed-phase C18-column (Dynamax C18, 300 Å, Rainin) and the following conditions: A = NH_4OAc buffer (50 mM), pH 6.5; B = MeCN; gradient = 0−4% B in 8 min and subsequent 4−10% in 60 min. After separation, the peptide-containing fractions were lyophilized, dissolved in MeOH, and described by UV/vis spectroscopy (Hewlett-Packard Diode array spectrometer 8452) and ESI mass spectrometry; the DNA-containing fraction was analyzed by mass spectrometry (MALDI-TOF). In control experiments where **1**, $[Ru(phen)_2(dppz)]Cl_2$, $[Ru(NH_3)_6]Cl_3$, or Lys-Trp-Lys was omitted, no oxidized product was detected.

Results and Discussion

Transient Absorption Studies. The formation of the tryptophan radical was initially pursued in flash-quench reactions of duplex **1** with noncovalently bound $[Ru(phen)_2(dppz)]^{2+}$,[37] using $[Ru(NH_3)_6]^{3+}$ as a quencher, and with increasing concentrations of Lys-Trp-Lys. Since the tripeptide binds well to poly(dA·dT), an alternating AT sequence was incorporated at one end of duplex **1** to ensure that intercalation of Lys-Trp-Lys could occur (Scheme 2).[16] In fact, a small change in the UV/vis absorption of Lys-Trp-Lys could be detected in the presence of the DNA **1**, consistent with the stacking of the indolyl part of the tripeptide. The tryptophan radical cation has a pK_a of 4.3−4.5,[38] so at the experimental pH of 8.5, only the neutral tryptophan radical should be present; this latter species can be monitored at $\lambda = 510$ nm.[39]

Excitation of $[Ru(phen)_2(dppz)]^{2+}$ in the presence of **1** (30 μM) leads to a biexponential luminescence decay, with $\tau_1 = 82$ ns and $\tau_2 = 318$ ns.[20,37] This excited state is quenched efficiently by $[Ru(NH_3)_6]^{3+}$, with 81% quenching observed for 600 μM $[Ru(NH_3)_6]^{3+}$. Lys-Trp-Lys does not significantly quench the $[Ru(phen)_2(dppz)]^{2+}$ excited state, indicating the absence of direct electron transfer from tryptophan to *Ru(II). Furthermore, concentrations ≤300 μM do not diminish the quenching by $[Ru(NH_3)_6]^{3+}$.

It is noteworthy that, based on redox potentials and titrations in DMSO, it is thermodynamically feasible for both tryptophan and guanine to reductively quench the *Ru(II) excited state. Bound to DNA, however, no evidence for such reductive quenching has been detected. Possibly, the MLCT character of the *Ru(II) excited state, bound to DNA, precludes this reductive quenching.

Oxidative quenching of the photoexcited metallointercalator generates a Ru(III) species intercalated in DNA that can oxidize guanine to form the guanine radical cation. When $[Ru(phen)_2(dppz)]^{2+}$ is quenched by $[Ru(NH_3)_6]^{3+}$ while bound to **1**, a long-lived signal ($k = 2 \times 10^5$ s^{-1}) is observed at 440 nm via transient absorption spectroscopy. This signal is negative because it is dominated by the Ru(III)−Ru(II) absorbance change, corresponding to the electron transfer from $[Ru(NH_3)_6]^{2+}$ to the Ru(III) intercalator. Support for the formation of the guanine radical is seen in the positive absorbance at 373 nm, a wavelength near both a Ru(III)−Ru(II) isosbestic point and a maximum in the neutral guanine radical spectrum. At $\lambda = 510$ nm, a small positive signal persists at long times in the absence of Lys-Trp-Lys, also consistent with the presence of guanine radical.

Addition of Lys-Trp-Lys to the flash-quench sample gives rise to a positive signal at 510 nm (Figure 1). We assign this signal to the neutral tryptophan radical, which has its λ_{max} at 510 nm.[39] When $[Ru(phen)_2(dppz)]^{2+}$ is quenched by $[Ru(NH_3)_6]^{3+}$ in the presence of **1** and Lys-Trp-Lys, a transient absorption rises within 10 μs, concomitant with the decay of Ru(III). Once formed, the signal decays nearly completely within 0.4 ms. The observed signal is strongly dependent on the peptide concentration, since the addition of 10 equiv of Lys-Trp-Lys increases the maximum of the transient absorption significantly. In fact, the concentration of tryptophan radical, determined based upon its extinction coefficient, corresponds closely to the concentration of DNA-bound Lys-Trp-Lys, based upon its binding constant. Thus it appears that each intercalated tryptophan becomes oxidized. The deprotonation of the initially formed tryptophan radical cation appears to be too fast to be monitored by our method, since there is no significant transient absorption detectable at $\lambda = 560$ nm, the absorbance maximum[39] of the tryptophan radical cation.

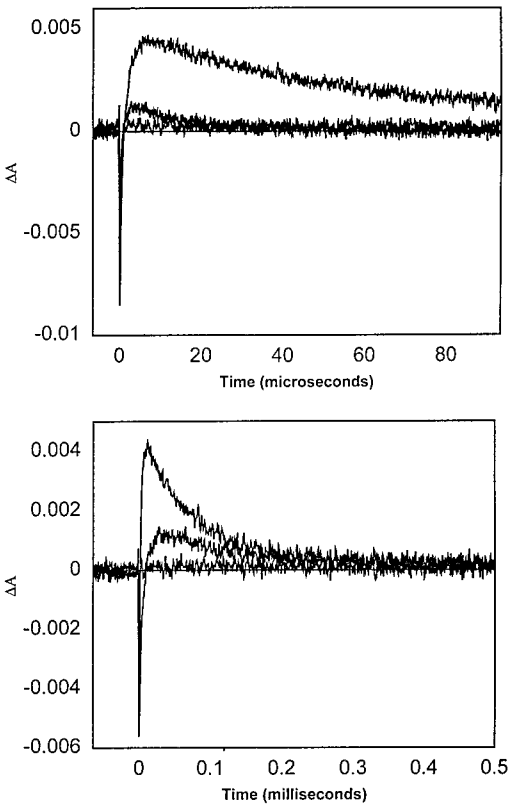

Figure 1. Transient absorption at $\lambda = 510$ nm for the sample containing oligonucleotide **1**, observed on two different time scales (top, 100 μs; bottom, 0.5 ms), in the absence (bottom line) and in the presence of Lys-Trp-Lys (30 μM, middle line, and 300 μM, upper line). Samples contained $[Ru(phen)_2(dppz)]^{2+}$ (30 μM), **1** (30 μM), and $[Ru(NH_3)_6]^{3+}$ (600 μM) in phosphate buffer (5 mM), pH 9.0, $\lambda_{exc} = 480$ nm.

The decay of the observed tryptophan radical signal is well-described by a monoexponential function (Table 1). The lifetime of the radical is in agreement with the earlier published observation that the reaction of the tryptophan radical with oxygen is too slow to be monitored by pulse radiolysis ($k = 5 \times 10^6$ s^{-1}).[40] No absorption at $\lambda = 510$ nm was observed in control experiments omitting either the oligonucleotide, quencher, or tripeptide. The absence of any one factor precluded electron transfer from the peptide to the intercalated Ru(III) complex, thus indicating that the reaction requires DNA.

To define better the intercalation site for the Ru(II) complex, we synthesized a second DNA substrate (**2**), bearing [Ru(phen)-

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(39) $\epsilon = 2.0 \times 10^3$ M^{-1} cm^{-1} for the tryptophan radical according to: Prütz, W. A.; Land, E. J. *Int. J. Radiat. Biol.* **1979**, *36*, 513. We assume a similar value for the DNA-bound radical.

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Table 1. Quenching Efficiencies, Decay of the Transient Absorption Signals at $\lambda = 373$ nm (guanine radical) and 510 nm (tryptophan radical) and Yields of the Generated Tryptophan Radical Using Different Oligonucleotides[a]

oligonucleotide	% quenched[b]	guanine radical ($\lambda = 373$ nm) decay	tryptophan radical ($\lambda = 510$ nm) decay	$conc_{max}$ (μM)[c]	yield (%)[d]
1	81	*e*	$k = 1.3 \times 10^4\ s^{-1}$	2.8 ± 0.1	1.0 ± 0.1
2	76	*e*	$k = 4.2 \times 10^3\ s^{-1}$	1.9 ± 0.2	0.8 ± 0.2
poly (dA·dT)	87	not observable	not observable	not observable	not observable
poly (dG·dC)	93	$k = 2.2 \times 10^6\ s^{-1}$	$k = 9.9 \times 10^3\ s^{-1}$	1.0 ± 0.2	0.4 ± 0.1

[a] [DNA] = 30 μM (for **1** and **2**), [nucleotide] = 600 μM (for poly(dA·dT and poly(dG·dC)), $[[Ru(phen)_2(dppz)]^{2+}] = 30\ \mu$M, [Q] = $[[Ru(NH_3)_6]^{3+}]$ = 600 μM, [Lys-Trp-Lys] = 300 μM, 5 mM P_i buffer, pH 9.0, $\lambda_{Exc} = 480$ nm. [b] $1 - I_0/I$, I and I_0 monitored at 610 nm. [c] $\epsilon = 2.0 \times 10^3\ M^{-1}cm^{-1}$ at $\lambda = 510$ nm.[19] [d] Corrected by the quenching efficiency. [e] Due to overlapping absorbances of Ru(III) and the guanine radical, the decay kinetics could not be determined.

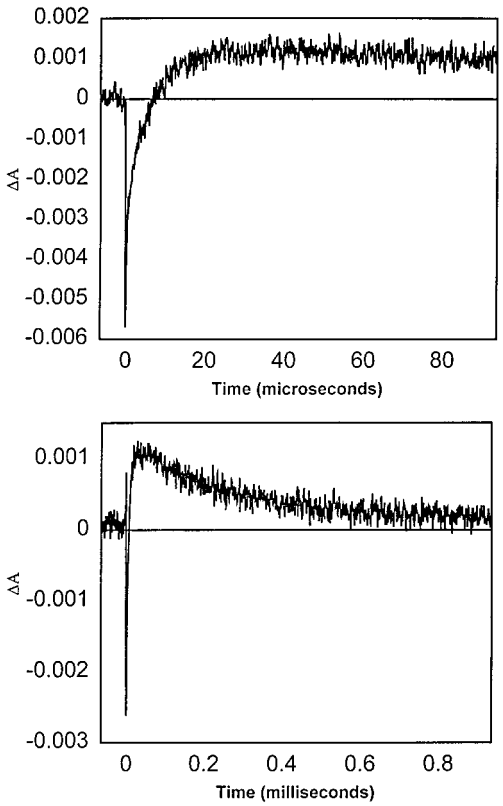

Figure 2. Transient absorption at $\lambda = 510$ nm for the sample containing oligonucleotide **2**, observed on two different time scales (top, 100 μs; bottom, 1 ms) in the presence of Lys-Trp-Lys (100 μM); the sample contained **2** (10 μM) and $[Ru(NH_3)_6]^{3+}$ (200 μM) in phosphate buffer (5 mM), pH 9.0, $\lambda_{exc} = 480$ nm.

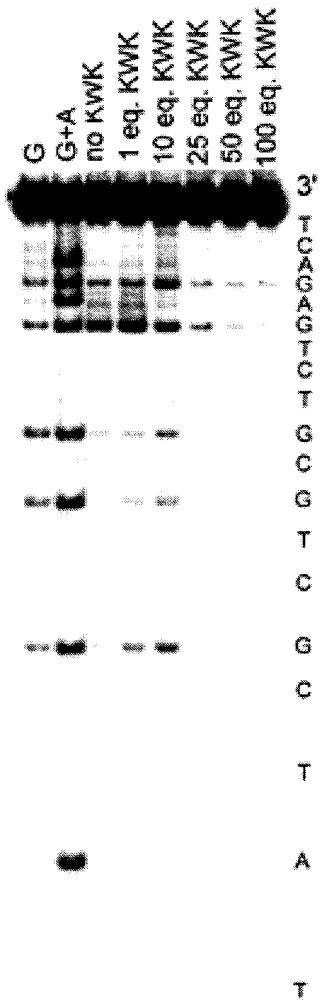

Figure 3. Gel electrophoretic analysis of oligonucleotide **2** after irradiation and treatment with piperidine at elevated temperature in the presence of different concentrations of Lys-Trp-Lys. Samples contained **2** (1 μM) and MV^{2+} (15 μM) in triethanolamine buffer (75 mM), pH 8.5. Samples were 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.

$(bpy')(dppz)]^{2+}$ covalently tethered to the 5′-end distal to the Lys-Trp-Lys-binding region (Scheme 2).[25,41] Irradiation of this duplex in the presence of $[Ru(NH_3)_6]^{3+}$ and Lys-Trp-Lys yielded comparable transient absorption signals at $\lambda = 510$ nm (Table 1), although the radical exhibited a longer rise-time (30−40 μs) and also a longer lifetime ($k = 4.2 \times 10^3\ s^{-1}$) than observed with noncovalently bound reactants (Figure 2).

Gel Electrophoretic Analysis. To explore how the oxidation of the intercalated tryptophan affects the intervening DNA bases, the yield of permanent damage associated with the flash-quench experiment was analyzed by gel electrophoresis (Figure 3). For this experiment, the complement to the $[Ru(phen)(bpy')(dppz)]^{2+}$-tethered strand in duplex **2** was radioactively labeled at the 5′ end.[36] The low oxidation potential for guanine dictates that, within canonical B-form DNA, guanine is the base which is most often the target of oxidative damage. Oxidative damage to guanine has traditionally been visualized using gel electrophoresis in measurements of strand breaks after piperidine treatment at elevated temperature.[42] Such digestive analysis reveals not only the precise location of damage but reflects also the extent to which it has occurred. In fact, a mixture of

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oxidative products occurs,[43] only some of which can be revealed by piperidine treatment. Digestion instead with enzymes which recognize damaged sites, such as formamidopyrimidine glycosylase, has previously been carried out and gives proportional results.[44] Thus we can measure the effect of tryptophan radical formation on the irreversible guanine damage.

When duplex **2** was irradiated at $\lambda = 436$ nm in the presence of differing concentrations of Lys-Trp-Lys, it was observed thattripeptide concentrations up to 10 equiv enhanced oxidative guanine damage progressively further down from the site of metal binding. This observation may be attributed to a competition between guanine and tryptophan for the electron hole. Oxidation of guanine (1.3 V)[22] is less favorable than that of tryptophan (0.6−1.1 V),[22,26] so it is expected that the radical will primarily reside on the tryptophan, localizing the electron hole near the binding region of Lys-Trp-Lys. The guanine radical cation is expected to be present only in small proportions relative to the tryptophan radical cation, because the resulting neutral, deprotonated guanine radical is more reactive and converts to irreversible oxidation products more readily than does the neutral tryptophan radical.[45] This redistribution would lead to an increase in radical concentration in the vicinity of Lys-Trp-Lys and, if associated with guanine, irreversible damage could occur. Certainly, it appears from the gel electrophoretic results that the presence of the tryptophan radical cation affects the distribution of guanine radical cations along the DNA helix but not the overall yield of it. This distribution occurs on a time scale that is fast compared to the irreversible trapping of the neutral guanine radical to yield piperidine-labile oxidized guanine nucleotides. As a result, the oxidative guanine damage is facilitated near and at the binding region of Lys-Trp-Lys, but only in the presence of the tripeptide. Also consistent with this explanation, the short-range damage relative to that seen with the Ru complex without peptide is diminished. We had earlier observed a competition between the oxidative repair of thymine dimers and guanine damage in DNA.[7c]

At higher concentrations of Lys-Trp-Lys (more than 25 equiv), the peptide competes with the quencher (MV^{2+}) for binding to DNA. In that case no DNA damage is observed at all, since contact between the quencher and DNA is required to generate the Ru(III) complex and initiate DNA-mediated electron transfer.[25] Alternatively, it is possible that, at high peptide concentrations, the peptide interferes with the ability of $[Ru(phen)(bpy')(dppz)]^{2+}$ to intercalate efficiently within **2**.

It is noteworthy that the generated tryptophan radical appears unreactive toward adduct formation with any of the DNA bases. No cross-linking product of the reaction between Lys-Trp-Lys and the DNA could be detected either by gel electrophoresis or mass spectrometry (MALDI-TOF).

Irreversible Product Analysis. After irradiation at $\lambda = 436$ nm for 30 min, we were able to separate an oxidized product of Lys-Trp-Lys by HPLC and characterize it by ESI mass spectrometry and UV/vis spectroscopy (Figure 4). Importantly, its mass (*m*/*z* 493.3) is 32 mass units heavier than native peptide (*m*/*z* 461.2), indicating that two oxygen atoms are inserted per peptide molecule. The different UV/vis spectrum of the oxidized product in comparison to the original peptide additionally suggests that the indolyl aromatic system has been modified.

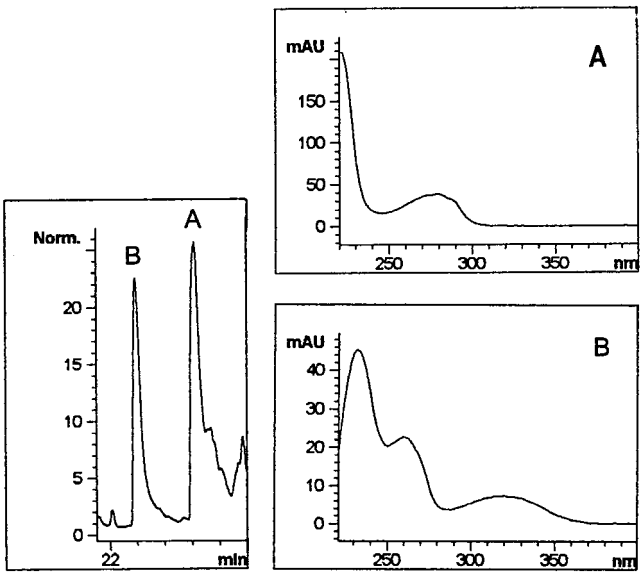

Figure 4. HPLC analysis (left) and UV/vis spectra (right) of the original peptide Lys-Trp-Lys (A) and the oxidized product (B) of the irradiated samples. The sample contained $[Ru(phen)_2(dppz)]^{2+}$ (30 μM), **1** (30 μM), $[Ru(NH_3)_6]^{3+}$ (600 μM), and Lys-Trp-Lys (300 μM) in phosphate buffer (5 mM), pH 9.0, and was irradiated at $\lambda_{exc} = 436$ nm for 30 min and then analyzed by HPLC.

This is based on a comparable spectral change observed upon oxidation of guanine to 8-oxo-7,8-dihydroguanine through DNA-mediated electron transfer.[46] On the basis of these results we suggest that *N*-formylkynurenine, shown as the major photoproduct of tryptophan,[47] is also the product of the peptide-to-DNA electron-transfer reaction. This product has appropriate mass and UV/visible absorbance. The described product formation with the peptide is strongly oxygen dependent, since the same reaction carried out in deaerated buffer and under argon does not yield any oxidized product. Instead, a dimeric peptide is obtained (*m*/*z* 919.7) under anaerobic conditions. In all experiments there were no cross-linking products between Lys-Trp-Lys and the oligonucleotides detectable by mass spectrometry (MALDI-TOF).

The pattern of reactivity that we find is consistent with spin density calculations of the neutral tryptophan radical, which showed that the unpaired electron is mainly located on the five-membered ring of the indolyl system.[48] Steric considerations would suggest that tryptophan is intercalated mainly with the benzene part of the indolyl aromatic residue. As a result, dimerization of the peptide would be expected to occur outside the DNA base stack at higher peptide concentrations, as, in fact, was observed under anaerobic reaction conditions.

Studies with Poly(dG·dC) and Poly(dA·dT). To explore whether the in situ generated and intercalated Ru(III) complex oxidizes the tryptophan residue directly or the electron-transfer reaction occurs via electron migration through the π-stack, we repeated the transient absorption experiments using either poly(dA·dT) or poly(dG·dC). These experiments test the intermediacy of the guanine radical cation. Studies with poly(dA·dT) provide an important control, since emissive Ru(II) complexes are generated only in DNA-bound samples in H_2O. Without DNA, the *Ru(II) complex is quenched by water on a fast time

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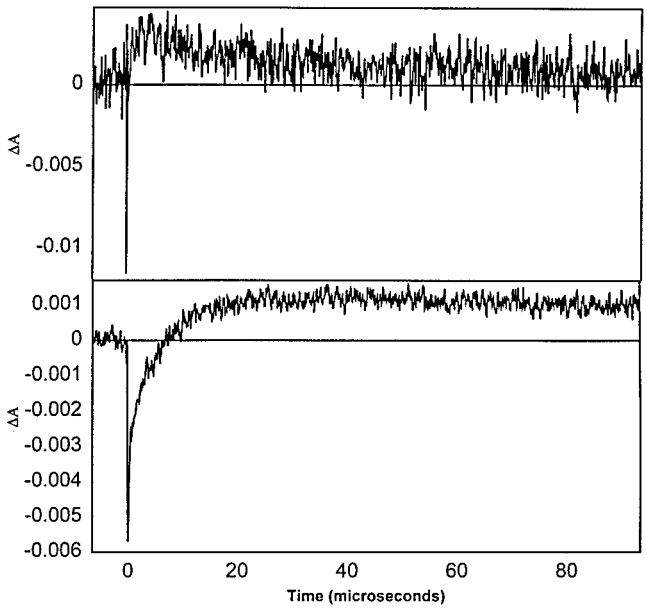

Figure 5. Transient absorption at $\lambda = 373$ nm (top) and 510 nm (bottom) of the assay containing poly(dG·dC) in the presence of Lys-Trp-Lys (300 μM) observed on the same time scale (100 μs). Samples contained $[Ru(phen)_2(dppz)]^{2+}$ (30 μM), poly(dG·dC) (600 μM nucleotides), and $[Ru(NH_3)_6]^{3+}$ (600 μM) in 5 mM phosphate buffer, pH 9.0, $\lambda_{exc} = 480$ nm.

scale (200 ps).[19,20] Thus experiments carried out without DNA are not sufficient controls. It is important to note also that poly(dA·dT) provides favorable binding sites for Lys-Trp-Lys.

Using poly(dG·dC) as the substrate, the signal at $\lambda = 510$ nm was obtained only in the presence of Lys-Trp-Lys. It exhibits a similar kinetic behavior compared to the results observed with the oligonucleotides **1** and **2** (Table 1). The yield of observed tryptophan radical per *Ru quenched is somewhat lower with poly(dG·dC), since this oligonucleotide does not provide the favored alternating AT binding sequence (Table 1). Nonetheless, in addition to the tryptophan signal at $\lambda = 510$ nm, a new signal could be observed at $\lambda = 373$ nm, albeit on a shorter time scale. The tryptophan signal at $\lambda = 510$ nm concomitantly rises with the decay of the signal observable at $\lambda = 373$ nm (Figure 5). From its fast decay (within 50 μs; Table 1), the 373 nm signal can be attributed to the neutral guanine radical and suggests that the guanine radical is an intermediate of the electron-transfer reaction from the peptide to the intercalated Ru complex.[8]

In contrast, with poly(dA·dT) as the DNA substrate, no signal at $\lambda = 510$ nm was observed in the presence of Lys-Trp-Lys, although the quenching process was efficient (Table 1). This observation is more remarkable still given that the alternating AT sequence of this substrate provides many favorable sites for tripeptide intercalation. Importantly, this result rules out the formation of the tryptophan radical via direct contact of the tripeptide with $[Ru(phen)_2(dppz)]^{3+}$. Once formed, the Ru(III) complex does not appear to oxidize tryptophan in poly(dA·dT); the back reaction with the quencher is apparently faster. Moreover, it strongly suggests that guanine bases within the DNA duplex are in fact necessary for electron transfer from tryptophan to $[Ru(phen)(bpy')(dppz)]^{3+}$ in the covalent assembly.

Implications. We have demonstrated that the flash-quench methodology can be used to elucidate electron-transfer reactions between peptides and DNA. This methodology could be valuable in detailing the intercalation in DNA of aromatic side chains of DNA-binding proteins. In this context, it is also noteworthy that the tryptophan radical, in its bound form, shows no significant reactivity toward adduct formation with any of the DNA bases. This lack of reactivity is remarkable in terms of the oxidative DNA damage that arises within the cell; intercalated tryptophan residues would be expected, then, not to participate in this damage. It is tempting to consider, instead, that bound tryptophan residues could serve as valuable electron-transfer relays between protein redox cofactors and DNA. Indeed, given the facility of DNA-mediated charge transfer and of DNA−peptide electron transfer, it is tempting to suggest that such redox reactions might play some role in DNA repair processes.

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