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ORIGINAL PAPER

A novel method for the analysis of calf thymus DNA based on CdTe quantum dots-Ru(bpy)$_3^{2+}$ photoinduced electron transfer system

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Abstract A method has been developed for the rapid determination of calf thymus (ct) DNA that is based on the photoinduced electron transfer (PET) that occurs between CdTe quantum dots and the ruthenium(II)tris-bipyridyl complex. The latter quenches the photoluminescence (PL) of the quantum dots through PET. The Stern-Volmer quenching constant is 2,500 L mol^{-1}. The intensity of the PL the system is recovered in the presence of ct DNA, and relative recovered PL intensity is linearly proportional to the concentration of ct-DNA. The dynamic range is from 17 μM to 1.5 mM of DNA, and the detection limit (at S/*N*=3) is 5.7 μM. The relative standard deviation (at 0.5 mM of ct-DNA) is 4.1% (*n*=11). A possible reaction mechanism is discussed.

Keywords Quantum dots · Calf thymus DNA · Photoinduced electron transfer · Ru(bpy)$_3^{2+}$

Introduction

Deoxyribonucleic acid (DNA) is a kind of very important bimolecular as the carrier of genetic information and the material basis of gene expression. Due to its significant functions in life process, several methods have been founded to the determination of DNA including fluorescence [1, 2], electrochemistry [3–5], chemiluminescence [6] etc. Among them, fluorescence-based analytical method has been widely used because of its high sensitivity, good repeatability and accuracy. For example, Xu and co-workers report a cationic red-region fluorescent dye (Nile Blue) for the determination of DNA and RNA [7]. Li et al. have designed a series of pi-conjugated fluorescent dyes sensing DNA based on the fluorescence resonance energy transfer mechanism [8]. However, fluorescent dyes have some obvious shortcomings, such as narrow excitation spectra, broad emission spectra and poor photostability, which limit their analytical applications.

Compared with organic fluorescent dyes, quantum dots (QDs) have some excellent fluorescence properties, such as their narrow emission spectra, broad absorption spectra and stability against photo-bleaching [9–12]. Therefore, QDs-based fluorescent probe has attracted much attention in DNA analysis. For example, Wang and co-workers have designed an ultrasensitive nanosensor to detect DNA based on fluorescence resonance energy transfer between quantum dots and dye [13]. He et al. have developed a new fluorescent ensemble probe for the determination of complementary double-stranded DNAs (dsDNA) by using thioglycolic acid (TGA) capped CdTe QDs [14]. And recently, Raymo's group has developed a photoinduced electron transfer (PET)-based QDs sensors [15]. The fluorescence of QDs is quenched by a quencher absorbed on the surface of the QDs through electrostatic association, and the intensity would be restored when added a receptor which could react with and remove the quencher.

Based on the above motif, we aim to develop a QDs sensor to detect calf thymus (ct) DNA. The photoluminescence (PL) intensity of QDs was quenched by a Ru(bpy)$_3^{2+}$ complex, a simple and commercial reagent, through PET

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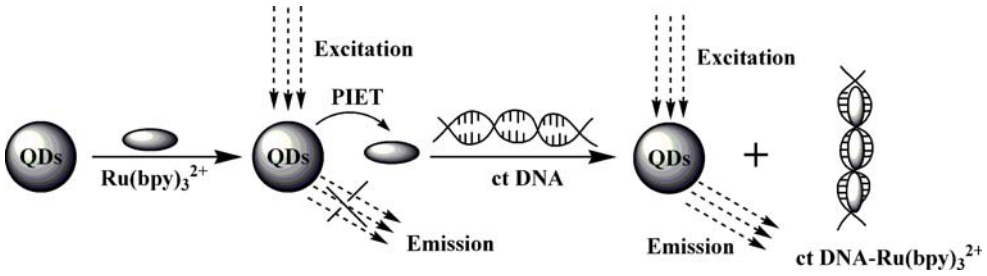

Scheme 1 Schematic diagram of the proposed mechanism to detect ct DNA

process. ct DNA can react with a $Ru(bpy)_3^{2+}$ and remove it from the surface of QDs. Then, the PL intensity of the QDs-$Ru(bpy)_3^{2+}$ system is restored. On the basis of this interesting phenomenon, the CdTe QDs-$Ru(bpy)_3^{2+}$ system to detect ct DNA has been designed (Scheme 1).

Experimental

Apparatus

The ultraviolet and visible (UV-vis) absorption spectra were obtained with 1.0 cm×1.0 cm quartz cuvette on a Thermo Nicolet Corporation Model evolution 300 (America; http://www.thermonicolet.com). All PL spectra were recorded by a Perkin-Elmer LS-55 fluorescence spectrometer (America; http://www.perkinelmer.com) equipped with a 20 kW xenon discharge lamp as a light source.

Reagents

ct DNA and $Ru(bpy)_3^{2+}$ (bpy=2.2-bipyridine) complex were purchased from Sigma-Aldrich (America; http://www.sigmaaldrich.com/china-mainland.html). $CdCl_2$•$2.5H_2O$ (99.0%) and $NaBH_4$ (96.0%) were obtained from Tianjin Chemical Reagent Plant (Tianjin, China; http://www.reagent-1.com). Glutathione (GSH) was got from Sanland-chem. International Inc. (Xiamen, China; http://www.sanland-chem.com.cn). All other common chemicals used were of analytical reagent grade. All solutions were prepared with doubly deionized water.

Preparation of GSH-capped CdTe QDs

In a typical synthesis, $CdCl_2$•$2.5H_2O$, (2.5×10^{-4}mol) was dissolved in 25 mL water in a three-necked flask, and GSH (3.0×10^{-4}mol), trisodium citrate dihydrate, Na_2TeO_3 and $NaBH_4$ (2.4×10^{-4}mol) were added at pH10.5 with vigorous stirring. The mixture was refluxed at 100°C and the growth of GSH-capped CdTe QDs took place immediately. All reactions were carried out under an air atmosphere.

Procedures for detection of ct DNA using CdTe QDs-Ru(bpy)$_3^{2+}$ system

In our experiments, the PL spectra were recorded with excitation and emission slits of 10 nm and excitation wavelength of 390 nm. First, GSH-capped CdTe QDs was diluted with phosphate buffer solution, and a certain volume of $Ru(bpy)_3^{2+}$ solution was added into the diluted colloids. Then ct DNA with different concentrations was added into above system for PL detection.

Results and discussion

Characterization of GSH-capped CdTe QDs

The UV-vis absorption (a) and PL (b) spectra of GSH-capped CdTe QDs are presented in Fig. 1. The curve (a) indicates that the absorbance maximum of GSH-capped CdTe QDs is centered at 565 nm. The maximum emission is observed at 617 nm (b). Simultaneously, a good monodisperse of GSH-capped CdTe QDs can be obtained from the symmetric and narrow PL peak.

The particle size was described by the following formula:

$$D = \left(9.8127\times10^{-7}\right)\lambda^3 - \left(1.7147\times10^{-3}\right)\lambda^2 + (1.0064)\lambda - 194.84$$

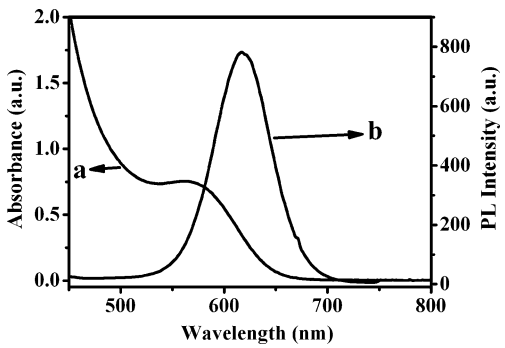

Fig. 1 UV-vis absorption (**a**) and PL (**b**) spectra of GSH-capped CdTe QDs in 10 mmol L^{-1} phosphate buffer solution of pH9.0

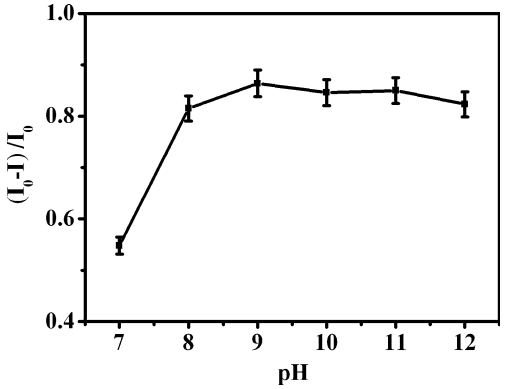

Fig. 2 Effect of pH on the PL intensity of GSH-Capped CdTe QDs-$Ru(bpy)_3^{2+}$ system

Where D (nm) is the particle size of CdTe QDs, λ (nm) is the wavelength of the first excitonic absorption peak of the corresponding sample [16], and the particle size of the CdTe QDs was calculated to be ca. 3.4 nm.

Effect of pH value

The pH value played an important role in the interaction of CdTe QDs with other molecules [17–19]. Therefore, the effect of pH value of phosphate buffer solution on the PL intensity of GSH-capped CdTe QDs-$Ru(bpy)_3^{2+}$ system was investigated. It was found that GSH-capped CdTe QDs showed extraordinarily weak PL signals under acidic conditions. Further research indicated that the PL signals of the reaction between $Ru(bpy)_3^{2+}$ and GSH-capped CdTe QDs were also very weak. As shown in Fig. 2, the relative decreased PL intensity increased at the range of pH7.0–9.0, after the pH value of 9.0, the relative decreased PL intensity was almost stable. So in the experiment, 10 mmol L^{-1} phosphate buffer solution of pH9.0 has been chosen for further experiments.

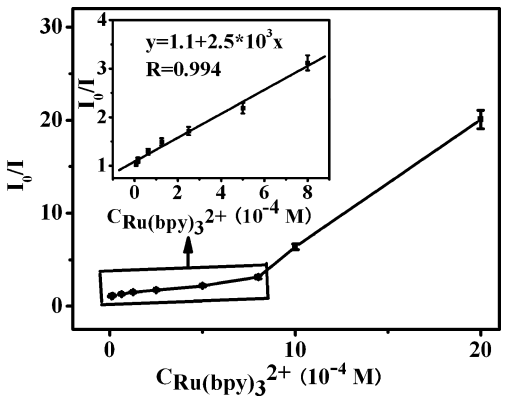

Fig. 3 Stern-Volmer plot of QDs quenched by $Ru(bpy)_3^{2+}$ in phosphate buffer solution

Effect of reaction time

The reaction time of GSH-capped CdTe QDs and $Ru(bpy)_3^{2+}$ was investigated. The results demonstrated that the reaction between $Ru(bpy)_3^{2+}$ and QDs was finished within 3 min and the PL intensity was stable for more than 30 min. In this study, a 10-min reaction time was adopted.

Effect of $Ru(bpy)_3^{2+}$ complex concentrations on the PL intensity of GSH-capped CdTe QDs

In this system, the effect of Ru(bpy)32+ complex concentration on the PL intensity of CdTe QDs has been investigated. With increasing amount of $Ru(bpy)_3^{2+}$ concentration in the GSH-capped CdTe QDs solution, an obvious and regular PL quenching with a slight blue shift was observed (Fig. S-1).

The quenching behavior of $Ru(bpy)_3^{2+}$ complex on the PL intensity of GSH-capped CdTe QDs is found to follow a conventional "Stern-Volmer" equation (Fig. 3):

$$I_0/I = 1+K_{sv}[M]$$

I_0 and I are the PL intensity of GSH-capped CdTe QDs in the absence and presence of $Ru(bpy)_3^{2+}$, respectively; [M] is the concentration of $Ru(bpy)_3^{2}$+, and Ksv is the quenching constant. The "Stern-Volmer" plot is shown in Fig. 3 inset, the K_{sv} of $Ru(bpy)_3^{2+}$ is 2,500 $L mol^{-1}$ and the linear range is from 7.8×10^{-6} to 8.0×10^{-4} mol L^{-1}. A correlation coefficient of 0.994 is acquired.

CdTe quantum Dots-$Ru(bpy)_3^{2+}$ system to detect ct DNA

The quenched PL intensity of GSH-capped CdTe QDs is recovered gradually with the accession of ct DNA.

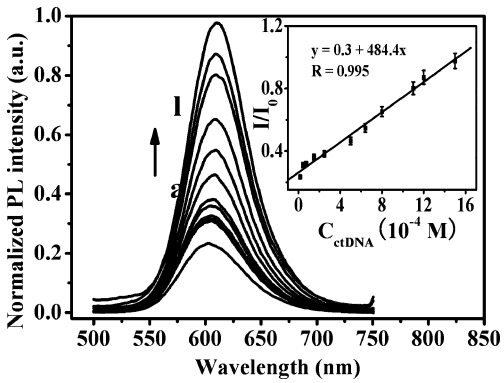

Fig. 4 PL spectra represent the restoration of QDs-$Ru(bpy)_3^{2+}$ system by the addition of ct DNA with different concentrations of 0, 1.7×10^{-5}, 4.6×10^{-5}, 7.4×10^{-5}, 1.5×10^{-4}, 2.5×10^{-4}, 5.0×10^{-4}, 6.4×10^{-4}, 8.0×10^{-4}, 1.1×10^{-3}, 1.2×10^{-3}, 1.5×10^{-3} mol L^{-1}(a-l) in the 10 mmol L^{-1} phosphate buffer solution of pH9.0. Inset: the relationship between I/I_0 (I is the recovered PL intensity of QDs in the presence of ct DNA and I_0 is the PL intensity of QDs at 617 nm, respectively) of QDs and $C_{ct\ DNA}$

Opposite to the quenching process, it is accompanied by a red shift (Fig. 4). It was found that the PL intensity restoration occurred immediately the ct DNA was added into the QDs-Ru$(bpy)_3^{2+}$ system. The PL intensity of pure GSH-capped CdTe QDs has no obvious change in the presence of ct DNA (Fig. S-2). Thus, the PL intensity recovery of GSH-capped CdTe QDs-Ru$(bpy)_3^{2+}$ system ascribed to the interaction between ct DNA and Ru$(bpy)_3^{2+}$.

Under optimum conditions, a good linear relationship (Fig. 4 insert) which is in the range of 1.7×10^{-5} to 1.5×10^{-3} mol L^{-1} with a correlation coefficient of 0.995 have been obtained between I/I_0 (I was the recovered PL intensity of QDs in the presence of ct DNA, and I_0 was the PL intensity of QDs at 617 nm) and the concentration of ct DNA.

The mechanism of reaction

The mechanisms for quenching the PL intensity of QDs may happen by energy transfer and electron or whole transfer. Firstly, we considered the possibility of energy transfer from smaller to bigger QDs. However, in the present system, a slight blue shift was observed upon quenching by Ru$(bpy)_3^{2+}$ of the PL of QDs (Fig. S-1) which suggested that the quenching was not due to the aggregation of QDs. We also investigated the feasibility of fluorescence resonance energy transfer from QDs to Ru$(bpy)_3^{2+}$. As shown in Fig. S-3, the excitation spectrum of the Ru$(bpy)_3^{2+}$ only slightly overlapped with the emission spectrum of QDs. however, on the basis of Förster formalism, efficient overlap between emission spectrum of donor and absorption spectrum of acceptor is needed. Obviously, the observed PL intensity quenching of QDs by Ru$(bpy)_3^{2+}$ is hardly caused by fluorescence resonance energy transfer.

The observed quenching therefore likely originated from electron or hole transfer process. In principle [20], upon photoexcitation of QDs, the electrons from the valence band are excited to the conduction band. The excited electron and the oppositely charged "hole" attract one another. When the excited electron recombines with the hole, a photon is emitted in the form of PL. In the experiment, there are negative charges on the surface of GSH-capped CdTe quantum dots, whereas Ru$(bpy)_3^{2+}$ have positive charges, therefore Ru$(bpy)_3^{2+}$ can work as an electron acceptor. In this case, they can form an ionic conjugate due to electrostatic attraction. The static association between the Ru$(bpy)_3^{2+}$ and GSH-capped CdTe QDs induces PET from GSH-capped CdTe QDs to the electron acceptor which prevents the normal recombination of the electron and the hole in QDs. This would lead to a decrease in the PL intensity of QDs with increasing Ru$(bpy)_3^{2+}$ concentration, as what was experimentally observed. The blue shift occurred in the experiment is another proof of the PET [21–23].

As mentioned above, the PL intensity of GSH-capped CdTe QDs-Ru$(bpy)_3^{2+}$ system was recovered with the addition of ct DNA. It is because ct DNA can bind with Ru$(bpy)_3^{2+}$ [24, 25] and suppressed the PET process. Therefore, the electron and the hole in QDs could recombine together resulting in the restoration of the system.

Conclusions

In conclusion, a novel method for the determination of ct DNA has been established based on CdTe QDs-Ru$(bpy)_3^{2+}$ PET system. Ru$(bpy)_3^{2+}$ as a novel electron acceptor is observed. Due to the electrostatic interactions between ct DNA and Ru$(bpy)_3^{2+}$, the PL intensity of CdTe QDs-Ru$(bpy)_3^{2+}$ system is restored. The relative recovered PL intensity (I/I_0) is linearly proportional to the concentration of ct DNA between 1.7×10^{-5} mol L^{-1} and 1.5×10^{-3} mol L^{-1}, with a detection limit of 5.7×10^{-6} mol L^{-1}. The proposed method is simple and fast, and has a potential application in other DNA analysis.

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