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Low-Background, Highly Sensitive DNA Biosensor by Using an Electrically Neutral Cobalt(II) Complex as the Redox Hybridization Indicator

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Abstract: An electrically neutral cobalt complex, $[Co(GA)_2(phen)]$ (GA = glycollic acid, phen = 1,10-phenathroline), was synthesized and its interactions with double-stranded DNA (dsDNA) were studied by using electrochemical methods on a glassy carbon electrode (GCE). We found that $[Co(GA)_2(phen)]$ could intercalate into the DNA duplex through the planar phen ligand with a high binding constant of $6.2(\pm0.2)\times10^5\,M^{-1}$. Surface studies showed that the cobalt complex could electrochemically accumulate within the modified dsDNA layer, rather than within the single-stranded DNA (ssDNA) layer. Based on this feature, the complex was applied as a redox-active hybridization indicator to detect 18-base oligonucleotides from the CaMV35S promoter gene. This biosensor presented a very low background signal during hybridization detection and could realize the detection over a wide kinetic range from 1.0×10^{-14} M to 1.0×10^{-8} M, with a low detection limit of 2.0 fM towards the target sequences. The hybridization selectivity experiments further revealed that the complementary sequence, the one-base-mismatched sequence, and the non-complementary sequence could be well-distinguished by the cobalt-complex-based biosensor.

Keywords: biosensors · cobalt · DNA · electrochemistry · intercalation

Introduction

The development of sensitive and accurate testing technology for DNA is of great importance in the fields of biomedical research, clinical diagnosis, forensic and environmental analyses, drug screening, and gene mapping.[1] Electrochemical methods that are mainly based on electrochemical DNA biosensors have been widely used for DNA analysis over the past decade, owing to their high sensitivity, small dimensions, 1ow cost, and good compatibility with microfabrication technology.[2]

In a typical electrochemical DNA biosensor scheme, the target DNA sequence is detected by monitoring the hybridization reaction with the single-stranded DNA (ssDNA) probe anchored onto a solid-support platform. To sensitively transfer the hybridization event into the readable electrochemical signals, various strategies, such as biocoding,[3] molecular labeling with a redox-active reporter,[4] and enzyme amplification,[5] have been developed. However, these methods are often restricted in terms of their practical application, owing to their need for complicated treatment processes. Alternatively, procedures that involve electroactive substances with different affinities towards ssDNA and double-stranded DNA (dsDNA) are more convenient and practical in DNA-hybridization detection, owing to their high electroactivity and facile treatment process. Currently, the most-widely used electroactive substances include metal complexes,[6] anticancer agents,[7] and organic dyes.[8] Of these substances, transition-metal complexes attract much attention because of their excellent redox activity, controllable coordination geometry, and good chemical stability. The exploited indicators include polypyridyl–transitional-metal complexes,[9] threading complexes,[10] and polymeric complexes.[11] However, apart from their interactions with dsDNA, through specific groove-binding and/or intercalation, these metal complexes also exhibit binding to ssDNA through non-specific electrostatic forces, owing to the common presence of positive charges. As a result, inferior selectivity and high background signal are frequently observed during the hybridization detection.

To avoid this disadvantage, herein, we designed and applied a readily obtained electrically neutral cobalt(II) complex with the mixed ligands of glycollic acid (GA) and 1,10-phenathroline (phen). First, the cobalt ion was chosen as the central ion because it was readily available and less harmful than the osmium and ruthenium ions that have often been used for DNA analysis. In addition, the electron-transfer process of the cobalt(II)/cobalt(III) couple can be easily achieved[12] and its moderate redox potential (close to 0 V)

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can effectively avoid side reactions and oxidative DNA damage during testing. The polypyridyl moiety of the phen ligand is a versatile chelating reagent that has strong coordinative ability to many metal ions and the polycyclic planar structure of phen facilitates the interactions of its metal complex with DNA through specific intercalative modes. Therefore, many phen-based metal complexes have been exploited as electrochemical and optical probes in DNA analysis.[6f,9d,11b,13] However, the typical phen-chelated metal complexes are positively charged, because the phen ligand is neutral. Therefore, in this work, to neutralize the positive charge from the central metal ion and then eliminate the nonspecific electrostatic interaction with ssDNA, two anionic ligands of GA were introduced into the complex as charge-controlling ligands. Thus, the complex is charge-neutral as a whole, thus avoiding electrostatic interactions with ssDNA, but retaining specific intercalation with dsDNA. The electrochemical characterization experiments, as well as the differential-binding studies of the complex with dsDNA and ssDNA reported herein, confirmed this speculation. When the complex was utilized as an electrochemical hybridization indicator in a DNA biosensor, we found that the biosensor showed extremely low background response. A wide kinetic range from 1.0×10^{-14} M to 1.0×10^{-8} M and a low detection limit of 2.0 fM were obtained for the target-sequence detection, thus showing that the rational design of the hybridization indicator is conducive for exploiting high-performance DNA biosensors.

Results and Discussion

The $[Co(GA)_2(phen)]$ complex was characterized by X-ray crystal-structure analysis. Detailed crystallographic information and selected bond lengths and angles are listed in the Supporting Information, Tables S1 and S2, respectively. Figure 1 shows the crystal structure of the complex. The complex has a mononuclear structure, in which one cobalt(II) atom is coordinated by two nitrogen atoms from one phen ligand and four oxygen atoms from two GA, thus displaying a distorted trigonal geometry. As a whole, the molecular unit is electrically neutral, owing to the compensation of the positive charges on the cobalt(II) ion by the negative charges from two GA ligands. In addition, the good planarity of the phen ligand in the complex makes it easy for $[Co(GA)_2(phen)]$ to intercalate between adjacent base pairs of the DNA duplex.

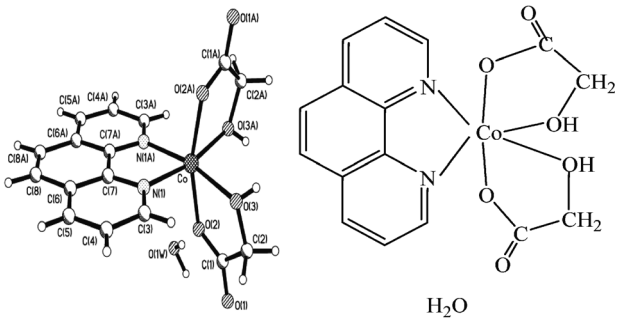


Figure 1. ORTEP (left) and chemical structure (right) of $[Co(GA)_2(phen)]\cdot H_2O$.

Typical cyclic voltammograms (CVs) of a 4.0×10^{-4} M solution of $[Co(GA)_2(phen)]$ in 20 mM pH 5.0 B-R buffer in the absence and presence of dsDNA are shown in Figure 2. The cobalt complex has a pair of redox peaks at +0.078 V and −0.122 V (Figure 2a), which could be ascribed to the Co^{II}/Co^{III} redox couple.[14] This moderate redox potential range is very suitable for DNA biosensing without affording the oxidative damage of DNA. After interaction with a 1.7×10^{-4} M solution of dsDNA in 20 mM pH 5.0 B-R buffer, we observed that the redox peak currents of the complex obviously decreased, accompanied by a positive shift of the formal potential (Figure 2b), in good agreement with the characteristics of an intercalative mode.[15] To determine the intercalative mode between the two species, a melting temperature (T_m) experiment was performed and the results showed that, after interaction with $[Co(GA)_2(phen)]$, the T_m of the dsDNA increased dramatically from 76.4 °C to 81.6 °C (see

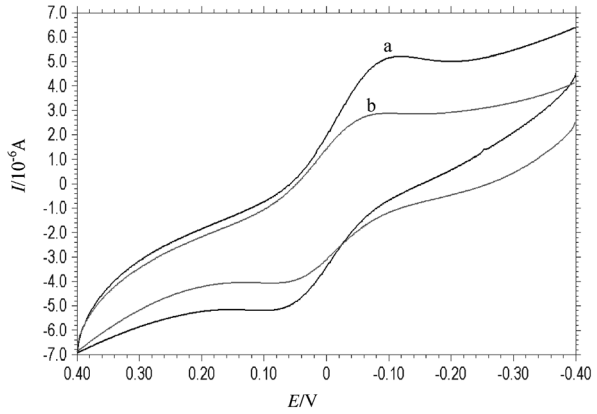


Figure 2. Cyclic voltammograms of a 4.0×10^{-4} M solution of $[Co(GA)_2(phen)]$ before (a) and after interaction with 1.7×10^{-4} M DNA (b) in 20 mM pH 5.0 B-R buffer.

Abstract in Chinese:
以羟基乙酸(GA)和1,10-邻菲咯啉(phen)作为配体，合成了一种新型的电中性钴(II)配合物$[Co(GA)_2(phen)]$。采用电化学方法研究了其与DNA的相互作用，结果表明二者之间以嵌插模式相互作用，结合常数为$6.2(\pm0.2)\times10^5\ M^{-1}$。将该配合物作为杂交指示剂应用于DNA传感器中对转基因植物 CaMV35S 启动子基因特征片段进行检测，结果表明，该传感器在杂交检测中呈现很低的背景信号，而且在1.0×10^{-14} M ~ 1.0×10^{-8} M 浓度范围内能实现对目标序列的定量检测，检测限为2.0 fM。选择性实验进一步表明该传感器对互补序列，单碱基错配序列和非互补序列具有很好的识别能力。

the Supporting Information, Figure S1), thus further suggesting that $[Co(GA)_2(phen)]$ had bound to dsDNA through intercalation.[16] On the other hand, it has been reported that, for a Nernstian electron-transfer process, if both the oxidized and reduced forms of a complex interacts with dsDNA, the shift of the formal potential ($\Delta E^{0\prime}$) of the complex can be used to estimate the ratio of equilibrium binding constants (K_{Co}^{II}/K_{Co}^{III}), according to Equation (1),[15] where $E_b^{0\prime}$ and $E_f^{0\prime}$ are the formal potentials of the dsDNA bound and unbound forms of the complex, respectively, and K_{Co}^{II} and K_{Co}^{III} are the corresponding binding constants of the reduction and oxidation species to dsDNA, respectively.

$$\Delta E^{0\prime} = E_b^{0\prime} - E_f^{0\prime} = 0.059 \log (K_{Co}^{II}/K_{Co}^{III}) \quad (1)$$

From the value of $\Delta E^{0\prime}$ (from −0.022 to −0.010 V) as determined herein, the K_{Co}^{II}/K_{Co}^{III} value was calculated to be 1.6, thus suggesting that the reduced state of the cobalt complex has a stronger affinity with dsDNA than the oxidized state, which also agrees with the characteristic of the intercalative binding mode.[17]

The technique of normal pulse voltammetry (NPV) was further applied to determine the binding constant (K) between $[Co(GA)_2(phen)]$ and dsDNA, because the limiting current in NPV can precisely reflect the mass transfer between free-metal complexes and their dsDNA-bound form.[6e,18] The NPV results of $[Co(GA)_2(phen)]$ on interaction with an increasing amount of dsDNA are shown in Figure 3A. It can be clearly seen that, on increasing the concentration of dsDNA in the $[Co(GA)_2(phen)]$ solution, the limiting current of the complex decreases gradually and finally reaches steady-state minimum values. Then, the binding constant of the cobalt complex with dsDNA can be calculated according to Equations (2)–(4), as deduced by Welch and Thorp,[18b] where X_b is the molar binding fraction, I_w the observed current with the addition of dsDNA, $I_{w,0}$ is the initial current before the addition of dsDNA, $I_{w,sat}$ is the expected current upon saturated binding, C_t is the total concentration of $[Co(GA)_2(phen)]$, [DNA] is the concentration of added dsDNA, and s is the site size of base pairs with a bound $[Co(GA)_2(phen)]$ molecule.

$$X_b = \{b-(b^2-2K^2C_t[DNA]/s)^{1/2}\}/2KC_t \quad (2)$$

$$X_b = (I_w^2 - I_{w,0}^2)/(I_{w,sat}^2 - I_{w,0}^2) \quad (3)$$

$$b = 1+KC_t+K[DNA]/2s \quad (4)$$

The points in Figure 3B represents the calculated X_b values from the limiting current of $[Co(GA)_2(phen)]$ upon titrating with different concentrations of dsDNA. Through the nonlinear fitting analysis according to Eqs. (1)-(3) with *Origin* 7.0 software, the value of K was yielded to be $6.2(\pm0.2)\times10^5\,M^{-1}$, which is well in agreement with the dsDNA binding intensity of the other metal intercalators such as $[Co(phen)_2IP]^{2+}$ (IP = imidazo[f]1,10-phenanthroline, $K = 3.7\times10^5$),[19a] $[Ru(NH_3)_4dppz]^{2+}$ (dppz = dipyridophenazine, $K = 1.2\times10^5$),[19b] $[Co(phen)_2(HPIP)]^{3+}$ (HPIP = 2-(2-hydroxyphenyl)imidazo[4,5-f][1,10]phenanthroline, $K = 4.1\times10^5$).[19c] Additionally, the value of s was obtained to be 1.7-(±0.2), which indicated that each $[Co(GA)_2(phen)]$ molecule covered about two base pairs of dsDNA in average.

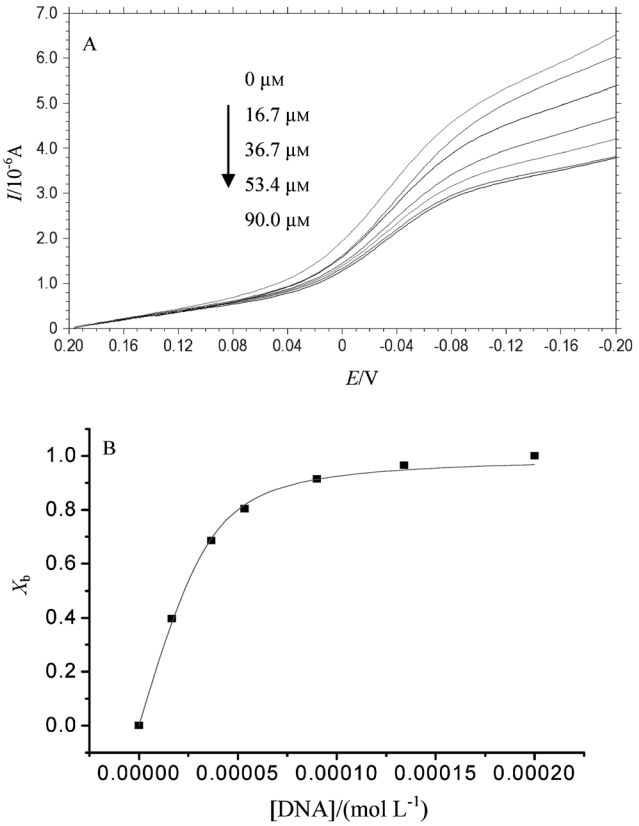


Figure 3. A) NPVs of 1.0×10^{-5} M $[Co(GA)_2(phen)]$ in 20 mM pH 5.0 B-R buffer during titration with increasing amounts of dsDNA. B) Plot of the bound molar fraction (X_b) of 1.0×10^{-5} M $[Co(GA)_2(phen)]$ upon titration with dsDNA in 20 mM pH 5.0 B-R buffer and the associated nonlinear least-squares fits to Equations (1)–(3).

As well known, the intercalative-binding is a specific mode that only happens for dsDNA rather than ssDNA, and meanwhile, the non-specific electrostatic interaction of the studied complex with ssDNA should not exist because the complex was electrically neutral. In order to testify this speculation, the selective interaction of $[Co(GA)_2(phen)]$ with dsDNA and ssDNA was investigated through the surface-based method. In this way, the ssDNA and dsDNA can be confined on the electrode surface to maintain their natural conformation states. Especially for ssDNA, the possibility of self-intertwining or local self-hybridization that might happen in the homogeneous solution can be effectively prevented.

Figure 4 shows the multi-sweep cyclic voltammograms of ssDNA/GCE (A) and dsDNA/GCE (B) in 4.0×10^{-4} M $[Co(GA)_2(phen)]$. It is observed that on ssDNA/GCE, the redox signals are small and the voltammetric curves are hardly changed with the increase of scan cycles. In contrast, when dsDNA/GCE were tested under the same conditions,

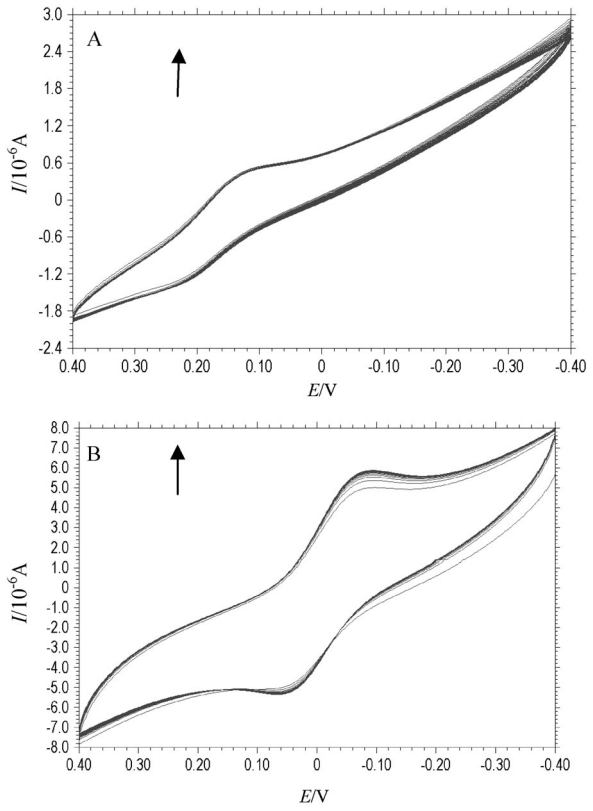


Figure 4. Multi-sweep cyclic voltammograms of 4.0×10^{-4} M $[Co(GA)_2(phen)]$ on ssDNA/GCE (A) and dsDNA/GCE (B). Scan rate: 0.1 Vs^{-1}. Arrows in (A) and (B) indicate the increasing trend in the peak current with an increasing number of scan cycles.

it is found that the redox peak of $[Co(GA)_2(phen)]$ has the lower redox potential and the peak currents enhance gradually with the increase of the scan cycles, showing that more and more $[Co(GA)_2(phen)]$ molecules have been accumulated within dsDNA/GCE surface. This also indicates that the complex of $[Co(GA)_2(phen)]$ has different interaction with ssDNA and dsDNA, and meanwhile presents different electrochemical response on ssDNA and dsDNA modified interface. The essential reason might lie in the different secondary structure and electric conductivity of dsDNA and ssDNA. On ssDNA/GCE, the weak electrochemical response might be caused from the hydrophobic or π stacking interaction of $[Co(GA)_2(phen)]$ with ssDNA, and this interaction can be finished in a short time since all the bases on ssDNA are exposed to the solution. However, on dsDNA/GCE, the cobalt complex are adsorbed to the electrode surface through an intercalative behavior with dsDNA, and this interaction mode need more time to finish because the base pairs are deeply buried inside dsDNA.[12a,20] On the other hand, because ssDNA has inferior electric conductivity as compared with dsDNA owing to the disruption of the base-pairing configuration,[21] the $[Co(GA)_2(phen)]$ accumulated on ssDNA/GCE is hard to communicate electrons with the electrode interface, so in comparison with the case of dsDNA/GCE, the $[Co(GA)_2(phen)]$ presents the higher redox potential to overcome the resistance from the insulating layer of ssDNA.

It has also been reported that the strength of the intercalation of small molecules into dsDNA is typically stronger than electrostatic, hydrophobic, and π-stacking interactions.[22] In our experiments, the different interactions of $[Co(GA)_2(phen)]$ with ssDNA and dsDNA were further confirmed by desorption experiments, in which $[Co(GA)_2(phen)]$ was first equilibrated with ssDNA/GCE and dsDNA/GCE under saturated conditions, followed by transferring the electrodes into a blank supporting electrolyte solution for the electrochemical measurements after rinsing with water. Figure 5A,B shows the corresponding time-dependent differential pulse voltammograms (DPVs) of the adsorbed $[Co(GA)_2(phen)]$ onto dsDNA/GCE and ssDNA/GCE, respectively. It can be clearly seen that, at $t=0$, the bound complex shows a strong reduction peak on dsDNA/GCE ($I_{pc}=8.9\times10^{-7}$ A; Figure 5A, top curve). However, on ssDNA/GCE, the reduction peak current is very small ($I_{pc}=9.0\times10^{-8}$ A), which also indicates that a much smaller amount of $[Co(GA)_2(phen)]$ has accumulated on ssDNA/GCE as compared with dsDNA/GCE. In addition, on increasing the dissociation time, the reduction peaks become less and less intense on both electrodes, owing to the gradu-

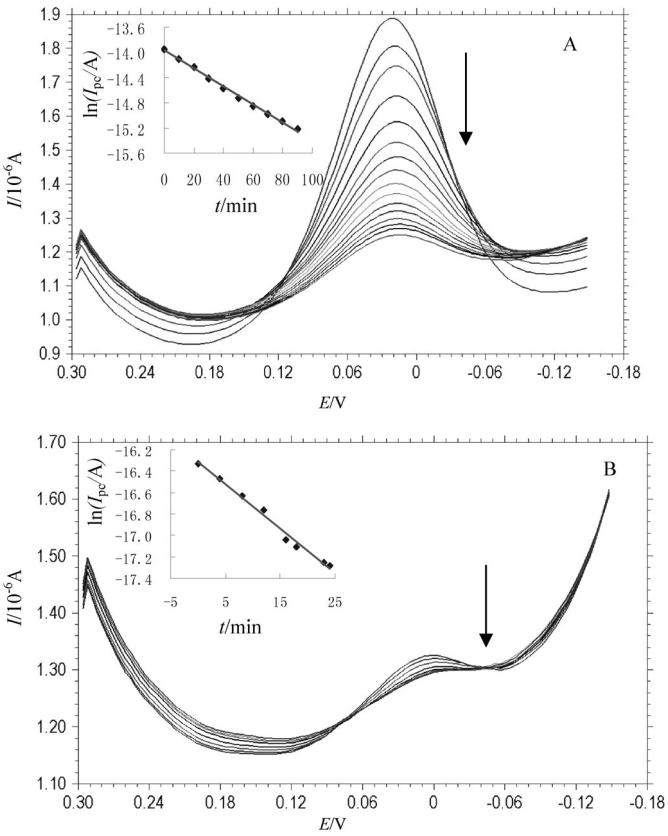


Figure 5. Time-dependent DPVs in 20 mM pH 5.0 B-R solution at dsDNA/GCE (A) and ssDNA/GCE (B). Inset: Time dependence of the logarithm of the peak current. Arrows show the trends in the dissociation process with increasing dissociation time (t).

al dissociation of $[Co(GA)_2(phen)]$ from dsDNA/GCE or ssDNA/GCE in the blank solution. The logarithm values of the reduction peak currents, $\ln(I_{pc}/A)$, on both dsDNA/GCE and ssDNA/GCE linearly depend on the dissociation time (t), as showed in Figure 5, inset, thus illustrating that the dissociation process of the complex from the two electrodes obeys first-order kinetics, according to Equation (5).[23]

$$[Co(GA)_2(phen)]-DNA/GCE \xrightarrow{k} DNA/GCE+[Co(GA)_2(phen)] \quad (5)$$

Thus, the first-order kinetics follow Equation (6).

$$\ln(I_{pc}/A) = -kt+\text{“constant”} \quad (6)$$

The dissociation rate constants (k) of $[Co(GA)_2(phen)]$ from dsDNA/GCE and ssDNA/GCE were determined to be $1.4\times10^{-2}\,min^{-1}$ and $4.1\times10^{-3}\,min^{-1}$, respectively. By comparing the values of k on the two electrodes, it can be clearly seen that $[Co(GA)_2(phen)]$ dissociated more slowly (3.4-fold) from dsDNA/GCE than from ssDNA/GCE. This difference in kinetics was very useful for using the complex as an indicating probe in DNA-biosensing analysis.[12a,20]

Figure 6 shows DPVs that were obtained in 20 mM B-R buffer (pH 5.0) on GCEs that were modified with oligonucleotide probe DNA (S1, that is, S1/GCE) before (Figure 6a) and after hybridization with complementary sequence S2 (Figure 6b), one-base-mismatched sequence S3 (Figure 6c), and non-complementary sequence S4 (Figure 6d). In Figure 6, there is a very small DPV-peak response of $[Co(GA)_2(phen)]$ on S1/GCE (Figure 6a), with $I_{pc}=3.0\times10^{-8}$ A, which is clearly lower than the reported values of many positively charged indicators, such as $[Os(DA\text{-}bpy)_2dppz]^{2+}$ (DA-bpy = 4,4′-diamino-2,2′-bipyridine, 1.3×10^{-7} A),[6e] brilliant cresyl blue (about 7.0×10^{-7} A),[24] and PVP–$[Os(5,6\text{-}dmphen)_2Cl]^{2+}$ (PVP = poly(4-vinylpyridine), 5,6-dmphen = 5,6-dimethyl-1,10-phenanthroline, about 1.9×10^{-7} A),[11b] thus suggesting that the $[Co(GA)_2(phen)]$-based DNA biosensor has a lower background signal, which can be attributed to very weak affinity between the electrically neutral $[Co(GA)_2(phen)]$ indicator and ssDNA. Furthermore, a similar DPV response is observed on an electrode that is hybridized with a non-complementary sequence (S4-S1/GCE; Figure 6d), thus suggesting that the biosensing surface remains in the same state after hybridization with a non-complementary sequence. However, a significantly increased DPV signal is seen on S2-S1/GCE, thus indicating that much more $[Co(GA)_2(phen)]$ molecules are concentrated on the S2-S1-duplex-modified electrode because of the specific intercalation of $[Co(GA)_2(phen)]$ within dsDNA. However, on an electrode that is hybridized with a one-base-mismatched sequence (S3-S1/GCE), a significantly decreased DPV signal is observed (Figure 6c). This result can be ascribed to two reasons: 1) When base-mismatched sequences are hybridized, an incomplete duplex structure is formed, thus leading to the a decrease in the intercalative site, as well as the binding amount of $[Co(GA)_2(phen)]$; 2) the base mismatch also blocks the effective long-range electron transfer of intercalated $[Co(GA)_2(phen)]$ in the DNA duplex.[25] In summary, these results show that the biosensor has good hybridization selectivity to recognize the complementary, non-complementary, and base-mismatched sequences, depending on the different interactions of $[Co(GA)_2(phen)]$ with dsDNA and defective DNA.

By using $[Co(GA)_2(phen)]$ as an electroactive hybridization indicator, the sensitivity of the DNA biosensor was further investigated by varying the concentration of the target sequence. As shown in Figure 7, the peak currents that were obtained from DPV measurements increased with increasing concentration of the target sequences, thus showing that more and more perfect DNA duplexes were formed through hybridization reactions. The peak currents of $[Co(GA)_2(phen)]$ show excellent correlation with the logarithm of C_{S2} ($Lg\,C_{S2}$), from 1.0×10^{-14} M to 1.0×10^{-8} M (Figure 7, inset), according to regression Equation (7).

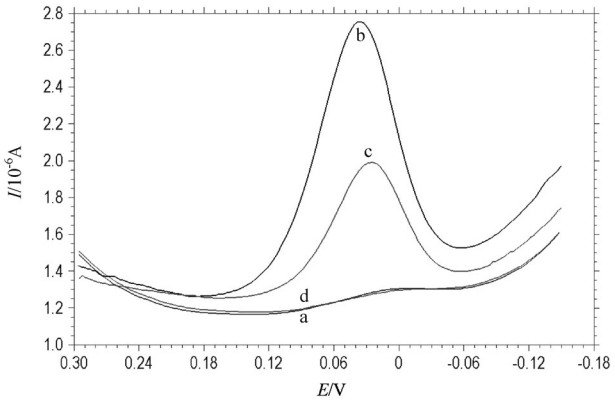


Figure 6. DPVs of 5.0×10^{-3} M $[Co(GA)_2(phen)]$ in 20 mM pH 5.0 B-R buffer at the S1/GCE before (a) and after hybridization with complementary sequence S2 (b), one-base-mismatched sequence S3 (c), and non-complementary sequence S4 (d). All of the concentrations of the target sequences were 1.0×10^{-8} M.

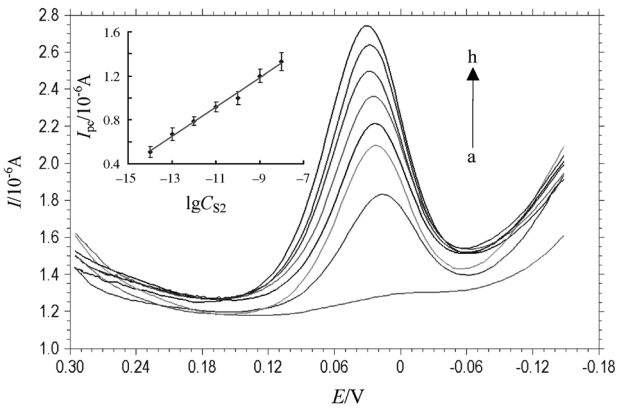


Figure 7. DPVs of 5×10^{-3} M $[Co(GA)_2(phen)]$ in 20 mM pH 5.0 B-R buffer at the S1/GCE before (a) and after hybridization with 1.0×10^{-14} M, (b), 1.0×10^{-13} M (c), 1.0×10^{-12} M (d), 1.0×10^{-11} M (e), 1.0×10^{-10} M (f), 1.0×10^{-9} M (g), and 1.0×10^{-8} M of sequence S2 (h).

$$I_{pc}(\mu A) = 0.1332\,Lg C_{S2}(M) + 2.3825,\quad R^2 = 0.9931 \qquad (7)$$

The detection limit was estimated to be 2.0 fM, based on the signal-to-noise ratio (S/N = 3:1), which was lower than that of many reported positively charged indicators, such as $[Co(phen)]^{3+}$ (4.0 nM),[6a] chitosan–Cd^{2+} (4.2 nM),[11c] and $[Co(phen)_2IP]^{2+}$ (27 pM, IP = imidazo[f]1,10-phenanthroline),[19a] thus suggesting that our developed biosensors were more sensitive.

The reproducibility of any biosensor is extremely important for practical applications. Thus, the reproducibility of our biosensor was tested by repeated hybridization with a target sequence and interaction with $[Co(GA)_2(phen)]$. A relative standard deviation (RSD) of 3.8% ($n = 5$) was estimated from five parallel measurements, thus showing the high reproducibility of this DNA biosensor.

The regeneration of the biosensor was performed by thermal denaturation in 90 °C TE buffer for 10 min and then cooling with ice water. These results show that the biosensor can be used in repeated hybridization/denaturation/hybridization cycles 10 times with a decrease of only 4.2% in current response.

Conclusions

DNA-recognition molecules have found widespread applications in various biological fields. Herein, a new cobalt complex was synthesized as an electroactive indicator and its interactions with dsDNA were carefully investigated by using electrochemical methods. The binding constant $K = 6.2(\pm 0.2) \times 10^5\ M^{-1}$ was obtained from NPV-based titration experiments. Moreover, by using $[Co(GA)_2(phen)]$ as a new electroactive indicator, the constructed electrochemical DNA biosensor can selectively detect the target ssDNA fragment with a very low background signal. The target ssDNA can be quantified over a linear range from 1.0×10^{-14} M to 1.0×10^{-8} M, with a detection limit of 2.0 fM. This electrochemical DNA biosensor, based on the cobalt complex, holds great promise in transducing DNA hybridization.

Experimental Section

Reagents and Instruments

Native salmon fish-sperm DNA (dsDNA) was purchased from Beijing Biodee Biotechnology Company (China) and used as received. The concentration of dsDNA was determined spectrophotometrically at 260 nm by using the well-known molar extinction coefficient of 6600 $L\,mol^{-1}\,cm^{-1}$.[26] ssDNA was prepared by a typical thermally denaturation process, as reported in the literature.[12a] In brief, a solution of dsDNA (0.5 $g\,L^{-1}$) in doubly distilled water was heated in a bath of boiling water for 8 min, followed by rapid cooling in an ice bath. Other reagents, such as tris(hydroxymethyl)aminomethane (Tris) and ethylenediamine tetraacetate (EDTA), were of analytical grade and purchased commercially. Britton–Robinson (B-R) buffer solution was prepared by mixing 40 mM H_3PO_4, 40 mM H_3BO_3, and 40 mM acetic acid and 0.2 M NaOH was used to adjust the pH value. Doubly distilled water (DDW) was used to prepare all of the solutions.

The 18-base synthetic oligonucleotides that were related to the CaMV35S promoter sequence were purchased from Shanghai Sangon Bioengineering Ltd. Company (Shanghai, China). Their base sequences were as follows:

Amino-modified probe sequence (S1): 5′-NH_2-$(CH_2)_6$-TCT TTG GGA CCA CTG TCG-3′

Complementary sequence (S2): 5′-CGA CAG TGG TCC CAA AGA-3′

One-base-mismatch sequence (S3): 5′-CGA CAG TGG TCC CAA CGA-3′

Non-complementary sequence (S4): 5′-GCA TCG AGC GAG CAC GTA-3′

Stock solutions (10 μM) of all of the oligonucleotides were prepared in TE buffer solution (10 μM Tris-HCl, 1.0 mM EDTA, pH 8.0) and kept frozen.

Elemental analysis of the cobalt complex was performed on a Perkin–Elmer 1400C analyzer (USA). IR spectra were recorded on a Nicolet 170SX spectrometer (USA) by using pressed KBr pellets within the range 4000–400 cm^{-1}. Single-crystal X-ray diffraction measurements were recorded on an Enraf–Nonius CAD-4 diffractometer with graphite-monochromatic $Mo_{K\alpha}$ radiation ($\lambda = 0.071073$ nm) at 293(2) K. Electrochemical experiments were measured on a CHI 650C electrochemical workstation (China), which consisted of a traditional three-electrode system: A bare or DNA-modified glassy carbon working electrode (GCE, Φ = 3 mm), a Ag/AgCl reference electrode, and a Pt-wire counter electrode.

Melting Temperature (T_m) Experiments

DsDNA-melting experiments were performed by monitoring the absorbance of a 6.0×10^{-5} M solution of dsDNA in B-R buffer at 260 nm at various temperatures in the absence and presence of 4.0×10^{-5} M $[Co(GA)_2(phen)]$ with a temperature interval of 5 °C.

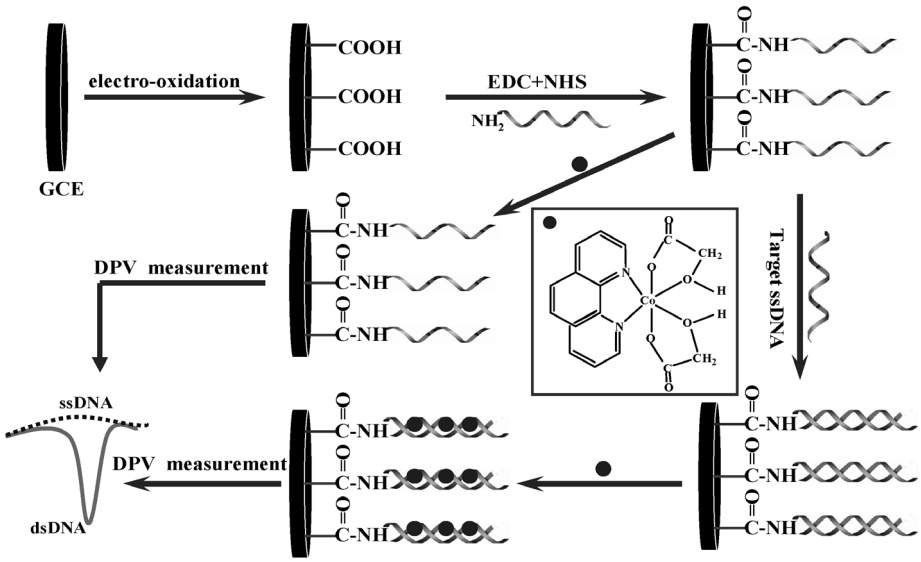
Electrochemical Studies on the Interactions Between $[Co(GA)_2(phen)]$ and dsDNA

Different amounts of fish-sperm dsDNA were added to a B-R buffer solution (pH 5.0) that contained an appropriate amount of $[Co(GA)_2(phen)]$. The interaction characterization of $[Co(GA)_2(phen)]$ with dsDNA was performed by cyclic voltammetry (CV) and normal pulse voltammetry (NPV). The CV was scanned within the potential range −0.40 to +0.40 V at a scan rate of 0.1 $V\,s^{-1}$. The NPV measurements were performed with a potential increment of 0.004 V, a pulse width of 0.05 s, a pulse period of 0.2 s, and a potential range of +0.20 to −0.20 V.

Surface studies of ssDNA and dsDNA with $[Co(GA)_2(phen)]$ were performed according to the following procedures: First, ssDNA- or dsDNA-modified GCEs were prepared by using a physical-adsorption method, as reported in the literature.[20] Then, the ssDNA/GCE or dsDNA/GCE were immersed in a 20 mM B-R buffer solution that contained 5 mM $[Co(GA)_2(phen)]$ and electrochemically detected until steady-state voltammetric curves were obtained. The dissociation experiments were performed by placing the dsDNA/GCE or ssDNA/GCE with fully absorbed $[Co(GA)_2(phen)]$ into a cell that contained a blank solution (also 20 mM pH 5.0 B-R buffer) and monitored by DPV at regular time intervals until the electrochemical signals became steady. The DPV measurements were performed with a potential increment of 0.004 V, a pulse amplitude of 0.05 V, a pulse width of 0.05 s, a pulse period of 0.2 s, and a potential range of +0.30 to −0.15 V.

Covalent Immobilization of Probe DNA on a GCE and its Hybridization Reaction

To obtain the higher hybridization efficiency and stability of the biosensor, a covalent method was employed to immobilize the probe DNA (S1) onto a GCE.[27] Briefly, a cleaned GCE was polarized at +0.50 V for 3 min in 50 mM PBS buffer (pH 7.1) to afford functional carboxy groups on the electrode surface. After rinsing with DDW, 50 mM PBS buffer solution that contained 5 mM NHS and 8 mM EDC (20 μL) was cast onto the electrode surface to activate the functional oxygen-containing groups. Afterwards, a 1 μM solution of sequence S1 (10 μL) was dropped onto the



Scheme 1. Schematic representation of the application of $[Co(GA)_2(phen)]$ as a hybridization indicator in a DNA biosensor. NHS = *N*-hydroxysuccinimide, EDC = 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide).

electrode surface for the reaction and then rinsed with DDW to eliminate the nonspecifically bound DNA when the surface was dried. Thus, a GCE that was covalently modified with sequence S1 (S1/GCE) was obtained.

The hybridization reactions were performed by immersing the S1/GCE into hybridization solutions that contained different target sequences (S2, S3, or S4) for 1 h at 42 °C with shaking. Then, the hybridized electrode was washed with TE buffer to remove the unhybridized DNA and the obtained electrode was denoted as S2-S1/GCE, S3-S1/GCE, and S4-S1/GCE, respectively.

Indicator Binding and Electrochemical Measurements

The accumulation of $[Co(GA)_2(phen)]$ on the electrode surface was performed by immersing the different electrodes into a stirring 20 mM B-R buffer solution that contained 5 mM $[Co(GA)_2(phen)]$ without applying any potential. Under the optimal conditions, the concentration of $[Co(GA)_2(phen)]$ was set at 5 mM and the accumulation time of $[Co(GA)_2(phen)]$ was chosen as 5 min for the analytical tests. Then, the electrode was rinsed with 20 mM B-R to remove any non-specifically bound $[Co(GA)_2(phen)]$ and subjected to electrochemical measurements. The detailed fabrication and detection procedures for this DNA biosensor are shown in Scheme 1.

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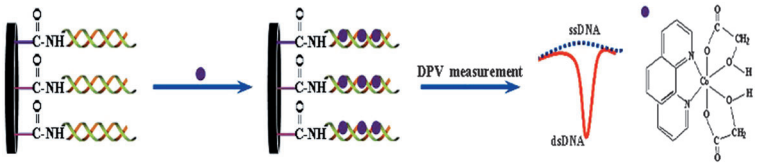

Die another DNA: An electrically neutral cobalt complex, $[Co(GA)_2(phen)]$ (GA = glycollic acid, phen = 1,10-phenathroline), could intercalate into the DNA duplex through the planar phen ligand with a binding constant of 6.2-$(\pm 0.2) \times 10^5\ M^{-1}$.

Biosensors

Qingxiang Wang, Yingtao Ding, Liheng Wang, Jiancong Ni, Zhanglong Yu, Haibin Lin, Feng Gao* ____ ▮▮▮▮–▮▮▮▮

Low-Background, Highly Sensitive DNA Biosensor by Using an Electrically Neutral Cobalt(II) Complex as the Redox Hybridization Indicator