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Direct Electrochemiluminescence Detection of Oxidized DNA in Ultrathin Films Containing [Os(bpy)$_2$(PVP)$_{10}$]$^{2+}$

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Abstract: Direct electrochemiluminescence (ECL) involving oxidized DNA was demonstrated in ultrathin
films of cationic polymer [Os(bpy)$_2$(PVP)$_{10}$]$^{2+}$ [PVP = poly(vinyl pyridine)] assembled layer-by-layer with
DNA or oligonucleotides. Electrochemically oxidized Os^{II} sites generated ECL from films containing oxo-
guanines on DNA formed by chemical oxidation using Fenton reagent. Films combining DNA, [Ru(bpy)$_2$-
(PVP)$_{10}$]$^{2+}$, and [Os(bpy)$_2$(PVP)$_{10}$]$^{2+}$ had Os^{II} sites that produced ECL specific for oxidized DNA, and Ru^{II}
sites gave ECL from reaction with oxo-adenines, chemically damaged DNA, and possibly from cleaved
DNA strands.

Oxidative stress in mammals damages DNA,[1] generating
lesions that may contribute to aging and mutagenesis.[2–5] Many
oxidatively formed DNA adducts have been characterized.[6]
Oxidative DNA damage occurs from chemical reactions, ir-
radiation, and reactive oxygen species (ROS) generated during
metabolism,[7] including singlet oxygen, superoxide, and hydroxyl
radicals.[7c] Reaction of DNA with hydroxyl radicals[7,8] causes
single base modifications, yielding products including 8-oxo-
guanine (8-oxoG), 8-oxoadenine, thymine glycol and 8-hy-
droxycytosine, strand breaks, and cross-links.[9]

Hydroxyl radicals are generated from Fe^{II} and hydrogen
peroxide in the Fenton reaction.[10] Iron-mediated reactions may

contribute substantially to H$_2$O$_2$-mediated damage to DNA.[11,12]
Such DNA damage is thought to involve Fenton chemistry
generated by Fe^{II} associated with DNA.[13,14]

8-OxoG is a major product of nucleobase oxidation for which
specific cellular repair enzymes exist. It has been suggested as
a clinical biomarker for oxidative stress.[15] When present in
DNA, 8-oxoG caused G-to-T transversions and A-to-C substitu-
tions.[16,17] While guanine is the most easily oxidized of the
natural nucleobases,[18] 8-oxoG has a much lower oxidation
potential[19] and is itself oxidized more easily than guanine. The
oxidation products of 8-oxoG are guanidinohydantoin and
2-amino-4,5,6-trioxypyrimidine.[20] Guanidinohydantoin in ss-
DNA was recently found to be highly mutagenic and caused
G-to-T transversions.[21]

8-OxoG can be determined in hydrolyzed DNA by liquid
chromatography (LC) coupled to a mass spectrometer (MS) or
an electrochemical (EC) detector.[15,22] We recently used LC–

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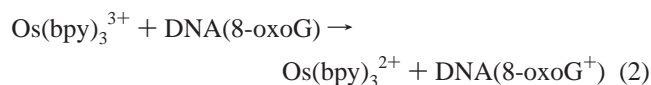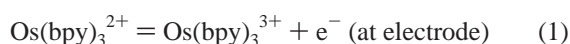§ University of Connecticut Health Center.
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EC to observe the time course of 8-oxoG generation by reacting hydroxyl radicals with DNA in vitro.[23] Requirements for hydrolysis and workup of DNA make LC-based methods too labor intensive and expensive for routine clinical measurements. A long-term goal of our work is to develop biosensor alternatives that can detect DNA damage without hydrolysis or complex instrumentation.

Electroanalysis provides instrumentally simple, sensitive, and inexpensive approaches to detect DNA hybridization and damage.[24–29] We recently reported that oxidation of guanines in DNA by electrochemically generated $[Ru(bpy)_2(PVP)_{10}]^{3+}$ {PVP=poly(vinylpyridine)} in ultrathin films leads to photoexcited $[Ru(bpy)_2^{2+}]^*$ sites that decay to generate electrochemiluminescence (ECL).[30] No sacrificial reductant is required. The reaction is initiated by electrochemical catalytic oxidation of guanines in DNA similar to that reported by Thorp et al.[31] for soluble $Ru(bpy)_3^{2+}$. This thin-film ECL approach was utilized to detect DNA damage from styrene oxide.[30]

Ropp and Thorp showed that $Os(bpy)_3^{3+}$ selectively oxidizes 8-oxoG in the presence of guanine.[32] $Os(bpy)_3^{2+}$ has a much lower redox potential ($\sim$0.62 V vs SCE) than $Ru(bpy)_3^{2+}$ and therefore does not oxidize guanine. $Os(bpy)_3^{2+}$ catalyzes the oxidation of 8-oxoG in eqs 1 and 2[33]

$$Os(bpy)_3^{2+} = Os(bpy)_3^{3+} + e^- \text{ (at electrode)} \quad (1)$$

$$Os(bpy)_3^{3+} + DNA(8\text{-}oxoG) \rightarrow$$
$$Os(bpy)_3^{2+} + DNA(8\text{-}oxoG^+) \quad (2)$$

This pathway was used to probe telomerase function using 8-oxoG at specific DNA sites[32] and to investigate intermolecular vs intramolecular reactivity of Os-labeled oligonucleotides.[34]

Dissolved $Os(bpy)_3^{2+}$ is capable of generating ECL if oxidized in the presence of a sacrificial reductant such as oxalate.[35] Electrochemical catalytic oxidation of 8-oxoG with $Os(bpy)_3^{2+}$ is analogous to the oxidation of guanine with metallopolyion $[Ru(bpy)_2(PVP)_{10}]^{2+}$ that generates ECL from DNA directly without a sacrificial reductant.[30] We suspected that catalytic oxidation of 8-oxoG in DNA with $[Os(bpy)_2(PVP)_{10}]^{2+}$ could lead to photoexcited Os^{II*} sites and provide

ECL signals. We report here that ultrathin films containing $[Os(bpy)_2(PVP)_{10}]^{2+}$ and oligonucleotides on electrodes directly generate ECL from oxidized DNA without using a sacrificial reductant. Films combining $[Ru(bpy)_2(PVP)_{10}]^{2+}$ and $[Os(bpy)_2(PVP)_{10}]^{2+}$ can be used to detect DNA oxidation and nucleobase adducts from chemical damage.

Experimental Section

Chemicals and Materials. Bis-$[Ru(bpy)_2(PVP)_{10}](ClO_4)_2$ and bis-$[Os(bpy)_2(PVP)_{10}]Cl_2$ were prepared, purified, and characterized as described previously.[36–38] Standard time-resolved luminescence gave quantum yields and luminescence lifetimes,[39] respectively, as follows: (1) $[Ru(bpy)_2(PVP)_{10}]^{2+}$ in cast films in pH 5.5 buffer: 0.87%, 61 ns, dissolved in solution 5.9%, 45 ns. (2) $[Os(bpy)_2(PVP)_{10}]^{2+}$ in films 0.03%, 22 ns, in solution 0.47%, 14 ns. Quantum yields and luminescence lifetimes did not show a large influence of pH.

Calf Thymus (CT) double-stranded (ds) DNA (Sigma, type XV, 13 000 av base pairs, 41.9% G/C), CT single-stranded (ss)-DNA, Salmon Testes (ST) ds-DNA (Sigma, $\sim$2000 av base pairs, 41.2% G/C), ST ss-DNA, polyguanadylic acid (5′) (Poly [G]), polycytidylic acid (5′) (Poly [C]), styrene oxide, and toluene were from Sigma, and polyadenylic acid (5′) (Poly [A]) was from ICN Biomedical Research Products. Water was purified with a Hydro Nanopure system to specific resistance >18 mΩ cm. All other chemicals were reagent grade.

ECL Voltammetry. Simultaneous square wave voltammetry (SWV)/ ECL measurements were made in a three-electrode cell at 37.0 ± 0.5 °C using a CH Instruments model 660a electrochemical analyzer. The cell employed a saturated calomel reference electrode (SCE), a Pt wire counter electrode, and a 4-mm diameter disk of ordinary basal plane pyrolytic graphite (PG, Advanced Ceramics) as working electrode placed close to the glass cell bottom. The electrolyte solution was 10 mM acetate buffer, pH 5.5, containing 50 mM NaCl. SWV conditions were 4 mV step height, 25 mV pulse height, and frequency 5 Hz. The cell was covered with a black cloth to avoid external light. Solutions were purged with pure nitrogen for 15 min prior to each series of experiments, and a nitrogen atmosphere was maintained during data collection. A different electrode was used for each analysis of DNA oxidation.

A Labmaster Coherent Ultima photomultiplier/monochromator/data collection system was used to record light from the electrode (764 and 610 nm) via an optical fiber positioned directly underneath the electrode outside the flat bottom of the glass electrochemical cell.

Film Assembly. DNA/metallopolymer films were constructed by layer-by-layer alternate electrostatic assembly.[40–42] Basal plane PG electrodes were polished with 400 grit SiC paper and then with 0.3 μm α-alumina slurries on Buehler Microcloth, washed with water and sonicated in ethanol for 15 min, and then sonicated in water for 15 min. Layers were constructed by placing 30 μL drops of 0.2% aqueous $[Os(bpy)_2(PVP)_{10}]^{2+}$ or 1:1 $[Ru(bpy)_2(PVP)_{10}]^{2+}/[Os(bpy)_2(PVP)_{10}]^{2+}$ onto each PG electrode, allowing 15 min to achieve saturated adsorption,[42] and then washing with water. Subsequently, 30 μL of

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DNA or oligonucleotide solution (2 mg mL^{-1} DNA in 5 mM pH 5.5 acetate buffer +0.05 M NaCl) was placed on this PG surface, allowed to adsorb 15 min, and then washed with water. This sequence was repeated to obtain films with two metallopolymer/DNA bilayers. Films containing ss-DNA and polynucleotides were also assembled in this way.

Assembly of films was assessed at each step with a quartz crystal microbalance (QCM, USI Japan) using 9 MHz QCM resonators (AT-cut, International Crystal Mfg.). To mimic the carbon electrode surface, a negative monolayer was made by treating gold-coated (0.16 ± 0.01 cm^2) quartz resonators with 0.7 mM 3-mercapto-1-propanol and 0.3 mM 3-mercaptopropionic acid in ethanol.[40] Films were assembled as for PG electrodes. Resonators were dried in a stream of nitrogen before the frequency change (ΔF) was measured. Absorbed mass was estimated with the Sauerbrey equation,[42] for 9 MHz quartz resonators, giving dry film mass per unit area M/A as:

$$M/A \ (\text{g cm}^{-2}) = -\Delta F \ (\text{Hz})/(1.83 \times 10^8) \qquad (3)$$

The nominal thickness (d) of dry films was estimated with an expression confirmed by high-resolution electron microscopy:[43]

$$d \ (\text{nm}) \approx (-0.016 \pm 0.002)\Delta F \ (\text{Hz}) \qquad (4)$$

DNA Oxidation. Films were incubated in a stirred reactor at 37.0 ± 0.5 °C. A 100 μL volume of 15 mM FeSO$_4$ and 1 mL of H$_2$O$_2$ (Fenton reagent) were added to 9 mL of acetate buffer, pH 5.5 and 50 mM NaCl, to give final concentrations 0.15 mM FeSO$_4$ and 5.0 mM H$_2$O$_2$. PG electrodes coated with polynucleotide/metallopolymer films were incubated in the stirred solutions, then rinsed with water and transferred to the electrochemical cell containing fresh pH 5.5 buffer for SWV/ECL analysis. pH 5.5 allows efficient ECL production.[44] Alternative hydrolysis and 8-oxoG measurement by LC−UV−EC was done by a previously described method.[23]

Results

QCM Monitoring of Film Assembly. On the basis of previous studies of the influence of number of layers on ECL efficiency in [Ru(bpy)$_2$(PVP)$_{10}$]/DNA films,[30] we chose two bilayers of ds-DNA/[Os(bpy)$_2$(PVP)$_{10}$]$^{2+}$ (Os−PVP) or two bilayers of DNA and 1:1 [Ru(bpy)$_2$(PVP)$_{10}$] (Ru−PVP) and (Os−PVP), denoted (RuOs−PVP/DNA)$_2$. Integrity and reproducibility of film formation was characterized by measuring QCM frequency shifts (ΔF) after each layer of film growth, as illustrated for films of DNA and mixed metallopolymers (Figure 1). $-\Delta F$ values varied linearly with layer number for ss-DNA, and ds-DNA alternated with RuOs−PVP layers, suggesting regular film growth with reproducible layer formation. ΔF values with eqs 3 and 4 were used to estimate weights of metallopolymers and DNA and average nominal thickness of the films (Table 1). Total film thicknesses were ∼16 nm, with each film containing ∼3 μg cm^{-2} DNA. A slightly larger amount of ss-DNA than ds-DNA was incorporated into films, as also found for DNA in films with other polycations.[40]

ECL/SWV of Os−PVP/Oligonucleotide Films after Oxidation. We first examined films containing homogeneous polynucleotides and Os−PVP. Upon incubation of electrodes with Fenton's reagent to oxidize the oligonucleotides, a significant increase in the ECL and SWV responses for (Os−PVP/Poly G)$_2$ films was found (Figure 2). The SWV peak occurred at 0.58 V vs SCE, and the maximum ECL signal was

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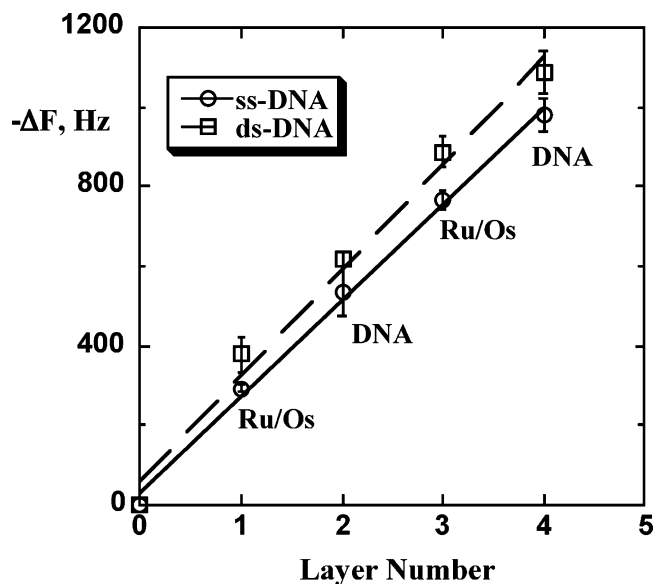

Figure 1. QCM frequency shifts monitoring film formation on gold−quartz resonators coated first with mixed monolayers of mercaptopropionic acid/mercaptopropanol for alternate adsorption of 1:1 [Os(bpy)$_2$(PVP)$_{10}$]$^{2+}$/[Ru-(bpy)$_2$(PVP)$_{10}$]$^{2+}$ and salmon testes DNA (average values for three replicate films).
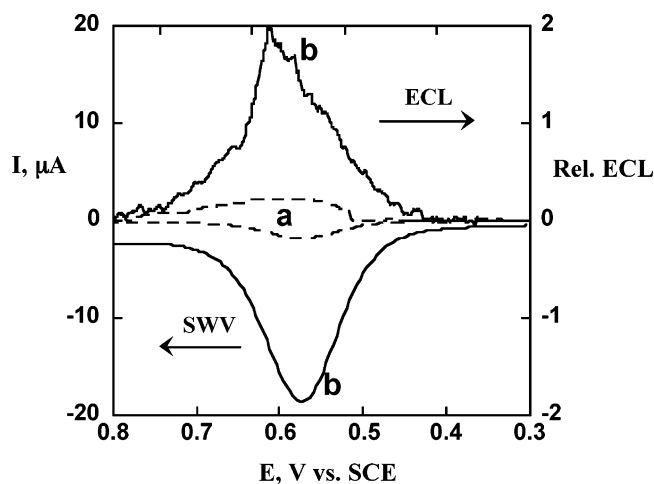

Figure 2. SWV and ECL for (Os−PVP/Poly G)$_2$ films on PG electrodes in pH 5.5 buffer and 50 mM NaCl (a) before and (b) after 20 min incubation at 37 °C with Fenton reagent. ECL emission monitored at 764 nm.

Table 1. Average Characteristics of Metallopolyion/DNA Films from QCM

film	nominal thickness, nm	mass DNA, μg cm^{-2}	mass RuOs−PVP, μg cm^{-2}
(RuOs−PVP/ST-ds-DNA)$_2$	15 ± 2	2.9 ± 0.5	1.8 ± 0.1
(RuOs−PVP/ST-ss-DNA)$_2$	17 ± 2	3.4 ± 0.4	2.0 ± 0.3

slightly positive of this at ∼0.60 V. Fenton's reagent oxidizes guanines to 8-oxoG,[17,23,45,46] which has a lower oxidation potential than guanine. 8-OxoG is presumably oxidized by [Os-(bpy)$_2$]$^{3+}$ centers in the film, similar to reactions with soluble osmium complexes.[32,47]

Fenton's reagent also oxidizes adenine and cytosine to products including 7-deazaadenine, 8-oxoadenine, and 5-hy-

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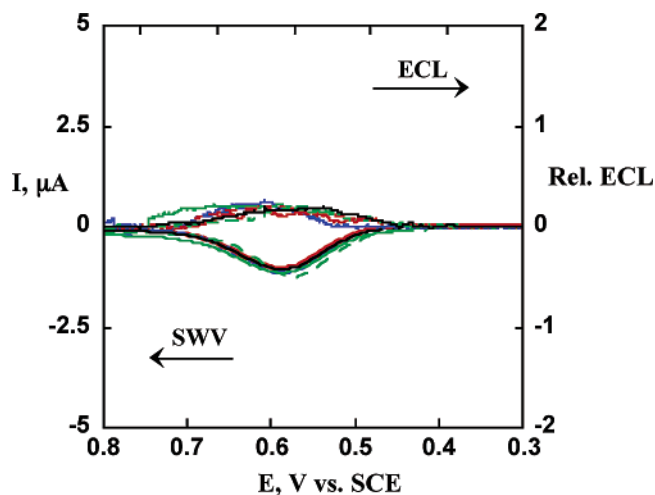
Figure 3. SWV/ECL for films containing Os−PVP but no DNA on PG electrodes in pH 5.5 buffer before and after 20 min incubation with Fenton reagent. Os−PVP alone (black, solid); (Os−PVP/PSS)$_2$ before (blue, dashed) and after (green, solid) incubation; (Os−PVP/Poly A)$_2$ before (red, solid) and after (red dashed) incubation; (Os−PVP/Poly C)$_2$ before (blue solid) and after (green, dashed) incubation. ECL emission monitored at 764 nm.
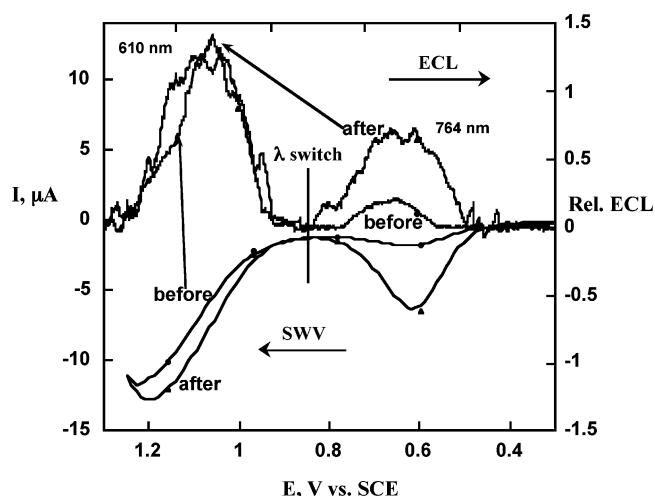

Figure 4. SWV/ECL for (OsRu−PVP/Poly G·Poly C)$_2$ films on PG electrodes in pH 5.5 buffer before and after 20 min incubation at 37 °C with Fenton reagent. ECL emission at ∼764 nm and then switched to ∼610 nm at 0.8 V at marker.

droxycytosine.[17,45,46] Figure 3 shows ECL/SWV for control films containing the Os−PVP alone, as well as Os−PVP with PSS, poly A, and poly C, before and after incubation with Fenton's reagent. ECL and SWV curves are nearly superimposable for all these films; no significant changes were found after incubation under our oxidation conditions. Curves were similar to those for films containing Os−PVP only. The Os^{II}/Os^{III} peak reflecting the formal potential in the films is at 0.58 V vs SCE. However, increasing the H$_2$O$_2$ concentration to 50 mM in the Fenton reagent resulted in the detection of small increases in Os^{II} SWV and ECL peaks in films containing Poly A but not poly C. If oxidized forms of cytosine are formed, their oxidation potentials may be too positive[48] for them to be catalytically oxidized by [Os(bpy)$_2$(PVP)$_{10}$]$^{2+}$.

Figure 4 shows the responses obtained for films made from the two metallopolymers and prehybridized poly G/poly C, i.e., (Os−Ru−PVP/PolyG·Poly C)$_2$. The Os^{II} catalytic peak appears

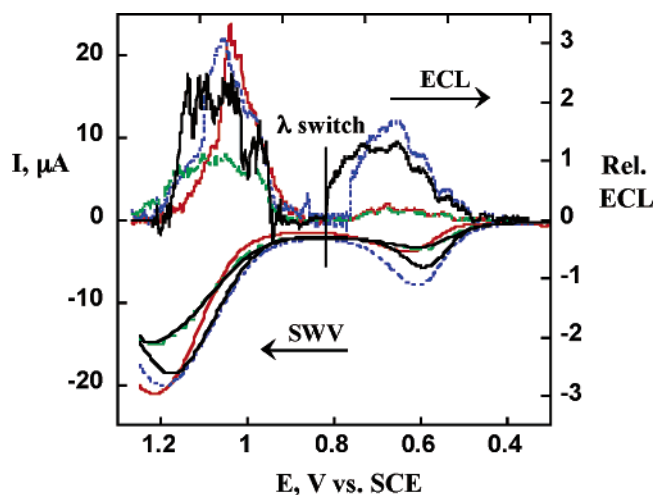(48) Jovanaic, S. V.; Simic, M. G. *J. Phys. Chem.* **1986**, *90*, 974−978.

Figure 5. SWV/ECL for (OsRu−PVP/ds-ST DNA)$_2$ films on PG electrodes in pH 5.5 buffer before (red = ss-DNA; green = ds-DNA) and after 20 min incubation at 37 °C (blue = ss-DNA; black = ds-DNA) ECL emission monitored at ∼764 nm and then switched to ∼610 nm at the 0.8 V as labeled.

at ∼0.6 V, and the Ru^{II} peak is at ∼1.2 V, reflecting the redox potentials of these polymers. Before incubation, the Ru peak is 6- to 7-fold larger than the Os peak in both ECL and SWV, reflecting the fact that catalytic oxidation of guanine by Ru centers can take place in the hybridized oligonucleotide and that there will be little catalysis by the Os sites if the DNA is not oxidized. After incubation with Fenton's reagent, there is a dramatic increase in both the ECL and SWV peaks for the Os^{II}/Os^{III} couple in the (Os−Ru−PVP/PolyG·Poly C)$_2$ films because of catalytic oxidation of 8-oxoG formed in the incubation. However, there is only a small increase in the Ru ECL and SWV peaks.

Qualitatively, the increase in the Os peaks after incubation was larger for the films containing unhybridized Poly G (Figure 2) than for the (Os−Ru/PolyG·Poly C)$_2$ film (Figure 4). This may result from better accessibility of the unhybridized bases to the reactants and the metallopolymers. Thus, we compared the films of ds- and ss-DNA for which Figure 1 was obtained (Figure 5), with known amounts of DNA and metallopolymers (Table 1). Before incubation, the ds- and ss-DNA gave nearly identical results for the Os peak. The ss-DNA gave a >2-fold larger peak at the Ru potential because of enhanced oxidation by the polymer due to greater accessibility of the guanines.[30] After incubation, the ss-DNA gave somewhat larger signals than ds-DNA for both the Os and the Ru peaks. However, the differences were much smaller than that of the Ru peak for non-oxidized ss- and ds-DNA.

When (OsRu−PVP/ds-DNA)$_2$ films were incubated with Fenton reagent for various times, increases in the ratios of ECL and SWV (final/initial) Os^{II} peaks were observed for up to 20 min. (Figure 6). At longer times, dampened oscillations in these signals were observed (see Supporting Information, Figure S2, for original SWV and ECL traces). Controls consisting of the same types of films incubated with FeSO$_4$ alone, H$_2$O$_2$ alone, or only buffer had peak ratios within experimental error of unity

We previously found similar oscillating concentration−time profiles for 8-oxoG during oxidation of DNA and guanine solutions by Fenton reagent under different conditions, determining 8-oxoG after DNA hydrolysis by using LC−EC.[23] Thus,
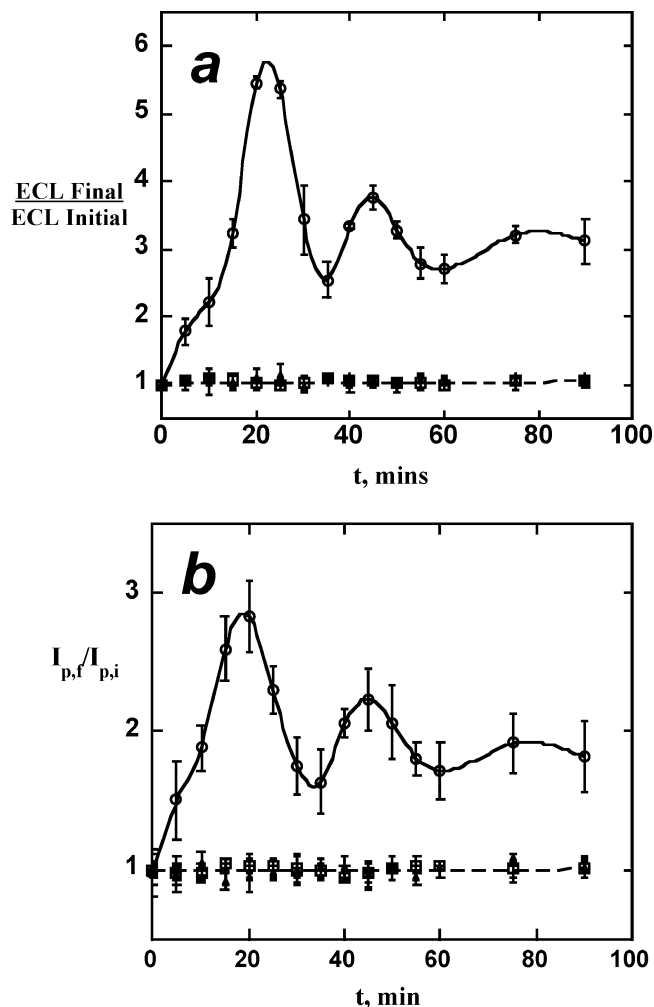
Figure 6. Influence of incubation of (OsRu−PVP/ds-ST DNA)$_2$ films with Fenton reagent (○), with FeSO$_4$ alone (▲), H$_2$O$_2$ alone (□), and only pH 5.5 buffer (+) on (a) average ECL signals and (b) average SWV catalytic peak currents for the Os^{II}/Os^{III} redox couple. Error bars represent standard deviations for three trials; one electrode was used per trial.

we repeated this LC−EC analysis on DNA in solution that was oxidized under exactly the same conditions as our electrodes for ECL/SWV. Concentration profiles for 8-oxoG obtained by LC−EC are given in Figure 7. The error bars of the LC−EC results for the small amounts of [8-oxoG] found are somewhat larger than those for ECL and SWV, but the same concentration profile was found during 100 min of reaction. An initial increase in [8-oxoG] leads to a clear maximum at about 20 min, followed by dampened oscillations in [8-oxoG] at longer times.

The Ru^{II} ECL and SWV peak ratios (Figure S3) for the same films for which the Os^{II} peaks were discussed (cf. Figure 6) showed increases in the first 20 min of incubation with Fenton's reagent. These ratios reached limiting values slightly larger than 2 at $t > 20$ min. The increase may be attributed to the formation of oxidized adenine derivatives or possible strand breaks, either of which can result from the action of the Fenton reagents on DNA. No significant increases or trends for the Ru^{II} peaks were found when the films were incubated in buffer only, buffer with FeSO$_4$ only, or buffer with H$_2$O$_2$ only.

To assess the contribution to the ECL signals from the formation of adenine or cytosine derivatives upon oxidation, films containing both the metallopolymers and either Poly A or Poly C were incubated with Fenton reagent (Supporting
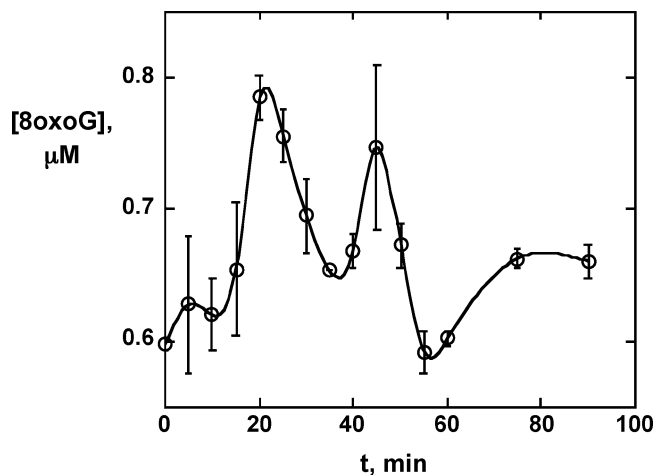

Figure 7. Concentration profile of [8oxoG] determined by LC−EC during oxidation of 1.2 mg mL^{-1} ds-DNA in pH 5.5 by Fenton reagent under same conditions as for incubation of DNA electrodes. (Average of three determinations. DNA hydrolyzed before analysis.)
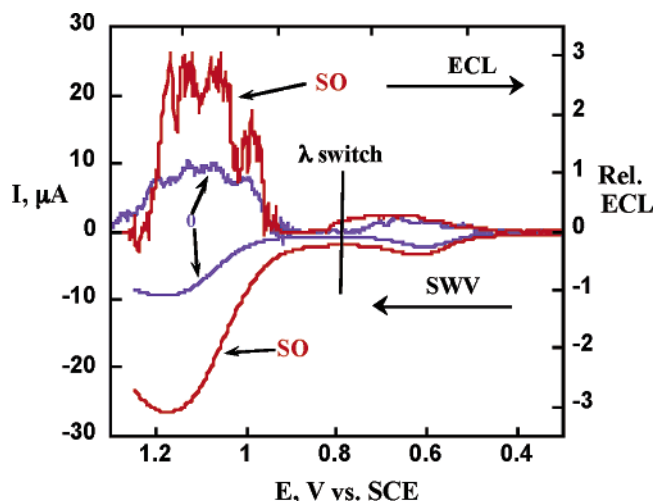

Figure 8. SWV and ECL responses for (Os−Ru/ds-ST DNA)$_2$ films on PG electrodes in pH 5.5 before (purple) after (red) 25 min incubations at 37 °C with saturated styrene oxide (SO). ECL emission monitored at ∼764 nm and then switched to ∼610 nm at the 0.8 V marker.

Information, Figure S4). For the Poly C films, there was no increase in either the Os or Ru peaks upon incubation. For the film containing Poly A, there was no increase in the Os peaks, but there was ∼7% increase in the Ru peaks in both ECL and SWV after 20 min incubation. This may reflect the oxidation of oxo-adenines by the electrogenerated Ru^{III} sites in the films.

ECL and SWV of (OsRu−PVP/Poly G)$_2$ films before and after incubation (Supporting Information, Figure S5) resembled those of (Os−Ru/Poly G·Poly C)$_2$ in Figure 4. The ECL and SWV signals for the Os peaks increased upon incubation with Fenton's reagent, but the peaks for the Ru sites remained about the same.

Chemically Damaged OsRu−PVP/DNA Films. The influence of chemical damage of DNA was investigated by treating films with styrene oxide, which forms covalent adducts with DNA in films mainly at the nitrogens of guanine.[40] OsRu−PVP/DNA films incubated with styrene oxide showed increases in the Ru^{II} SWV and ECL peaks (Figure 8), as previously reported for Ru−PVP/DNA films.[30] The films were also incubated in toluene, which does not react with DNA, and with

buffer alone, and they showed no increase or trend for the Ru or Os peaks. Films incubated with styrene oxide for 25 min gave only very small increases in the Os^{II} SWV or ECL peak signals (Figure 8).

Discussion

Our results demonstrate for the first time that ECL can be achieved by direct reaction of an osmium metallopolymer with oxidized DNA in ultrathin films (Figures 2, 4, and S5). The ECL detection equipment is simple and inexpensive, featuring a conventional voltammetric cell combined with an optical fiber that delivers light from the electrode to a monochromator/PMT detector. Osmium and ruthenium complex metallopolymers can be incorporated together in films with DNA to detect both DNA oxidation and chemical DNA damage (Figure 8). Alternate layer-by-layer electrostatic assembly on PG electrodes provided metallopolymer/DNA films ~16-nm thick (Table 1), presumably featuring intimate mixing of the metallopolymers and ds-DNA in a tiny reaction volume. Extensive intermixing of layers in polycation/polyanion and protein/polycation films assembled by this method has been established by neutron reflectivity studies with deuterium-labeled polyions.[42a,b,49] Such mixing facilitates intimate contact between the DNA and metallopolymers to achieve efficient catalytic oxidation of the DNA to produce ECL.
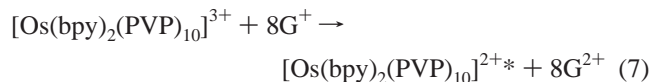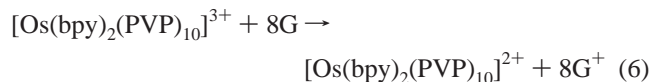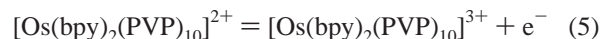
Among the homogeneous polynucleotides, only films containing poly G (Figures 4 and S5) gave significant increases in the Os^{II} SWV and ECL peaks upon oxidation by Fenton's reagent containing 5 mM H_2O_2. However, small increases in these peaks were also found in Poly A films when $[H_2O_2]$ was increased to 50 mM. Increases in the Os^{II} SWV and ECL peaks upon DNA oxidation by Fenton's reagent with 5 mM H_2O_2 appear to result mainly from oxidized guanines, presumably 8-oxoG. Oxidized adenines may contribute to the signal at higher $[H_2O_2]$ that presumably provides more extensive DNA oxidation. In general, the Os^{II} ECL in these films seems to be mainly specific for 8-oxoG, with minor contributions from oxidized adenines.

Again for the homogeneous polynucleotides, only films containing poly A gave increases for the Ru^{II} SWV and ECL peaks after oxidation (Figure S4). Films containing poly G (Figure S5) gave increases in the Os^{II} peaks but not in the Ru^{II} peaks. However, oxidized ss-DNA gave larger peaks at both Os^{II} and Ru^{II} potentials compared to oxidized ds-DNA (Figure 5). This is consistent with better accessibility of active oxidant sites in the film for their DNA-based reaction partners. In fact, oxidized ss-DNA gave peaks of about the same height as non-oxidized ss-DNA at the Ru^{II} potentials. On the basis of these observations, we rationalize the increase in the Ru^{II} peaks upon DNA oxidation (Figure 5) as related to oxidized adenines and strand breaks[9] that could produce an increased amount of single DNA strands in the films.
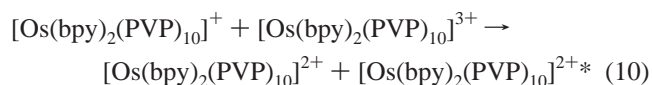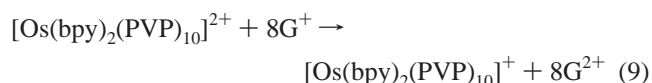
The formation of chemical adducts of guanine and adenine bases of DNA in films by reaction with styrene oxide under the conditions used in Figure 8 has been confirmed by LC−MS.[50] With the OsRu−PVP/DNA films, we observed minimal increases in the Os^{II} ECL and SWV (Figure 8) upon reaction with styrene oxide, but large increases were found for the Ru^{II}

peaks as reported previously.[30] This result suggests that the Os^{II} ECL is not sensitive to formation of the DNA base adducts in this experiment.

On the basis of our results and previous proposals for pathways for ECL generation,[35,37,44] the initial step is likely to involve reaction of electrochemically generated $[Os(bpy)_2\text{-}(PVP)_{10}]^{3+}$ with a reductant on DNA. We suggest that that the osmium metallopolymer generates ECL signals following pathways represented in eqs 5−10 (8G = 8-oxoguanine site):

$$[Os(bpy)_2(PVP)_{10}]^{2+} = [Os(bpy)_2(PVP)_{10}]^{3+} + e^- \quad (5)$$

$$[Os(bpy)_2(PVP)_{10}]^{3+} + 8G \rightarrow$$
$$[Os(bpy)_2(PVP)_{10}]^{2+} + 8G^+ \quad (6)$$

$$[Os(bpy)_2(PVP)_{10}]^{3+} + 8G^+ \rightarrow$$
$$[Os(bpy)_2(PVP)_{10}]^{2+*} + 8G^{2+} \quad (7)$$

$$[Os(bpy)_2(PVP)_{10}]^{2+*} \rightarrow [Os(bpy)_2(PVP)_{10}]^{2+} + h\nu \quad (8)$$

Or alternatively:

$$[Os(bpy)_2(PVP)_{10}]^{2+} + 8G^+ \rightarrow$$
$$[Os(bpy)_2(PVP)_{10}]^+ + 8G^{2+} \quad (9)$$

$$[Os(bpy)_2(PVP)_{10}]^+ + [Os(bpy)_2(PVP)_{10}]^{3+} \rightarrow$$
$$[Os(bpy)_2(PVP)_{10}]^{2+} + [Os(bpy)_2(PVP)_{10}]^{2+*} \quad (10)$$

Initial oxidation of the metallopolymer by the electrode gives the Os^{III} oxidant (eq 5), which reacts mainly with 8-oxoG (8G) in DNA to give $8G^+$ (eq 6), which may produce photoexcited $[Os(bpy)_2(PVP)_{10}]^{2+*}$ sites (eq 7), by directly reducing the Os^{III} sites in the film. This excited-state Os^{II} complex decays to the ground state by emission at ~764 nm (eq 8).[38] An alternative pathway is shown in eqs 9 and 10, where G^+ may reduce the Os^{II} sites to Os^I, which can then produce Os^{II*} by reacting with Os^{III} sites. Further studies are underway to distinguish these possibilities.

In the early stages of the Fenton oxidation of the DNA films, both the Os^{II} and Ru^{II} ECL and SWV peak ratios increase with reaction time (Figures 6 and S3). After about 20 min reaction of the films, the Ru^{II} signals reach a steady state, but the Os^{II} peaks showed dampened oscillations up to 100 min (Figure 6). LC−EC analysis of DNA after it had been oxidized under identical conditions and then hydrolyzed showed an almost identical pattern of oscillations in concentration of 8-oxoG (Figure 7).

We previously described oscillations of 8-oxoG concentrations during Fenton oxidation of both free guanine and DNA in solution under several conditions by LC−EC−UV analysis.[23] In the present work, our Fenton's reagent contains 10-fold less H_2O_2 than previously used. Nevertheless, oxidation of DNA in solution analyzed by LC−EC gave oscillations in [8-oxoG] (Figure 7) similar to those observed with 10-fold larger H_2O_2 concentrations. Detection of oscillations by the independent Os^{II} ECL/SWV method presented here (Figure 6) that is mainly selective for oxidized guanines confirms our earlier observation of this phenomenon. Out results are consistent with a competitive consecutive process in which guanine is oxidized to 8-oxoG, which is then rapidly oxidized to guanidinohydantoin. The latter

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was confirmed as a major product of 8-oxoG oxidation in our reactions by LC−MS.[23] Here, the common oxidant •OH reacts with starting reactant guanine as well as the initial reaction product 8-oxoG. While this simple consecutive pathway must certainly be featured in the oscillations, it would typically lead to only one maximum in the concentration of the initial product.[51] Oscillatory reactions often have very complex pathways featuring several interactive catalytic cycles and multiple elementary steps.[52,53] This particular case is now under further study using additional DNA oxidants.

In summary, ECL can be obtained directly from the reaction of oxidized guanines in DNA in thin films with the catalytic metallopolymer $[Os(bpy)_2(PVP)_{10}]^{2+}$. ECL and SWV peaks of the Os sites in the films are sensitive to oxidative damage that results in the formation of 8-oxoG. The ECL measurement is simple and inexpensive, and the approach may be useful for the detection of oxidized DNA as a biomarker for oxidative stress. The combination of ruthenium and osmium metallopolymers in the films can allow for future applications to the simultaneous detection of chemical and oxidative DNA damage.

Supporting Information Available: Five additional figures presenting reversible cyclic voltammetry of mixed metallopolymer films and ECL/SWV results on oligonucleotide films reacted with Fenton reagent. This material is available free of charge via the Internet at http://pubs.acs.org.

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