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ORIGINAL PAPER

A Novel Fluorescent Silver Ion Biosensor Based on Nucleic Acid Molecular "Light Switch"

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Abstract As one of nucleic acid molecular "light switch", $Ru(bipy)_2(dppx)^{2+}$ is a good probe for the determination of double-helical DNA, which displays intense fluorescence when double-helical DNA is present. However, the fluorescence of $Ru(bipy)_2(dppx)^{2+}$ is quenched when Ag^+ is added to the $Ru(bipy)_2(dppx)^{2+}$-DNA system. Based on the quenching of the fluorescence of $Ru(bipy)_2(dppx)^{2+}$-DNA system by Ag^+, a simple, rapid and specific method for Ag^+ determination was proposed. In the optimum conditions, Ag^+ concentration versus $Ru(bipy)_2(dppx)^{2+}$ fluorescence intensity gave a linear response in the range from 0.2 to 6.0 μM with a detection limit (3σ) of 3.2×10^{-8} M. The proposed method has been applied to determine the Ag^+ in water samples and sulfadiazine silver cream successfully. Because of the intense fluorescence of $Ru(bipy)_2dppx^{2+}$ when DNA is present, the interaction between Ag^+ and DNA was confirmed by fluorescence microscopy and the phenomenon of the fluorescence images agreed well with the results. The possible mechanism of the reaction was also discussed by circular dichroism spectra and isothermal titration calorimetry.

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Keywords $Ru(bipy)_2(dppx)^{2+}$ · DNA · Silver · Fluorescence image · Circular dichroism · Isothermal titration calorimetry

Introduction

Nucleic acid molecular "light switch" means some ruthenium complexes [1, 2], which show no fluorescence in aqueous solution due to quenching by hydrogen bonding between water and phenazine nitrogens of the ligand at ambient temperature, but display intense fluorescence in the double-helical DNA [3–8] because of the interaction between the ligand and the DNA duplex which protects the phenazine nitrogens from water [9, 10]. Their luminescence intensity can be enhanced more than 10^4 by duplex DNA [11, 12], so they have been used widely in DNA detection [13–16]. As a new kind of DNA probe, they have some advantages such as the merits of high sensitivity, litter interference from RNA, BSA and metal ions, etc [17–19]. The interaction between nucleic acid molecular "light switch" and DNA has been extensively investigated, which showed that ruthenium complexes intercalated into the double-helical DNA with the extended dppz or dppx ligand [20–23]. Our group had synthesized a series of derivatives of molecular "light switch" ($Ru(phen)_2dppz^{2+}$, $Ru(phen)_2dppx^{2+}$, $Ru(bipy)_2dppz^{2+}$, $Ru(bipy)_2dppx^{2+}$, phen=1,10-phenanthroline, bipy=2,2′-bipyridine, dppz=dipyrido[3,2-*a*:2′,3′-*c*]phenazine, dppx=7,8-dimethyl-dipyrido[3,2-*a*:2′,3′-*c*]phenazine) and applied them to detect trace amount of DNA, multi-mismatched oligonucleotides, single-mismatch, triplex DNA and DNA damage [24–28]. Because of the intense fluorescence of $Ru(phen)_2dppx^{2+}$ when DNA is present, we have been studied the photobleaching and luminescence recovery of $Ru(phen)_2dppx^{2+}$ using fluorescence microscopy [29]. The results of that work enabled the better understanding and wider application of the

Ru(II) DNA light switch probes in the study of DNA, such as DNA visible detection, DNA imaging, DNA dynamic processes study and so on [30–33].

Silver is an important element that is widely used in many fields. Because of its bacteriostatic properties, silver compounds are often used in filters and other equipments to purify water of swimming pool and drinking water, and used in the processing of foods, drugs, and beverages. So, it is very important to detect silver from real samples for the environment and human health. Up to now, various methods have been used for the determination of Ag^+, which include atomic absorption spectrometry [34], inductively coupled plasma mass spectrometry [35], colorimetric solid-phase extraction [36], membrane electrode [37] and square-wave voltammetry [38]. However, some of them are very expensive, time consuming and hard to automate. In comparison, simple and rapid optical sensors for Ag^+ determination with simple instrumental implementation and easy-operation have received a lot of attention. Recent years, fluorescence analysis has been widely utilized for Ag^+ determination because of their unique advantages of simplicity, rapidity, high sensitivity and low cost of instrumentation and maintenance [39–42]. Taki and co-workers have reported a rosamine-based fluorescent chemosensor for selective detection of Ag^+ in aqueous solution based on the increment of the fluorescence intensity with Ag^+ under physiological conditions [39]. Zhang et al. have also developed the selective ratiometric fluorescence sensors and fluorescence "turn on" chemosensors for Ag^+ [40, 41]. Recently, Marques and his co-workers have prepared novel indole derivatives and further used it for the determination of Ag^+ and Hg^{2+} [42]. However, these methods were low-sensitivity and were not applied to Ag^+ determination in real samples. Thus, there is an intense demand for simple, rapid sensor for the detection of Ag^+ with high sensitivity and specificity in real samples.

The interactions of metal ions (Ag^+, Hg^{2+}, Zn^{2+} etc) with nucleic acids, nucleosides, and nucleobases have been investigated extensively, because some positive ions may interact with DNA noncovalently as counter ions to balance the negative charge of the phosphate backbone and/or coordinatively at a number of different binding sites including the nitrogen centres in nucleobases, sugars and the phosphates. Because of the selective binding between Hg^{2+} ions and thymine-thymine (T-T) base pairs in DNA duplexs, Ono et al. could selectively detect Hg^{2+} ions in aqueous solution that contained excess amount of other heavy metal ions [43]. Hossain et al. have also reported that there was a strong interaction between Ag^+ and the DNA strands [44]. As far as we know, few paper has concerned about detecting Ag^+ by using the interaction between Ag^+ and the DNA strands. Based on that situation, a new kind of method was developed for the quantitative and selective determination of Ag^+ based on the quenching of the fluorescence of $Ru(bipy)_2(dppx)^{2+}$-DNA system. The proposed method has been successfully applied to the determination of Ag^+ in water samples and medicine sample. The possible mechanism of the reaction was also discussed by circular dichroism spectra and isothermal titration calorimetry.

Materials and methods

Materials

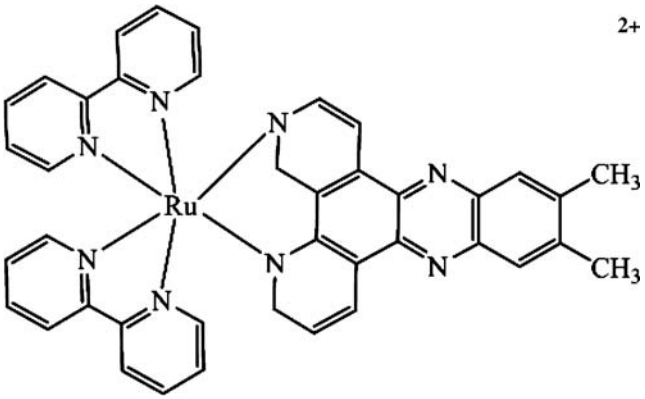
The calf thymus DNA (ctDNA) was purchased from Hua Mei Biochemical Co. (China). The concentration of ctDNA was calculated according to the absorption at 260 nm ($\varepsilon(\lambda_{260nm})=6{,}600\ L\ mol^{-1}\ cm^{-1}$). $Ru(bipy)_2(dppx)(BF_4)_2{\cdot}2H_2O$ was synthesized according to the references [12] and identified by ^1H NMR, elemental analysis and spectroscopy. The stock solution of $Ru(bipy)_2(dppx)^{2+}$ (0.1 mM) was prepared by dissolving 18.7 mg $Ru(bipy)_2(dppx)(BF_4)_2{\cdot}2H_2O$ in 200 mL water. The stock solution of Ag^+ was prepared by dissolving 1 g of $AgNO_3$ in 100 mL water and was further diluted with water whenever necessary. A citric acid buffer solution (0.1 mol L^{-1} citric acid, pH 6.0) was used in the experiments. All other chemicals were of analytical-reagent grade and used as received from Sigma (St. Louis, USA). Ultrapure water with a resistivity of 18.2 MΩ cm was produced by passing through a RiOs 8 unit followed by a Millipore-Q Academic purification set (Millipore, USA).

Apparatus

The absorption spectra were measured on a TU-1900 UV-vis spectrophotometer (Beijing Purkinje General Instrument Co., Ltd., China). All fluorescence measurements were recorded with a Perkin-Elmer Model LS-55 luminescence spectrometer (Perkin-Elmer, USA) equipped with a 20 kW xenon discharge lamp as a light source. All pH measurements were made with a Model pHS-3C meter (Shanghai Leici Equipment Factory, China). The fluorescence images were obtained by the ChemiDoc XRS (Bio-Rad, USA). The circular dichroism (CD) spectra were recorded on the Jasco J-810-150S automatic recording spectropolarimeter (Jasco, Japan). The isothermal titration calorimetry (ITC) results were made with a Model Nano-ITC 2G biocalorimetry instrument (TA, USA).

Fluorescence spectroscopy measurements

0.5 mL of 23 μM ctDNA solutions, 1.0 mL of citric acid buffer (pH 6.0) and certain amounts of Ag^+ were sequentially added to a 5-mL calibrated test tube. The mixture was diluted to volume with ultrapure water after addition of 0.5 mL 10 μM $Ru(bipy)_2(dppx)^{2+}$ solution.



Scheme 1 The chemical structure of $Ru(bipy)_2(dppx)^{2+}$

After 10 min reaction at room temperature, the fluorescence intensity was recorded at 601 nm with the excitation wavelength of 450 nm. Both slit widths of excitation and emission were 10 nm. When samples were determined, Ag^+ standard solution was substituted by the prepared sample solution described in "Sample treatment".

CD measurements

The CD profiles were obtained employing a scan speed of 1,000 nm/min and response time of 0.5 s. Each spectrum was the average of three successive scans and was corrected by citric acid buffer solution. Appropriate baseline corrections in the CD spectra were made. For the CD experiment, the concentration of ctDNA was 0.23 mM and the concentration of $Ru(bipy)_2(dppx)^{2+}$ was 0.1 mM. Far-CD spectra were recorded from 205 to 350 nm at 25°C in citric acid buffer solution.

ITC measurements

For the ITC experiment, ctDNA and $Ru(bipy)_2(dppx)^{2+}$ solutions were properly degassed prior to the titrations to avoid the formation of bubbles in the calorimeter cell. The reference cell contained the same volume of ultrapure water. In a standard experiment, the ctDNA (0.4 mM) in the 1.0 mL calorimeter cell was titrated by the $Ru(bipy)_2(dppx)^{2+}$ (1 mM) solutions by up to 20 successive automatic injections of 5 μL each. The first injection was ignored in the final data analysis. Integration of peaks corresponding to each injection and correction for the baseline were carried out using NanoAnalyze software provided by the manufacturer. Fitting the data to various interaction models results in the stoichimetry (n), equilibrium binding constant (K_b), and enthalpy of complex formation (ΔH). The other thermodynamic parameters were calculated according to the formulas:

$$\Delta G = \Delta H - T\Delta S \quad (1)$$

and

$$\Delta G = -RT \ln K_b \quad (2)$$

T is the absolute temperature (in the current experiment T=298 K), and R=8.3151 J mol^{-1} K^{-1}.

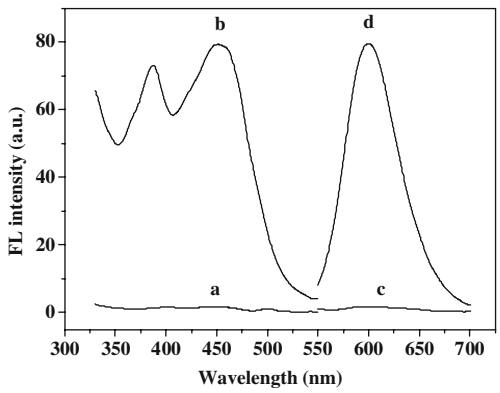


Fig. 1 The excitation (**a**, **b**) and emission (**c**, **d**) spectra of Ru $(bipy)_2(dppx)^{2+}$ (1.0 μM) in the absent (**a**, **c**) and present (**b**, **d**) of ctDNA (2.3 μM)

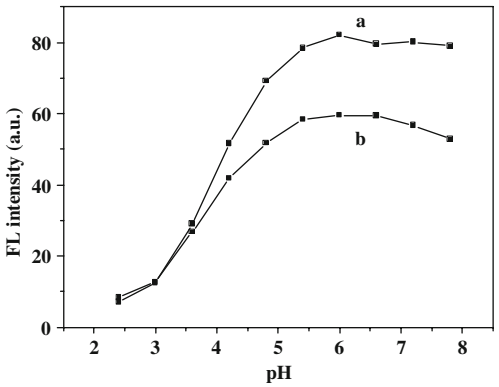


Fig. 2 The effect of pH on the fluorescence of $Ru(bipy)_2(dppx)^{2+}$-ctDNA system in the absent (**a**) and present (**b**) of Ag^+. The concentration of $Ru(bipy)_2(dppx)^{2+}$ and Ag^+ are all 1.0 μM and the concentration of ctDNA is 2.3 μM

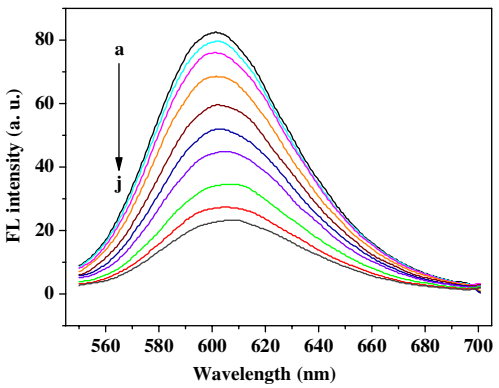


Fig. 3 The emission spectra of the system in the presence of Ag^+ in 0.1 mol L^{-1} citric acid buffer solution at pH 6.0. The final Ag^+ concentration is: (a) 0, (b) 0.2, (c) 0.4, (d) 0.6, (e) 1.0, (f) 1.5, (g) 2.0, (h) 3.0, (i) 4.5 and (j) 6.0 μM, respectively. The concentration of Ru $(bipy)_2(dppx)^{2+}$ is 1.0 μM and the concentration of ctDNA is 2.3 μM

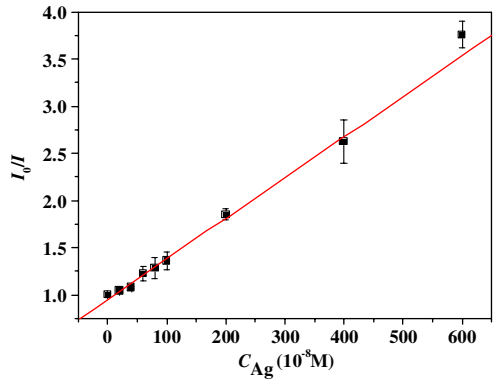


Fig. 4 The linear relationship between I_0/I and Ag^+ concentration in the range of 0.2~6.0 μM with a 0.997 correlation coefficient. The concentration of $Ru(bipy)_2(dppx)^{2+}$ is 1.0 μM and the concentration of ctDNA is 2.3 μM

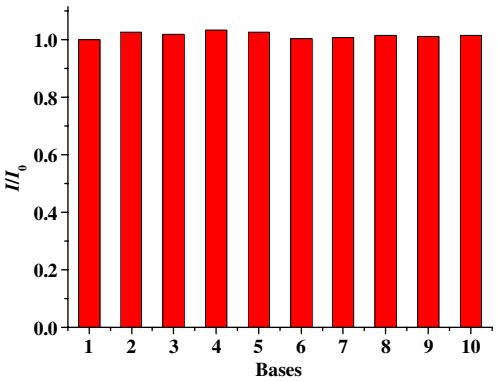


Fig. 6 Effect of several foreign substance on the fluorescence of Ru $(bipy)_2(dppx)^{2+}$-ctDNA system. 1 control, 2 stearic acid, 3 cellulose acetate, 4 glycerin, 5 PEG 2000, 6 glutin, 7 lanolin, 8 liquid paraffin, 9 triethanolamine, 10 vaselin. The concentration of $Ru(bipy)_2(dppx)^{2+}$ is 1.0 μM and the concentration of ctDNA is 2.3 μM

Sample treatment

For the water samples analysis, different water samples were selected. These water samples were Yangtze River water, East Lake water and Hanjiang River water. The water samples were purified by centrifugation and filtration. Then, 1 mL of the water samples were thoroughly mixed with 4 mL of the $Ru(bipy)_2(dppx)^{2+}$-ctDNA system and the fluorescence measurements were performed.

For the pharmaceutical analysis, 0.7142 g sulfadiazine silver (SD-Ag) cream was weighed. The content of SD-Ag was calculated. The cream was dissolved in 5 mL diluted HNO_3 solution and the solution was boiled for 5 min. After the cream was completely dissolved, the mixture was allowed to cool to the room temperature. The insoluble components were removed from solution by filtration. Then, the cream samples were added in the $Ru(bipy)_2(dppx)^{2+}$-ctDNA system and the fluorescence of the mix solutions were recorded.

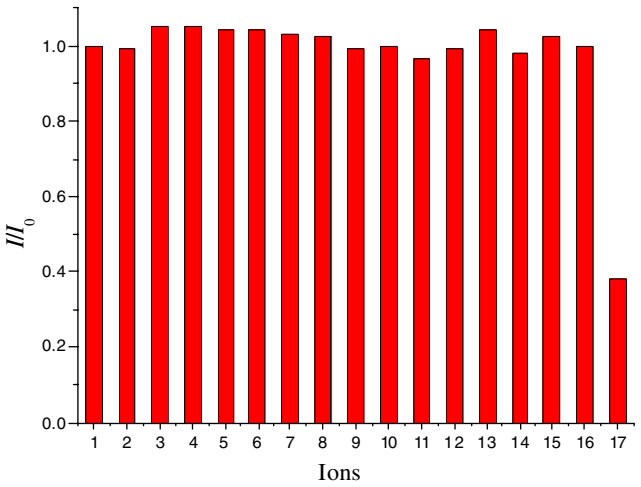


Fig. 5 The effect of foreign ions on the fluorescence of the Ru $(bipy)_2(dppx)^{2+}$-ctDNA system. (1) control, (2) Na^+, (3) K^+, (4) NH_4^+, (5) Ca^{2+}, (6) Fe^{3+}, (7) Mg^{2+}, (8) Zn^{2+}, (9) Co^{2+}, (10) Cu^{2+}, (11) Pb^{2+}, (12) Ba^{2+}, (13) Al^{3+}, (14) Hg^{2+}, (15) Mn^{2+}, (16) Ni^{2+}, (17) Ag^+. The concentrations of all ions are 5 μM. The concentration of Ru $(bipy)_2(dppx)^{2+}$ is 1.0 μM and the concentration of ctDNA is 2.3 μM

Results and discussion

Spectral characteristics of $Ru(bipy)_2(dppx)^{2+}$

The chemical structure of $Ru(bipy)_2(dppx)^{2+}$ was shown in Scheme 1 and the excitation and emission spectra of Ru $(bipy)_2(dppx)^{2+}$ were shown in Fig. 1. There was no excitation and emission peak in the spectra of Ru $(bipy)_2(dppx)^{2+}$ when ctDNA was absent. While there were two excitation peaks at 390 and 450 nm in the excitation spectrum of $Ru(bipy)_2(dppx)^{2+}$ when ctDNA was added into the solution. Since the intensity at 450 nm was higher than that of 390 nm, and the peak of 450 was from the absorption of metal-ligand charge transfer (MLCT), so

Table 1 Determination of Ag^+ in different water samples (n=5)

Samples	Added ($\times 10^{-7}$ M)	Found ($\times 10^{-7}$ M)	Recovery (%)	R. S. D. (%)
Yangtze River water	0	0	–	–
	8.0	7.45–7.76	93.1–97.0	4.2
	25.0	23.15–23.98	92.6–95.9	3.5
East Lake water	0	0	–	–
	8.0	7.48–7.69	93.5–96.1	2.8
	25.0	22.85–23.82	91.4–95.3	3.8
Hanjiang River water	0	0	–	–
	8.0	7.53–7.87	94.1–98.4	4.3
	25.0	23.88–24.81	95.5–99.2	2.3

Table 2 Determination of Ag^+ in SD-Ag cream by the proposed method

Samples	SD-Ag cream
Labeled values (%)	1.0
Found (%)	1.004
R. S. D.[a] (%)	4.4
E_r (%)	0.4

[a] The R. S. D. value is mean of five determinations

450 nm was selected as the excitation wavelength. When excited at 450 nm, an emission peak at the wavelength of 601 nm was occurred. These results indicated that ctDNA could enhance the fluorescence of $Ru(bipy)_2(dppx)^{2+}$ greatly, which was in accordance with the intercalative binding mode reported previously [26].

Effect of $Ru(bipy)_2(dppx)^{2+}$ concentration

Initial experiments demonstrated that when the ratio of the concentrations of $Ru(bipy)_2(dppx)^{2+}$ to the ctDNA was about 1:6, the maximum fluorescence intensity was obtained, which was in agreement with the result reported previously [4]. In the following experiments, the concentration ratio of 1:2.3 was selected to ensure the saturation of $Ru(bipy)_2(dppx)^{2+}$ intercalation.

Effect of reaction time

Pre-experiments indicated that the reaction between Ag^+ and ctDNA was very quick and finished within 3 min. When $Ru(bipy)_2(dppx)^{2+}$ was added into the solution, the reaction was also finished within 5 min and the fluorescence signals were stable more than 1 h, which indicated that this system exhibited rapid reaction and good stability. So we recorded the fluorescence intensity of the system after 10 min reaction.

Effect of pH

The effect of pH on the reaction was investigated from pH 2.4 to 7.8. As shown in Fig. 2, the pH of the solution had great effect on the fluorescence intensity of the system. The fluorescent intensity was stable and high in the range of pH 5.4~6.6. When the pH value was lower than 5.4, the change of the fluorescence intensity of the system decreased significantly. In general, the interaction between $Ru(bipy)_2(dppx)^{2+}$ and ctDNA might be weakened when the pH decreased to a certain value, which resulted in a significant decrease in the fluorescent intensity of the system. When the pH value was higher than 6.6, the formation of Ag^+ hydrate might decrease the fluorescence intensity of the system, which would lower the sensitivity of this method. To obtain high fluorescence intensity with good precision, pH 6.0 was chosen for the further experiments.

Calibration and sensitivity

Figure 3 showed the emission spectra of the system in various concentrations of Ag^+. It was found that Ag^+ quenched the fluorescence of $Ru(bipy)_2(dppx)^{2+}$-ctDNA system in a concentration dependence that was best described by a Stern-Volmer-type equation:

$$\frac{I_0}{I} = 1 + K_{SV} C_{Ag}$$

I and I_0 were the fluorescent intensity of the system at a given Ag^+ concentration and in the absent of Ag^+, respectively. C_{Ag} was the Ag^+ concentration. K_{SV} was found to be 4.5×10^5 L mol^{-1}. The calibration plot (Fig. 4) of I_0/I with concentration of Ag^+ was linear in the range 0.2~6.0 μM. The correlation coefficient was 0.997. Based on the three times standard deviation of 11 measurements of $Ru(bipy)_2(dppx)^{2+}$-ctDNA system without Ag^+, the limit of detection (3σ) for Ag^+ was up to 3.2×10^{-8} M.

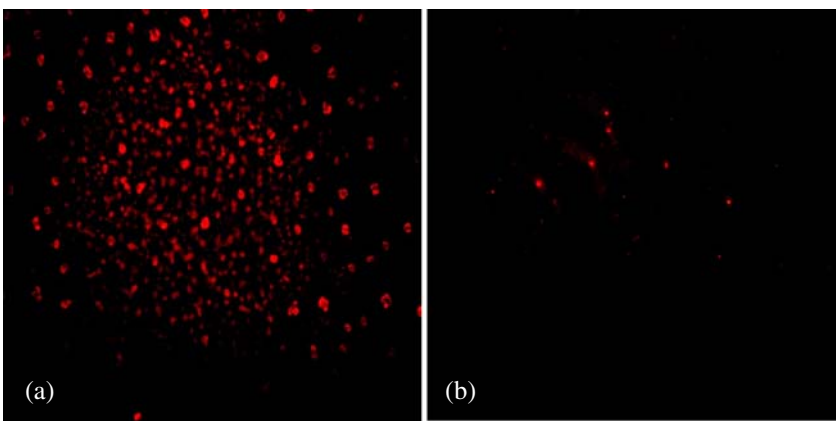


Fig. 7 Fluorescence images of ctDNA **a** without Ag^+ and **b** with Ag^+ using Ru $(bipy)_2(dppx)^{2+}$ as probe. The excitation wavelength was 470 nm and the magnification of the microscope is 400×

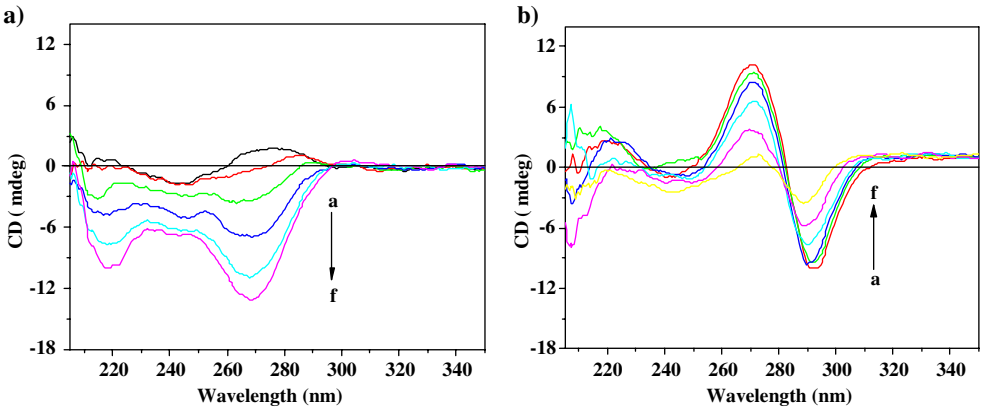


Fig. 8 CD spectra of ctDNA (a) and $Ru(bipy)_2(dppx)^{2+}$-ctDNA system (b) with different concentrations of Ag^+. The final Ag^+ concentration is: (a) 0, (b) 20, (c) 40, (d) 60, (e) 80 and (f) 100 μM, respectively. The concentration of Ru $(bipy)_2(dppx)^{2+}$ is 0.1 mM and the concentration of ctDNA is 0.23 mM

Effect of foreign substance

The fluorescence of $Ru(bipy)_2(dppx)^{2+}$-ctDNA system with various metal ions was conducted to examine the selectivity. As shown in Fig. 5, Na^+, K^+, NH_4^+, Ca^{2+}, Fe^{3+}, Mg^{2+}, Zn^{2+}, Co^{2+}, Cu^{2+}, Pb^{2+}, Ba^{2+}, Al^{3+}, Hg^{2+}, Mn^{2+}, Ni^{2+} had hardly effect on $Ru(bipy)_2(dppx)^{2+}$ fluorescence at the same concentration of Ag^+, which demonstrated that this method had high selectivity.

The cream often contained the following bases (stearic acid, cellulose acetate, glycerin, polythylene glycol 2000 (PEG 2000), glutin, lanolin, liquid paraffin, triethanolamine, and vaseline, et al.) [http://100md.com/html/DirDu/2006/04/12/96/23/43.htm]. But most of bases such as vaseline, lanolin, stearic acid, et al. are hardly dissolved in water, their saturated solutions in water are selected to study the effects. The concentration of some liquid bases were 50% (v : v), and the concentration of PEG 2000 was 20 mg/mL. As shown in Fig. 6, stearic acid, cellulose acetate, glycerin, PEG 2000, glutin, lanolin, liquid paraffin, triethanolamine and vaseline had hardly effect on fluorescence of $Ru(bipy)_2(dppx)^{2+}$-ctDNA system.

Application

The usefulness of the proposed method was evaluated for the determination of traces of Ag^+ in water samples. The results were shown in Table 1 and the recovery was 91.4~99.2%, which indicated the suitability of determination of Ag^+ in the real water samples.

The developed method was further applied to the determination of Ag^+ in SD-Ag cream. As shown in Table 2, the RSD was 4.4%, and the results obtained by the present method agreed with the labeled values for SD-Ag cream.

The fluorescence image

Figure 7 showed the fluorescence images of ctDNA without Ag^+ (Fig. 7a) and with Ag^+ (Fig. 7b) using $Ru(bipy)_2(dppx)^{2+}$ as probe. The fluorescence of $Ru(bipy)_2(dppx)^{2+}$ was clearly observed when Ag^+ was absent (Fig. 7a), which indicated the strong interaction between $Ru(bipy)_2(dppx)^{2+}$ and ctDNA. After Ag^+ adding into the ctDNA solution (Fig. 7b), it was found that the fluorescence intensity of Ru $(bipy)_2(dppx)^{2+}$ decreased dramatically. The phenomenon agreed well with the results of the fluorescence spectra.

The possible mechanism of the reaction

The possible mechanism of the reaction was first discussed by CD spectra methods. The interaction of Ag^+ with ctDNA was studied by CD spectra and the results were shown in Fig. 8a. A positive CD signal apparent in the range of 260~295 nm for ctDNA with a maximum amplitude at approximately 277 nm was due to base stacking [45–47]. The negative CD signal apparent in the range of 225~260 nm for ctDNA with a maximum amplitude at approximately 247 nm was due to the right-handed helicity of ctDNA [48]. When Ag^+ was added into the solutions, the positive CD signal of ctDNA decreased, indicating the conformation of ctDNA disturbed by Ag^+. Figure 8b showed the interaction of Ag^+ with the Ru

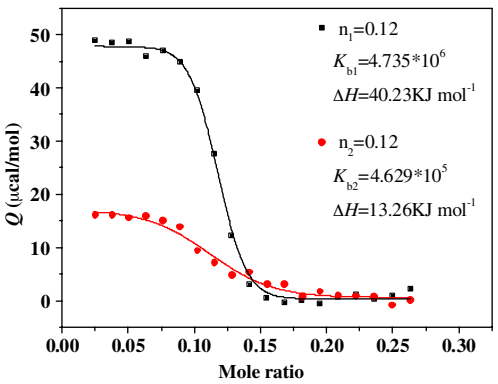


Fig. 9 The integrated ITC curve of $Ru(bipy)_2(dppx)^{2+}$ binding to the isolated ctDNA (*black wire*, ■) or ctDNA-Ag^+ complex (*red wire*, ◆). The final concentration of $Ru(bipy)_2(dppx)^{2+}$ is 0.1 mM and the concentration of ctDNA is 0.4 mM

$(bipy)_2(dppx)^{2+}$-ctDNA system. The enhancement of both the bands was due to the intercalation of $Ru(bipy)_2(dppx)^{2+}$ and ctDNA which strengthened the base-stacking and stabilized the right-handed B conformation of ctDNA [49]. When Ag^+ was added, the positive CD signal of Ru $(bipy)_2(dppx)^{2+}$-ctDNA system decreased and the negative CD signal of $Ru(bipy)_2(dppx)^{2+}$-ctDNA system increased. These results indicated that the intercalation of Ru $(bipy)_2(dppx)^{2+}$ and ctDNA was disturbed by Ag^+.

In order to investigate the possible mechanism of the reaction in more detail, titrations of ctDNA or ctDNA-Ag^+ samples with $Ru(bipy)_2(dppx)^{2+}$ were performed using isothermal calorimetry [50–52]. The Fig. 9 showed the integrated heats of reaction plotted against mole ratio of Ru $(bipy)_2(dppx)^{2+}$ to ctDNA after the correction for dilution effects of the $Ru(bipy)_2(dppx)^{2+}$. The exothermic heats accompanied the binding of $Ru(bipy)_2(dppx)^{2+}$ to ctDNA or ctDNA-Ag^+ complex. The enthalpies at 25°C were 40.23 KJ mol^{-1} for ctDNA and 13.26 KJ mol^{-1} for ctDNA-Ag^+ complex. A large enthalpy change (26.97 KJ mol^{-1}) and a large equilibrium binding constant change (almost one order of magnitude) were observed when $Ru(bipy)_2(dppx)^{2+}$ bound to isolated ctDNA and ctDNA-Ag^+ complex, respectively. However, the stoichimetry did not change when Ru $(bipy)_2(dppx)^{2+}$ bound to isolated ctDNA or ctDNA-Ag^+ complex, which demonstrated the invariant binding sites of $Ru(bipy)_2(dppx)^{2+}$ and ctDNA. These results verified the strong interaction between Ag^+ and ctDNA which resulted in the hindrance of the intercalation of $Ru(bipy)_2(dppx)^{2+}$ and ctDNA.

Dickson et al. found that the high affinity of Ag^+ for DNA bases [53]. Šponer and his co-workers have reported that Ag^+ formed coordinative bonds with N3 of cytosine and N7 of adenine distributed in an almost linear fashion [54]. So, the quenching of the fluorescence of Ru $(bipy)_2(dppx)^{2+}$-ctDNA system was due to the conformation change of ctDNA resulted by Ag^+.

Conclusions

A novel method for determination of Ag^+ has been developed based on the quenching of the fluorescence of $Ru(bipy)_2(dppx)^{2+}$-ctDNA system. In the optimum conditions, calibration graph was linear in the range 0.2~6.0 μM. The correlation coefficient was 0.997. The limit of detection (3σ) was 3.2×10^{-8} M. The present method was used to determine Ag^+ in SD-Ag cream and the results agreed with the claimed values. The phenomenon of the fluorescence images was also agreed well with the result of the emission spectra. The possible quenching mechanism was due to the conformation change of ctDNA resulted by Ag^+.

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