Studies on the Interaction Mechanism of 1,10-Phenanthroline Cobalt(II) Complex with DNA and Preparation of Electrochemical DNA Biosensor

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Abstract: Fluorescence spectroscopy and ultraviolet (UV) spectroscopy techniques coupled with cyclic voltammetry (CV) were used to study the interaction between salmon sperm DNA and 1,10-Phenanthroline cobalt(II) complex, [Co(phen)$_2$(Cl)(H$_2$O)]Cl·H$_2$O, where phen = 1,10-phenanthroline. The interaction between [Co(phen)$_2$(Cl)(H$_2$O)]$^+$ and double-strand DNA (dsDNA) was identified to be intercalative mode. An electrochemical DNA biosensor was developed by covalent immobilization of probe single-strand DNA (ssDNA) related to human immunodeficiency virus (HIV) on the activated glassy carbon electrode (GCE). With [Co(phen)$_2$(Cl)(H$_2$O)]$^+$ being the novel electrochemical hybridization indicator, the selectivity of ssDNA-modified electrode was investigated and selective detection of complementary ssDNA was achieved using differential pulse voltammetry (DPV).

Keywords: 1,10-Phenanthroline, Cobalt(II), Voltammetry, Intercalative, Electrochemical DNA biosensor

1. Introduction

Nowadays, considerable attention has been paid on electrochemical DNA biosensor in the diagnosis of genetic diseases and the detection of pathogenic biological species due to the highly sensitive, rapid yet accurate, simple and inexpensive detection technique it offers [1-5]. Electrochemical DNA biosensor is generally based on an electrode with oligonucleotide immobilization. The sequence-specific hybridization of nucleic acids could be detected directly [6-9] or by DNA intercalator [10-13], and thus different forms of electrochemical DNA biosensors have been developed now. Among them,
the later fashion, using an intercalator as redox label or indicator for the hybridization events, attracts great interest.

Owing to the potential of interacting with the nitrogenous bases of DNA, metal complexes, especially transition metal complexes, have received considerable attention in recent years as remarkable and unique DNA-binding compounds. Researches conducted also showed that transition metal complexes have important biological activities. It was reported that the metal coordination compounds of 1,10-phenanthroline (phen) and its derivatives could inhibit tumor growth by interacting with DNA. In order to obtain more insight into the design of highly sensitive reactive probes, diagnostic reagents and new drugs, detailed understanding of the interaction between such complexes and DNA is potentially useful. Yang [14] reported that [Ni(phen)$_2$dppz]$^{2+}$ and [Co(phen)$_2$dppz]$^{3+}$ could bind strongly to the DNA and make DNA split in the light, which made instructive suggestions in the designing of new anticancer drugs. Although interaction between cobalt(III) complexes and DNA have been reported in many researches [15-17], interaction between 1,10-Phenanthroline cobalt(II) complex and DNA is seldom studied. To our best knowledge, research on the interaction between [Co(phen)$_2$(Cl)(H$_2$O)]Cl·H$_2$O and DNA has not been reported.

In the present paper, fluorescence spectroscopy and ultraviolet (UV) spectroscopy techniques combined with cyclic voltammetry (CV) were used to study the interaction between [Co(phen)$_2$(Cl)(H$_2$O)]Cl·H$_2$O and model DNA, salmon sperm DNA. Results showed that [Co(phen)$_2$(Cl)(H$_2$O)]$^+$ could bind to double-strand DNA (dsDNA) through intercalative binding mode. After probe single-strand DNA (ssDNA) related to human immunodeficiency virus (HIV) was covalently immobilized on the activated glassy carbon electrode (GCE), an electrochemical DNA biosensor using [Co(phen)$_2$(Cl)(H$_2$O)]$^+$ as the electrochemical hybridization indicator was developed and it demonstrated potential in selective detection of the complementary ssDNA. The work might bring further insight on the interaction mechanism between transition metal complexes and DNA and be helpful for further research for designing novel anti-tumor drugs and diagnosis disease.

2. Experimental Section

2.1. Instrumentation

The electrochemical measurement was carried out with Model CHI 832B Voltammetric Analyzer (ChenHua Instruments, China). A three-electrode system was employed with Pt wire as the auxiliary electrode, Ag/AgCl/KCl (sat) as reference electrode, and GCE or modified GCE as working electrode. Spectroscopic experiments were conducted on Hitachi F-4500 fluorospectrometer (Hitachi, Japan) and Cary 50 UV/Vis spectrometer (Varian, Australian).

2.2 Reagents

Salmon sperm DNA (10 mg mL$^{-1}$, A$_{260}$/A$_{280}$>1.8) were purchased from Huashun Biological Engineering Company (Shanghai, China) with the concentration being determined by the ultraviolet absorption at 260 nm ($\varepsilon$ = 6600 L mol$^{-1}$ cm$^{-1}$). Ethidium bromide (EB), tris (hydroxymethyl)amnmomethane (Tris), ethylenediamine tetraacetic acid solution (EDTA), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide solution (EDC) and N-hydroxysuccinimide solution (NHS) were
also obtained from Huashun Biological Engineering Company (Shanghai, China). The [Co(phen)$_2$(Cl)(H$_2$O)]Cl·H$_2$O was synthesized according to Zhao et al [18]. Three 21-base oligonucleotides of HIV gene were purchased from SBS Genetech Company (Beijing, China). The base sequences were as follow: S$_1$: 5'-ACT GCT AGA GAT TTT CCA CAT-3'; S$_2$: 3'-TGA CGA TCT CTA AAA GGT GTA-5'; S$_3$: 3'-AAA AGG TGT AAG CGT TTG CCG-5'. Amongst, S$_1$ is complementary to S$_2$. All oligonucleotide stock solutions of the three 21-base oligomers (100 µg mL$^{-1}$) were prepared with Tris-HCl-EDTA buffer solution (TE solution, 1.00 × 10$^{-2}$ mol L$^{-1}$ Tris-HCl plus 1.00 × 10$^{-3}$ mol L$^{-1}$ EDTA, pH 8.0). Other chemicals employed were of analytical grade and doubly deionized water (DDW) was used throughout.

2.3 Spectroscopic study on the interaction between [Co(phen)$_2$(Cl)(H$_2$O)]$^+$ and DNA

Fluorescence spectroscopy measurements were performed by maintaining a constant concentration of the [Co(phen)$_2$(Cl)(H$_2$O)]$^+$ while varying concentration of salmon sperm DNA. 0.10 mL of 1.00 × 10$^{-2}$ mol L$^{-1}$ [Co(phen)$_2$(Cl)(H$_2$O)]$^+$ solution and different amounts of salmon sperm DNA solution were added to 10 mL colorimetric tubes. The mixture was diluted to the mark using 0.1 mol L$^{-1}$ phosphate buffer solution (PBS) at pH 6.0 and reacted for 12 min at room temperature. Afterward, the measurement of fluorescence was performed using 298 nm as the excited wavelength. To investigate the binding mode between [Co(phen)$_2$(Cl)(H$_2$O)]$^+$ and DNA, the fluorescence spectrums of EB-DNA system in the presence of [Co(phen)$_2$(Cl)(H$_2$O)]$^+$ were recorded by maintaining a constant concentration of EB and [Co(phen)$_2$(Cl)(H$_2$O)]$^+$ while varying concentration of salmon sperm DNA. The measurement of fluorescence was performed using 490 nm as the excited wavelength.

UV spectroscopy was also used to investigate the interaction between [Co(phen)$_2$(Cl)(H$_2$O)]$^+$ and salmon sperm DNA. DNA solution and [Co(phen)$_2$(Cl)(H$_2$O)]$^+$ was mixed and reacted for 12 min at room temperature. Afterwards, UV spectrum of the mixture was recorded with a scanning range from 200 nm to 400 nm.

2.4. Electrochemical study on the interaction between [Co(phen)$_2$(Cl)(H$_2$O)]$^+$ and DNA

The changes on characteristics of cyclic voltammograms (CVs) and the differential pulse voltammograms (DPVs) of the [Co(phen)$_2$(Cl)(H$_2$O)]$^+$ solutions in the absence and presence of salmon sperm DNA were investigated. For cyclic voltammetric scanning, the potential scanning ranged from -0.4 V to 0.6 V and the scanning rate was 0.10 V s$^{-1}$. The sample interval was set as 0.001 V and the quiet time was 2 s. For DPV measurement, the initial potential was 0.8 V. The high potential was 0.8 V and the low potential was -0.4 V. The pulse scope was set as 0.004 V and the pulse extent was 0.05 s. The pulse cycle was 0.2 s. The sample interval and the quiet time were 0.001 V and 2 s respectively.

2.5. Preparation of the electrochemical DNA sensor

The developed electrochemical DNA biosensor was based on the covalent immobilization of human immunodeficiency virus (HIV) probe single-strand DNA (S$_1$) on the activated electrode. Prior to experiment, the GCE was firstly polished using 1.0 µm, 0.3 µm and 0.05 µm α-Al$_2$O$_3$ suspension respectively and then extensively rinsed in DDW with ultrasonic. Afterwards, the electrode was
oxidized at +0.50 V for 1 min in 5.00 × 10^{-2} \text{ mol} \text{ L}^{-1} \text{ PBS} \text{ at pH} \ 7.4 \text{ followed by thoroughly rinsed with DDW. As previously reported [19], four-step procedure was used for the preparation of the DNA biosensor. Firstly, the electrode was activated by dropping 20 \mu \text{L} \ 5.00 \times 10^{-2} \text{ mol} \text{ L}^{-1} \text{ PBS (pH} \ 7.4) \text{ containing} \ 5.0 \times 10^{-3} \text{ mol} \text{ L}^{-1} \text{ EDC and} \ 8.0 \times 10^{-3} \text{ mol} \text{ L}^{-1} \text{ NHS on the electrode surface. After the solution was air-dried, the activated electrode was rinsed with DDW for several times. Secondly, ssDNA (S_1) was immobilized on the electrode surface by dropping ssDNA solution on the activated GCE. After dried under an infrared lamp, the electrode was rinsed with DDW to eliminate the DNA adsorbed. Thirdly, the S_1-immobilized GCE was immersed in 20 \text{ mmol} \text{ L}^{-1} \text{ Tris-HCl buffer (pH} \ 7.0) \text{ containing complementary S_2 or uncomplementary S_3 respectively to obtain S_1-S_2 hybridized GCE and S_1-S_3 hybridized GCE respectively. Such DNA interaction was allowed at 40 \degree \text{C} \text{ for 1 h with stirring. Afterwards, the obtained electrodes were thoroughly rinsed with the same Tris-HCl buffer and DDW successively to eliminate the adsorbed S_2 or S_3 and then dried at room temperature. Fourthly, \text{[Co(phen)_2(Cl)(H_2O)]}^+ \text{ was accumulated onto the surface by immersing the resulted electrode in the Tris-HCl buffer solution containing \text{[Co(phen)_2(Cl)(H_2O)]}^+ \text{ at room temperature for 5 min with stirring. After rinsed with the same Tris-HCl buffer and DDW successively, the DPV measurements were performed.}

3. Results and Discussion

3.1. Fluorescence spectroscopic studies of the interaction between \text{[Co(phen)_2(Cl)(H_2O)]}^+ \text{ and DNA}

Fluorescence spectroscopy was used to investigate the interaction between \text{[Co(phen)_2(Cl)(H_2O)]}^+ \text{ and salmon sperm DNA. Figure 1 displayed the fluorescence features of \text{[Co(phen)_2(Cl)(H_2O)]}^+ \text{ in the absence and presence of DNA. It was observed from curve 1 that \text{[Co(phen)_2(Cl)(H_2O)]}^+ \text{ had the maximal emission at 366 nm. We could find that the fluorescence intensity of \text{[Co(phen)_2(Cl)(H_2O)]}^+ \text{ enhanced gradually with the amount of added DNA increased (curve 2-4), indicating the interaction between \text{[Co(phen)_2(Cl)(H_2O)]}^+ \text{ and salmon sperm DNA. The phenomenon might be ascribed to the hydrophobic circumstance of DNA, which could enhance the fluorescence quantum yield of \text{[Co(phen)_2(Cl)(H_2O)]}^+ \text{ and the corresponding fluorescence intensity.}}

To investigate the binding mode between \text{[Co(phen)_2(Cl)(H_2O)]}^+ \text{ and dsDNA, the fluorescence spectrum of EB-DNA system in the presence of \text{[Co(phen)_2(Cl)(H_2O)]}^+ \text{ was studied. As shown in Figure 2, EB itself emitted weak fluorescence emission (curve 1). After it intercalated into the double helix of DNA molecule, a significant increase in fluorescence intensity was observed (curve 2). However, distinct quenching of fluorescence occurred when \text{[Co(phen)_2(Cl)(H_2O)]}^+ \text{ was added (curve 3-5). Moreover, the more the \text{[Co(phen)_2(Cl)(H_2O)]}^+ \text{ added, the lower the fluorescence emitted. Such fluorescence quenching might be due to the competition between \text{[Co(phen)_2(Cl)(H_2O)]}^+ \text{ and EB for the binding sites of dsDNA, which made the fluorescence intensity of EB-DNA system weaken [20]. Accordingly, the binding mode of \text{[Co(phen)_2(Cl)(H_2O)]}^+ \text{ to dsDNA might be similar with EB. So, the intercalative binding between \text{[Co(phen)_2(Cl)(H_2O)]}^+ \text{ and DNA was supposed.}
Figure 1. Fluorescence spectrum of \([\text{Co(phen)}_2(\text{Cl})(\text{H}_2\text{O})]^{+}\) in the absence and presence of different concentration of salmon sperm DNA. \([\text{Co(phen)}_2(\text{Cl})(\text{H}_2\text{O})]^{+}\) concentration: \(1.00 \times 10^{-4}\) mol L\(^{-1}\); DNA concentration (mol L\(^{-1}\)): (1): 0; (2): \(3.12 \times 10^{-4}\); (3): \(4.68 \times 10^{-4}\); (4): \(5.46 \times 10^{-4}\).

Figure 2. Fluorescence spectrum of EB-DNA system in the absence and presence of \([\text{Co(phen)}_2(\text{Cl})(\text{H}_2\text{O})]^{+}\). (1): \(6.35 \times 10^{-5}\) mol L\(^{-1}\) EB; (2): (1) + \(1.56 \times 10^{-3}\) mol L\(^{-1}\) salmon sperm DNA; (3): (2) + \(6.67 \times 10^{-5}\) mol L\(^{-1}\) \([\text{Co(phen)}_2(\text{Cl})(\text{H}_2\text{O})]^{+}\) (4): (2) + \(1.33 \times 10^{-4}\) mol L\(^{-1}\) \([\text{Co(phen)}_2(\text{Cl})(\text{H}_2\text{O})]^{+}\) (5): (2) + \(2.67 \times 10^{-4}\) mol L\(^{-1}\) \([\text{Co(phen)}_2(\text{Cl})(\text{H}_2\text{O})]^{+}\).

3.2. UV spectroscopic studies of the interaction between \([\text{Co(phen)}_2(\text{Cl})(\text{H}_2\text{O})]^{+}\) and DNA

The hypochromic effect and the bathochromic effect were the identifying marks of the intercalation, as reported by Long et al [21]. To confirm our hypothesis, UV spectroscopy was used to further study the interaction between \([\text{Co(phen)}_2(\text{Cl})(\text{H}_2\text{O})]^{+}\) and DNA. The variation of UV spectrum for \([\text{Co(phen)}_2(\text{Cl})(\text{H}_2\text{O})]^{+}\) in the absence and presence of salmon sperm DNA was shown in Figure 3. When the amount of added DNA increased, the absorption peaks of \([\text{Co(phen)}_2(\text{Cl})(\text{H}_2\text{O})]^{+}\) at 202.0,
224.0 and 268.0 nm decreased and a slight red shift was observed, indicating both the hypochromic effect and bathochromic effect. Therefore, the intercalative interaction between \([\text{Co(phen)}_2(\text{Cl})(\text{H}_2\text{O})]^+\) and DNA was identified.

**Figure 3.** UV spectrum of \([\text{Co(phen)}_2(\text{Cl})(\text{H}_2\text{O})]^+\) in the absence and presence of salmon sperm DNA. (1): \(2.00 \times 10^{-5}\) mol L\(^{-1}\) \([\text{Co(phen)}_2(\text{Cl})(\text{H}_2\text{O})]^+\); (2): (1) + \(1.87 \times 10^{-5}\) mol L\(^{-1}\) DNA; (3): (1) + \(3.74 \times 10^{-5}\) mol L\(^{-1}\) DNA.

3.3. Electrochemical study on the interaction between \([\text{Co(phen)}_2(\text{Cl})(\text{H}_2\text{O})]^+\) and DNA

To explore the application of \([\text{Co(phen)}_2(\text{Cl})(\text{H}_2\text{O})]^+\) in electrochemical DNA biosensors, electrochemical study on \([\text{Co(phen)}_2(\text{Cl})(\text{H}_2\text{O})]^+\) and its interaction with DNA were performed. CVs of \([\text{Co(phen)}_2(\text{Cl})(\text{H}_2\text{O})]^+\) in the absence and presence of salmon sperm DNA were shown in Figure 4. We could see that there were a couple of quasi-reversible redox voltammetric peaks for \([\text{Co(phen)}_2(\text{Cl})(\text{H}_2\text{O})]^+\) (curve 1), indicating the electrochemical activity of \([\text{Co(phen)}_2(\text{Cl})(\text{H}_2\text{O})]^+\). The cathodic peak potential (\(E_{pc}\)) and the anodic peak potential (\(E_{pa}\)) were 0.010 V and 0.077 V respectively. Curves 2-3 were the CVs of \([\text{Co(phen)}_2(\text{Cl})(\text{H}_2\text{O})]^+\) in the presence of different concentration of DNA. When the DNA concentration in the solution increased, the peak currents, both \(I_{pc}\) and \(I_{pa}\), decreased slightly and small shift to positive potentials for both \(E_{pc}\) and \(E_{pa}\) occurred. The phenomena mentioned above were further studied by DPV and results were shown in Figure 5. Curve 1 was the differential pulse voltammogram of the \([\text{Co(phen)}_2(\text{Cl})(\text{H}_2\text{O})]^+\) solution, while curve 2-3 were the voltamgramms when different amounts of DNA were added to the solution. As can be seen, the peak currents also decreased with increasing of DNA. Therefore, intercalative binding mode of \([\text{Co(phen)}_2(\text{Cl})(\text{H}_2\text{O})]^+\) to DNA drawn from electrochemical measurement was consistent with that obtained through spectroscopic studies [22].
Figure 4. Cyclic voltammograms of \([\text{Co(phen)}_2(\text{Cl})(\text{H}_2\text{O})]^+\) in the absence and presence of different concentration of DNA. \([\text{Co(phen)}_2(\text{Cl})(\text{H}_2\text{O})]^+\) concentration: \(1.00 \times 10^{-3}\) mol L\(^{-1}\); DNA concentration (mol L\(^{-1}\)): (1): 0; (2): \(2.10 \times 10^{-4}\); (3): \(6.23 \times 10^{-4}\).

Figure 5. Differential pulse voltammograms of \([\text{Co(phen)}_2(\text{Cl})(\text{H}_2\text{O})]^+\) in the absence and presence of different concentration of DNA. \([\text{Co(phen)}_2(\text{Cl})(\text{H}_2\text{O})]^+\) concentration: \(1.00 \times 10^{-3}\) mol L\(^{-1}\); DNA concentration (mol L\(^{-1}\)): (1): 0; (2): \(5.21 \times 10^{-4}\); (3): \(1.53 \times 10^{-3}\).

The experimental conditions of the interaction between \([\text{Co(phen)}_2(\text{Cl})(\text{H}_2\text{O})]^+\) and DNA, e.g. pH, reaction time, scan rate and the concentration of DNA, were optimized. Phosphate buffer solution (PBS) at pH 6.0 was chosen as the reaction medium and 12 min as the reaction time. \(I_{pc}\) is directly in proportion to the square root of the scan rate in the range from \(0.02\) V s\(^{-1}\) to \(0.23\) V s\(^{-1}\). The regression equation is \(I_{pc} = 17.339 \nu^{1/2} + 0.6043\) with a correlation coefficient \(\gamma = 0.9991\). This indicated that the electrochemical process of \([\text{Co(phen)}_2(\text{Cl})(\text{H}_2\text{O})]^+\) was controlled by the diffusion of \([\text{Co(phen)}_2(\text{Cl})(\text{H}_2\text{O})]^+\). It was also found that \(\Delta I_{pc}\) is in linear proportion to the concentration of DNA in the range from \(2.10 \times 10^{-4}\) mol L\(^{-1}\) to \(2.77 \times 10^{-3}\) mol L\(^{-1}\), with a regression equation \(\Delta I_{pc} = 0.072 C_{DNA} - 0.0165\), and a correlation coefficient \(\gamma = 0.9997\).
3.4. The electrochemical characterization of modified GCE

The DNA biosensor using \([\text{Co(phen)}_2\text{(Cl)}\text{(H}_2\text{O})]^{+}\) as electrochemical hybridization label was studied. The electrochemical probe molecules, \(\text{Fe(CN)}_6^{3-}/\text{Fe(CN)}_6^{4-}\), were chosen to characterize the modified electrode. The bare GCE, S1-immobilized GCE, and S1-S2 hybridized GCE had been used as the working electrode respectively. The CVs of \(5 \times 10^{-3} \text{mol L}^{-1} \text{K}_4\text{Fe(CN)}_6\) in 0.01 mol L\(^{-1}\) KCl solution were shown in Figure 6. Compared to bare GCE, redox peak currents of \(\text{Fe(CN)}_6^{3-}/\text{Fe(CN)}_6^{4-}\) on S1-immobilized GCE, and S1-S2 hybridized GCE decreased significantly. The negative charge of \(\text{Fe(CN)}_6^{4-}\) and DNA immobilized on the electrode surface resulted in the static repulsive force and weakened the electrochemical reaction of \(\text{Fe(CN)}_6^{3-}/\text{Fe(CN)}_6^{4-}\) on the electrode, which lead to the decrease of the redox peak currents.

![Figure 6. Cyclic voltammograms of Fe(CN)\(_6\)\(^{3-}/\)Fe(CN)\(_6\)\(^{4-}\) on different electrodes. (1): Bare GCE; (2): S\(_1\)-immobilized GCE; (3): S\(_1\)-S\(_2\) hybridized GCE.](image)

3.5. The selectivity of the DNA electrochemical biosensor

Differential pulse voltammetry was used to study the selectivity of the prepared electrochemical DNA biosensor. Figure 7 showed the representative differential pulse voltammograms obtained in PBS (pH 6.0) with the S\(_1\)-immobilized GCE (curve 1), S\(_1\)-S\(_2\) hybridized GCE (curve 2) and S\(_1\)-S\(_3\) hybridized GCE (curve 3) being the working electrode respectively. S\(_1\)-S\(_2\) hybridized GCE represented that S\(_1\)-immobilized GCE hybridized with the complimentary DNA segment S\(_2\) and S\(_1\)-S\(_3\) hybridized GCE referred to the electrode obtained after S\(_1\)-immobilized GCE hybridized with uncomplimentary DNA segment S\(_3\). Before DPV measurement, \([\text{Co(phen)}_2\text{(Cl)}\text{(H}_2\text{O})]^{+}\) accumulation step for the three electrodes were all performed. No voltammetric response for the S\(_1\)-immobilized GCE was found in curve 1. However, the reduction peak of the S\(_1\)-S\(_2\) hybridized GCE appeared at about -0.144 V, as shown in curve 2, which was the same potential as the bare GCE in the \([\text{Co(phen)}_2\text{(Cl)}\text{(H}_2\text{O})]^{+}\) solution. The peak current was \(1.378 \times 10^{-7} \text{A}\). The phenomenon indicated the intercalation of the indicator, \([\text{Co(phen)}_2\text{(Cl)}\text{(H}_2\text{O})]^{+}\), into the base pairs of the dsDNA resulted from S\(_1\)-S\(_2\) hybridization. In the case of S\(_1\)-S\(_3\) hybridized GCE, no dsDNA was formed because S\(_3\) was uncomplimentary to S\(_1\) and
no indicator could intercalate. As expected, S₁-S₃ hybridized GCE displayed no voltammetric response, as shown in curve 3, which indicated the favorable selectivity of the ssDNA modified GCE.

Figure 7. Differential pulse voltammograms of different working electrode modified with [Co(phen)₂(Cl)(H₂O)]⁺ accumulation step. (1): S₁-immobilized GCE; (2): S₁-S₂ hybridized GCE; (3): S₁-S₃ hybridized GCE.

In summary, the interaction between [Co(phen)₂(Cl)(H₂O)]⁺ and the probing DNA, salmon sperm DNA, was studied by fluorescence spectroscopy, ultraviolet spectroscopy and voltammetry. [Co(phen)₂(Cl)(H₂O)]⁺ can bind to dsDNA by intercalative binding mode. By using [Co(phen)₂(Cl)(H₂O)]⁺ as the electrochemical hybridization indicator, the electrochemical DNA biosensor was prepared base on the covalent immobilization of ssDNA and it showed high selectivity in detection of complementary ssDNA.

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