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Highly sensitive fluorescent sensor for mercury ion based on photoinduced charge transfer between fluorophore and π-stacked T–Hg(II)–T base pairs

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ABSTRACT

A novel and simple oligodeoxyribonucleotide-based sensor with single fluorophore-labeled for mercury ion sensing was reported. An oligodeoxyribonucleotide poly(dT) was labeled with fluorescein as donor. Based on the specific binding of Hg(II) to T–T mismatch base pairs, the formation of π-stacked [T–Hg(II)–T] with "sandwich" structure on the addition of Hg(II) ions facilitates the electron transfer via photoinduced charge transfer (PCT), which creates an additional nonradiative decay channel for excited fluorophore and triggers the fluorescence to be quenched. The π-stacked [T–Hg(II)–T] functioned not only as mercury ion recognition but also as an electron acceptor to quench the donor. A linear relationship was observed over the range of 0–1.0 μM with the detection limit of 20 nM for mercury ions. The fluorescence quenching phenomenon and quenching mechanism, reliability and selectivity of the system were investigated in detail.

1. Introduction

Recently, the interactions of metal ions with nucleic acids, proteins are recognized as an important topic [1], as they are involved in RNA folding, mechanisms of ribozymes action [2], gene mutations [3] and the design of biomolecular devices with metal cofactors [4]. Recent efforts to align metal ions along DNA helix have demonstrated that metal ions, such as Cu(II), Zn(II), Ag(I), Ni(II), Co(II), bind to nucleobases, deoxyribose, or phosphodiester backbone and have provided potential applications of the metallised DNA (M-DNA) to nanomaterials and biosensors [5–7]. In this regard, mercury ions preferentially bind to thymine–thymine (T–T) mismatch base pairs, in which imino protons of the bases are replaced by Hg(II), to generate stable mercury-mediated base pairs [T–Hg(II)–T] [8–11]. The formation of stable DNA π-stacks containing mercury-mediated thymine–thymine pairs suggests a new approach to the development of M-DNA.

In the past few years this preferential binding property of thymidines has successfully been applied in the design of oligonucleotide-based mercuric ion fluorescent sensors [12–19]. Combining with the high extinction coefficient and unique distance dependent optical properties of DNA–Au nanoparticles, colorimetric sensing systems [20–24] for mercury have also been demonstrated in aqueous media. Furthermore, UO_2^{2+}-dependent DNAzyme [25] and self-cleaving DNAzyme [26] for mercury ions have also been reported. In these sensing systems, the thymidine–Hg(II)–thymidine coordination chemistry only played a role in mercury ion recognition.

Till now, research related to mercury-mediated thymine–thymine pairs [T–Hg(II)–T] has mostly been focused on the structure confirmation [8–11] and application of this coordination chemistry to recognise mercury ions [12–26]. Nevertheless, effect of [T–Hg(II)–T] on DNA-mediated charge transfer (CT) efficiency has rarely been explored [27–29]. During our studies of thymine derivatives we unexpectedly found that the fluorescence of single fluorophore-labeled oligodeoxyribonucleotide containing consecutive thymines can be quenched on the addition of mercury ion. Encouraged by this finding, we designed an oligodeoxyribonucleotide-based sensor for mercury ions in aqueous solution with high sensitivity and selectivity. At the presence of Hg(II) ions, the formation of π-stacked [T–Hg(II)–T] with "sandwich" structure facilitates the electron transfer via photoinduced charge transfer (PCT), which creates an additional nonradiative channel for the excited fluorophore and triggers the fluorescence to be quenched. The π-stacked [T–Hg(II)–T] functioned not only as mercury ion recognition but also as an electron acceptor to quench the fluorescence. The fluorescence quenching phenomenon and quenching mechanism, reliability and selectivity of the system were investigated in detail in this study. To the best of our knowledge, this is the first example of single fluorophore-labeled oligodeoxyribonucleotide to study fluorescence phenomena in M-DNA and making use of M-DNA as electron acceptor for metal ions sensing.

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Table 1
Sequence of oligonucleotides used in this study.

$(T)_7$–F	Fluorescein-5′-TTTTTTT-3′
C-DNA	5′-TTTTTTT-3′

2. Experimental

2.1. Chemicals

All oligonucleotides were obtained from Shanghai Sangon Biological Engineering Technology & Services Co., Ltd. of China. The sequence of oligonucleotides was listed in Table 1. The $Hg(NO_3)_2$ stock solution (1.0×10^{-3} M) was prepared by measuring the appropriate amount of $Hg(NO_3)_2{\cdot}H_2O$ into a 250 mL volumetric flask and adding 2 or 3 drops of concentrated nitric acid, followed by deionized water. All chemicals used were analytical grade or better. All buffers were prepared by using metal free reagents and water was purified with MilliQ purification system (Millipore).

2.2. Fluorescence measurements

The fluorescence measurements were performed at room temperature on a Varian Cary Eclipse Fluorescence Spectrophotometer except specific indication. Before conducting fluorescence measurements, aliquots of Hg(II) aqueous(0–50.0 μM) were added separately to 10 mM 3-(N-morpholino) propanesulfonic acid (MOPS, pH 6.8) buffer containing 20 nM $(T)_7$–F and equilibrated for 24 h. The emission spectra were collected from 500 to 600 nm with the excitation wavelength of 480 nm.

2.3. Fluorescence lifetime measurements

Fluorescence lifetime measurements were obtained using an Edinburgh Analytical Instruments FL/Fs920 combined TCSPC lifetime spectrometer and spectrofluorimeter system (Edinburgh Instruments Ltd.). Fluorescence lifetime data were obtained with a free running discharge flash lamp (nF900 Nanosecond Flash lamp) operating at 7.0 kV, 0.40 bar, and a frequency of 40 kHz. The decay profiles were collected over 1024 channels of the multichannel analyzer with a time-channel of 0.048 ns until 1100 counts were collected in the peak channel. The instrument response function (IRF) was measured after recording the sample nanosecond emission graph collection (1100 counts in the peak channel) with a light scattering solution (LUDOX Colloidal silica, Sigma) in the sample compartment. The IRF and decay profiles were collected with a bandwidth of 1.0 nm for both the excitation and emission monochromators. The fluorescence decay data were performed with 273 nm excitation wavelength and 519 and 527 nm emission wavelength for $(T)_7$–F and Hg(II)-mediated $(T)_7$–F, respectively and were fitted to give the fluorescence lifetimes of $(T)_7$–F and Hg(II)-mediated $(T)_7$–F. The reduced χ^2 and residual plots were used to judge the quality of the fits. The χ^2 values of these fittings were kept as close to 1 as possible.

3. Results and discussion

3.1. Strategy of sensing

The sensor, $(T)_7$–F, consists of 7-mer thymine carrying a fluorescent residue (fluorescein, F) at the 5′-termini with an intervening six-carbon spacer. As a proof-of-concept experiment, the number of thymine in the prototype is seven but not limited to seven. Fluorescein was selected as the fluorophore due to its superior brightness ($\Phi \approx 1$, high ε) and water solubility [30]. Fluorescence spectra of $(T)_7$–F at the absence and presence of $Hg(NO_3)_2$ are shown in Fig. 1.

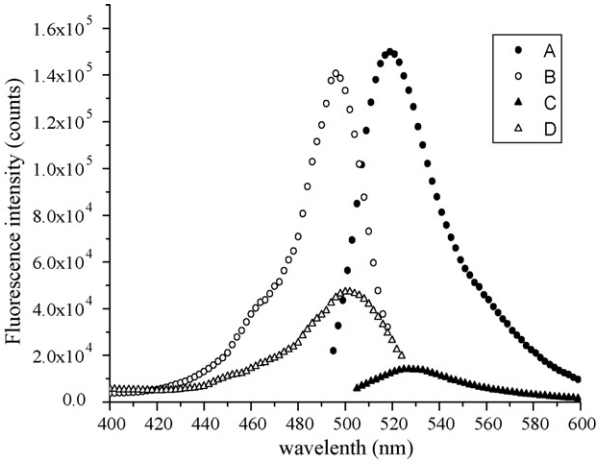

Fig. 1. Fluorescence spectra of $(T)_7$–F at the absence and presence of $Hg(NO_3)_2$ curves (A) and (B) are emission and excitation spectra of 20 nM $(T)_7$–F at the absence of $Hg(NO_3)_2$, respectively; curves (C) and (D) are emission and excitation spectra of 20 nM $(T)_7$–F at the presence of 10 μM $Hg(NO_3)_2$, respectively.

The emission profile of $(T)_7$–F is identical to that of free fluorescein, except a spectral shift [31] to longer wavelength about 6 nm and a decrease in quantum yield [32]. When $Hg(NO_3)_2$ was added, the fluorescence decreased with the red shift of the excitation and emission wavelength from 496 to 501 nm and 519 to 527 nm, respectively.

For the sensor does not contain guanosine residue, the fluorescence quenching via photoinduced electron transfer due to the good electron-donating property of guanosine [33] could be excluded. Furthermore, the absorption of [T–Hg(II)–T] pairs (at 240–300 nm [9]) does not overlap with the emission of fluorescein, the quenching by Förster fluorescence resonance energy transfer could also be ruled out. In addition, in the same condition Hg(II) ions do not quench the fluorescence of free fluorescein which was not incorporated into the oligodeoxyribonucleotide. For mercury ions preferentially bind to T–T pairs, when $(T)_7$–F was exposed to Hg(II) ions, Hg(II) ions bind directly to N_3 of thymidine in place of the imino proton and bridge two thymidine residues [8–11] to form the [T–Hg(II)–T] pairs between two molecules of the sensor. The structure of [T–Hg(II)–T] [8–11] is shown in Fig. 2(a). Therefore, two molecules of $(T)_7$–F form "sandwich" structure with Hg(II) ions with the help of [T–Hg(II)–T] pairs. The interaction of neighboring [T–Hg(II)–T] base pairs considerably increased the electron hole transfer efficiency [27] and facilitates electron transfer over long distances along the helix [34–36]. Thus π-stacked [T–Hg(II)–T] accepts and mediates the electron transfer along the DNA, creates an additional nonradiative decay channel via PCT for the excited

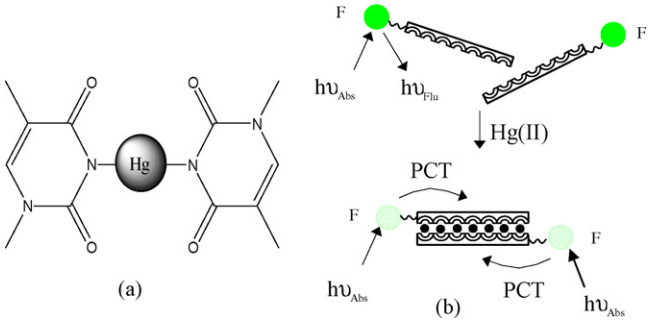

Fig. 2. (a) The structure of [T–Hg(II)–T]. (b) The rationale of the prototype (F: fluorescein; $h\upsilon_{Abs}$: fluorophore was excited at absorption wavelength; $h\upsilon_{Flu}$: fluorescence emission; PCT: photoinduced charge transfer).