Oxidation of 7-Deazaguanine by One-Electron and Oxo-Transfer Oxidants: Mismatch-Dependent Electrochemistry and Selective Strand Scission

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Addition of oligonucleotides containing 7-deazaguanine (Z) to solutions containing $Ru(dmb)_3^{2+}$ (dmb = 4,4′-dimethyl-2,2′-bipyridine) produces an enhancement in the oxidative current in the cyclic voltammogram of the metal complex that can be used, through digital simulation, to determine the rate of oxidation of 7-deazaguanine by $Ru(dmb)_3^{3+}$. The measured rate constants are about 10 times higher than those for oxidation of guanine by $Ru(bpy)_3^{3+}$, even though the redox potential of $Ru(dmb)_3^{3+/2+}$ is 200 mV lower. A potential of 0.75 V (vs Ag/AgCl) can therefore be estimated for the oxidation of 7-deazaguanine, which can be selectively oxidized over guanine when $Ru(dmb)_3^{3+}$ is the oxidant. The rate of oxidation was much faster in single-stranded DNA, and the difference between rates of single-stranded and duplex DNA was higher than for guanine. The oxidation rate was also sensitive to the presence of a single-base mismatch at the 7-deazaguanine in the order Z·C < Z·T < Z·G ∼ Z·A < single-stranded. The Z·T mismatch was much more readily distinguished than the G·T mismatch, consistent with the overall greater sensitivity to secondary structure for Z. The oxidation reaction was also probed by monitoring piperidine-labile cleavage at the Z nucleotide, which could be generated by treatment with either photogenerated $Ru(bpy)_3^{3+}$ or the thermal oxidant $Ru(tpy)(bpy)O^{2+}$ (tpy = 2,2′,2″-terpyridine). These oxidants gave qualitatively similar selectivities to the electron-transfer rates from cyclic voltammetry, although the magnitudes of the selectivities were considerably lower on the sequencing gels.

Electron-transfer reactions of guanine and its derivatives are of interest for understanding the mechanism of natural mutagenesis,[1,2] probing electron-transfer reactions in DNA,[3−14] developing detection schemes for nucleic acids,[15] and probing nucleic acid secondary structure.[16−21] Guanine electron-transfer reactions have been studied extensively using photochemically generated one-electron oxidants.[3−14] These studies suggest that long-range electron transfer in DNA can be realized when guanines are available between the remote sites to mediate the transfer via hole hopping.[13,22,23]

We have developed an alternative approach to studying guanine electron transfer in which generation of $Ru(bpy)_3^{3+}$ during a cyclic voltammogram produces a catalytic current enhancement that is related to the reactivity of the guanine nucleobase (bpy = 2,2′-bipyridine).[15,24] The main advantage of electrochemistry is the ability to collect kinetic data on relatively small quantities of material compared to spectrophotometric methods. Digital simulation of cyclic voltammetry data allows study of the mechanism and acquisition of rate constants for guanine oxidation in different DNA environments.[16,25−28] At high ionic strength, binding of the metal complex to the polyanion is negligible and the simulation requires a two-step EC′ mechanism:

$$Ru(bpy)_3^{2+} \leftrightharpoons Ru(bpy)_3^{3+} + e^- \quad (1)$$

$$Ru(bpy)_3^{3+} + DNA \rightarrow Ru(bpy)_3^{2+} + DNA' \quad (2)$$

High sensitivity of the rate constant of eq 2 to the solvent

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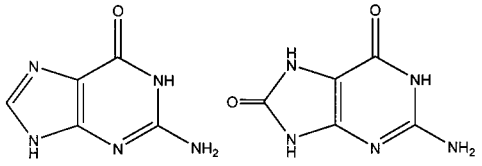
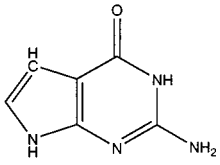
accessibility of guanine nucleobases has enabled us to identify and distinguish all possible single nucleotide mismatches of guanine.[25]

Greater versatility in the design of DNA electron-transfer experiments is afforded by other readily oxidized guanine derivatives. Modified guanine bases that have been identified as more easily oxidized lesions than guanine itself are 8-oxoguanine (8G)[29] and 7-deazaguanine (Z).[8] The 8-oxoguanine (or





more precisely, 8-oxo-7,8-dihydroguanine) base has been under extensive scrutiny because it is a product of oxidative DNA damage in double-stranded DNA[30] and is responsible for some biological G → T transversions.[31] The effect of guanine stacking on the oxidation of 8G[32] and selective oxidation of 8G by Ir(IV) have been reported,[33] in addition to the use of 8-oxoguanine as a site-selective electrochemical probe for DNA secondary structure.[34]

Compared to 8-oxoguanine, considerably less attention has been paid to the 7-deazaguanine derivative. The 7-deazaguanine base is of interest not only because it is a facile electron donor and therefore useful in DNA electron-transfer studies but also because substitution of the 7-deazaguanine base disrupts noncanonical secondary structures. Thus, 7-deazaguanine is used to amplify highly structured DNAs that are difficult to amplify with native guanine.[35] For electron transfer, previous investigations on the 7-deazaguanine derivative were centered on using this lesion to probe electronic coupling in the DNA π stack;[8,9] these studies give an estimate of the potential of the 7-deazaguanine base of 0.8 ± 0.1 V (all potentials vs Ag/AgCl). The 7-deazaguanine base has also been used to probe the electronic structure of guanine multiplets using our electrochemical approach.[27]

Here we report a broad investigation of the redox activity of 7-deazaguanine. The derivative is rapidly oxidized during the redox cycling of $Ru(dmb)_3^{2+}$ (dmb = 4,4′-dimethyl-2,2′-bipyridine), which exhibits a potential 200 mV lower than that of the $Ru(bpy)_3^{2+}$ complex we have used to study guanine. The rate of the reaction of 7-deazaguanine with $Ru(dmb)_3^{3+}$ is still significantly faster than the analogous rate for the reaction of guanine with $Ru(bpy)_3^{3+}$, and we estimate the potential of the 7-deazaguanine base to be about 0.75 V in accord with earlier estimates.[8,9] In addition, we report here that the 7-deazaguanine base exhibits a larger attenuation in electron-transfer rate upon hybridization to the complementary strand than in the guanine derivative. This observation is probably related to the greater stability of DNA duplexes with 7-deazaguanine.[35] Accordingly, a ZT mismatch is much more readily distinguished in the cyclic voltammetry than a GT mismatch. Finally, we have shown that oxidation of 7-deazaguanine by outer-sphere electron transfer or by an oxo transfer nuclease, $Ru(tpy)(bpy)O^{2+}$ (tpy = 2,2′,2″-terpyridine), produces a piperidine-labile lesion. The strand scission is formed selectively at 7-deazaguanine compared to guanine and at single-stranded 7-deazaguanine compared to duplex 7-deazaguanine. Thus, the 7-deazaguanine chemistry can be used to probe the secondary structure selectively at individual nucleotides by electrophoresis. These studies therefore provide a broad picture of the utility of 7-deazaguanine in studying nucleic acid structure and DNA electron transfer.

Experimental Section

Materials. Synthetic oligonucleotides were purchased from the Nucleic Acid Core Facility at the Lineberger Comprehensive Cancer Center at the University of North Carolina at Chapel Hill and purified by double ethanol precipitation. Water was purified with a Milli-Q purification system (Millipore). Reagents for buffer preparation were purchased from Gibco BRL or Mallinckrodt. $[Ru(bpy)_3]Cl_2$ (bpy = 2,2′-bipyridine) was purchased from Aldrich and purified by recrystallization from methanol. $K_3[Fe(CN)_6]$ and L-ascorbic acid were purchased from Aldrich and Sigma, respectively, and used as received. $[Ru(dmb)_3]Cl_2$ (dmb = 4,4′-dimethyl-2,2′bipyridine), $[Fe(dmb)_3]Cl_2$, and $[Ru(tpy)(bpy)O](ClO_4)_2$ (tpy = 2,2′,2"-terpyridine) were prepared according to published procedures[36−38] with the only modification being the use of Cl_2 instead of Br_2 to oxidize $[Ru(tpy)(bpy)OH_2](ClO_4)_2$ to $[Ru(tpy)(bpy)O](ClO_4)_2$.

Measurements. All solution concentrations were determined spectrophotometrically using a Hewlett-Packard HP 8452 diode array spectrophotometer, with the exception of $K_3[Fe(CN)_6]$ where molecular weight was used. The extinction coefficients used were $\epsilon_{452} = 14\,600$ M^{-1} cm^{-1} for $Ru(bpy)_3^{2+}$ and $\epsilon_{528} = 8400$ M^{-1} cm^{-1} for $Fe(dmb)_3^{2+}$.[36,37] The concentration of $[Ru(tpy)(bpy)O](ClO_4)_2$ was determined by taking the difference in absorbance at 476 nm before and after addition of ascorbic acid and using the extinction coefficient $\epsilon_{476} = 9600$ M^{-1} cm^{-1} for $[Ru(tpy)(bpy)OH_2](ClO_4)_2$.[38] Extinction coefficients for oligonucleotides were calculated using the nearest-neighbor equation, giving the concentration of nucleic acid in strand concentration.[39] Solutions of double-stranded oligonucleotides were prepared by mixing 1:1.3−1:1.5 guanine- or 7-deazaguanine-containing and complementary oligonucleotides in the desired buffer, heating the mixture at 90 °C for 5 min, and cooling the mixture to room temperature over a period of 3 h. Cyclic voltammograms and simulations were performed as described previously.[28]

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Table 1. List of Oligonucleotide Sequences **1**−**7**[a]

name	length, bases	sequence (5′ → 3′ direction)
1	33	**Z**TT CA**Z** ACC AT **Z** AAT ACC ATT TTT TCC **Z**CT C**Z**C
2	32	**G**TT CA**G** ACC AT**G** AAT ACC ATT TTT TCC **G**CT C**G**
3	32	CIA ICI IAA AAA ATI ITA TTC ATI ITC TIA AC
4	21	GTA GAT TAA T**Z**A TAT AAG ATG
5C	21	CAT CTT ATA TCA TTA ATC TAC
5T	21	CAT CTT ATA TTA TTA ATC TAC
5G	21	CAT CTT ATA TGA TTA ATC TAC
5A	21	CAT CTT ATA TAA TTA ATC TAC
6	26	GCT TAT **G**TA TAT **Z**TT ATC AT**G** TAT TA
7	19	TGA TAA CAT ATA CAT AAG C

[a] I = inosine; Z = 7-deazaguanosine.

DNA Cleavage Reactions. Oligonucleotides were labeled as described previously[40] and treated with piperidine to remove any lesions that may have been generated during synthesis or exposure to air by heating in 9 M piperidine at 90 °C for 30 min. The solutions were lyophilized to remove piperidine, and the pellets were washed with two 20 μL aliquots of water and dissolved in 80% formamide. Fragments were separated on a 20% polyacrylamide gel with 7 M urea under denaturing conditions. The bands corresponding to full-length products were cut out of the gel, and DNA was extracted by shaking in water overnight at 4 °C followed by ethanol precipitation. Oligonucleotides were dissolved in water and diluted to 5 μM strand concentration solutions for radiolabeling. The 5′-[^{32}P]-labeled oligonucleotide was heated at 90 °C for 5 min in the presence or the absence of the excess complement in 20 mM sodium phosphate, pH 7. Double-stranded samples were allowed to cool slowly to room temperature over a period of 4−5 h, while single-stranded oligonucleotides were placed on ice immediately. Unlabeled $(GAA)_7$ oligonucleotide was added to a final concentration between 4 and 10 μM in strand concentration. For reactions with $Ru(tpy)(bpy)O^{2+}$, a 10 μL aliquot of 1−4 μM $[Ru(tpy)(bpy)O](ClO_4)_2$ in 10 mM sodium phosphate, pH 7, was added to 10 μL of a DNA stock solution containing 50−100 000 cpm labeled oligonucleotide. The reaction was allowed to proceed for 10 min at room temperature and subsequently quenched with ethanol. Following ethanol precipitation, DNA pellets were dissolved in 60 μL of 0.7 M piperidine and heated at 90 °C for 30 min. The solution was lyophilized, and the pellets were washed with 10 μL of water. For the flash-quench reactions, a solution containing 10 μL of 4 μM $[Ru(bpy)_3]Cl_2$ and 10 μL of 40 μM $K_3[Fe(CN)_6]$, both in 10 mM sodium phosphate, pH 7 buffer, was added to 20 μL of DNA stock solution containing 50−100 000 cpm labeled oligonucleotide. Reaction mixtures were photolyzed for 10 min with an Oriel arc Hg lamp at 300 W and a 350 nm cutoff filter in 100 μL glass polyspring inserts (Fisher). Oligonucleotides were ethanol-precipitated and piperidine-treated in the same manner as for $[Ru(tpy)(bpy)O](ClO_4)_2$.

Electrophoresis. Native gel electrophoresis was performed by adding a sample of 25−50 000 cpm of radiolabeled 7-deazaguanine-containing oligonucleotide to a 100 μM solution of unlabeled oligonucleotide alone or with 130 μM of one of the four complementary oligonucleotides in 50 mM NaP_i/800 mM NaCl buffer. Oligonucleotides were annealed, loaded on a 20% nondenaturing polyacrylamide gel, and electrophoresed at 4 °C at 250 V for 5−7 h. The gel was exposed on a phosphorimager screen overnight and scanned using the Storm 840 system (Molecular Dynamics). Sequencing gels were performed as described previously.

Results

Electrochemical Oxidaiton of 7-Deazaguanine. The electrocatalysis of one-electron 7-deazaguanine oxidation was investigated by cyclic voltammetry of the oligonucleotides given in Table 1. Oligonucleotides **1** and **2** each have the same sequence except that the five 7-deazaguanines in **1** are replaced by native guanines in **2**. The sequences were designed to avoid GG steps because of the enhanced reactivity of the 5′ guanine in the GG sequence compared to guanines that are 5′ to other bases, including 7-deazaguanine.[27,41] In the complementary oligonucleotide **3**, inosine was used in place of guanosine so that no additional electrochemical reactivity would be contributed by the complementary strand. Inosine is frequently used as a redox-silent guanine analogue.[42] Use of inosine in the complementary strand simplifies kinetic analysis and allows for direct comparison of reactivity patterns in single- and double-stranded oligonucleotides.[40]

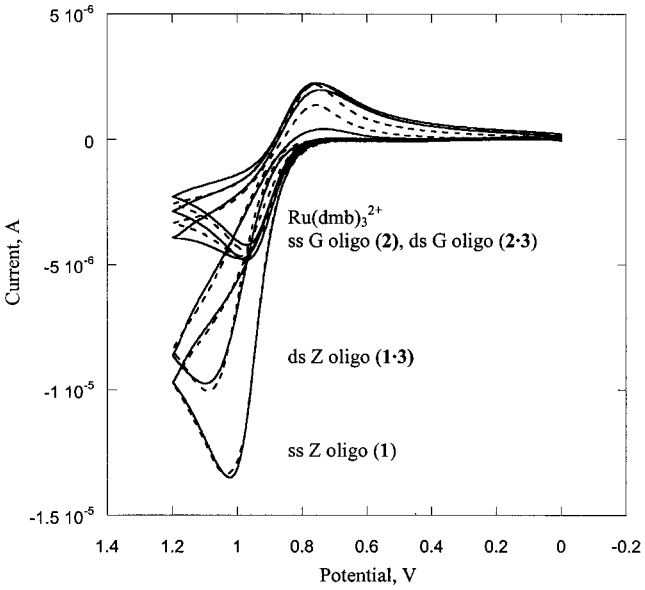

Figure 1. Cyclic voltammograms (solid) and simulations (dashed, using eqs 3−7) of 50 μM $Ru(dmb)_3^{2+}$ in the presence of 25 μM **1** or **2** alone or hybridized to **3** in 50 mM sodium phosphate, pH 7, with 800 mM NaCl at 250 mV/s (A = 0.32 cm^2). Concentrations are in strand.

Because we suspected that 7-deazaguanine was easier to oxidize than native guanine,[8,9] initial experiments were carried out with $Ru(dmb)_3^{2+}$, which exhibits a lower redox potential ($E_{1/2}$ = 0.86 V vs Ag/AgCl) than $Ru(bpy)_3^{2+}$ ($E_{1/2}$ = 1.05 V). The voltammetry was performed at high ionic strength (880 mM Na^+, pH 7) where binding of the metal complex to the nucleic acid is negligible.[26,43] The cyclic voltammograms of 50 μM $Ru(dmb)_3^{2+}$ in the presence of 25 μM **1** or **2**, alone and hybridized to oligonucleotide **3**, are shown in Figure 1. Large current enhancements were observed in the presence of both single- and double-stranded Z oligonucleotides, while their G counterparts showed very little enhancement. This result is

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Table 2. Second-Order Rate Constants for Oxidation of Guanine or 7-Deazaguanine by $Ru(dmb)_3^{3+}$ in Oligonucleotides **1** and **2** Alone or Hybridized to **3**

oligonucleotide	scan rate, mV/s	k, $M^{-1}\ s^{-1}$ [a]	k', $M^{-1}\ s^{-1}$	k'', $M^{-1}\ s^{-1}$
ss Z (**1**)	25	$(3.0 \pm 1.8) \times 10^6$	$(6.8 \pm 4.5) \times 10^4$	$(1.0 \pm 0.30) \times 10^4$
ss Z (**1**)	250	$(3.2 \pm 1.9) \times 10^6$	$(1.3 \pm 0.40) \times 10^5$	$(1.1 \pm 0.60) \times 10^4$
ss G (**2**)	25	$(2.4 \pm 0.80) \times 10^3$	0	0
ss G (**2**)	250	$(3.7 \pm 1.3) \times 10^3$	0	0
ds Z (**1·3**)	25	$(8.2 \pm 5.2) \times 10^4$	$(2.1 \pm 1.4) \times 10^4$	$(2.3 \pm 0.20) \times 10^3$
ds Z (**1·3**)	250	$(2.0 \pm 1.0) \times 10^5$	$(3.9 \pm 1.8) \times 10^4$	0
ds G (**2·3**)	25	410 ± 20	0	0
ds G (**2·3**)	250	$(1.9 \pm 0.60) \times 10^3$	0	0

[a] Rate constants are per Z or G in 50 mM sodium phosphate, pH 7, with 800 mM sodium chloride.

consistent with a lower redox potential for 7-deazaguanine than that for guanine. In addition, single-stranded 7-deazaguanine gives significantly more current enhancement than double-stranded 7-deazaguanine at high ionic strength. This finding parallels our results on the reactions of native guanine with $Ru(bpy)_3^{3+}$ [25] and 8-oxoguanine with $Os(bpy)_3^{3+}$.[34] As observed previously,[28] the selectivity toward secondary structure is best observed at high ionic strength where solvent accessibility is the only parameter governing the reactivity.

Rate information for oxidation of 7-deazaguanine by $Ru(dmb)_3^{3+/2+}$ was obtained by digital simulation of cyclic voltammograms according to the mechanism that we have established for oxidation of guanine by $Ru(bpy)_3^{3+/2+}$.[26,28] The mechanism accounts for slow spontaneous conversion of the oxidized metal complex back to the reduced form, which is known to occur at neutral pH for the $Ru(bpy)_3^{3+}$ complex[44] and is likely to occur to a smaller extent for $Ru(dmb)_3^{3+}$. As with many of our studies of guanine,[28] two overoxidation steps (i.e., where the product of the one-electron oxidation is oxidized by one more electron) were necessary to fit the data well. Many guanine oxidation products are more easily oxidized than guanine itself,[30] which is presumably the case for 7-deazaguanine as well. The complete mechanism used to extract rate constants is shown in eqs 3−7:

$$Ru(dmb)_3^{2+} \leftrightarrows Ru(dmb)_3^{3+} + e^- \quad (3)$$

$$Ru(dmb)_3^{3+} \leftrightarrows Ru(dmb)_3^{2+} \quad (4)$$

$$Ru(dmb)_3^{3+} + DNA \xrightarrow{k} Ru(dmb)_3^{2+} + DNA' \quad (5)$$

$$Ru(dmb)_3^{3+} + DNA' \xrightarrow{k'} Ru(dmb)_3^{2+} + DNA'' \quad (6)$$

$$Ru(dmb)_3^{3+} + DNA'' \xrightarrow{k''} Ru(dmb)_3^{2+} + DNA''' \quad (7)$$

The digital simulations obtained using the mechanism in eqs 3−7 are shown as the dashed lines Figure 1, and the average rate constants k, k', k'' are summarized in Table 2. The rate constants for oxidation of 7-deazaguanine by $Ru(dmb)_3^{3+/2+}$ in both double- and single-stranded sequences are 10−50 times higher than those for oxidation of G by $Ru(bpy)_3^{3+/2+}$ under identical conditions.[28] This enhanced reactivity most likely reflects a higher driving force for the reaction of $Ru(dmb)_3^{3+/2+}$ with 7-deazaguanine compared to the guanine/$Ru(bpy)_3^{3+/2+}$ reaction where $\Delta G^\circ \approx 0$.[25] Using the published estimate for the redox potential of 7-deaza-dGTP of ~0.8 V[8] gives a driving force for the 7-deazaguanine/$Ru(dmb)_3^{3+/2+}$ reaction of about 0.1 eV. This value is only an estimate because the redox potential of 7-deazaguanine may be different in oligonucleotides than in the free mononucleotide triphosphate.

Direct comparison of rates of oxidation of the two substrates by $Ru(dmb)_3^{3+/2+}$ is also informative. The first oxidation step (k) for single-stranded 7-deazaguanine is ~1000 times faster than the reaction of $Ru(dmb)_3^{3+}$ with single-stranded G. For the double-stranded forms, the reaction with 7-deazaguanine is ~100 times faster than that of guanine. The driving force for the reaction of guanine with $Ru(dmb)_3^{3+}$ is unfavorable by 0.2 eV. In addition, 7-deazaguanine is oxidized 20−40 times faster in single-stranded than in duplex DNA. This ratio of reactivities is slightly higher than that for oxidation of guanine by $Ru(bpy)_3^{3+/2+}$ in oligonucleotides containing multiple guanines, where the ratio is closer to 10,[28] suggesting that the 7-deazaguanine/$Ru(dmb)_3^{3+}$ reaction may provide even better sensitivity for examining DNA secondary structure than the guanine/$Ru(bpy)_3^{3+}$ reaction.

Another notable feature of the rate constants in Table 2 is the lack of scan rate dependence of the initial oxidation step (k). For cyclic voltammograms of $Ru(bpy)_3^{2+}$ taken in the presence of guanine, the simulated rate constant increases with the scan rate.[28,43] We have attributed this scan rate dependence to the biphasic nature of the reaction where the faster phase of the reaction contributes more to the apparent rate constant at shorter reaction times observed at higher scan rates.[28,43] Because the reaction of $Ru(dmb)_3^{3+}$ with 7-deazaguanine is faster than the reaction of guanine with $Ru(bpy)_3^{3+}$, we suspect that the lack of scan rate dependence arises from the fact that the fast phase of the reaction is too fast to contribute to the electrochemical response. This idea is supported by separate experiments where the reaction of 7-deaza-dGTP with $Ru(dmb)_3^{3+}$ is too fast to be monitored even by stopped-flow spectrophotometry.[45] If only a single, slower reaction phase contributes to the current enhancement, no scan dependence for the simulated rate constant would be expected.[43]

Effect of Single-Base Mismatches. We next sought to determine whether the rate of 7-deazaguanine would exhibit a dependence on the identity of the complementary base, as we have observed with the guanine/$Ru(bpy)_3^{2+}$[25] and 8-oxoguanine/$Os(bpy)_3^{2+}$[34] reactions. The reaction of 7-deazaguanine with $Ru(dmb)_3^{3+/2+}$ can be monitored at a potential that would not interfere with either one of these reactions. Oligonucleotides with different mismatches opposite the 7-deazaguanine lesion were therefore examined by cyclic voltammetry. The oligonucleotide containing one 7-deazaguanine and four guanines (oligonucleotide **4** in Table 1) was hybridized to its complements containing four different bases in the position opposite the 7-deazaguanine (oligonucleotides **5C**, **5T**, **5G**, and **5A**; Table 3). Complete hybridization of all of the complementary oligo-

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Table 3. Second-Order Rate Constants for 7-Deazaguanine Oxidation by $Ru(dmb)_3^{3+}$ in Oligonucleotide **4** Hybridized to **5C**, **5T**, **5G**, or **5A** in 50 mM Sodium Phosphate, pH 7, with 800 mM Sodium Chloride at 25 mV/s

oligonucleotide	k, $M^{-1}\ s^{-1}$ [a]	k', $M^{-1}\ s^{-1}$ [a]	k'', $M^{-1}\ s^{-1}$ [a]
Z·C (**4·5C**)	$(5.6 \pm 1.0) \times 10^4$	$(1.8 \pm 0.80) \times 10^4$	0
Z·T (**4·5T**)	$(9.3 \pm 2.2) \times 10^4$	$(6.6 \pm 1.2) \times 10^4$	$(3.8 \pm 3.1) \times 10^4$
Z·G (**4·5G**)	$(2.4 \pm 0.50) \times 10^5$	$(3.9 \pm 2.6) \times 10^5$	$(4.6 \pm 1.9) \times 10^5$
Z·A (**4·5A**)	$(2.4 \pm 0.30) \times 10^5$	$(1.5 \pm 0.40) \times 10^5$	$(2.6 \pm 0.50) \times 10^5$

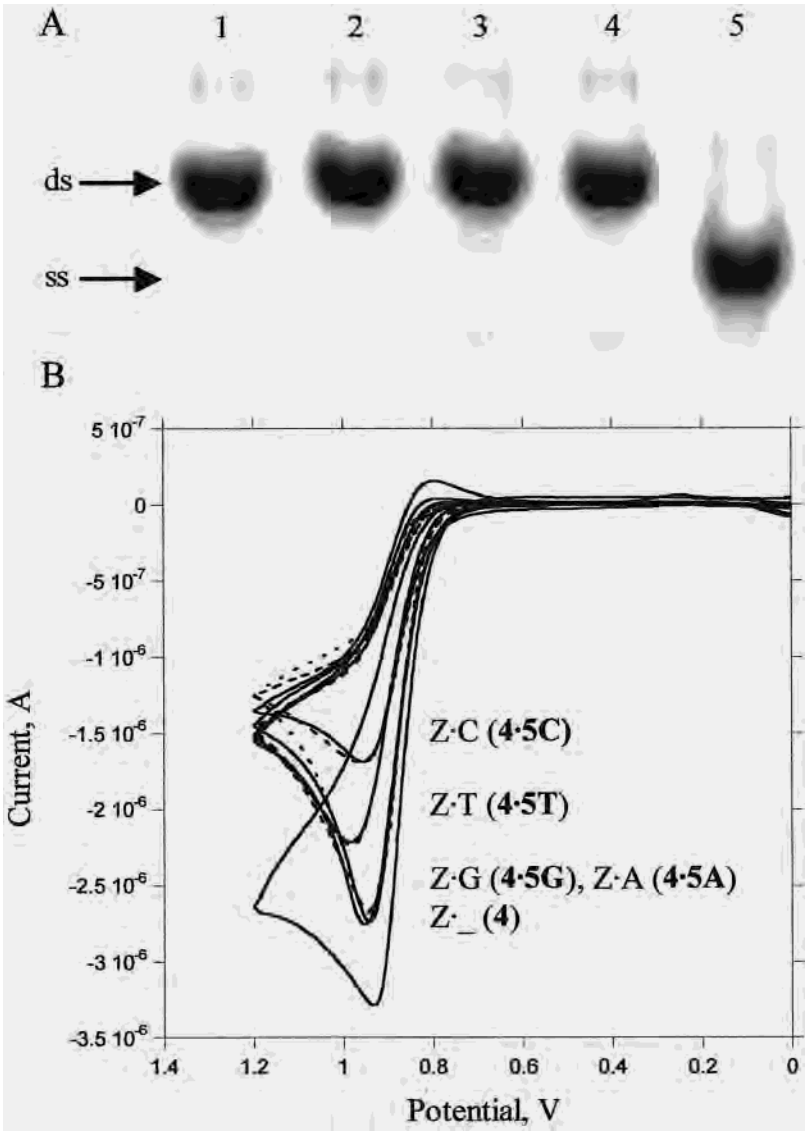

Figure 2. (A) Nondenaturing polyacrylamide gel of **4** hybridized to complements **5X** visualized by phosphorimagery: (1) **4·5C**, (2) **4·5T**, (3) **4·5G**, (4) **4·5A**, (5) **4** (no complement). (B) Cyclic voltammograms (solid) and digital simulations (dashed, using eqs 3−7) of 25 μM $Ru(dmb)_3^{2+}$ in the presence of 7-deazaguanine mismatches at 25 mV/s ($A = 0.11$ cm^2). Conditions for (A) and (B) are the following: 125 μM **4**, 163 μM **5X**, 50 mM sodium phosphate, pH 7 with 800 mM NaCl.

nucleotides to **4** was confirmed by native gel electrophoresis (Figure 2A), which ensures that any difference in current enhancement for different duplexes can be ascribed to the effect of the mismatched nucleotide *in the intact duplex*.

Cyclic voltammograms of $Ru(dmb)_3^{3+/2+}$ in the presence of the 7-deazaguanine mismatches are shown in Figure 2B. The value of the rate constant for oxidation of 7-deazaguanine with its match (**4·5C**) is slightly lower than the rate for oxidation of double-stranded 7-deazaguanine in the hybrid **1·3** (Table 2). Acceptable simulations for the single-stranded 7-deazaguanine oligonucleotide could not obtained because the amount of simulated current was always smaller than the observed current enhancement. The relative order of reactivities Z·C < Z·T < Z·G ~ Z·A < Z·_ (single stranded) is similar to that of guanine and 8-oxoguanine mismatches (Table 4).[25,34] For 7-deazaguanine, the rates were similar for Z·G and Z·A, which is in contrast to our studies with G and 8G.[25,34] In the latter cases, G(or 8G)·A was more reactive than G(or 8G)·G (Table 4), a trend that follows the thermodynamic stabilities of the mismatches.[46,47] The most significant result, however, is the unique rate of the mismatch of 7-deazaguanine with thymine. The mismatches of thymine with guanine and 8-oxoguanine were much more difficult to distinguish from the match with cytosine.[34] Therefore, 7-deazaguanine enables the detection for

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Table 4. Rate Constants for Oxidation of 7-Deazaguanine, Guanine, and 8-Oxoguanine by $Ru(dmb)_3^{3+}$, $Ru(bpy)_3^{3+}$, and $Os(bpy)_3^{3+}$, Respectively

oligonucleotide	k $Ru(dmb)_3^{3+}$/Z, M^{-1} s^{-1}	k $Ru(bpy)_3^{3+}$/G,[a] M^{-1} s^{-1}	k $Os(bpy)_3^{3+}$/8G,[b] M^{-1} s^{-1}
G·C	$(5.6 \pm 1.0) \times 10^4$	1.2×10^3	2.2×10^4
G·T	$(9.3 \pm 2.2) \times 10^4$	5.1×10^3	3.8×10^4
G·G	$(2.4 \pm 0.50) \times 10^5$	1.0×10^4	6.3×10^4
G·A	$(2.4 \pm 0.30) \times 10^5$	1.9×10^4	1.6×10^5
G·_		1.8×10^5	1.4×10^6

[a] Rate constants for $Ru(bpy)_3^{3+}$/G are for sequence 5′-AAA TAT AGT ATA AAA hybridized to 5′-TTT TAT AXT ATA TTT, where X is C, T, G, A, or _ (single-stranded).[25] [b] Rate constants for $Os(bpy)_3^{3+}$/8G are for sequence 5′-ATG AAG T8GA AGT TTT hybridized to 5′-AAA ACT TXA CTT CAT, where X is C, T, G, A, or _ (single-stranded).[34] The rate constants for Z are from Table 3. The rate for single-stranded Z is too fast to be determined.

the first time of the mismatch with thymine in the presence of native guanines in the strand.

DNA Cleavage Studies. The reactivity of 7-deazaguanine in oligonucleotides was further examined by studying the cleavage at 7-deazaguanine lesions in radiolabeled oligonucleotides. Guanine modification can be achieved via an inner-sphere electron-transfer pathway with oxoruthenium metallonucleoases such as $Ru(tpy)(bpy)O^{2+}$,[18,20,40,48] or by one-electron outer-sphere oxidation by photochemically activated $Ru(bpy)_3^{2+}$.[16,49] Strand scission at modified sites is afforded by base treatment with piperidine, and the products are detected and analyzed by high-resolution polyacrylamide gel electrophoresis.

We first investigated oxidation of 7-deazaguanines by $Ru(tpy)(bpy)O^{2+}$ in oligonucleotide **1** and compared it to guanine oxidation in oligonucleotide **2**. Conclusions drawn from the study of these particular sequences are only qualitative in nature because we do not have an internal control to normalize for variations in amounts of radiolabeled DNA in different lanes on the gel. A representative phosphorimage of the cleavage patterns in both single- and double-stranded oligonucleotides is shown in Figure 3. Reaction with oxoruthenium produces piperidine-labile lesions at 7-deazaguanine sites (lanes 3−6 and 8−11) compared to background cleavage by piperidine alone (lanes 2 and 7). In contrast, no reaction of 7-deazaguanine is observed with dimethyl sulfate (DMS) in the Maxam−Gilbert G lane (lane 1). This result is consistent with the mechanistic proposal that oxoruthenium oxidizes the guanine base, whereas DMS reacts with N7 of guanine.[48,50] The replacement of the nitrogen by a carbon at the 7 position renders the base inactive toward modification with DMS, but the oxidation reaction is apparently not inhibited. As expected, the amount of products visualized on the gel increased with the increase in RuO^{2+} concentration both for Z (lanes 2−6 and 7−11) and G (lanes 13−17 and 18−22),[20,40] and the reaction of 7-deazaguanine was more pronounced than that of native guanine.

To quantify the extent of cleavage at 7-deazaguanine sites, we designed a 26-mer oligonucleotide that contains one Z and two G bases (**6**). When hybridized to the complementary 19-mer oligonucleotide **7**, G7 and Z13 become a part of the double helix while G21 remains single-stranded. The reactivity of G21 in the overhang will be the same in both the single- and double-stranded forms, so this site can be used as an internal control to normalize for variations in the amount of radiolabel in different lanes on the gel. Oligonucleotides **6** and **7** form a stable duplex structure, as shown in the native gel in Figure 4A.

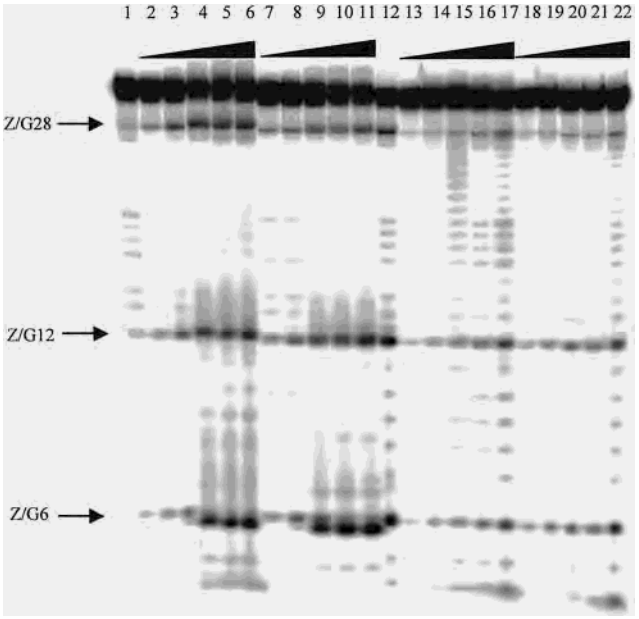

Figure 3. Phosphorimage of the denaturing gel showing cleavage by $Ru(tpy)(bpy)O^{2+}$ of **1** and **2** alone or hybridized to **3**: (1) Maxam−Gilbert G reaction for **1**; (2−6) reaction of $Ru(tpy)(bpy)O^{2+}$ with **1**; (7−11) reaction of $Ru(tpy)(bpy)O^{2+}$ with **1·3**; (12) Maxam−Gilbert G reaction for **2**; (13−17) reaction of $Ru(tpy)(bpy)O^{2+}$ with **2**; (18−22) reaction of $Ru(tpy)(bpy)O^{2+}$ with **2·3**. Concentrations of $Ru(tpy)(bpy)O^{2+}$ are 0, 0.5, 1, 1.5, and 2 μM; unlabeled [DNA] = 2 μM.

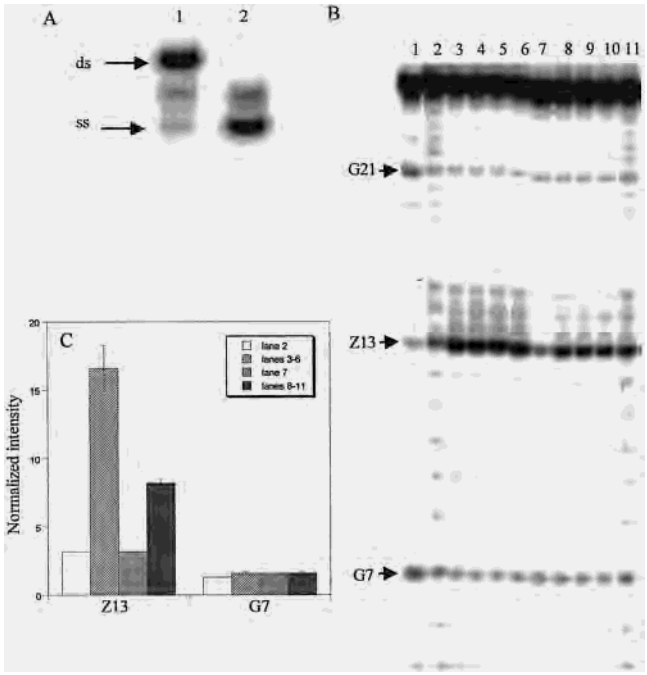

Figure 4. (A) Nondenaturing polyacrylamide gel of the duplex **6·7** (1) and single-stranded **6** (2) visualized by phosphorimagery. (B) Phosphorimage showing cleavage by $Ru(tpy)(bpy)O^{2+}$ of the oligonucleotide **6** alone or hybridized to **7**: (1) Maxam−Gilbert G reaction; (2) **6** + 0 μM $Ru(tpy)(bpy)O^{2+}$; (3−6) **6** + 1 μM $Ru(tpy)(bpy)O^{2+}$; (7) **6·7** + 0 μM $Ru(tpy)(bpy)O^{2+}$; (8−11) **6·7** + 1 μM $Ru(tpy)(bpy)O^{2+}$. Unlabeled [DNA] = 5 μM. (C) Relative intensities for the gel in (B) showing the extent of cleavage at Z13 and G7 normalized to G21.

Oxidation of 7-deazaguanine by oxoruthenium(IV) in the single- and double-stranded forms of **6** yielded results similar to those for **1**. A representative gel and histogram of cleavage

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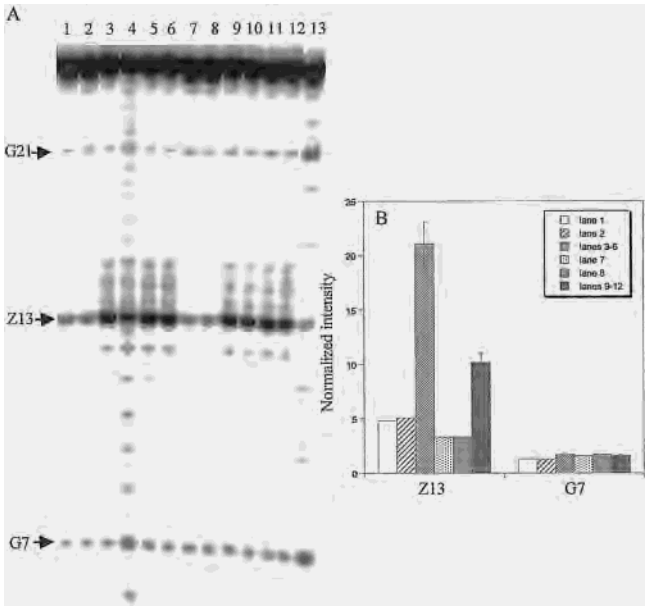

Figure 5. (A) Phosphorimage showing cleavage of the oligonucleotide **6** alone or hybridized to **7** with $Ru(bpy)_3^{3+}$ generated by the flash-quench technique: (1) **6** + 0 μM $Ru(bpy)_3^{2+}$ with 0 μM $Fe(CN)_6^{3-}$; (2) **6** + 0 μM $Ru(bpy)_3^{2+}$ with 10 μM $Fe(CN)_6^{3-}$; (3−6) **6** + 1 μM $Ru(bpy)_3^{2+}$ with 10 μM $Fe(CN)_6^{3-}$; (7) **6·7** + 0 μM $Ru(bpy)_3^{2+}$ with 0 μM $Fe(CN)_6^{3-}$; (8) **6·7** + 0 μM $Ru(bpy)_3^{2+}$ with 10 μM $Fe(CN)_6^{3-}$; (9−12) **6·7** + 1 μM $Ru(bpy)_3^{2+}$ with 10 μM $Fe(CN)_6^{3-}$; (13) Maxam−Gilbert G reaction. Unlabeled [DNA] = 5 μM. (B) Relative intensities for the gel in (A) showing the extent of cleavage at Z13 and G7 normalized to G21.

intensities normalized to G21 are shown in parts B and C of Figure 4. The 7-deazaguanine site displays high reactivity relative to guanine in both single-stranded and duplex DNA. The histogram of cleavage intensities shows that more cleavage is observed for single- than for double-stranded Z. We suspect that this enhanced reactivity reflects higher solvent accessibility and perhaps a qualitatively different structure of Z in single-stranded DNA and parallels our previous findings for the reaction of guanine with RuO^{2+}.[20,40] No cleavage above background was observed at guanine probably because all of the oxidant molecules were consumed by the faster reaction with Z.

Oligonucleotide cleavage at 7-deazaguanine lesions by the outer-sphere electron-transfer oxidant $Ru(bpy)_3^{3+}$ was also investigated. Since the lower redox-potential analogue $Ru(dmb)_3^{3+}$ oxidizes 7-deazaguanine rapidly, we suspected that piperidine-labile lesions could result from oxidation of 7-deazaguanine by photochemically generated $Ru(bpy)_3^{3+}$. For the cleavage studies, the active $Ru(bpy)_3^{3+}$ species was generated using the "flash-quench technique" where photolysis of $Ru(bpy)_3^{2+}$ is performed in the presence of the quencher $Fe(CN)_6^{3-}$.[16,49] This reaction provides effective cleavage of guanines in native DNA.[16,49]

Shown in Figure 5A are the results of cleavage of oligonucleotide **6** and duplex **6·7** by $Ru(bpy)_3^{3+}$ generated via flash quench. Photolysis of DNA in the presence of both $Ru(bpy)_3^{2+}$ and $Fe(CN)_6^{3-}$ results in large amounts of piperidine-induced strand scission at Z13 and no reaction above background at G7 (lanes 3−6 and 9−12). As expected, very little cleavage is observed in control reactions where DNA is photolyzed in the absence of $Ru(bpy)_3^{2+}$ and $Fe(CN)_6^{3-}$ (lanes 1 and 7) or in the presence of $Fe(CN)_6^{3-}$ only (lanes 2 and 8). As with the $Ru(tpy)(bpy)O^{2+}$ reaction, the extent of cleavage was quantitated and normalized to the single-stranded guanine (G21) (Figure 5B). As with $Ru(tpy)(bpy)O^{2+}$, the cleavage was much higher for 7-deazaguanine than for native guanine. From the data normalized to G21, the selectivity for single-stranded 7-deazaguanine compared to single-stranded guanine, double-stranded guanine, and double-stranded 7-deazaguanine could be calculated. These are shown with error limits in Table 5. Surprisingly, the selectivities were similar for oxidation by both $Ru(bpy)_3^{3+}$ and $Ru(tpy)(bpy)O^{2+}$.

Table 5. Relative Ratios of Cleavage Intensities for Z13 and G7 in Oligonucleotides **6** and **6·7** by $Ru(tpy)(bpy)O^{2+}$ and $Ru(bpy)_3^{3+}$ Metallonucleases

ratio	$Ru(tpy)(bpy)O^{2+}$ [a]	$Ru(bpy)_3^{3+}$ [a]
ss Z/ss G	12 ± 1.8	15 ± 2.8
ss Z/ds G	5.5 ± 0.57	5.8 ± 0.66
ss Z/ds Z	2.6 ± 0.57	2.4 ± 1.1

[a] Each reported value is an average with one standard deviation of 12 lanes on the gel from three separate experiments.

Discussion

Deazaguanine Electron-Transfer Kinetics. The studies reported here are consistent with those showing 7-deazaguanine to be a more facile electron donor than guanine.[8,9,27] Electron transfer from 7-deazaguanine to $Ru(dmb)_3^{3+}$ occurs 10−50 times faster than oxidation of guanine by $Ru(bpy)_3^{3+}$, even though the redox potential of $Ru(dmb)_3^{2+}$ is 200 mV lower than that of $Ru(bpy)_3^{2+}$. The generally faster rate implies a driving force for the reaction of 7-deazaguanine with $Ru(dmb)_3^{3+}$ that is about 0.1 eV higher than that for the reaction of guanine with $Ru(bpy)_3^{3+}$ according to Marcus theory arguments we have used for similar reactions.[25,34] This reasoning implies a redox potential for 7-deazaguanine of about 0.75 V, which is in agreement with estimates from Barton and co-workers.[8,9] As expected from these findings, the $Ru(dmb)_3^{3+}$ complex oxidizes 7-deazaguanine 100−1000 times faster than guanine, depending on the sequence. This high selectivity for 7-deazaguanine allows for selective interrogation of the modified base in the presence of native guanines in the strand, similar to our observations for 8-oxoguanine.[34]

The effect of secondary structure on the rate of 7-deazaguanine is similar to that for guanine[25,28] and 8-oxoguanine[34] where the higher solvent accessibility of single-stranded bases gives faster rates than bases protected inside the double helix. Single-stranded oligonucleotides containing multiple 7-deazaguanines are 20−40 times more reactive than their double-stranded counterparts under high ionic strength conditions. For similar sequences with guanine, the rate for single-stranded oligonucleotides is only about 10 times higher than for the duplex.[28] Thus, the 7-deazaguanine modification offers a more sensitive probe of secondary structure than native guanine.

The greater sensitivity of the rate of 7-deazaguanine oxidation to secondary structure is also apparent in the reactions of single-base mismatches. As with guanine and 8-oxoguanine,[25,34] the duplex forms are more reactive in the order Z·C < Z·T < Z·G ~ Z·A < Z·_. However, the differentiation of Z·C from Z·T was poor with both guanine and 8-oxoguanine while with 7-deazaguanine, the Z·T mismatch was easy to distinguish. This finding is consistent with the overall effect of hybridization on the rate constant, which could originate from three factors. First, hybridization may cause greater steric occlusion of the 7-deazaguanine compared to native guanine; no structural studies of 7-deazaguanine duplexes are available to evaluate this point. Second, the methyl groups on $Ru(dmb)_3^{2+}$ may affect either

the distance or orientation in the encounter complex. Finally, the driving force for oxidation of the paired 7-deazaguanine may be slightly different than for guanine because of differences in the hydrogen bonding. These points will be subject to future investigations.

DNA Cleavage. The observation of piperidine-labile strand scission upon oxidation of 7-deazaguanine by either $Ru(bpy)_3^{3+}$ or $Ru(tpy)(bpy)O^{2+}$ provides a useful new probe of nucleobase oxidation chemistry. Strand scission reactions have been observed for oxidation of 8-oxoguanine,[33,51] suggesting that modified lesions that are redox-active will exhibit piperidine-labile cleavage; however, 8-oxoguanine still contains an electron-rich N7 atom. Strand scission for 7-deazaguanine cannot occur via direct involvement of the N7 group in either the outer-sphere or inner-sphere reactions. Accordingly, reaction of 7-deazaguanine with dimethyl sulfate did not produce strand scission because this reagent methylates N7 of guanine to produce the piperidine-labile lesion.[50]

In previous studies, when oxidation of guanine by $Ru(bpy)_3^{3+}$ was studied in parallel by electrochemical kinetics and gel electrophoresis, greater selectivities were observed for the measured rate constants compared to ratios of strand scission determined from quantitating strand scission.[27] For example, the enhancement in the rate of guanine oxidation in a GG doublet appears much higher from the measured electrochemical rate constants than from the ratio of cleavage at the 5′-guanine to the 3′-guanine.[27] Similar effects are observed here where the selectivities observed on the sequencing gels are qualitatively similar to those observed in the electrochemical rate constants, but the quantitative ratios are generally lower than those observed in the direct rates. As we have discussed elsewhere,[27] this observation suggests that the followup chemistry proceeds with lower or even opposite selectivity to the initial electron transfer.

A final noteworthy point concerns the similarity in the cleavage selectivities for both $Ru(bpy)_3^{3+}$ and $Ru(tpy)(bpy)O^{2+}$. The reaction of $Ru(bpy)_3^{3+}$ can only involve outer-sphere electron transfer because there are no reactive ligands on the metal that allow an inner-sphere reaction. However, $Ru(tpy)(bpy)O^{2+}$ has a number of reaction pathways available.[52−54] This complex is reactive toward oxo-transfer reactions such as oxidation of stilbene to stilbene oxide[54] and hydrogen-transfer reactions such as oxidation of 2-propanol to acetone.[53] With nucleic acids, $Ru(tpy)(bpy)O^{2+}$ oxidizes both the sugar moiety and guanine.[55] The complex was found to oxidize xanthine, which is a more reactive derivative of guanine, with a faster rate than guanine.[40] In addition to hydrogen-transfer and oxo-transfer pathways, $Ru(tpy)(bpy)O^{2+}$ is also capable of outer-sphere oxidation according to[38]

$$Ru(tpy)(bpy)O^{2+} + H^+ + e^- \rightarrow Ru(tpy)(bpy)OH^{2+} \quad (8)$$

This reaction exhibits an $E_{1/2} = 0.62$ V at pH 7,[38] which is only 130 mV below our estimate for the redox potential of 7-deazaguanine. Thus, an outer-sphere electron transfer could contribute to oxidation of 7-deazaguanine by $Ru(tpy)(bpy)O^{2+}$, provided the deprotonation of the oxo ligand, which has profound kinetic implications,[54] could be facilitated. This feature stands in contrast to reactions of guanine, where the outer-sphere electron transfer with $Ru(tpy)(bpy)O^{2+}$ would be uphill by 0.43 eV and almost certainly does not contribute to guanine-based strand scission. The large electron-transfer component could enable the similar selectivity ratios observed for $Ru(bpy)_3^{3+}$ and $Ru(tpy)(bpy)O^{2+}$.

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