[Ru(bpy)$_2$dpdz]$^{2+}$ Electrochemiluminescence Switch and Its Applications for DNA Interaction Study and Label-free ATP Aptasensor

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[Ru(bpy)$_2$dpdz]$^{2+}$ electrochemiluminescence (ECL) was studied, and it was used to investigate DNA interaction and develop a label-free ATP aptasensor for the first time. ECL of [Ru(bpy)$_2$dpdz]$^{2+}$ is negligible in aqueous solution, and increases ~1000 times when [Ru(bpy)$_2$dpdz]$^{2+}$ intercalates into the nucleic acid structure. The ECL switch behavior of [Ru(bpy)$_2$dpdz]$^{2+}$ is ascribed to the intercalation that shields the phenazine nitrogens from the solvent and results in a luminescent excited state. The ECL switch by DNA was applied to investigate the interaction of [Ru(bpy)$_2$dpdz]$^{2+}$ with herring sperm DNA. The calculated equilibrium constant ($K$) is $1.35 \times 10^6$ M$^{-1}$, and the calculated binding-site size ($s$) is 0.88 base pair, which is consistent with the reported values. Moreover, ATP can dramatically affect ECL of the [Ru(bpy)$_2$dpdz]$^{2+}$/ATP aptamer complex. As a result, a label-free, sensitive, and selective [Ru(bpy)$_2$dpdz]$^{2+}$ ECL method for ATP detection was developed. The detection limit is 100 nM for ATP (with a signal-to-noise ratio, S/N, of 3) with a linear range of 0–1 μM. The result demonstrates that [Ru(bpy)$_2$dpdz]$^{2+}$ ECL holds great promise in aptasensors.

Electrogenerated chemiluminescence (ECL), which is also called electrochemiluminescence, refers to the phenomenon where luminescence is produced in a redox reaction of electrogenerated reactants. ECL has now become a powerful analytical technique, because of its inherent features, such as high sensitivity, low background, simple instrumentation, and fast sample analysis. It has been widely used in the areas of immunoassay,†† pharmaceutical study,‡‡ and environmental detection. The interaction between metal complexes and DNA has received much attention, because of the use of metal complexes as diagnostic probes, reactive agents, and the therapeutics. Bard and colleagues pioneered the ECL method for studying the DNA interaction with metal complexes including Ru(phen)$_2$ and Os(bpy)$_2$ and Ru(phen)$_2$ and Os(bpy)$_2$. These metal complexes are popular “light switch” molecules, because they show no photoactivity, because of their extended aromatic structure. These metal complexes are popular “light switch” molecules, because they show no photoactivity, because of their extended aromatic structure.22,24

Metal complexes that contain the dipyrido[3,2-a:2',3'-c]phenazine (dpdz) ligand, such as [Ru(bpy)$_2$dpdz]$^{2+}$, [Os(bpy)$_2$dpdz]$^{2+}$, and Ru(phen)$_2$dpdz, have been found to intercalate into DNA with high affinity ($K_a \approx 10^6$ M$^{-1}$), because of their extended aromatic structure.22–24 These metal complexes are popular “light switch” molecules, because they show no photoactivity.

Figure 1. Scheme of ECL switch, based on [Ru(bpy)$_{2}$dppz]$^{2+}$ and DNA.

luminescence in aqueous solution, but they do display intense photoluminescence in the presence of DNA. They have been widely used as promising fluorescent probes in DNA detection and structure analysis.\textsuperscript{25–28} However, their ECL properties have never been studied.

In this study, ECL of [Ru(bpy)$_{2}$dppz]$^{2+}$ was investigated and was applied to study the interaction of [Ru(bpy)$_{2}$dppz]$^{2+}$ with DNA. In contrast to Ru(phen)$_{3}$$^{2+}$ ECL and Os(bpy)$_{2}$ECL, which decreased $\sim$50% in the presence of DNA, [Ru(bpy)$_{2}$dppz]$^{2+}$ ECL increased $\sim$1000 times in the presence of DNA (see Figure 1). The remarkable increase in ECL intensity in the presence of DNA not only allows easier investigation of DNA interaction, but also provides a sensitive method for the determination of analytes that compete for intercalation into double-stranded DNA segments.

Aptamers can interact with target molecules with strong affinity and excellent specificity. They usually fold into unique three-dimensional structures to ensure their specific binding to targets.

EXPERIMENTAL SECTION

Reagents. The ATP aptamer used in the experiment (5'-ACCTG GGGGA GTATT CCGGA GGAAG GT-3') and the thymin DNA aptamer (5'-GGTTG GTGTG GTTGG-3') were purchased from Shanghai Sangon Biotechnology Company. Herring Sperm DNA, ATP, CTP, UTP, and GTP were purchased from Sigma. [Ru(bpy)$_{2}$dppz]$^{2+}$ was synthesized according to the published procedure. Other chemicals were analytical-reagent grade and used as received. All solutions were prepared with doubly distilled water. The concentrations of oligonucleotides were determined based on 260-nm ultraviolet (UV) absorbance and the corresponding sequence.

Instrumentation. Electrochemical measurements were performed in a conventional three-electrode cell (volume of 0.5 mL) with a CH Instruments Model 800B (CHI, Inc.), using an Ag/AgCl reference electrode (saturated KCl) and a platinum wire counter electrode. Prior to each electrochemical experiment, the working electrodes were regularly polished with an aqueous slurry of 0.05 $\mu$m alumina particles on a Microcloth polishing cloth, sonicated in water, and then rinsed thoroughly with water. All experiments were performed at room temperature. ECL intensities were monitored through the bottom of three-electrode cell with a BPCL Ultra-Weak luminescence analyzer. Unless otherwise noted, the PMT was biased at 800 V. Fluorescence measurements were performed on a Perkin–Elmer Model LS-55 luminescence spectrometer. The emission spectra were recorded upon excitation at 482 nm. The sample cell was a 1-mL quartz cuvette. The slits for both excitation and emission were set at 15 nm.

RESULTS AND DISCUSSION

Cyclic Voltamgrams and ECL of [Ru(bpy)$_{2}$dppz]$^{2+}$ in the Presence of DNA. Interaction of metal complexes with DNA has been studied by cyclic voltammetry based on the change of the voltammograms of the metal complex after binding with DNA.\textsuperscript{34–36} Generally, the peak current decreased due to slower mass diffusion of the much larger DNA/metal complex adduct.\textsuperscript{34,37} As shown in Figure 2, the peak current decreases 25% after the addition of 0.16 mM DNA, demonstrating that [Ru(bpy)$_{2}$dppz]$^{2+}$ binds strongly to DNA.

Figure 3A shows the fluorescence spectra of [Ru(bpy)$_{2}$dppz]$^{2+}$ in the absence and presence of 0.16 mM DNA. Fluorescence is detectable only when [Ru(bpy)$_{2}$dppz]$^{2+}$ has been intercalated into the nucleic acid structure. The ECL behavior of [Ru(bpy)$_{2}$dppz]$^{2+}$ is similar to its fluorescence behavior. A typical ECL intensity–potential curve for the [Ru(bpy)$_{2}$dppz]$^{2+}$-oxalate system at a glassy carbon electrode is shown in Figure 3B. [Ru(bpy)$_{2}$dppz]$^{2+}$ shows negligible ECL in 5 mM oxalate solution, but its ECL intensity increases by a factor of $\sim$1000
after adding 0.16 mM DNA. In previous ECL studies of the interactions of metal complexes with DNA, >1 mM DNA was needed, because of the slight decrease in ECL intensity. For instance, 2.4 mM calf thymus DNA was required to produce ∼50% decrease in ECL of 0.1 mM Os(bpy)$_3^{3+}$. In this study, the remarkable increase in [Ru(bpy)$_2$dpdz]$_{2+}$ ECL in the presence of DNA makes the study of DNA interaction more sensitive and allows the use of much lower DNA concentrations.

**ECL Switch Mechanism.** [Ru(bpy)$_2$dpdz]$_{2+}$ shows no photoluminescence in aqueous solution, because its triplet metal-to-ligand charge transfer (MLCT) excited state is effectively quenched by hydrogen bonding between water and the phenazine nitrogen of the ligand. When it binds to DNA, the interaction between the ligand and the base pairs of duplex nucleic acid protects the phenazine nitrogen from water, leading to intense emission.$^{38}$ The ECL properties of [Ru(bpy)$_2$dpdz]$_{2+}$ may be similar to its photoluminescence, because the ECL emission may generate via the same excited state as photoluminescence. In aqueous solution, the ECL of [Ru(bpy)$_2$dpdz]$_{2+}$ is quenched by the protonation of the phenazine N atoms in the excited state. When [Ru(bpy)$_2$dpdz]$_{2+}$ binds to DNA, the N atoms are protected, because of the intercalation of the planar phenazine ligand between the base pairs of DNA, resulting in intense ECL emission. [Ru(bpy)$_2$dpdz]$_{2+}$ ECL in the presence of DNA is produced as described by eqs (1−7),$^{39,40}$ where Ru(II)-DNA and Ru(III)-DNA represent [Ru(bpy)$_2$dpdz]$_{2+}$ or [Ru(bpy)$_2$dpdz]$_{3+}$ bound to DNA.

\[
\text{Ru(II)-DNA} \rightarrow \text{Ru(III)-DNA} \quad (1)
\]
\[
\text{Ru(III)-DNA} + \text{C}_2\text{O}_4^{2-} \rightarrow \text{Ru(II)-DNA} + \text{C}_2\text{O}_4^{-} \quad (2)
\]
\[
\text{C}_2\text{O}_4^{-} \rightarrow \text{CO}_2^{-} + \text{CO}_2 \quad (3)
\]
\[
\text{Ru(II)-DNA} + \text{CO}_2^{-} \rightarrow \text{Ru(I)-DNA} + \text{CO}_2 \quad (4)
\]
\[
\text{Ru(III)-DNA} + \text{CO}_2^{-} \rightarrow \text{Ru(II)*-DNA} + \text{CO}_2 \quad (5)
\]

or

\[
\text{Ru(III)-DNA} + \text{Ru(I)-DNA} \rightarrow \text{Ru(II)-DNA} + \text{Ru(II)*-DNA} \quad (6)
\]

\[
\text{Ru(II)*-DNA} \rightarrow \text{Ru(II)-DNA} + hv \quad (7)
\]

**ECL Investigation of the Interaction of [Ru(bpy)$_2$dpdz]$_{2+}$ with DNA.** Figure 4 shows a titration of 60 µM DNA with [Ru(bpy)$_2$dpdz]$_{2+}$ in 5 mM oxalate solution. The potential was stepped from 0.2 V to 1.35 V.

![Figure 2](image1.png)

**Figure 2.** Cyclic voltammograms at a glassy carbon electrode in 5 mM pH 5.5 oxalate solution containing (a) 0.1 mM [Ru(bpy)$_2$dpdz]$_{2+}$ and (b) 0.1 mM [Ru(bpy)$_2$dpdz]$_{2+}$ + 0.16 mM DNA.

![Figure 3](image2.png)

**Figure 3.** (A) Photoluminescence and (B) electrochemiluminescence intensities in 5 mM pH 5.5 oxalate solution containing 0.1 mM [Ru(bpy)$_2$dpdz]$_{2+}$ (curve a) and 0.1 mM [Ru(bpy)$_2$dpdz]$_{2+}$ + 0.16 mM DNA (curve b).

![Figure 4](image3.png)

**Figure 4.** ECL titration of 60 µM DNA with [Ru(bpy)$_2$dpdz]$_{2+}$ in 5 mM pH 5.5 oxalate solution. The potential was stepped from 0.2 V to 1.35 V.
off at high [Ru(bpy)$_2$dpzp]$^{2+}$ concentrations, suggesting that the DNA is saturated with [Ru(bpy)$_2$dpzp]$^{2+}$ molecules. The ratio of [Ru(bpy)$_2$dpzp]$^{2+}$ and DNA bases in the saturated solution is estimated to be $\sim$1:2.

Figure 5 shows results of a titration of 10 $\mu$M [Ru(bpy)$_2$dpzp]$^{2+}$ with DNA. No ECL was observed in the 5 mM oxalate solution containing 10 $\mu$M [Ru(bpy)$_2$dpzp]$^{2+}$ in the absence of DNA. The ECL intensity increases with DNA concentration linearly up to 20 $\mu$M, and then it reaches a plateau at higher DNA concentrations, because all [Ru(bpy)$_2$dpzp]$^{2+}$ molecules have become bound to DNA. Because only the bound metal complex contributes to ECL, independent determination of free and DNA-bound complex can be achieved. The ECL titration data in Figure 5 allow the use of the classical Scatchard equation to estimate the equilibrium constant and binding-site size, under the assumption that all DNA sites are equivalent and independent. The calculation based on the Scatchard model yields $K = 1.35 \times 10^6$ M$^{-1}$ and $s = 0.88$ bp. The value of equilibrium constant for the ECL titration data agrees with that determined by Barton.25 The binding-site size is consistent with the saturated ratio of [Ru(bpy)$_2$dpzp]$^{2+}$ to DNA 1:2 and comparable to the previous report value of $s = 0.70$ bp for [Os(bpy)$_2$dpzp]$^{2+}$.

Aptamer-Based ATP Assay Using [Ru(bpy)$_2$dpzp]$^{2+}$ as an ECL Probe. To further exploit the application of [Ru(bpy)$_2$dpzp]$^{2+}$ as an ECL probe in DNA-related analysis, an aptamer-based target assay was developed using ATP as a model. A 27-nt DNA aptamer against ATP that has two stacked G-quartets and two short double-helix stems was used in this work.24 As shown in Figure 6, [Ru(bpy)$_2$dpzp]$^{2+}$ alone shows very low ECL in oxalate solution. Its ECL intensity increases greatly ($\sim$100 times) after the addition of the ATP aptamer. The molar ratio of [Ru(bpy)$_2$dpzp]$^{2+}$ to aptamer was 20:1 to ensure the saturation of [Ru(bpy)$_2$dpzp]$^{2+}$ intercalation. The increase in ECL intensity indicates that [Ru(bpy)$_2$dpzp]$^{2+}$ intercalated into the folded aptamer. The ECL intensity of the [Ru(bpy)$_2$dpzp]$^{2+}$/aptamer decreased markedly ($\sim$50% decrease) when 10 $\mu$M ATP was added. To confirm the ATP-dependent ECL signal decrease, a 15-nt DNA aptamer against thrombin was used for a control experiment.43 The thrombin aptamer has two stacked G-quartets similar to the ATP aptamer, but it has no double-helix stem; thus, the ECL of the thrombin aptamer with [Ru(bpy)$_2$dpzp]$^{2+}$ is much lower than that of the ATP aptamer.26 As ATP could not bind with the thrombin aptamer, the addition of 10 $\mu$M ATP to the [Ru(bpy)$_2$dpzp]$^{2+}$/thrombin DNA aptamer solution essentially has no effect on ECL intensity (see Figure 6). This experiment supported the notion that the binding of ATP is the reason for the decrease in ECL of the [Ru(bpy)$_2$dpzp]$^{2+}$/aptamer.

The selectivity of the signaling aptamer for ATP has also been tested by comparing the ECL changes of the [Ru(bpy)$_2$dpzp]$^{2+}$/ aptamer solution upon the addition of ATP and three ATP analogues, UTP, CTP, and GTP. When 5 $\mu$M ATP, UTP, CTP, and GTP were added to the [Ru(bpy)$_2$dpzp]$^{2+}$/aptamer solution, only ATP causes a marked decrease in ECL intensity. In contrast, UTP, CTP, and GTP have little effect on ECL intensity (see Figure 7). This proves that the ECL aptasensor was specific for ATP determination, because of the inherent specificity of the aptamer toward ATP.

Titration experiments were performed by adding increasing amounts of ATP to [Ru(bpy)$_2$dpzp]$^{2+}$/aptamer to examine whether the ECL change could be used for ATP detection. Figure 8 shows that the ECL intensity decreases as the ATP concentrations first increase and then it levels off at high ATP concentrations. There is a good linear relationship between the ECL decrease and ATP concentrations from 0 $\mu$M to 1 $\mu$M with the correlation coefficient of 0.995. The detection limit for ATP is 0.1 $\mu$M (with a signal-to-noise ratio (S/N) of $\geq$3). The sensitivity is comparable to the common ATP assays.44–46 Yao et al. recently have reported a more-sensitive ECL aptasensor for ATP determination.25 In their method, a double-stranded DNA formed from

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the hybridized of ATP aptamer and its cDNA was immobilized on the electrode surface, with a ruthenium complex labeled at the 3’ terminus of cDNA as an ECL probe for ATP sensing. Perhaps Yao et al.’s immobilized system increases the ECL S/N value to give greater sensitivity. Compare with their system, our new solution-based ECL detection strategy is simple and needs neither the labeling nor the immobilization of aptamers and the capture probes. In comparison with the commonly used DNA-binding fluorescing molecular switch ethidium bromide, [Ru(bpy)$_2$dpdz]$^{2+}$ exhibits a photoluminescence enhancement of $\sim 10^4$ upon DNA binding and ethidium bromide exhibits a photoluminescence enhancement of only $\sim 20$ upon DNA binding. The dramatic ECL enhancement of [Ru(bpy)$_2$dpdz]$^{2+}$ upon DNA binding and the nice sensitivity of the [Ru(bpy)$_2$dpdz]$^{2+}$ ECL approach demonstrate that [Ru(bpy)$_2$dpdz]$^{2+}$ is a versatile and powerful molecular switch.

CONCLUSIONS

[Ru(bpy)$_2$dpdz]$^{2+}$ displays a remarkable increase in ECL upon intercalation into the nucleic acid structure. The origin of the ECL switch effect is attributed to the removal of the water molecules surrounding the [Ru(bpy)$_2$dpdz]$^{2+}$ complex by intercalation of its dpdz ligand between DNA base pairs. The interaction of [Ru(bpy)$_2$dpdz]$^{2+}$ with herring sperm DNA was investigated using the ECL technique. The dramatic increase in ECL intensity upon intercalation makes the investigation of interaction more sensitive and allows the use of much lower DNA concentrations. Moreover, a label-free, sensitive, and selective ECL platform for ATP detection was developed. The strategy takes advantage of the sensitive ECL intensity change when ATP binds to the folded aptamer where Ru(bpy)$_2$dpdz$^{2+}$ intercalates. Our studies demonstrate that Ru(bpy)$_2$dpdz$^{2+}$ ECL holds great promise for aptamer-based target detection.

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