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ORIGINAL PAPER

Studies on the Fluorescence Fiber-Optic DNA Biosensor Using *p*-Hydroxyphenylimidazo[f]1,10-phenanthroline Ferrum(III) as Indicator

Shu-yan Niu · Shao-juan Wang · Chao Shi · Shu-sheng Zhang

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Abstract A complex $Fe(phen)_2 \cdot PHPIP \cdot 3ClO_4 \cdot 2H_2O$, where phen = 1,10-phenanthroline and PHPIP = *p*-hydroxyphenylimidazo[f]1,10-phenanthroline, was synthesized and acted as a good fluorescence indicator based on its interaction with double-duplex DNA. Then a fiber-optic DNA biosensor of fluorimetric detection was developed based on the recognition of target DNA in DNA hybridization assays. A probe ssDNA was covalently immobilized onto the surface of quartz optical fibers and then the probe ssDNA hybridized with complementary ssDNA introduced into the local environment of the sensor. The hybridization with complementary strands was monitored in real time by fluorimetric detection. Several factors affecting the probe immobilization, target DNA hybridization, and indicator binding reactions were optimized to maximize the sensitivity and shorten the assay time. Using this method, a sequence of the 16-mer oligonucleotides could be quantified over the range from 4.98×10^{-7} to 4.88×10^{-6} M and a detection limit of 1.08×10^{-7} M. And the designed optic-fiber biosensor could be conveniently regenerated by thermal denature. The utility of the novel hybridization indicator could provide a simple, rapid, low toxicity and reusable detection.

Keywords $Fe(phen)_2 \cdot PHPIP \cdot 3ClO_4 \cdot 2H_2O$ · Fiber-optic biosensor · Fluorimetric detection · Oligonucleotides · DNA hybridization

Abbreviations

PHPIP	*p*-hydroxyphenylimidazo[f]1,10-phenanthroline
phen	1,10-phenanthroline
GCE	glassy carbon electrode
PBS	phosphate buffer solution
EB	ethidium bromide
DPV	differential pulse voltammetry
NHS-LC-biotin	sulfosuccinimidyl-6-(biotinamido) hexanoate
APTS	(3-aminopropyl) triethoxysilane
SEM	scanning electron microscopy

S.-y. Niu · S.-j. Wang · C. Shi · S.-s. Zhang (✉)
College of Chemistry and Molecular Engineering,
Qingdao University of Science and Technology,
Qingdao 266042, People's Republic of China
e-mail: shushzhang@126.com

Introduction

DNA biosensors based on nucleic acid hybridization are currently under intense investigation owing to their increasing importance in the diagnosis of disease [1, 2]. DNA biosensors are largely based on piezoelectric [3–5], electrochemical [6–8], and optical transducer [9, 10] recently. An optical fiber-based biosensor employs an optical fiber or optical fiber bundle as a platform for the biological recognition element and a conduit for excitation light and/or the resultant signal. The incorporation of an optical fiber into a biosensor results in several advantages. A light guide can carry more information than electric wire. The temperature-dependence of the fiber is lower than that of electrodes. The fiber-optic biosensor can be easily miniaturized at low cost, thus finding application for in-vivo measurements. Lately there has been a growing interest in using optical fibers for biosensing purposes [11, 12].

Nucleic acid optical fiber-based biosensing applications typically require immobilization of a single-stranded probe sequence to the surface of the optical substrate. This probe sequence is traditionally allowed to bind with a fluorescently label. As an example, an evanescent wave fiber-optic sensor for detection of 16-mer oligonucleotides in

DNA hybridization assays has been described by Abel et al. [13].

Recent techniques using intercalating dyes present the possibility of sensing without a label step. In these biosensors, an intercalating dye was attached to the probe sequence via a molecular tether [14]. Intercalating dyes have been demonstrated to exhibit an increase in their quantum yield upon integration into dsDNA. The advantage of dye tethering that no labeling step is prior to analysis of a sample, and selectivity is based on the structure of the hybrid. Using ethidium bromide (EB) as a fluorescent DNA stain, a fiber-optic DNA biosensor for fluorometric nucleic acid determination was described by Piunno et al. [15]. The intercalating dye thiazole orange also was used for detection of hybridization [14].

Most dyes including EB are all acute poison, so one recent focus in our laboratory is the development of novel fluorescent probes that are for directly monitoring of oligonucleotides. $Fe(phen)_2{\cdot}PHPIP{\cdot}3ClO_4{\cdot}2H_2O$ (*p*-hydroxyphenylimidazo[f]1,10-phenanthroline, PHPIP; 1,10-phenanthroline, phen) attracts our much attention for its low toxicity [16]. It was synthesized and used as a fluorescence indicator in our experiment. The interaction mechanism between $Fe(phen)_2{\cdot}PHPIP{\cdot}3ClO_4{\cdot}2H_2O$ and dsDNA was studied by fluorescence spectroscopy and electrochemical behavior. The ssDNA may be covalently immobilized with a highly specific orientation onto the surface of quartz optical fibers and can undergo hybridization with complementary DNA introduced into the local environment of the sensor. The hybridization with complementary strands was monitored in real time by fluorescence indicator $Fe(phen)_2{\cdot}PHPIP{\cdot}3ClO_4{\cdot}2H_2O$. The double strands formed by hybridization could be dissociated by thermal denaturalization. The specificity of nucleic acids for the target analyte can be controlled more easily than that of other biological recognition elements. Oligonucleotides are particularly well suited as biological recognition elements. They can be synthesized in a very short time and are available as single-stranded molecules. Every desired base sequence can be chosen for synthesis, making gene probe based tests widely applicable to any complementary sequence of interest. The approach using $Fe(phen)_2{\cdot}PHPIP]^{3+}$ as a fluorescence probe must lead to a particular inexpensive, simple and sensitive system. The fluorescence fiber-optic biosensor for the determination of oligonucleotides is simple and low cost.

Experimental

Apparatus and reagents

A Hitachi F-4500 fluorescence spectrophotometer (Japan) equipped with 1 cm quartz cells was used for recording and making fluorescence measurements. CHI 832 electrochemical analyzer was obtained from Shanghai Chenhua Instrument Company of China, the three electrode system consisted of the glassy carbon electrode as the working electrode, Ag/AgCl as the reference electrode and a platinum wire as the auxiliary electrode. 3 mL plastic cuvette and Y style quartz optical fiber (Φ 3.6 mm) was purchased from Peking Glass Research Institute R&D Center. JSM-6700F Field Emission Scanning Electron Microscope. The pH of all solutions was measured by a Model pHS-3D digital acidometer (Shanghai Leici Factory).

Sulfosuccinimidyl-6-(biotinamido) hexanoate, the affinity purified avidin, (3-aminopropyl) triethoxysilane (APTS) and Tween-20 were the products of Sigma. Salmon sperm DNA was purchased from Shanghai Huashun Biological Engineering Company ($A_{260}/A_{280}>1.8$), the concentration was determined by the ultraviolet absorption at 260 nm ($\varepsilon=6{,}600\ M^{-1}{\cdot}cm^{-1}$). Oligonucleotides (as lyophilized powder) were purchased from Peking Sanboyuanzhi Biological with the following sequences:

Immobilized probe (16-mer sequence S1):
16*B biotin-5′-CAC AAT TCC ACA CAA C-3′
Target ssDNA (16-mer sequence S2):
16*C 5′-GTT GTG TGG AAT TGT G-3′
Mismatch ssDNA (20-mer sequence S3):
20*N 5′-CTG CAA CAC CTG ACA AAC CT-3′

All oligonucleotides were dissolved in doubly distilled water and kept frozen. Other chemicals were of analytical grade without further purification. All aqueous solutions were made up in doubly distilled water.

Preparation of $Fe(phen)_2{\cdot}PHPIP{\cdot}3ClO_4{\cdot}2H_2O$

The $Fe(phen)_2{\cdot}PHPIP{\cdot}3ClO_4{\cdot}2H_2O$ was synthesized and purified as followed. Firstly, PHPIP was synthesized according to the literature [17]. Then 0.080 g PHPIP was added to a 12 mL hot solution dissolved 0.121 g $NH_4Fe(SO_4)_2{\cdot}12H_2O$ and 0.100 g phen. The mixture was refluxed for 5 h with continuously stirring. It was allowed to cool at room temperature and 1 mL sodium perchlorate solution (2.5 M) was added. The color of the solution turned mahogany immediately and solid precipitate appeared. The precipitate was recrystallized from hot water and the mahogany crystal of $Fe(phen)_2{\cdot}PHPIP{\cdot}3ClO_4{\cdot}2H_2O$ was obtained finally. The formula of the complex was shown in Fig. 1.

Fluorescence spectroscopy studies on the interaction mechanism between $[Fe(phen)_2{\cdot}PHPIP]^{3+}$ and DNA

Two hundred microliters of 4.00×10^{-4} M $Fe(phen)_2{\cdot}PHPIP{\cdot}3ClO_4{\cdot}2H_2O$ solution was firstly transferred into

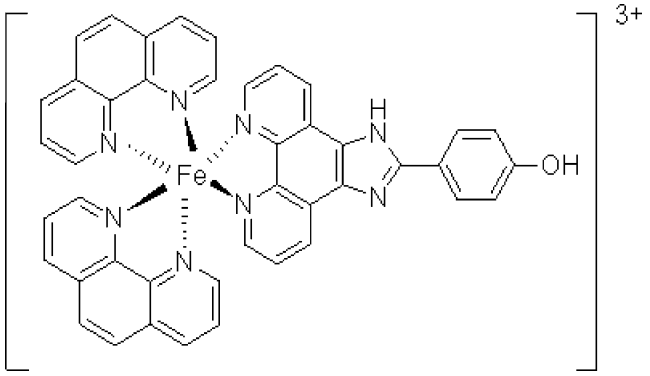

Fig. 1 Formula of $[Fe(phen)_2 \cdot PHPIP]^{3+}$

two 10 mL colorimetric tubes with 4 mL of pH 6.0 phosphate buffer solution (PBS) respectively. A certain amount of dsDNA standard solution was added into the second colorimetric tube, then diluted two colorimetric tubes to the graduation with doubly distilled water and mixed. The relative fluorescence intensity was measured at the wave length range from 320 to 500 nm with excitation wave at 270 nm. The factors that affected the fluorescent sensitivity of the method and the stability of the system were investigated.

Two milliliters PBS (pH 6.0) was transferred into two 1.0 cm quartz cells, then 5 μL of 2.54×10^{-2} M EB solution was added into one cell. 10 μL of 4.68×10^{-2} M DNA and different amounts of $[Fe(phen)_2 \cdot PHPIP]^{3+}$ solution were in turn added into two cells. The fluorescence intensities of EB, EB-DNA and different concentrations $[Fe(phen)_2 \cdot PHPIP]^{3+}$-EB-DNA were measured at the wave length range from 520 to 700 nm with excitation wave at 500 nm.

The measurements of fluorescence were made by Hitachi F-4500 fluorescence spectrophotometer. Background fluorescence has been subtracted for each value.

Electrochemical behavior of the interaction between $[Fe(phen)_2 \cdot PHPIP]^{3+}$ and DNA

One hundred microliters of 1.0×10^{-3} M $[Fe(phen)_2 \cdot PHPIP]^{3+}$ solution and 4 mL of 0.2 M PBS (pH 6.0) solution were transferred into each of the 10 mL colorimetric tubes, and then different amounts of DNA were in turn added respectively. The mixture was diluted to the mark, and the differential pulse voltammetry (DPV) of the solution were recorded using CHI 832 electrochemical analyzer; the initial potential was −0.07 V; the high potential was 1.3 V; the low potential was −0.07 V; the pulse scope was 0.05 V; the pulse extent was 0.05 s; the pulse cycle was 0.2 s; the sample interval was 0.001 V and the quiet time was 2 s.

Preparation of fiber-optic fluorescence DNA biosensor

Preparation of the DNA sensor was according to the literature [13]. Polished the surface of the Y style quartz optical fiber, then cleaned it by sonicating in 65% HNO_3 for 30 min, followed by washing steps in doubly distilled water until pH neutrality. The surface of the fiber was silanized with APTS to make it become an aminosilanized fiber. For the preparation of aminosilanized fiber, freshly cleaned fiber was immersed for 15 min in a stirred solution consisting of 600 μL of toluene, 60 μL of Tween-20, 600 μL of doubly distilled water, and 600 μL of APTS, followed rinsing it in doubly distilled water. The quartz fiber was biotinylated by immersing silanized fibers in 0.1 $mg \cdot mL^{-1}$ sulfosuccinimidyl-6-(biotinamido) hexanoate [18] in 0.1 M bicarbonate buffer (pH 8.5) for 3 h at room temperature. Then, avidin was bound to the fiber surface by incubating the biotinylated fibers overnight at 4 °C in 70 μL of a solution containing 2.5 $mg \cdot mL^{-1}$ avidin and 40 $mg \cdot mL^{-1}$ Tween-20 in 70 mM PBS (pH 7.0). Biotinylated capture probes were bound to immobilized avidin by incubating freshly prepared fiber overnight at 4 °C in 70 μL of a solution containing 100 $\mu g \cdot mL^{-1}$ 16*B in 70 mM PBS (pH 7.0), resulting in fiber surfaces as illustrated schematically in Fig. 2. Before performing an assay, the optical fibers with immobilized oligonucleotides were rinsed in doubly distilled water, blown dry with a stream of nitrogen and inserted into a plastic cuvette. The hybridization buffer is 0.2 M PBS (pH 6.0). The experimental instrumentation used to measure fluorescence intensity from quartz optical fibers coated with immobilized DNA was shown in the Fig. 3.

Hybridization and intercalation studies

The immobilized capture probe 16*B was hybridized with the different concentrations complementary strand 16*C (target DNA). The ssDNA-modified optical fiber was immersed in 0.2 M PBS (pH 6.0) containing target ssDNA for 1 h at 27 °C with stirring to form dsDNA at the fiber surface. After hybridization, the dsDNA fiber was washed with doubly distilled water to remove oligonucleotide bound nonspecifically. In order to detect nonspecific binding, a noncomplementary, 20-mer oligonucleotide 20*N was used.

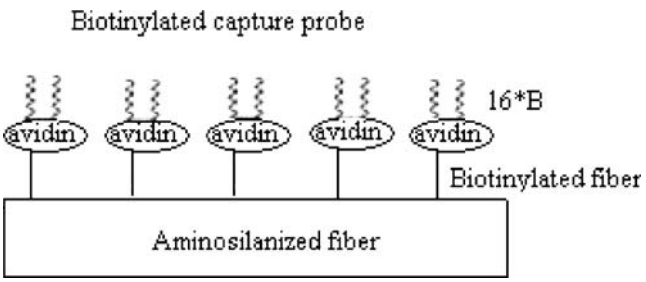

Fig. 2 Schematic diagrams of the possible arrangement of immobilized capture probes

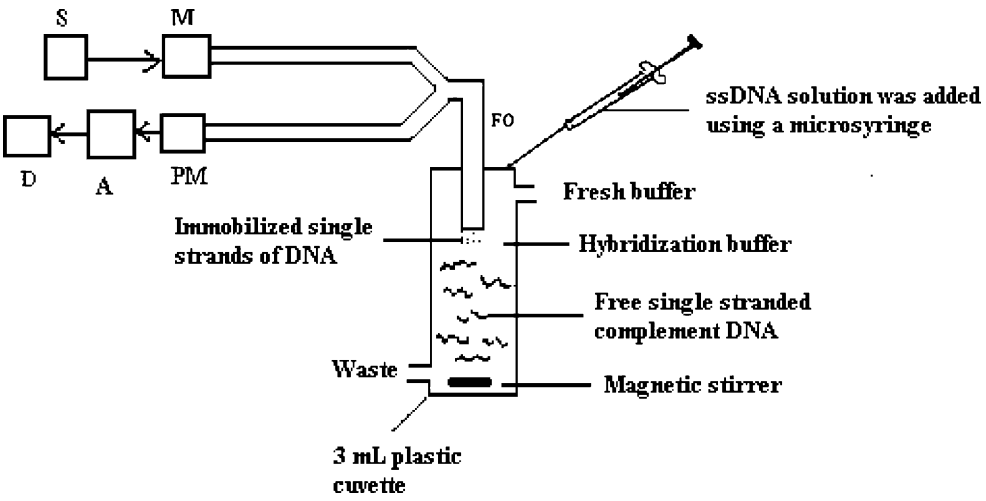

Fig. 3 Instrumentation used to measure fluorescence intensity from optic-fiber coated with immobilized DNA. (*S*: Lamp-house; *FO*: Y style quartz optical fiber; *M*: Excitation monochromator grating; *PM*: Emission monochromator grating; *A*: Monitor detector; *D*: PC)

The DNA-modified optical fiber was immersed in a 8×10^{-5} M $[Fe(phen)_2{\cdot}PHPIP]^{3+}$ aqueous solution for 15 min. Then, the optical fiber was rinsed with water, placed in a 3 mL plastic cuvette containing 0.2 M PBS (pH 6.0), and subsequently subjected to fluorescence spectroscopy studies.

Results and discussion

Spectral characteristics of $Fe(phen)_2{\cdot}PHPIP{\cdot}3ClO_4{\cdot}2H_2O$

By the infra-red spectrogram and the elemental analysis, the synthesized complex was confirmed, then fluorescence spectral characteristics were studied. $[Fe(phen)_2{\cdot}PHPIP]^{3+}$ displayed a relatively weak fluorescence emission in aqueous solution with the excitation and emission peaks at 270 nm and 368 nm, respectively. Figure 4 shows that the emission maximum peaks of the $[Fe(phen)_2{\cdot}PHPIP]^{3+}$-DNA system were similar to that of $[Fe(phen)_2{\cdot}PHPIP]^{3+}$ without DNA, but the fluorescence intensity was enhanced. These results indicated that $[Fe(phen)_2{\cdot}PHPIP]^{3+}$ was likely to enter the interior of DNA molecule and intercalate into the base pairs of DNA. The hydrophobic circumstance of DNA could help to enhance the fluorescence quantum yield of the $[Fe(phen)_2{\cdot}PHPIP]^{3+}$, which led to the fluorescence enhancement. In addition, the energetic transfer from DNA to $[Fe(phen)_2{\cdot}PHPIP]^{3+}$ could also induce the fluorescence enhancement of $[Fe(phen)_2{\cdot}PHPIP]^{3+}$ [19]. The energetic transfer from DNA to $[Fe(phen)_2{\cdot}PHPIP]^{3+}$ suggested the intercalative binding mode between $[Fe(phen)_2{\cdot}PHPIP]^{3+}$ and DNA. The experiment indicated that $Fe(phen)_2{\cdot}PHPIP{\cdot}3ClO_4{\cdot}2H_2O$ was suitable to be used as fluorescence probe for the determination of DNA.

To further investigate the possible intercalative binding of $[Fe(phen)_2{\cdot}PHPIP]^{3+}$ complex with DNA, the fluorescence spectra of EB-DNA system in the presence of $[Fe(phen)_2{\cdot}PHPIP]^{3+}$ was studied. As shown in Fig. 5, EB itself emitted weak fluorescence emission (curve 1), but a significant increase of EB fluorescence intensity was observed (curve 6) after it intercalated into the double helix of DNA molecule. Curve 2–5 showed the quenching fluorescence for EB-DNA system after different amounts of $[Fe(phen)_2{\cdot}PHPIP]^{3+}$ was added. It was likely that $[Fe(phen)_2{\cdot}PHPIP]^{3+}$ and EB compete for the same binding sites of DNA and weaken the fluorescence intensity of EB-DNA system, which further confirmed the intercalation mode of $[Fe(phen)_2{\cdot}PHPIP]^{3+}$ with DNA. It is concluded that the $[Fe(phen)_2{\cdot}PHPIP]^{3+}$ could enter the interior of DNA molecule and intercalate into the base pairs of DNA as the EB [20].

Factors that affect the fluorescent sensitivity of the method and the stability of the system were investigated. The experimental results indicated that the fluorescent

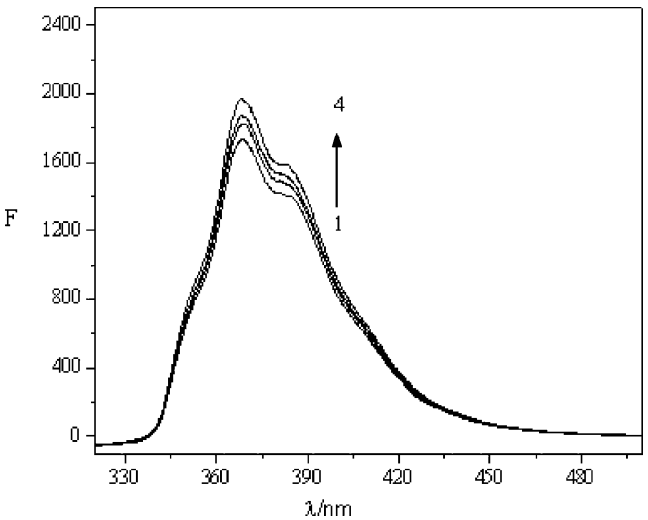

Fig. 4 Fluorescence spectra of $[Fe(phen)_2{\cdot}PHPIP]^{3+}$ with increasing concentrations of DNA. E_x=270 nm, $C_{PBS\ (pH\ 6.0)}$: 0.20 M, $C_{[Fe(phen)2{\cdot}PHPIP]3+}$: 8.00×10^{-6} M; C_{DNA}: (*1*) 0; (*2*) 3.00×10^{-8} M; (*3*) 6.00×10^{-8} M; (*4*) 1.00×10^{-7} M

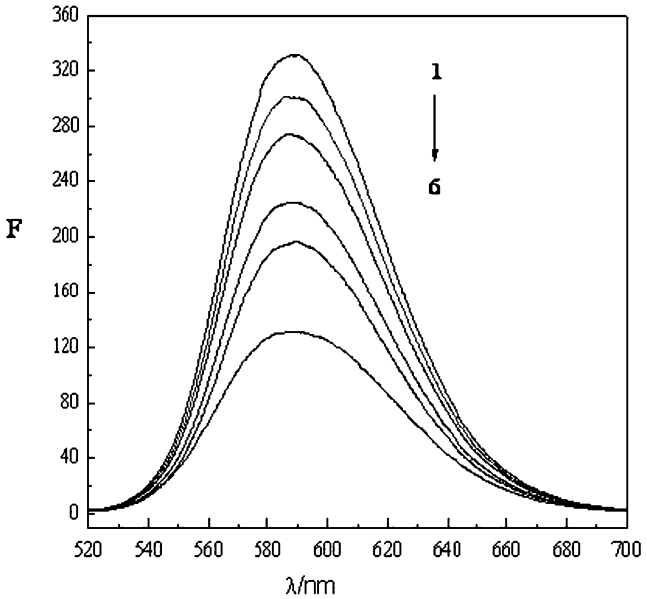

Fig. 5 The effect of $[Fe(phen)_2 \cdot PHPIP]^{3+}$ on the fluorescent intensity of EB-DNA. E_x=500 nm; $C_{PBS\ (pH\ 6.0)}$: 0.20 M; C_{DNA}: 2.34×10^{-4} M; C_{EB}: 6.35×10^{-5} M; (*1*) EB + DNA; (*2*) (*1*) + 2.00×10^{-6} M $[Fe(phen)_2 \cdot PHPIP]^{3+}$; (*3*) (*1*) + 4.00×10^{-6} M $[Fe(phen)_2 \cdot PHPIP]^{3+}$; (*4*) (*1*) + 8.00×10^{-6} M $[Fe(phen)_2 \cdot PHPIP]^{3+}$; (*5*) (*1*) + 1.20×10^{-5} M $[Fe(phen)^2 \cdot PHPIP]^{3+}$; (*6*) EB

intensity approached the maximum and constant value when the $[Fe(phen)_2 \cdot PHPIP]^{3+}$ concentration was in the range of 7.50×10^{-6}–8.50×10^{-5} M. The effect of pH on the fluorescence of the system also was studied. The fluorescence intensity reached a maximum value over the pH range 5.8–6.3. The sufficient time of the reaction was about 2 min. In this experiment, concentration of 8.00×10^{-6} M was selected and optimal pH value was at pH 6.0.

Electrochemical studies on interaction between $[Fe(phen)_2 \cdot PHPIP]^{3+}$ and DNA

The phenomena mentioned above were further studied by DPV, as shown in Fig. 6. Curve 1 in Fig. 6 was the voltammogram of the $[Fe(phen)_2 \cdot PHPIP]^{3+}$ solution, while curves 2 and 3 were results when $[Fe(phen)_2 \cdot PHPIP]^{3+}$ interacted with different concentrations of dsDNA for 2 min. The peak current decreased with increasing of dsDNA and came to a constant value and remained unchanged when dsDNA concentration came to some extent, indicating the interaction between $[Fe(phen)_2 \cdot PHPIP]^{3+}$ and dsDNA was completed.

To determine the composition of salmon sperm DNA with $[Fe(phen)_2 \cdot PHPIP]^{3+}$ and the equilibrium constant of the binding reaction, the experiments were performed as the previously literature [21, 22].

To study the binding ratio and binding constant between $[Fe(phen)_2PHPIP]^{3+}$ and DNA, it was assumed that interaction of DNA and $[Fe(phen)_2PHPIP]^{3+}$ only produced one single complex: DNA-n$[Fe(phen)_2PHPIP]^{3+}$, as shown in the following equation.

$$DNA + n[Fe(phen)_2PHPIP]^{3+} \rightleftharpoons DNA\text{-}n[Fe(phen)_2PHPIP]^{3+} \quad (n=1, 2, 3, \ldots \text{ or } 1/2, 1/3, \ldots)$$

The equilibrium constant β could be expressed as Eq. 1 and Eqs. 2–7 could be deduced, where C_{DNA} denoted the analytical concentration of DNA while [DNA] and [ML] represented the equilibrium concentration of DNA and the metal complex.

$$\beta = \frac{[DNA - nML]}{[DNA][ML]^n} \tag{1}$$

$$\Delta I_{pa,max} = KC_{DNA} \tag{2}$$

$$\Delta I_{pa} = K[DNA - nML] \tag{3}$$

$$[DNA] + [DNA - nML] = C_{DNA} \tag{4}$$

$$\Delta I_{pa,max} - \Delta I_{pa} = K(C_{DNA} - [DNA - nML]) \tag{5}$$

$$\Delta I_{pa,max} - \Delta Ipa = K[DNA] \tag{6}$$

$$\frac{1}{\Delta I_{pa}} = \frac{1}{\Delta I_{pa,max}} + \frac{1}{\beta \Delta I_{pa,max}[ML]^n} \tag{7}$$

According to the Eq. 7, different *n* might result in different curves of versus $[[Fe(phen)_2PHPIP]^{3+}]^{-n}$. With the suitable *n*, the curve of versus $[[Fe(phen)_2PHPIP]^{3+}]^{-n}$ should be a straight line if there was only one complex formed when $[Fe(phen)_2PHPIP]^{3+}$ bounded to DNA. From

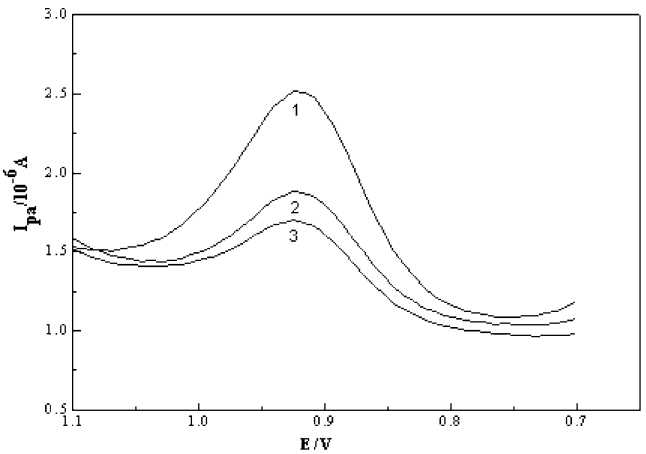

Fig. 6 Differential pulse voltammograms of $[Fe(phen)_2 \cdot PHPIP]^{3+}$. $C^{3+}_{[Fe(phen)2PHPIP]}$: 1.00×10^{-4} M; CDNA: (*1*) 0, (*2*) 4.00×10^{-4} M, (*3*) 1.00×10^{-3} M

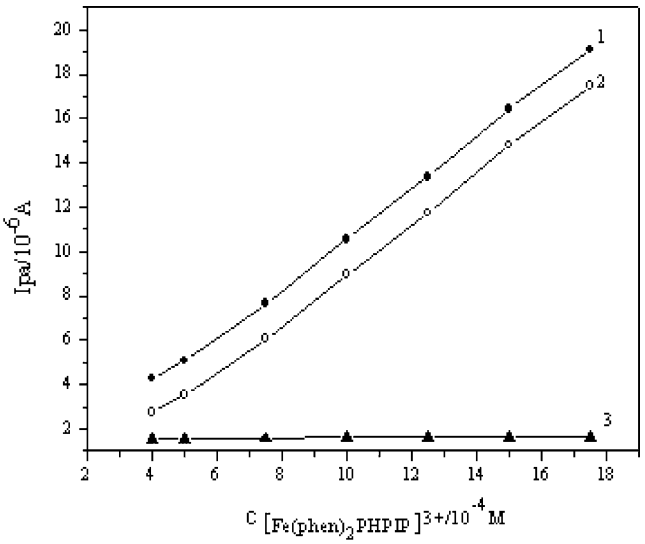

Fig. 7 Relationship between I_{pa1}, I_{pa2} and ΔI_{pa} with the concentration of $[Fe(phen)_2PHPIP]^{3+}$ (*1*) without DNA, (*2*) 1.00×10^{-4} M DNA, (*3*) $\Delta I_{pa}=I_{pa1}-I_{pa2}$

the slope and intercept of the straight line, the binding constant β could be calculated and the *n* could be regarded as the binding ratio.

The dependence of the oxidation peak current (I_{pa}) on the analytical concentration of $[Fe(phen)_2PHPIP]^{3+}$ in the absence (curve 1) and presence (curve 2) of DNA was shown in Fig. 7. The relationship between ΔI_{pa} ($\Delta I_{pa}=I_{pa1}-I_{pa2}$) and the analytical concentration of $[Fe(phen)_2PHPIP]^{3+}$ was also displayed (curve 3). The curves of ΔI_{pa}^{-1} versus $[[Fe(phen)_2PHPIP]^{3+}]^{-0.5}$, ΔI_{pa}^{-1} versus $[[Fe(phen)_2PHPIP]^{3+}]^{-1}$, ΔI_{pa}^{-1} versus $[[Fe(phen)_2PHPIP]^{3+}]^{-2}$ were displayed in

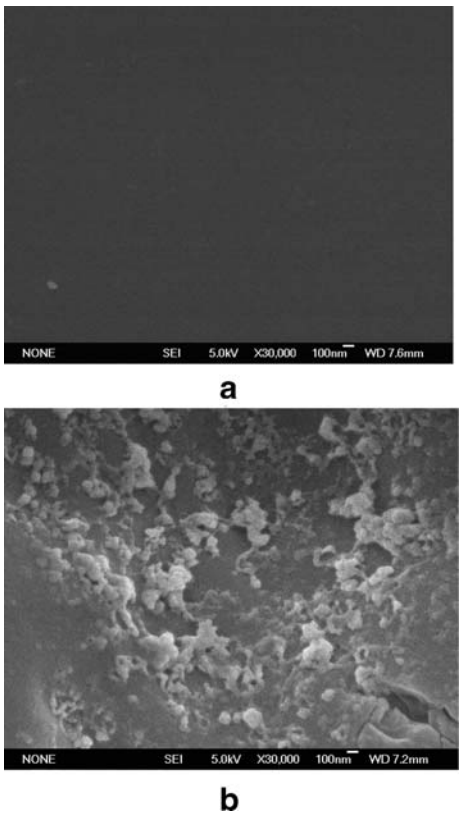

Fig. 9 Scanning electron microscopy micrograph of bare optical fiber (**a**, 100 nm) and optical fiber with biotinylated ssDNA via avidin. (**b**, 100 nm)

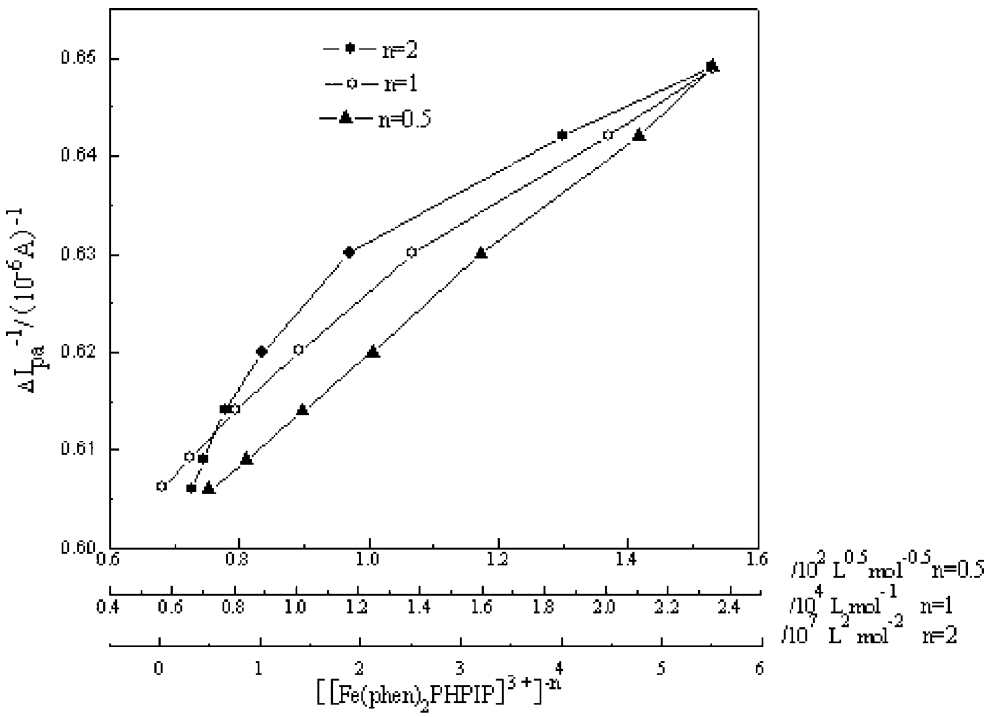

Fig. 8 The relationship curves of ΔI_{pa}^{-1} versus $[[Fe(phen)_2PHPIP]^{3+}]^{-n}$

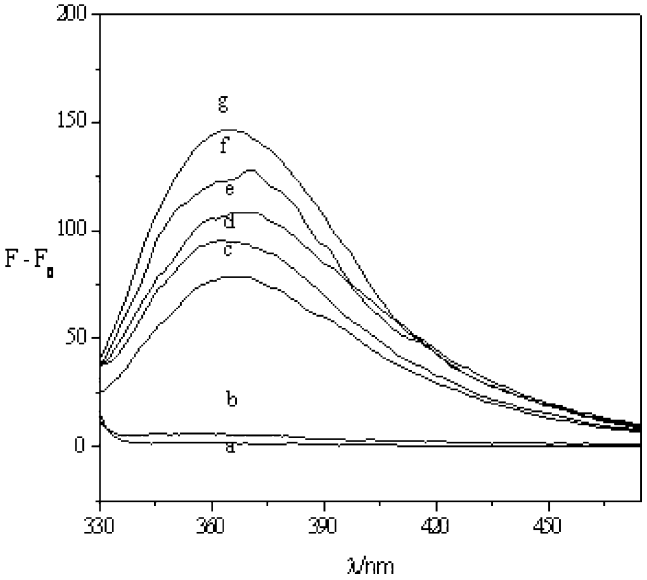

Fig. 10 Fluorescent intensity of $Fe(phen)_2 \cdot PHPIP \cdot 3ClO_4 \cdot 2H_2O$ on (*a*) S_1/optical fiber; (*b*) S_1–S_3/optical fiber; and (*c*–*g*) S_1–S_2/optical fiber. $C_{PBS\ (pH\ 6.0)}$: 0.20 M, $C^{3+}_{[Fe(phen)2PHPIP]}$: 8.0×10^{-6} M, (*c*) Cs_2: 1.98×10^{-6} M; (*d*) Cs^2: 2.96×10^{-6} M; (*e*) Cs^2: 3.92×10^{-6} M; (*f*) Cs^2: 4.89×10^{-6} M; (*g*) Cs^2: 58.25×10^{-6} M

Fig. 8, where $[[Fe(phen)_2PHPIP]^{3+}]$ represented the equilibrium concentration of $[Fe(phen)_2PHPIP]^{3+}$ and calculated from data in Fig. 1. For n=2 and 1, the curves bent down and up respectively. While for n=0.5, the curve was a straight line indicating the forming of a 1:2 association between $[Fe(phen)_2\ PHPIP]^{3+}$ and DNA. From the slope and intercept of the straight line, the binding constant β was calculated to be $1.02\times10^3\ M^{-0.5}$, which was corresponding to the equation below.

$$DNA + 1/2[Fe(phen)_2PHPIP]^{3+} \rightleftharpoons DNA - 1/2[Fe(phen)_2PHPIP]^{3+}$$

Scanning electron microscopy images of the DNA fluorescence biosensor surface at different steps

Scanning electron microscopy images show the difference between the bare optical fiber (Fig. 9a) and optical fiber with immobilized ssDNA via avidin (Fig. 9b). In Fig. 9a, the bare optical fiber appeared as polished and homogenous. In Fig. 9b, the white coloured area indicated that a biotinylated ssDNA was modified onto optical fiber's surface.

The selectivity of the DNA fluorescence biosensor

Since $[Fe(phen)_2 \cdot PHPIP]^{3+}$ could intercalate into the bases of dsDNA, the DNA biosensor using $[Fe(phen)_2 \cdot PHPIP]^{3+}$ as fluorescence hybridization indicator to detect target DNA was developed. Figure 10 shows the fluorescence intensity for the signal of $[Fe(phen)_2 \cdot PHPIP]^{3+}$ at ssDNA probe immobilized optical fiber (Fig. 10, curve a), after hybridization with some amount of complementary DNA

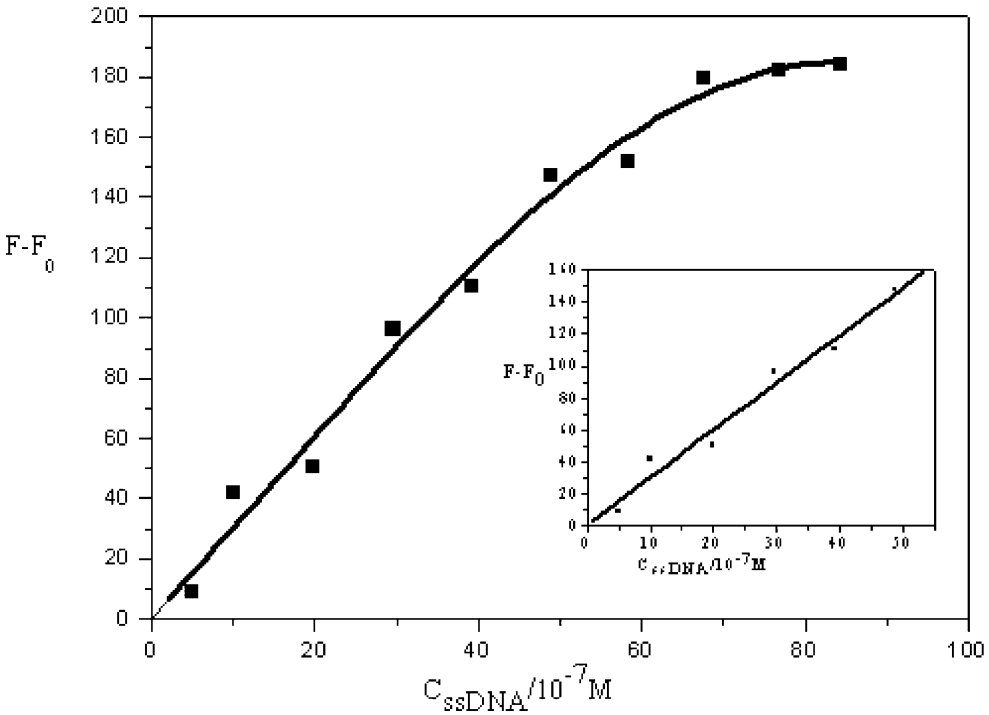

Fig. 11 Calibration curve for DNA. $C_{PBS\ (pH\ 6.0)}$: 0.20 M, $C^{3+}_{[Fe(phen)2PHPIP]}$: 8.0×10^{-6} M

sequence (Fig. 10, curve c,d,e,f & g), and the non-complementary sequence (Fig. 10, curve b).

There was no response at S_1/fiber-optic and S_1–S_3/fiber-optic, while the peak of the S_1–S_2/fiber-optic appeared at about 368 nm. The enhancement of fluorescence indicated that more $[Fe(phen)_2 \cdot PHPIP]^{3+}$ molecules were reconcentrated on the S_1–S_2/fiber-optic surface for the binding interaction between the immobilized dsDNA and the complex. These results suggested that the target sequence of the ssDNA fragment could be recognized using $[Fe(phen)_2 \cdot PHPIP]^{3+}$ as a fluorescence indicator.

The sensitivity of the hybridization assay was investigated by varying the target oligonucleotide concentration according to the procedure described. The different fluorescence intensities obtained after hybridization of probe with target DNA. The fluorescence intensities were in proportion to the concentration of the target sequence used over the range from 4.98×10^{-7} to 4.88×10^{-6} M (Fig. 11). The regression equation was $y=1.0024+2.9527x$ (y was the fluorescence intensity of $[Fe(phen)_2 \cdot PHPIP]^{3+}$, background fluorescence has been subtracted. x was the concentration of the target DNA, 10^{-7} M), and the regression coefficient of the linear curve γ was 0.9835. A detection limit of 1.08×10^{-7} M of the complementary oligonucleotides could be estimated using 3σ (where σ is the standard deviation of the blank solution, $n=11$).

Reusability of the fiber-optic biosensor

Besides sensitivity, reusability is also an important feature for biosensors. The designed fiber-optic biosensor could be conveniently regenerated. The regeneration was achieved by thermal denature. The temperature was adjusted to 27 °C for the hybridization, the fiber surface was heated to a temperature of 69 °C for 20 min for the thermal regeneration. After the regeneration, a new assay cycle was started after a 5 min equilibration step.

Conclusion

In this work, $Fe(phen)_2 \cdot PHPIP \cdot 3ClO_4 \cdot 2H_2O$ was used as a novel fluorescence hybridization indicator to detect oligonucleotides. This approach provides a measured detection limit of 1.08×10^{-7} M. That novel hybridization indicator could provide a simple, rapid, low toxicity and reusable detection. The desired oligonucleotides base sequence could be chosen for synthesis, making gene probe based tests widely. The use of $Fe(phen)_2 \cdot PHPIP \cdot 3ClO_4 \cdot 2H_2O$ as a fluorescence probe must lead to a particular inexpensive, simple and sensitive system.

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References

1. Wang J (1999) Electroanalysis and biosensors. Anal Chem 71 (12):328–332
2. Hashimoto K, Ito K, Ishimori Y (1994) Sequence-specific gene detection with a gold electrode modified with DNA probes and an electrochemically active dye. Anal Chem 66(21):3830–3833
3. Wang H, Zeng H, Liu ZhM, Yang YH, Deng T, Shen GL, Yu RQ (2004) Immunophenotyping of acute leukemia using an integrated piezoelectric immunosensor array. Anal Chem 76(8):2203–2209
4. Baltus RE, Carmon KS, Luck LA (2007) Quartz crystal microbalance (QCM) with immobilized protein receptors: Comparison of response to ligand binding for direct protein immobilization and protein attachment via disulfide linker. Langmuir 23 (7):3880–3885
5. Minunni M, Tombelli S, Fonti J, Spiriti MM, Mascini M, Bogani P, Buiatti M (2005) Detection of fragmented genomic DNA by PCR-free piezoelectric sensing using a denaturation approach. J Am Chem Soc 127(22):7966–7967
6. Millan KM, Spurmanis AJ, Mikkelsen SR (1992) Covalent immobilization of DNA onto glassy carbon electrodes. Electroanalysis 4(10):929–932
7. Millan KM, Mikkelsen SR (1993) Sequence-selective biosensor for DNA based on electroactive hybridization indicators. Anal Chem 65(17):2317–2323
8. Mlllan KM, Saraullo A, Mlkkelse SR (1994) Voltammetric DNA biosensor for cystic fibrosis based on a modified carbon paste electrode. Anal Chem 66(18):2943–2948
9. Piunno PAE, Krull UJ, Hudson RHE, Damha MJ, Cohen H (1994) Fiber optic biosensor for fluorimetric detection of DNA hybridization. Anal Chim Acta 288:205–214
10. Vo-Dinh T, Houck K, Stokes DL (1994) Surface-enhanced raman gene probes. Anal Chem 66(20):3379–3383
11. Wolfbeis OS (2004) Fiber-optic chemical sensors and biosensors. Anal Chem 76(12):3269–3284
12. Monk DJ, Walt DR (2004) Optical fiber-based biosensors. Anal Bioanal Chem 379:931–945
13. Abel AP, Weller MG, Duveneck GL, Ehrat M, Widmer HM (1996) Fiber-optic evanescent wave biosensor for the detection of oligonucleotides. Anal Chem 68(17):2905–2912
14. Wang X, Krull UJ (2002) Tethered thiazole orange intercalating dye for development of fibre-optic nucleic acid biosensors. Anal Chim Acta 470:57–70
15. Piunno PAE, Krull UJ (1995) Fiber-optic DNA sensor for fluorometric nucleic acid determination. Anal Chem 67(15): 2635–2643
16. Bochner BR, Huang HC, Schieven GL, Ames BN (1980) Positive selection for loss of tetracycline resistance. J Bacteriol 143(2): 926–933
17. Liu Y, Duan ZhY, Zhang HY, Jiang XL, Han JR (2005) Selective binding and inverse fluorescent behavior of magnesium ion by podand possessing plural imidazo[4,5-*f*]-1,10-phenanthroline groups and its Ru(II) complex. J Org Chem 70(4):1450–1455
18. Diamandis EP, Christopoulos TK (1991) The biotion-(strept) avidin system: principles and applications in biotechnology. Clin Chem 37(5):625–645
19. Haq I, Lincoln P, Suh D, Norden B, Chowdhry BZ, Chaires JB (1995) Interaction of Δ- and Λ-$[Ru(phen)_2DPPZ]^{2+}$ with DNA: a

calorimetric and equilibrium binding study. J Am Chem Soc 117 (17):4788–4796
20. Kang JW, Su BQ, Li ZF, Wu HX, Lu XQ (2006) Study on the interaction between rutin-Co(II) coordination compound and DNA. Chem Res Appl 18(4):360–364
21. Zhang SS, Niu SY, Qu B, Jie GF, Xu H, Ding CF (2005) Studies on the interaction mechanism between hexakis(imidazole) manganese(II) terephthalate and DNA and preparation of DNA electrochemical sensor. J Inorg Biochem 99:2340–2347
22. Niu SY, Zhang SS, Wang L, Li XM (2006) Hybridization biosensor using di(1,10-phenanthroline)(imidazo[f] 1,10-phenanthrolin) Cobalt(II) as electrochemical indicator for detection of human immunodeficiency virus DNA. J Electroanal Chem 597:111–118