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Simultaneous Direct Electrochemiluminescence and Catalytic Voltammetry Detection of DNA in Ultrathin Films

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Abstract: Direct electrochemiluminescence (ECL) involving DNA was demonstrated in 10 nm films of cationic polymer $[Ru(bpy)_2(PVP)_{10}]^{2+}$ assembled layer-by-layer with DNA. A square wave voltammetric waveform oxidized the Ru^{II} sites in the metallopolymer to Ru^{III}, and ECL was measured simultaneously with catalytic voltammetric peaks in a simple apparatus. Significant ECL generation occurred only when guanine bases were present on oligonucleotides in the films. This result along with knowledge of proposed ECL pathways suggests that guanine radicals initially formed by catalytic oxidation of guanines by Ru^{III} react with the metallopolymer to produce electronically exited Ru^{II}* sites in the film. ECL and catalytic SWV peaks were sensitive to oligonucleotide hybridization and chemical DNA damage. Simultaneous linear growth of ECL and SWV peaks occurred after incubation with known DNA damage agent styrene oxide over 20 min. The estimated detection limit was 1 damaged DNA base in 1000. Control incubations of metallopolymer/ds-DNA films in buffer containing unreactive toluene resulted in no significant changes of the ECL or SWV peaks.

Introduction

Sensitive, selective detection of DNA is central to clinical tests, pathogen detection, and other methods utilizing polymerase chain reaction (PCR), to genetic disease screening based on oligonucleotide hybridization, and to molecular genotoxicity studies.[1] Electrochemistry provides simple, sensitive, and inexpensive approaches to detecting DNA hybridization and damage.[2−5] One of the most sensitive approaches was first reported by Thorp et al., who showed that ruthenium tris(2,2′-bpyridyl) $[Ru(bpy)_3^{2+}]$ oxidizes guanine bases in DNA and oligonucleotides in an electrochemical catalytic pathway as in Scheme 1:[6]

Scheme 1

$$Ru(bpy)_3^{2+} \leftrightarrow Ru(bpy)_3^{3+} + e^- \quad \text{(at electrode)} \qquad (1)$$

$$Ru(bpy)_3^{3+} + DNA(guanine) \rightarrow Ru(bpy)_3^{2+} + DNA(guanine_{ox}) \qquad (2)$$

Here the one-electron oxidation product DNA($guanine_{ox}$) can be further oxidized.[7] Cycling $Ru(bpy)_3^{3+}$ to $Ru(bpy)_3^{2+}$ by the fast chemical step in eq 2 provides a greatly enhanced catalytic oxidation current in voltammetry. In solution, guanines reacted with $Ru(bpy)_3^{3+}$ at rate constants of 9×10^3 M^{-1} s^{-1} in double-stranded (ds) calf thymus (CT) DNA and 2×10^5 M^{-1} s^{-1} in single-stranded (ss) CT-DNA.[6] Guanines in various base-mismatched hybridized oligomers reacted at rate constants intermediate between the ds- and ss-DNA values. The rate of oxidation and the resulting catalytic peak current measured in voltammetry depend greatly on DNA structure and guanine sequence, providing selectivity in DNA analyses.[8−13] Recent results suggest that the initial oxidation of guanines in DNA by $Ru(bpy)_3^{3+}$ is a proton-coupled process leading to guanine radicals.[14]

Damage of DNA by the formation of chemical adducts of nucleobases also alters the native ds-DNA structure, resulting in an increased reaction rate with $Ru(bpy)_3^{3+}$. Capitalizing on this fact, we developed sensors to detect chemical DNA damage

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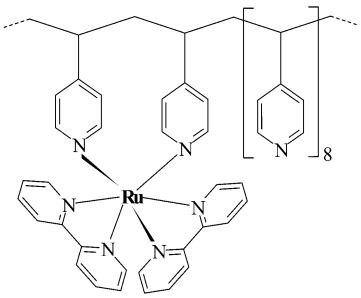
in ultrathin films using soluble[15] $Ru(bpy)_3^{2+}$ and a poly(vinylpyridine) (PVP)−$RuCl(bpy)_2^{+}$ polymer[16,17] as catalysts for square wave voltammetry.

Electrochemiluminescence (ECL) using $Ru(bpy)_3^{2+}$ attached to DNA provides a sensitive method of detection.[18−24] The $Ru(bpy)_3^{2+}$ labels on DNA are oxidized to $Ru(bpy)_3^{3+}$, and ECL is generated by using a sacrificial reductant, often tripropylamine. ECL depends on generation of photoexcited $[Ru(bpy)_3^{2+}]^*$ in a process involving reaction of a radical form of the reductant with electrochemically generated $Ru(bpy)_3^{3+}$.[23,25,26] Alternatively, $Ru(bpy)_3^{+}$ is formed by reduction of $Ru(bpy)_3^{2+}$ by the radical, followed by reaction of Ru^{I} and Ru^{III} species to give $[Ru(bpy)_3^{2+}]^*$.[27] Decay of $[Ru(bpy)_3^{2+}]^*$ to the ground state with luminescent emission at 610 nm is measured in the detection step. Bimetallic ruthenium complexes can give enhanced ECL intensity.[28]

One-electron oxidation of guanine in DNA is well-known to give guanine radicals,[29] which can also be oxidized by $Ru(bpy)_3^{3+}$.[7] Thus, we suspected that the reaction of guanine with $Ru(bpy)_3^{3+}$ or related species on a metallopolymer could also lead to a photoexcited Ru^{II}* moiety and generate ECL. Further, the rate and yield of this process might be enhanced by utilizing films on electrodes with high local concentrations of Ru catalyst and guanine. In this paper, we report that ultrathin films of the catalytic polymer $[Ru(bpy)_2(PVP)_{10}](ClO_4)_2$ and oligonucleotides on electrodes can generate direct ECL signals that are sensitive to hybridization and chemical damage of DNA. ECL can be monitored simultaneously with catalytic electrochemical voltammograms in a simple apparatus.

Experimental Section

Chemicals and Materials. *cis*-$[Ru(bpy)_2(H_2O)](ClO_4)_2$ was prepared and characterized by a published method.[30] The bis-substituted metallopolymer $[Ru(bpy)_2(PVP)_{10}](ClO_4)_2$ was prepared by refluxing *cis*-$[Ru(bpy)_2(H_2O)](ClO_4)_2$ with a 10-fold excess of poly(vinylpyridine) (PVP, MW 280 000) (i.e. 1 ruthenium/10 pyridine units) to give the structure below. Preparation and characterization were reported in detail previously.[31]



Calf thymus (CT) double-stranded (ds) DNA ((Sigma, type XV, 13 000 average base pairs, 41.9% G/C), CT ss-DNA, salmon testes (ST) ds-DNA (Sigma, ~2000 average base pairs, 41.2% G/C), ST ss-DNA, poly(guanadylic acid) (5′) (poly[G]), poly(cytidylic acid) (5′) (poly[C]), poly(adenylic acid) (5′) (poly[A]), styrene oxide, and toluene were from Sigma. Poly(dimethyldiallyammonium chloride) (PDDA, MW 200 000−350 000) was from Aldrich. Water was purified with a Hydro Nanopure system to specific resistance >16 MΩ cm. All other chemicals were reagent grade.

Apparatus. Simultaneous square wave voltammetry (SWV)−ECL measurements were made in a glass 3-electrode electrochemical cell at 37.0 ± 0.5 °C using a CH Instruments model 660 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 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 protected from light by coating with a black cloth to avoid external optical background and possible photodecomposition of the ruthenium metallopolymer. 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 catalytic film analysis, as the oxidative analysis modifies the nucleic acids in the films.

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

Film Assembly. DNA−metallopolymer films were constructed by the layer-by-layer electrostatic assembly method.[15,17,32] 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, sonicated in ethanol for 15 min, and then sonicated in water for 15 min. Layers were constructed by placing a 30 μL drop of 0.2% aqueous $[Ru(bpy)_2(PVP)_{10}](ClO_4)_2$ onto each PG electrode, allowing 15 min to achieve saturated adsorption[32] and then washing with water. Subsequently, 30 μL of DNA 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 2 metallopolymer/DNA bilayers. Films containing ss-DNA and other polynucleotides were also assembled in this way.

Assembly of films was monitored 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) resonators with 0.7 mM 3-mercapto-1-propanol and 0.3 mM 3-mercaptopropionic acid in ethanol.[15] Films were assembled as for

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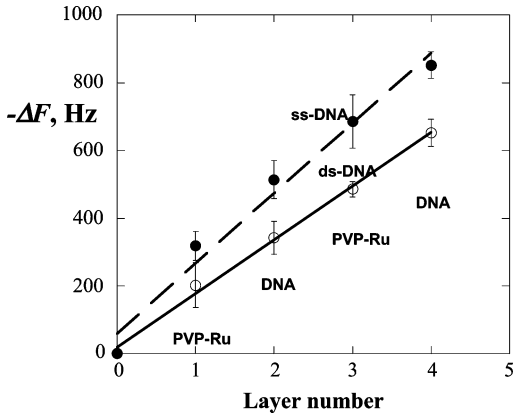

Figure 1. QCM frequency shifts for cycles of alternate PVP−Ru and CT-DNA layers on gold-quartz resonators coated first with mixed monolayers of mercaptopropionic acid/mercaptopropanol for ss-DNA (●) and ds-DNA (○) (average values for 3 replicate films).

PG electrodes. Resonators were dried in a stream of nitrogen before measuring the frequency change (ΔF). Absorbed mass was estimated with the Sauerbrey equation,[32] for 9 MHz quartz resonators, giving dry film mass/unit area M/A as

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

The nominal thickness (d) of dry films was estimated with an expression confirmed by high-resolution electron microscopy:[33]

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

Reactions with Styrene Oxide. Incubations of films were done in styrene oxide solutions in a stirred reactor at 37.0 ± 0.5 °C. A 120 μL volume of neat styrene oxide or toluene (as control) was added to 10 mL of acetate buffer, pH 5.5, + 50 mM NaCl to give saturated solutions.[15] pH 5.5 gave optimum reaction rates of DNA with styrene oxide[17] and also allowed efficient ECL production.[27] PG electrodes coated with polynucleotide or DNA films were incubated in the stirred emulsion and then rinsed with water and transferred to the electrochemical cell containing pH 5.5 buffer for SWV/ECL analysis.

Safety Note: *Styrene oxide is a suspected human carcinogen and somewhat volatile. Gloves should be worn, all manipulations done under a hood, and reactions done in closed vessels.*

Results

QCM Monitoring of Film Assembly. Films were constructed for SWV/ECL measurements by the layer-by-layer electrostatic assembly method[32] with two bilayers of CT-DNA and metallopolymer $[Ru(bpy)_2(PVP)_{10}]^{2+}$ (Ru−PVP), denoted $(PVP-Ru/DNA)_2$. These films were first characterized by measuring QCM frequency shifts during film growth. ΔF values varied linearly with layer number for ss-DNA and ds-DNA layers alternated with Ru−PVP (Figure 1), suggesting regular film growth with reproducible layers of DNA and proteins. ΔF values with eq 3 were used to obtain weights of Ru−PVP and DNA. Equation 4 was used to estimate the average nominal thickness of the films (Table 1). About 20% DNA and 30% more metallopolymer were incorporated in films when ss-DNA was used compared to ds-DNA. The mole ratio of guanine to ruthenium in these films was about 1.6:1.

ECL and SWV Measurements. One of us recently reported a method in which voltammetric or amperometric current was measured simultaneously with ECL at electrodes coated with $[Ru(bpy)_2(PVP)_{10}]^{2+}$ for oxalate detection.[34] We used a similar approach in the present work, here scanning by SWV from potentials at which the Ru^{II} redox centers in the metallopolymer are not oxidized through values at which Ru^{II} is oxidized to Ru^{III} ($E^{\circ\prime} \sim 1.15$ V vs SCE), activating the catalytic cycle in Scheme 1. Preliminary results showed that measurable ECL signals could be observed in pH 6 buffer containing dissolved $Ru(bpy)_3^{2+}$ and guanosine monophosphate, poly(guanylic acid) (poly[G]) or ds-DNA while scanning voltammograms through the potential region where $Ru(bpy)_3^{2+}$ is oxidized to $Ru(bpy)_3^{3+}$. Also, ECL signals were detected with nucleic acid species in solution by using a glassy carbon electrode with an adsorbed layer of $[Ru(bpy)_2(PVP)_{10}]^{2+}$. In this work, we utilized films designed to contain all the necessary components for ECL generation with DNA. These ultrathin films feature the reactive species in high concentrations in a tiny reaction volume, estimated from film thickness (Table 1) and electrode area at $\sim 2 \times 10^{-4}$ mm^3.

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

film	thickness, nm	DNA mass, μg cm^{-2}	RuPVP mass, μg cm^{-2}
$(PVP-Ru/ST\text{-}ds\text{-}DNA)_2$	10	1.6	1.9
$(PVP-Ru/ST\text{-}ss\text{-}DNA)_2$	14	1.9	2.6

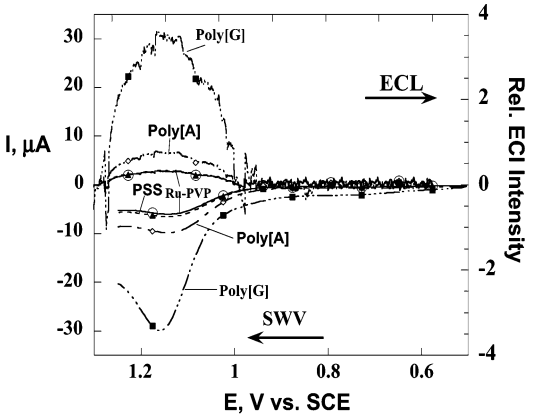

Figure 2. SWV and ECL response for films containing Ru−PVP on PG electrodes in pH 5.5 buffer + 50 mM NaCl. Films are Ru−PVP alone (○), $(Ru-PVP/PSS)_2$ (▲), $(Ru-PVP/poly[A])_2$ (◊), and $(Ru-PVP/poly[G])_2$ (■). Symbols are for curve identification only.

Similar to previous reports, $[Ru(bpy)_2(PVP)_{10}]^{2+}$ adsorbed on PG electrodes gave reversible redox peaks,[31,34] with formal potential ca. 1.15 V vs SCE at pH 5.5 measured by cyclic voltammetry and SWV and surface concentration of 6×10^{-11} mol cm^{-2} obtained for electroactive ruthenium by integration of CVs at 5 mV s^{-1}. Comparing this value with QCM results, we find that 84% of the ruthenium present in this first adsorbed layer is electroactive.

Figure 2 shows that combined ECL/SWV measurements on films containing Ru−PVP, alone or in $(PVP/PSS)_2$ films, gave the Ru^{II}/Ru^{III} oxidation peak and a very small amount of light. However, $(Ru-PVP/poly[G])_2$ films gave a significant ECL peak, as well as a catalytic current by SWV that was much larger than the noncatalytic Ru^{II}/Ru^{III} oxidation peak for Ru−PVP films not containing poly[G]. Figure 2 also shows that

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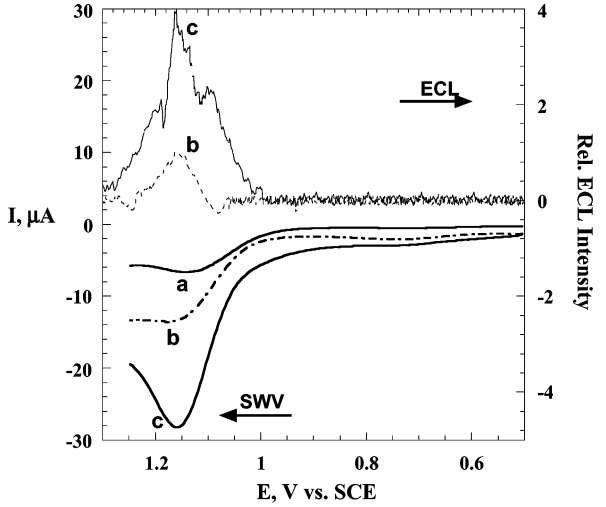

Figure 3. SWV and ECL response for (a) Ru−PVP, (b) (Ru−PVP/poly-[G]/poly[C])$_2$ and (c) (Ru−PVP/poly[G])$_2$ films on PG electrodes in pH 5.5 buffer + 50 mM NaCl.

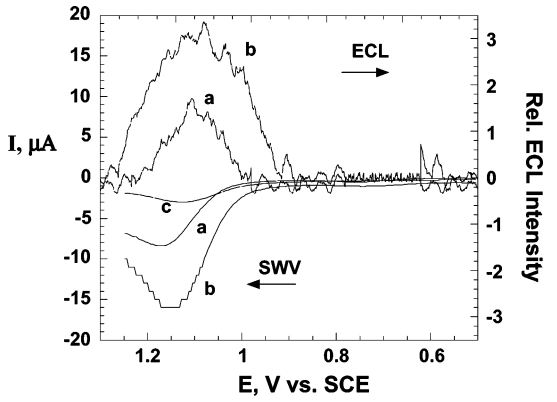

Figure 4. SWV and ECL for (a) (Ru−PVP/ds-CT DNA)$_2$ films and (b) (Ru−PVP/ss-CT DNA)$_2$ films on PG electrodes in pH 5.5 buffer + 50 mM NaCl and (c) SWV only for Ru−PVP film with no DNA.

(Ru−PVP/poly[A])$_2$ gave a small catalytic current and a very small ECL signal, slightly above the background for Ru−PVP films.

We investigated the influence of hybridization on the ECL signal by using films containing hybridized and unhybridized poly[G]. Figure 3 compares the ECL/SWV responses of films of (Ru−PVP/poly[G])$_2$ and (Ru−PVP/ poly[G]/poly[C])$_2$. The latter films were made by using a solution of poly[G] and poly-[C] for which UV−Vis spectra confirmed hybridization. Both ECL and SWV peaks are about 3-fold larger for films containing the hybridized poly[G]/poly[C] layers compared to the film with only the poly[G] layer (Figure 3).

Similar results were obtained when comparing ECL/SWV signals for films containing ss- and ds-DNA. (Figure 4). Films containing ss-DNA gave about twice the ECL signal as those made with ds-DNA. SWV peaks for the ss-DNA films were about 2.5-fold larger than their ds-DNA analogues. Similar results were found for calf thymus and salmon testes DNA. Films assembled with DNA and the polycation PDDA showed no significant ECL peaks.

There was a slight increase in the ECL signal when an additional Ru−PVP/ds-DNA bilayer was grown on top of (Ru−PVP/ds-DNA)$_2$ films, but this increase was about at the level of film-to-film variance of the 2-bilayer films. Films with

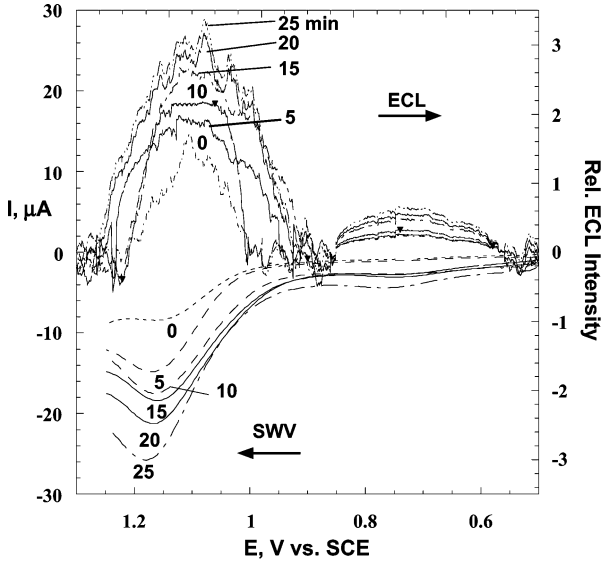

Figure 5. SWV and ECL responses for (Ru−PVP/ds-CT DNA)$_2$ films on PG electrodes in pH 5.5 buffer + 50 mM NaCl after incubations 37 °C with saturated styrene oxide. Numerical labels are incubation times in minutes.

4 bilayers, i.e., (Ru−PVP/ds-DNA)$_4$, gave smaller ECL peaks than 2-bilayer films, probably because of mass and electron transport limitations as film thickness increased similar to those observed with enzyme−polyion films.[35]

Reaction of Nucleic Acid Films with Styrene Oxide. The purine bases guanine and adenine in DNA form covalent adducts with styrene oxide, with the majority of reactions occurring at guanine.[36−42] Such adducts can serve as important markers of human exposure to mutagens and carcinogens.[43,44] Further, covalent adduct formation disrupts the double helical structure of DNA and makes the guanines more accessible for catalytic oxidation, even when the DNA is present in polyion films.[15] By using capillary electrophoresis and liquid chromatography−mass spectrometry to analyze enzyme- and acid-hydrolyzed DNA that had been reacted with styrene oxide, we previously confirmed that styrene oxide−guanine and styrene oxide−adenine adducts form under the incubation conditions used in this work.[45,46]

When (Ru−PVP/ds-DNA)$_2$ films were incubated with styrene oxide and then scanned by SWV, increases in the ECL and the SWV peaks were observed with increasing incubation time (Figure 5). Average peak currents for the ds-DNA films increased linearly with incubation time for about the first 20 min, followed by a slight decrease (Figure 6). When (Ru−PVP/

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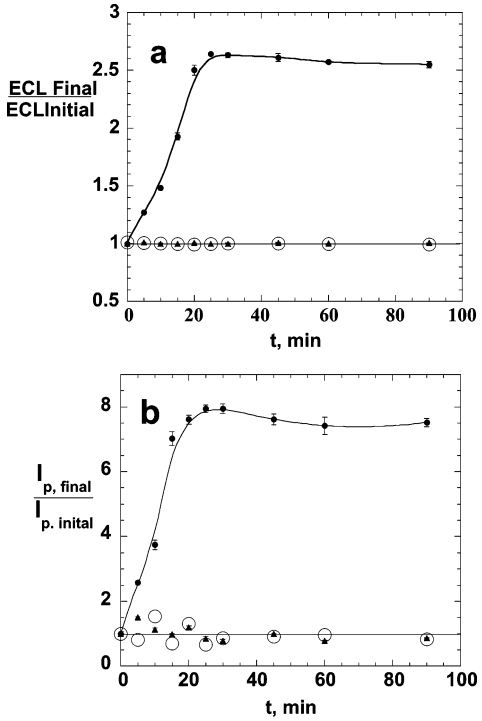

Figure 6. Influence of incubation time with styrene oxide (●), toluene (▲), and buffer alone (○) on (a) average ECL signals and (b) average SWV catalytic peak currents (final response/initial response) for $(Ru/ds\text{-}CT\ DNA)_2$ films. Error bars represent standard deviations for three trials, with 1 electrode/trial.

ds-$DNA)_2$ films were incubated with toluene, for which no chemical reactions with DNA have been reported, or in buffer only, ECL and SWV peaks remained within electrode-to-electrode variability and showed no trends with incubation time. Error bars in Figure 6 are mainly the result of electrode-to-electrode variability. However, both the error bars and scatter in the controls were smaller for the ECL ratios than for the SWV ratios.

In addition to catalytic oxidation of guanines, it is possible that adducts formed on DNA by reaction with styrene oxide could be catalytically oxidized by the ruthenium metallopolymer. To assess this possibility, styrene oxide was incubated with films containing individual polynucleotides and the metallopolymer. Figure 7 shows that both ECL and SWV peaks increased after 10 min incubation of $(Ru-PVP/poly[G])_2$ with styrene oxide. An 80% increase in SWV peak current and a 40% increase in ECL intensity was found. However, for films incubated with toluene, ECL and SWV peaks were nearly identical to initial values.

Similar experiments were done with films containing the other three polynucleotides. For intact poly[A] films, only a small SWV peak for catalytic oxidation was observed. After poly[A] was reacted with styrene oxide, a 25% increase in this peak was found. However, ECL signals for poly[A] treated with styrene oxide were indistinguishable from the baseline before treatment. SWV of poly[C] and poly[A] films showed no increase in oxidation peaks either before or after 10 min incubations with styrene oxide. Poly[A] or poly[C] films did not produce significant ECL before or after incubations with styrene oxide.

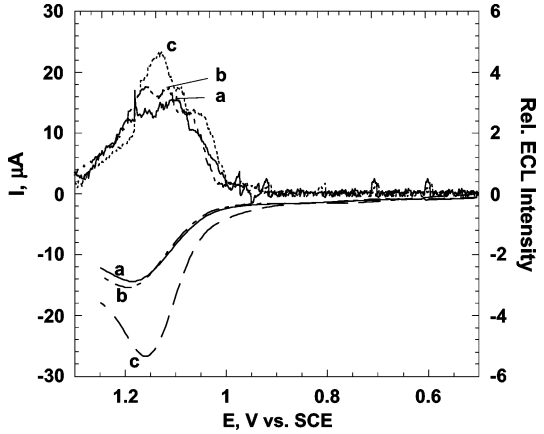

Figure 7. SWV and ECL response for $(Ru-PVP/poly[G])_2$ films on PG electrodes in pH 5.5 buffer + 50 mM NaCl: (a) no incubation; (b) incubated with saturated toluene control for 10 min.; (c) incubated with saturated styrene oxide for 10 min.

Discussion

Results described herein demonstrate for the first time, to our knowledge, that ECL can be achieved by direct reaction of a Ru^{III} complex with DNA, in this case by using $[Ru(bpy)_2(PVP)_{10}]^{2+}$ in films with DNA. Alternate layer-by-layer electrostatic assembly provided Ru−PVP/ds-CT DNA films about 10 nm thick with intimate mixing of Ru−PVP and DNA reactants in a tiny reaction volume. Extensive intermixing of neighboring layers in polycation/polyanion films assembled by this method has been established by neutron reflectivity studies.[32a,b,47]

The ECL response appears to involve mainly the guanine bases in DNA, since no other homopolymeric nucleotides besides poly[G] (Figure 2) gave significant ECL signals in films with Ru−PVP. ECL signals for Ru−PVP/poly[G] films were 3-fold larger than for films of Ru−PVP and hybridized poly-[G]/poly[C] (Figure 3). Films of metallopolymer and ss-DNA gave 2-fold larger ECL intensity than with ds-DNA (Figure 4), although the mass of ss-DNA in films was ∼18% larger than ds-DNA (Table 1). Thus, the ECL yield is sensitive to the hybridization state of oligonucleotides in the films, a key feature for detecting base mismatches.[2−5]

Figures 5 and 6 show that direct ECL in Ru−PVP/DNA films can be used to detect DNA damage. Figure 6a shows a nearly linear increase in ECL intensity over 20 min when Ru−PVP/ds-DNA films were reacted with styrene oxide under conditions which were confirmed to give styrene oxide−guanine and styrene oxide−adenine adducts in films and in solutions.[45,46] No enhancement of ECL signals was found when the films were incubated with toluene, which does not react with DNA (Figure 6). After 5 min of reaction with styrene oxide, the ECL peak ratio for ds-DNA films was more than 3-fold greater than the average peak ratio for controls.

We can correlate the increased ECL peak ratio for DNA films incubated with styrene oxide with previous capillary electrophoresis results showing that under our conditions about 1.2% damage/h occurs for the first several hours of reaction with styrene oxide.[46] In the present study, we found that a 5 min incubation time gave an ECL peak ratio more than 3-fold larger than the noise (Figure 6a). This signal corresponds to an

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estimated practical detection limit for ECL of about 0.1% damage or 1 damaged base in 1000.

Figures 5 and 6 also show increased catalytic SWV responses to DNA treated with styrene oxide but no current increases when the films are treated with unreactive toluene or buffer in control incubations. The development of the ECL and SWV peaks with time agree very well. A similar comparison as above leads to a detection limit for catalytic SWV similar to ECL. This is about the same as for polyion/DNA films with an underlayer of $[Ru(bpy)_2Cl(PVP)_{10}]^+$,[17] which has 5 N−Ru bonds/Ru instead of 6 as in $[Ru(bpy)_2(PVP)_{10}]^{2+}$ used for this work. $[Ru(bpy)_2Cl(PVP)_{10}]^+$ has a lower formal potential, about 0.75 V vs SCE as opposed to 1.15 V for $[Ru(bpy)_2(PVP)_{10}]$. However, $[Ru(bpy)_2Cl(PVP)_{10}]^+$ cannot produce significant emission at room temperature.

ECL generation involves initial reaction of electrochemically generated $[Ru(bpy)_2(PVP)_{10}]^{3+}$ with a reductant to give a radical. By analogy with previously proposed mechanisms,[27,34] the pathway in our films could be represented as in Scheme 2:

Scheme 2

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

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

$$G^{\bullet} + [Ru(bpy)_2(PVP)_{10}]^{3+} \rightarrow G_{2ox} + [Ru(bpy)_2(PVP)_{10}]^{2+*} \quad (7)$$

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

Initial oxidation by electron donation from the metallopolymer to the electrode at sufficiently positive potentials gives the Ru^{III} oxidant (eq 5), which reacts with guanines (G) in DNA to give guanine radical (eq 6), consistent with the proton-coupled catalytic DNA oxidation pathway recently proposed by Thorp et al.[14] This radical may produce Ru^{II}* sites (eq 7), representing the excited state complex, by directly reducing the Ru^{III} sites. G_{2ox} in eq 7 represents a guanine oxidized by two electrons, a reaction observed[7] in ss-DNA oxidized by dissolved $Ru(bpy)_3^{3+}$.

A viable alternative is shown in eqs 9 and 10, where the guanine radical may reduce the Ru^{II} complex to Ru^{I}, which can then produce Ru^{II}* by reacting with Ru^{III}:

$$G^{\bullet} + [Ru(bpy)_2(PVP)_{10}]^{2+} \rightarrow G_{2ox} + [Ru(bpy)_2(PVP)_{10}]^{+} \quad (9)$$

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

Ru^{II}* represents the electronically excited state which decays to ground state by emission at ~610 nm (eq 8), providing for the simultaneous detection of ECL along with SWV from the films. The onset of light emission occurs in a similar potential range as the appearance of the increased SWV current (Figures 2−5 and 7). Furthermore, similar development of final/initial peak ratios vs time of incubation with styrene oxide (Figure 6), as well as the dependence of ECL and SWV signals on hybridization of the oligonucleotides in the films (Figures 3 and 4), suggest that, as in purely voltammetric DNA oxidation with catalytic complexes,[7−9,11,12] the reaction of the Ru^{III} sites with guanines in the DNA is likely to be rate limiting (eq 6).

The increase in ECL and SWV peaks with time of incubation with styrene oxide probably reflects a larger average rate of reaction between Ru^{III}−PVP and the chemically damaged DNA compared to the reaction with intact ds-DNA, consistent with the structurally related rate effects found by Thorp et al.[7−9,11,12] As previous suggested when using SWV with soluble $Ru(bpy)_3^{2+}$ or the lower oxidation potential metallopolymer $[Ru(bpy)_2Cl(PVP)_{10}]^+$ to catalyze DNA oxidation in films, guanine in the ds-DNA structure must be less accessible to the oxidant than in damaged or ss-DNA. A smaller distance of closest approach of the Ru^{III} active sites in the polymer to the oxidizable bases presumably leads to faster reaction rates and larger catalytic peaks for damaged DNA as well as for unhybridized oligonucleotides. Covalent adducts of styrene oxide and guanines and adenines disrupt the double helix and allow closer contact between oxidizable moieties on the DNA and the active oxidizing agent. Figure 7 suggests that there may also be a small contribution to the ECL by styrene oxide−guanine adducts, but the exact nature or degree of this contribution is uncertain at present.

In summary, our results show that ECL can be obtained *directly* from the reaction of guanine bases in oligonucleotides in ultrathin films with the catalytic metallopolymer $[Ru(bpy)_2(PVP)_{10}]^{2+}$ without using a sacrificial reductant. ECL and SWV peaks are sensitive to oligonucleotide hybridization and chemical damage of ds-DNA. Both applications involve measurement of ratios, and the reproducibility of these ratios for DNA damage was slightly better for ECL (cf. Figure 6). Preliminary studies also showed that ECL can be detected for oligonucleotides in solution by using electrodes coated with $[Ru(bpy)_2(PVP)_{10}]^{2+}$. Thus, direct ECL as described herein may find future applications in films or solutions for DNA detection in applications such as hybridization or estimation of DNA damage. ECL might also be used in toxicity screening applications in films with DNA and metabolic enzymes[45] that produce metabolites that cause DNA damage. It is not necessary to measure the voltammetric signature along with the ECL output, and such approaches could simplify applications of electrode arrays for high throughput DNA analyses in which simultaneous digital imaging of ECL in multiple electrode arrays might be achieved.

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