Trans-Dioxorhenium(V)-Mediated Electrocatalytic Oxidation of DNA at Indium Tin−Oxide Electrodes: Voltammetric Detection of DNA Cleavage in Solution

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The oxidative electrochemistry of *trans*-$[Re(O)_2(4\text{-OMe-py})_4]^+$ in the presence of DNA has been studied. The complex exhibits a reversible oxidation at $E_{1/2}$(VI/V) = 1.00 V (vs Ag/AgCl) in buffer or in the presence of poly(dA)·poly(dT). However, in the presence of calf thymus DNA or poly(dG)·poly(dC), a dramatic catalytic enhancement is observed. An identical result is obtained with $Fe(5\text{-Cl-phen})_3^{2+}$ ($E_{1/2}$(III/II) = 1.02 V), but no electrocatalytic enhancement is observed with *trans*-$[Re(O)_2(py)_2(dmap)_2]^+$ ($E_{1/2}$ = 0.90 V). Electrophoresis of plasmids electrolyzed at 1.2 V in the presence of *trans*-$[Re(O)_2(4\text{-OMe-py})_4]^+$ show relaxation from form I to form II, and analogous reactions with 5′-end ^{32}P-labeled synthetic oligonucleotides show piperidine-labile cleavage specifically at guanine. The combined results point to an electrocatalytic mechanism where the oxidized metal complex oxidizes guanine in DNA by one electron via an efficient, outer-sphere mechanism. Moreover, the experiments demonstrate a potential for the one-electron oxidation of guanine in double-helical DNA at neutral pH of between 0.90 and 1.00 V. This result should provide insight into the mechanisms of DNA oxidation by chemical agents and by ionizing radiation.

Metal complexes that act as oxidative cleavage agents for DNA and RNA are under study with the concurrent goals of developing new techniques for determining complex nucleic acid structures[1−4] and understanding the action of pharmacologically active natural products that damage nucleic acids.[5−9] In addition, understanding the mechanism of oxidative DNA damage is important in the context of the biological activity of ionizing radiation.[10−12] Electrochemistry provides an attractive means of activating cleavage agents because it eliminates the need for an excess of a sacrificial oxidant and potentially allows for the quantitation of the extent of activation through coulometry.[13−15] In addition, voltammetric techniques can be used to determine the binding affinity of the cleavage agent, since the diffusion coefficient of the DNA-bound complex is much smaller than that of the free complex.[15−19] Voltammetry has been used to study oxygen activation by cleavage agents;[13,14] however, an unrealized goal in this area is the development of a system where voltammetric methods can be used to study the reaction mechanism of the DNA oxidation through the direct observation of a coupled chemical reaction of the nucleic acid with an electrochemically generated oxidant.

Recent work in our laboratory has demonstrated both stoichiometric and electrocatalytic oxidative cleavage of DNA using high-valent oxoruthenium complexes.[15,20,21] The active cleavage agent can be generated *in situ* via controlled potential electrolysis at E > 0.8 V (all potentials vs Ag/AgCl) of Ru(tpy)(bpy)OH_2^{2+}, which is converted to Ru(tpy)(bpy)O^{2+} via two reversible oxidations at $E_{1/2}$(III/II) = 0.49 V and $E_{1/2}$(IV/III) = 0.62 V (eqs 1 and 2).[22] The Ru(tpy)(bpy)O^{2+} complex

$$Ru(tpy)(bpy)OH_2^{2+} \rightarrow Ru(tpy)(bpy)OH^{2+} + H^+ + e^- \quad (1)$$

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

is reactive toward hydrogen atom and hydride abstraction from a wide range of organic substrates,[23−25] and generation of the oxo complex in the presence of DNA leads to sugar oxidation, as indicated by the release of free base from the DNA.[26] Despite the suitability of Ru(tpy)(bpy)O^{2+} for nucleic acid oxidation, cyclic voltammograms of Ru(tpy)(bpy)OH_2^{2+} in the presence of DNA show only a barely detectable amount of catalytic enhancement in the Ru(IV/III) wave that is attributable to DNA oxidation.[15] There are two principal difficulties with observing such an enhancement in these voltammograms. The first is that the bound complex has a diffusion coefficient that is 2 orders

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of magnitude lower than that of the free complex, and current due to bound complex is therefore difficult to observe.[14,16,17] The second difficulty is that the rate of hydrogen and hydride abstraction is too slow (10^{-2} M^{-1} s^{-1})[23,26] to regenerate the reduced form of the metal complex during a voltammetric scan. Thus, a low binding affinity and a high rate of reaction are crucial in designing efficient electrocatalytic DNA cleavage agents whose reaction mechanisms are amenable to study using voltammetric methods.

We have recently undertaken the study of *trans*-$[Re^{V}(O)_2L_4]^+$ (L = substituted pyridine) systems and their interaction with DNA. These complexes are oxidized to the corresponding Re(VI) complexes at potentials between 0.5 and 1.5 V, depending on L.[27,28] Previous work has demonstrated DNA oxidation by the Re(VI/V) couple of *trans*-$[Re(O)_2(py)_4]^+$;[29] however, the high potential of this couple ($E_{1/2}$ = 1.25 V) and the limited potential window of buffered aqueous solutions prohibited direct observation of catalytic current enhancement. We have found that the *trans*-$[Re(O)_2(4\text{-OMe-py})_4]^+$ complex (4-OMe-py = 4-methoxypyridine), which undergoes reversible one-electron electrochemistry ($E_{1/2}$(VI/V) = 1.00) in buffered aqueous solution,[27] displays dramatic catalytic current enhancement in the presence of DNA. As shown in Figure 1A, a reversible cyclic voltammogram is obtained for $[Re(O)_2(4\text{-OMe-py})_4]^+$ alone.[30] Upon addition of calf thymus DNA, a large catalytic enhancement is seen in the forward (anodic) wave and the return wave nearly retraces the forward wave. This typical catalytic wave is indicative of efficient oxidation of the DNA by $[Re^{VI}(O)_2(4\text{-OMe-py})_4]^{2+}$.[31] Further studies show that this large catalytic enhancement is not observed in the presence of poly(dA)·poly(dT), but is observed for poly(dG)·poly(dC) (Figure 1B). Only small currents are observed for poly(dG)·poly(dC) in the absence of the metal complex, indicating very little direct oxidation of guanosine at the electrode.

Guanine is the most easily oxidized of the nucleic acid bases, both by oxo transfer and by outer-sphere, one-electron oxidation.[11,12,32] The *trans*-$[Re^{V}(O)_2L_4]^+$ complexes do not participate in oxo-transfer reactions, even to readily oxidized substrates such as PPh_3;[33,34] thus, the oxidation mechanism likely involves one-electron oxidation of guanine. In addition, oxo transfer pathways are likely to be too slow to yield a detectable current enhancement,[35] and the dramatic current enhancement shown in Figure 1 strongly implicates an efficient oxidation pathway, such as outer-sphere electron transfer. This hypothesis is confirmed by the observation that $[Fe(5\text{-Cl-phen})_3]^{2+}$ ($E_{1/2}$(III/II) = 1.02 V), which cannot participate in inner-sphere reactions,

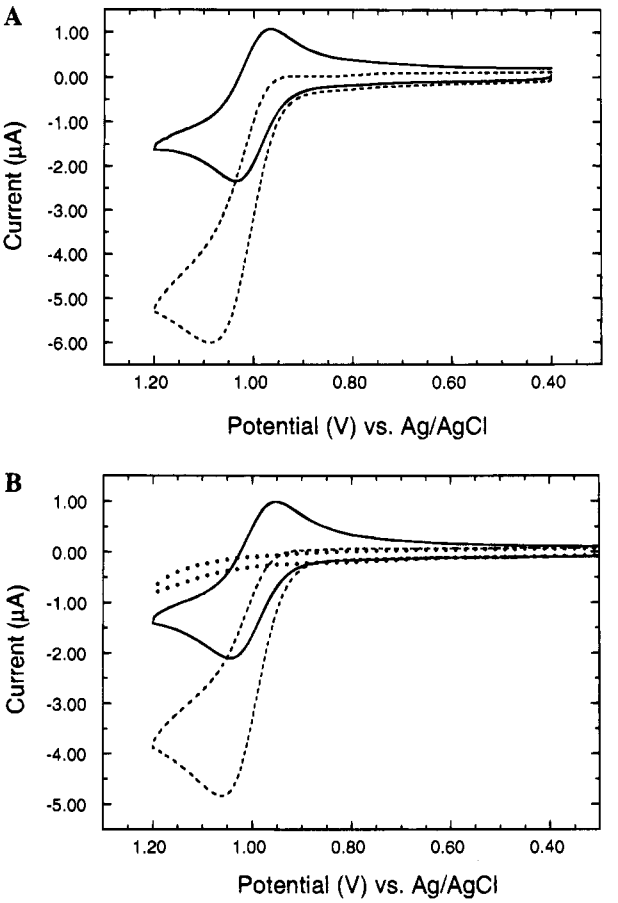

Figure 1. (A) Cyclic voltammogram of *trans*-$[Re(O)_2(4\text{-OMe-py})_4]^+$ (50 μM) with (dashed) and without (solid) 2.0 mM (in nucleotide phosphate) calf thymus DNA. The scan rate was 25 mV/s, and the buffer contained 100 mM NaCl and 5 mM phosphate buffer (pH 7). (B) Cyclic voltammogram of 250 μM poly(dG)·poly(dC) (dotted), *trans*-$[Re(O)_2(4\text{-OMe-py})_4]^+$ (50 μM) with 250 μM poly(dA)·poly(dT) (solid), *trans*-$[Re(O)_2(4\text{-OMe-py})_4]^+$ (50 μM) with poly(dG)·poly(dC) (dashed). Other experimental conditions same as for part A.

gives results identical to those shown in Figure 1 (5-Cl-phen = 5-chloro-1,10-phenanthroline).

Controlled-potential electrolysis at 1.2 V of *φX174* plasmid DNA in the presence of *trans*-$[Re(O)_2(4\text{-OMe-py})_4]^+$ induces relaxation of the plasmid DNA from form I (supercoiled) to form II (nicked circular).[36] More important, similar electrolysis using the single-stranded 5′-end-^{32}P-labeled oligonucleotide d(5′-ATACGCAAGGGCAT-3′) produces piperidine-labile lesions

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(30) (a) The complex *trans*-$[ReO_2(4\text{-OMe-py})_4](PF_6)$ was synthesized by the method of Brewer and Gray.[27] Due to its low solubility in water, all solutions were filtered immediately prior to use and only freshly prepared solutions of known concentration were used. Electrochemistry was performed using ITO electrodes, as described previously.[15] The ligand 5-Cl-phen was used as received from Aldrich and the $[Fe(5\text{-Cl-phen})_3](ClO_4)_2$ complex was prepared according to a literature procedure.[30b] (b) Musumeci, S.; Rizzarelli, E.; Fragala, I.; Sammartano, S.; Bonomo, R. P. *Inorg Chim. Acta* **1973**, *7*, 660.
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(36) A 300-μL solution consisting of 10 μM *trans*-$[Re(O)_2(4\text{-OMe-py})_4]^+$ and 100 μM *φX174* plasmid (Pharmacia) was electrolyzed at 1.2 V with mechanical stirring. During the 60 min of electrolysis, 10 μL samples were removed and analyzed by electrophoresis on a 1% agarose gel, as previously described.[15] A control electrolysis in the absence of *trans*-$[Re(O)_2(4\text{-OMe-py})_4]^+$ showed no significant isomerization.
(37) The synthetic oligonucleotides were synthesized at UNC and purified according to published procedures.[32] The 5′-terminal ^{32}P-labeling of d(5′-ATACGCAAGGGCAT-3′) was accomplished using γ-^{32}P-ATP and polynucleotide T4 kinase. The reaction mixture (320 μL) contained 3 μM unlabeled oligonucleotide, approximately 20 nCi 5-^{32}P-labeled oligonucleotide, 10 mM sodium phosphate buffer (pH 6.95) and 5 μM *trans*-$[Re(O)_2(4\text{-OMe-py})_4]^+$. At regular intervals during the electrolysis at 1.2 V, two 20 μL samples were removed and lyophilized to dryness. One sample was treated with piperidine, and both samples were analyzed using 20% polyacrylamide gel electrophoresis under denaturing conditions. Autoradiograms showed no cleavage without piperidine treatment and cleavage only at G after piperidine treatment. A control electrolysis in the absence of *trans*-$[Re(O)_2(4\text{-OMe-py})_4]^+$ showed no significant cleavage of the oligonucleotide with or without piperidine treatment.

specifically at guanine; no cleavage was observed without piperidine treatment.[37] Hybridization of the same ^{32}P-labeled oligonucleotide to the complementary strand produces the same result.[38] Recently, it has been shown that one-electron oxidation of guanine leads to the formation of 8-oxoguanine via hydrolysis of the guanine radical cation.[39] The presence of 8-oxoguanine is known to produce a base-labile lesion in DNA.[32,40] As discussed above, the observation of a catalytic enhancement also depends on a relatively low binding affinity for the metal complex, and we have determined the binding affinity of $[Re(O)_2(4\text{-OMe-py})_4]^+$ to be only 10 M^{-1} by emission titration using published procedures.[41] The $[Fe(5\text{-Cl-phen})_3]^{2+}$ complex is expected have a higher affinity for DNA (a binding constant on the order of 1.0×10^4 M^{-1} was measured[17] for $[Fe(phen)_3]^{2+}$), but this does not significantly affect the electrocatalytic oxidation.

Further evidence for the outer-sphere electron transfer mechanism is provided by the results of Federova and Podust,[42] who report the G-specific oxidation of small DNA fragments upon treatment with $[Ru(bpy)_3]^{3+}$ and subsequent hydrolysis in 1 M piperidine. The driving force for the oxidation of DNA by $[Ru(bpy)_3]^{3+}$ will be higher than that for *trans*-$[Re(O)_2(4\text{-OMe-py})_4]^+$ as the $Ru^{III/II}$ redox potential for $[Ru(bpy)_3]^{2+}$ in aqueous solution is 1.1 V vs Ag/AgCl. Federova and Podust do not speculate on possible mechanisms for the oxidation of DNA by Ru^{III} nor do they attempt to generate $[Ru(bpy)_3]^{3+}$ *in situ* electrochemically. Recent results in our laboratory demonstrate that cyclic voltammograms of $[Ru(bpy)_3]^{2+}$ in the presence of DNA display the same electrocatalytic enhancement observed with *trans*-$[Re(O)_2(4\text{-OMe-py})_4]^+$ and $[Fe(5\text{-Cl-phen})_3]^{2+}$.

A number of authors have determined the aqueous-phase redox potentials of guanine and guanosine, either by cyclic voltammetry or by pulse radiolysis experiments.[11,43] Due to differing pH and other conditions, the estimates of the one-electron oxidation potentials vary widely. In addition, the values obtained for free guanine or guanosine do not necessarily correspond to the oxidation potential of guanine in double-helical DNA. The results for *trans*-$[Re(O)_2(4\text{-OMe-py})_4]^+$ and other related dioxorhenium(V) complexes provide an indirect measure of the oxidation potential of guanosine *in double-helical DNA under physiological conditions*. As shown in Figure 1, the complex *trans*-$[Re(O)_2(4\text{-OMe-py})_4]^+$ oxidizes DNA rapidly with a potential of 1.00 V, as does the *trans*-$[Re(O)_2(py)_4]^+$ complex, which exhibits a higher potential.[29] Cyclic voltammograms of a similar complex, *trans*-$[Re(O)_2(py)_2(dmap)_2]^+$ (dmap = 4-(*N*,*N*-dimethylamino)pyridine, $E_{1/2} = 0.90$ V) show no catalytic enhancement of the anodic wave in the presence of DNA. This result implies that the effective potential for guanine oxidation in double-helical DNA in our buffer is between 0.90 and 1.00 V.

Detailed studies by Brabec[44,45] have used differential pulse voltammetry of DNA adsorbed to graphite electrodes to determine the approximate redox potentials of guanosine and adenosine in DNA. Their results indicate that the guanine residues are oxidized at potentials around 0.9 V vs SCE at neutral pH, in good agreement with our results. However, the electrochemical study of adsorbed DNA suffers from low sensitivity, especially for native DNA. In our work, the use of ITO electrodes is crucial as the DNA does not adsorb to the negatively-charged electrode as demonstrated by linear i_p vs $v^{1/2}$ plots for DNA/metal complex solutions where the metal complex does not oxidize the DNA.[15] In addition, the current observed for DNA alone is not significantly greater than background (see Figure 1B). Therefore, the use of ITO electrodes and a variety of metal complexes provide a sensitive and flexible system for the study of DNA oxidation.

The measurement of the potential of the guanine$^{+/0}$ couple in double-helical DNA provides insight into the possible mechanisms of guanine oxidation for particular oxidants. Guanine oxidation can be effected by outer-sphere electron transfer followed by hydrolysis of the radical cation,[39] inner-sphere oxo transfer,[32,40] or reaction with photosensitized singlet oxygen.[46] The applicability of the outer-sphere one-electron pathway vs inner-sphere oxo transfer pathways can now be evaluated on thermodynamic grounds. These considerations can be applied to oxidation of guanine by numerous methods, including Ni complexes,[47] copper–phenanthroline,[48] oxoruthenium(IV) complexes,[49] and ionizing radiation.[10] The second important implication of this work is that the potentials for oxidation of guanine in DNA under a variety of buffer conditions and in a range of base sequences can now be conveniently measured, since the potentials of the ReO_2^+, $Fe(L)_3^{2+}$, and $Ru(L)_3^{2+}$ complexes can be finely tuned over a wide range. Similarly, the potentials of other oxidizable bases, such as 8-oxo-G and adenine,[11] can be measured. This information should be invaluable with regard to understanding the mechanisms of oxidative nucleic acid damage.

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(38) (a) The double-stranded DNA was prepared by mixing a single-stranded oligonucleotide (d(5′-ATACGCAAGGGCAT-3′) (60 μM) with the 5′-^{32}P-labeled oligonucleotide (~170 kcpm), 100 mM sodium phosphate buffer (pH 6.98), and pure complementary single-stranded DNA d(5′-ATGCCCTTGCGTAT-3′) (66 μM). This mixture was heated at 90 °C for 8 min and then slowly cooled to room temperature over 4–5 h. The formation of the double-stranded DNA was confirmed using 20% nondenaturing polyacrylamide gel (19:1) using a published procedure.[38b] The reaction conditions for the electrolysis of the double-stranded DNA with *trans*-$[Re(O)_2(4\text{-OMe-py})_4]^+$ were similar to those for the single-stranded DNA listed above. (b) Sambrook, E. F. Fritsch, T. Maniatis, *Molecular Cloning—A Laboratory Manual*, 2nd ed., Cold Spring Harbor Laboratory Press: 1989, 6.39.

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