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ORIGINAL PAPER

Detection of DNA via the Fluorescence Quenching of Mn-doped ZnSe D-dots/Doxorubicin/DNA Ternary Complexes System

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Abstract This manuscript reports a method for the detection of double-stranded DNA, based on Mn:ZnSe d-dots and intercalating agent doxorubicin (DOX). DOX can quench the photoluminescence (PL) of Mn:ZnSe d-dots through photoinduced electron transfer process, after binding with Mn:ZnSe d-dots. The addition of DNA can result in the formation of the Mn:ZnSe d-dots-DOX-DNA ternary complexes, the fluorescence of the Mn:ZnSe d-dots-DOX complexes would be further quenched by the addition of DNA, thus allowing the detection of DNA. The formation mechanism of the Mn:ZnSe d-dots-DOX-DNA ternary complexes was studied in detail in this paper. Under optimal conditions, the quenched fluorescence intensity of Mn:ZnSe d-dots-DOX system are perfectly described by Stern–Volmer equation with the concentration of hsDNA ranging from 0.006 μg mL^{-1} to 6.4 μg mL^{-1}. The detection limit (S/N=3) for hsDNA is 0.5 ng mL^{-1}. The proposed method was successfully applied to the detection of DNA in synthetic samples and the results were satisfactory.

Keywords Mn:ZnSe d-dots · Doxorubicin · DNA · Fluorescence quenching

Introduction

DNA is the primary target molecule for most anticancer and antiviral therapies according to cell biology [1]. The photoluminescence (PL) intensity of DNA itself is very weak, and the direct use of its fluorescence emission to investigate its biological property is limited. Moreover, some fluorescent DNA probes are highly toxic and mutagenic, especially when the binding to DNA is irreversible. For example, ethidium bromide as a fluorescent DNA probe is well known as a carcinogenic compound. Therefore, the exploration of new and effective DNA probes with low toxicity and high selectivity and sensitivity is an active research field [2].

Synthesis of nonheavy metal-containing doped semiconductor quantum dots (d-dots) has recently attracted increased attention [3–6]. Li et al. demonstrated that d-dots had less destructivity for DNA, which may bring some potential applications, for example, as a new gene delivery agent [7]. Recently, Our group investigated the DNA damage induced by a series of water-soluble nanoparticles, including CdTe QDs, CdTe/SiO_2 QDs, and Mn:ZnSe d-dots. Mn:ZnSe d-dots resulted in the least DNA damage among the three kinds of nanoparticles. Therefore, Mn:ZnSe d-dots can be used as superior biocompatible fluorescent labels [8].

Doxorubicin (DOX) can interfere with the topoisomerase II-DNA complex, leading to the formation of double-stranded breaks of DNA or direct intercalation with DNA, which in turn inhibits DNA duplication and transcription to mRNA [9]. As one of the anthraquinone derivates that are well-known electron acceptors, DOX can quench the QDs PL through an electron-transfer process [10]. DOX can be chosen as an active molecular platform, which can serve as a quencher to QDs fluorescence and also possess the stronger binding ability with DNA.

In this paper, a method was developed for the detection of double-stranded DNA. The PL intensity of Mn:ZnSe d-dots is quenched when binding with DOX through an electron-transfer process. The PL intensity of Mn:ZnSe d-dots-DOX system would continue to be quenched by the addition of DNA, which is due to the formation of Mn:ZnSe d-dots-

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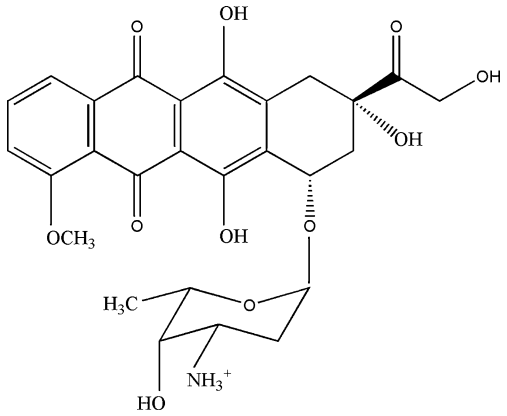

Fig. 1 Chemical structure of doxorubicin hydrochloride

DOX-DNA ternary complexes. The formation mechanism of the Mn:ZnSe d-dots-DOX-DNA ternary complexes was studied in detail in this paper. Under optimal conditions, the quenched fluorescence intensity of Mn:ZnSe d-dots-DOX system was directly proportional to the DNA concentration.

Experimental Section

Chemicals and Apparatus

All chemicals were of analytical reagent grade and were used without further purification. Mercaptopropionic acid (MPA) (99%), selenium powder (~200 mesh, 99.9%), Zn $(NO_3)_2{\cdot}6H_2O$ (99.9%), $MnCl_2{\cdot}4H_2O$ (99.9%), $NaBH_4$ (99%), Herring sperm DNA (hsDNA), and doxorubicin hydrochloride (DOX) were purchased from Sigma-Aldrich Chemical Co. The water used in all experiments had a resistivity higher than 18 $M\Omega{\cdot}cm^{-1}$. Fluorescence measurements were performed on a ShimadzuRF-5301 PC spectrofluorometer with a quartz cuvette. Fluorescence lifetimes were measured using the time-correlated single-photon counting technique with FL920-fluorescence lifetime spectrometer (Edinburgh instrument) with the instrument response of~1 ns. The pH value of the buffer solution was measured with a PHS-3 C pH meter (Tuopu Co., Hangzhou, China).

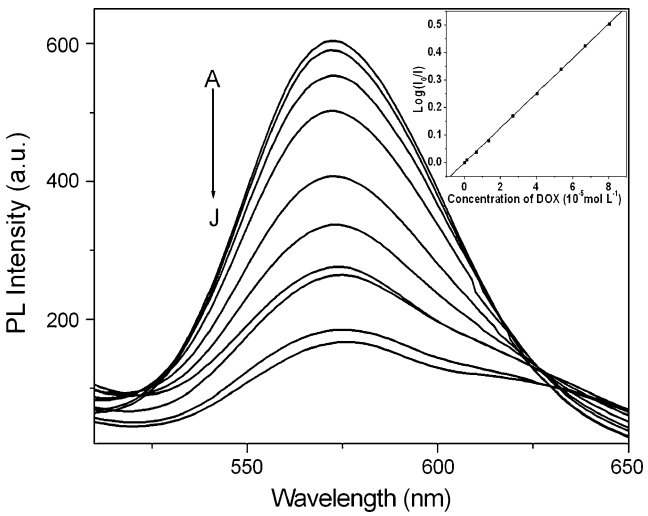

Fig. 2 The PL spectra of Mn:ZnSe d-dots with different concentrations of DOX. A-J represents the concentrations of DOX of 0, 0.13, 0.67, 1.34, 2.68, 4.02, 5.36, 6.7, 8.04 and 9.3×10^{-5} mol L^{-1}, respectively. The inset shows the relationship between Log (I/I_0) and the concentration of DOX. The Mn:ZnSe d-dots concentration is 8.3×10^{-5} mol L^{-1}

Preparation of Mn:ZnSe D-dots

Mn:ZnSe d-dots were prepared in aqueous solution by nucleation doping method based on our previous report [11]. The synthesis procedure of Mn:ZnSe d-dots was performed as follows: 0.2 mL $MnCl_2$ solution (1.25×10^{-2} M) and 90 mL H_2O were loaded into a 250 mL three neck flask and degassed for 30 min by bubbling with nitrogen. Fresh NaHSe solution was added to N_2-saturated $MnCl_2$ solution at pH 11.2 in the presence of MPA as stabilizing agent. The reaction was then switched from nitrogen bubbling to nitrogen flow and subjected to a reflux at 100 °C for 40 min. 10 mL of $Zn(NO_3)_2$ stock solution (1.25×10^{-2} M) was injected and the reaction was refluxed for 5 h. The Mn-to-Se-to-Zn and Zn-to-MPA precursor ratios were 1 : 25 : 50 and 1 : 24, respectively. Finally, the reaction mixture was allowed to cool down to room temperature and the as-prepared Mn:ZnSe d-dots were used directly without any post-treatments. The final concentration of Mn:ZnSe d-dots, as measured by the Zn^{2+} concentration, was 1.25×10^{-3} mol L^{-1}.

Fluorescence Determination

A certain concentration of Mn:ZnSe d-dots solution, varying amounts of DOX, and hsDNA were successively added into 2.0 mL calibrated test tube, then diluted to the mark with phosphate buffer solution (pH=7.0) and mixed thoroughly. At an excitation wavelength of 350 nm, the fluorescence spectra of the Mn:ZnSe d-dots-DOX system and the Mn:ZnSe d-dots-DOX-DNA system were recorded in the 510–650 nm emission wavelength range.

Results and Discussion

The Quenching Effect of DOX on the Fluorescence of Mn:ZnSe D-dots

It has been reported that electron acceptors adsorbed on the surface of QDs can quench the exciton emission of QDs. Figure 1 shows the chemical structure of DOX, the DOX

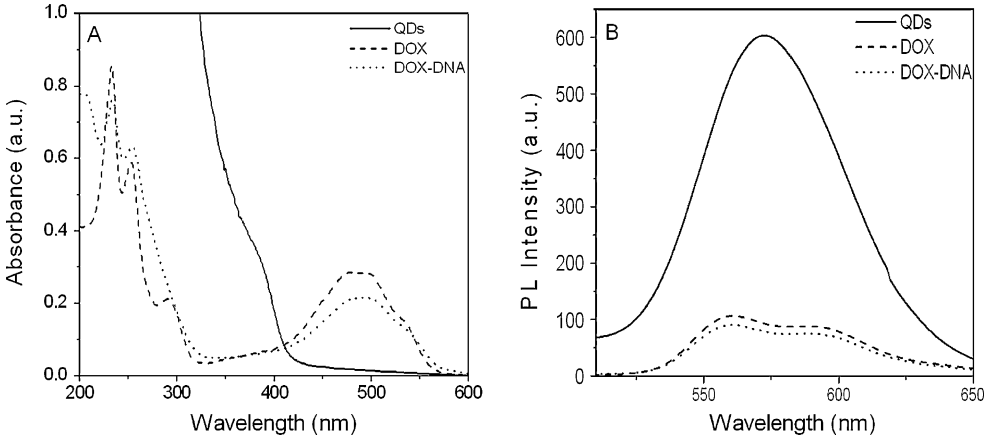

Fig. 3 (**a**) UV–vis absorption spectra of Mn:ZnSe d-dots, DOX and DOX+DNA, respectively. (**b**) The PL spectra of Mn:ZnSe d-dots, DOX and DOX+DNA, respectively. The DOX concentration is 2.68×10^{-5} mol L^{-1}, the DNA concentration is 4.14 μg mL^{-1}

molecule is positively charged in solution. As an anthraquinone derivative, DOX can adsorb on the surface of Mn:ZnSe d-dots, resulting in the quenching of QDs PL emission through an electron-transfer process [12]. As shown in Fig. 2, With the addition of DOX, the PL intensity of Mn:ZnSe d-dots at 570 nm will gradually decrease with the increase of the concentration of DOX. The fluorescence quenching did not fit well with the conventional Stern–Volmer equation. While using the modified Stern–Volmer equation

$$\log(I_0/I) = K_{SV}[Q] + C \quad (1)$$

where I_0 and I are the PL intensity of Mn:ZnSe d-dots in the absence and presence of the quencher (DOX). K_{SV} is the Stern–Volmer quenching constant, [Q] is the concentration of quencher (DOX) and C is the constant of equation. A good linear relationship was observed (see the inset in Fig. 1), which indicated that both dynamic and static quenching seem to act together [13]. The regression equation is $\log(I_0/I) = -0.0012+6.31\times10^{-3}C$ (mol L^{-1}) and the corresponding regression coefficient is 0.999. The linear relationship is observed for the concentration of DOX ranging from 1.3×10^{-6} mol L^{-1} to 8.0×10^{-5} mol L^{-1}, and the K_{SV} is calculated to be 6.31×10^{-3} L mol^{-1}.

The Quenching Effect of DNA on the Fluorescence of Mn:ZnSe D-dots–DOX System

Figure 3a shows the UV–vis absorption spectra of Mn:ZnSe d-dots, DOX and DOX-DNA, respectively. The absorption band of Mn:ZnSe d-dots appeared at around 385 nm.. An absorption band appeared at around 480 nm in the absorption spectra of DOX, and with the addition of DNA the absorption band showed an obvious decrease and red-shift. The red-shift of absorption band proved the binding mode between DOX and DNA was intercalation. Accordingly, it can be considered that DOX can intercalate between the pair-bases of double helix DNA. DOX fluorescence can be quenched upon DOX intercalation into the DNA, as illustrated in Fig. 3b. It also shows the interaction of DOX and DNA.

Figure 4a shows the UV–vis absorption spectra of Mn:ZnSe d-dots, Mn:ZnSe d-dots -DOX and Mn:ZnSe d-dots-DOX-DNA, respectively. The absorption spectra of the Mn:ZnSe d-dots-DOX complexes showed an obvious decrease with the addition of DNA. The decrease of absorption band indicated the interaction of DNA and Mn:ZnSe d-dots-DOX complexes. As shown in Fig. 4b, the quenched PL intensity of Mn:ZnSe d-dots by DOX can be further

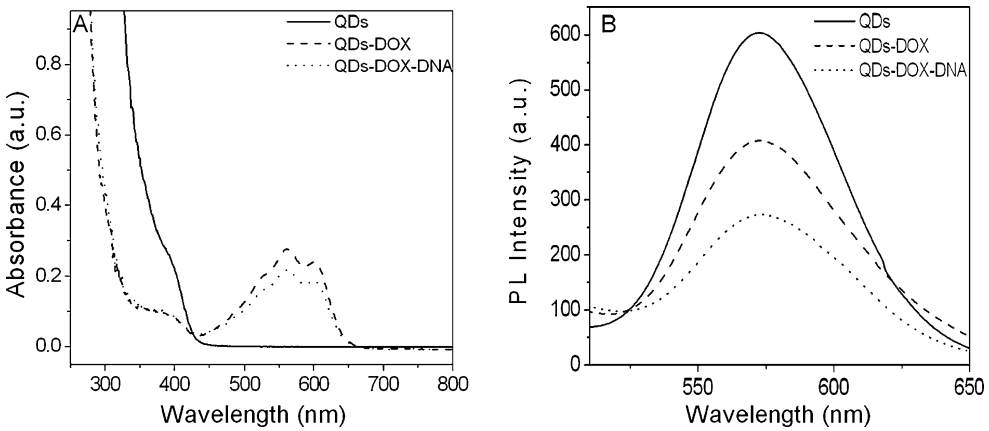

Fig. 4 (**a**) UV–vis absorption spectra of Mn:ZnSe d-dots, QDs+DOX and QDs+DOX+DNA, respectively. (**b**) The PL spectra of Mn:ZnSe d-dots, QDs+DOX and QDs+DOX+DNA, respectively. The Mn:ZnSe d-dots concentration is 8.3×10^{-5} mol L^{-1}, the DOX concentration is 2.68×10^{-5} mol L^{-1}, the DNA concentration is 4.14 μg mL^{-1}

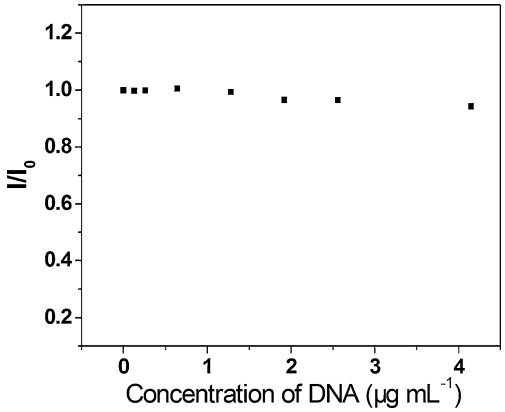

Fig. 5 The effect of DNA concentration on the PL intensity of Mn:ZnSe d-dots. I_0 and I are the PL intensity of Mn:ZnSe d-dots in the absence and presence of DOX. The Mn:ZnSe d-dots concentration is 8.3×10^{-5} mol L^{-1}, the DOX concentration is 2.68×10^{-5} mol L^{-1}

quenched by the presence of DNA. The addition of 2.68×10^{-5} mol L^{-1} DOX caused the quenching of Mn:ZnSe d-dots PL intensity to 67.6%, and the subsequent addition of 4.14 μg mL^{-1} DNA could further quench the PL intensity of Mn:ZnSe d-dots to 45.3%. Figure 5 shows that the PL intensity of Mn:ZnSe d-dots is almost not influenced by the addition of DNA in the concentration rang from 0.13 μg mL^{-1} to 4.14 μg mL^{-1}, so the PL quenching of Mn:ZnSe d-dots-DOX system caused by the addition of DNA should come from the interaction between DNA and DOX.

Figure 6 shows the quenching effect of DNA on the PL intensity of Mn:ZnSe d-dots-DOX system. It was found that

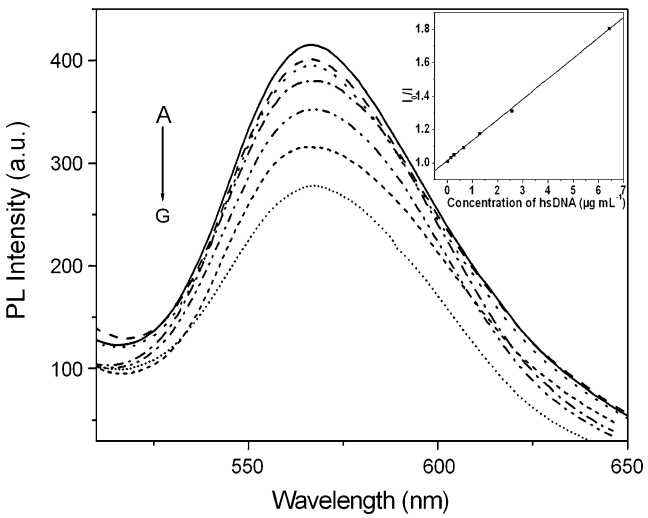

Fig. 6 The PL spectra represents the quenching effect of Mn:ZnSe d-dots-DOX system by the addition of hsDNA. A-G represents the concentrations of hsDNA of 0.006, 0.13, 0.26, 0.64, 1.28, 2.56 and 6.40 μg mL^{-1}, respectively. The Mn:ZnSe d-dots concentration is 8.3×10^{-5} mol L^{-1}, the DOX concentration is 2.68×10^{-5} mol L^{-1}. The inset shows the relationship between I/I_0 and the concentration of hsDNA

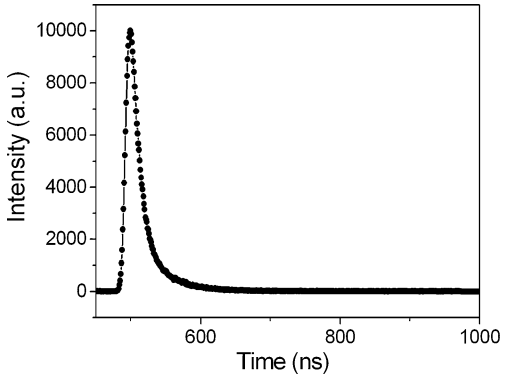

Fig. 7 The fluorescence decay curve of the Mn:ZnSe d-dots-DOX complexes

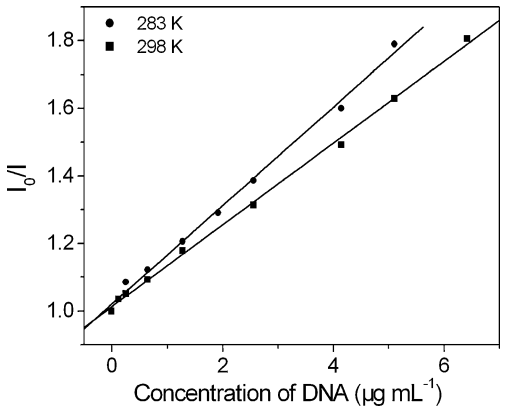

Fig. 8 Stern-Volmer plots of the Mn:ZnSe d-dots-DOX versus the concentration of hsDNA at different temperatures

Table 1 The interference of coexisting substances on the fluorescence quenching of Mn:ZnSe d-dots-DOX system by 5.0 μg mL^{-1} hsDNA

Coexisting substance	Tolerable concentration (μg mL^{-1})	ΔI/I (%)
Na^+	5,000	2.7
K^+	5,000	−2.1
Mg^{2+}	1,250	−3.3
Zn^{2+}	1,250	4.7
Ca^{2+}	500	4.2
Cd^{2+}	500	2.3
Cl^-	5,000	2.7
NO_3^-	1,250	4.7
Glucose	250	3.9
L-tyrosine	250	−2.7
Protein, BSA	250	1.7

$\Delta I=I_o - I$, where I_0 and I are the fluorescence intensity of Mn:ZnSe d-dots-DOX-DNA system in absence and presence of interfering species.

Table 2 Determination of hsDNA in synthetic samples

hsDNA concentration ($\mu g\ mL^{-1}$)	Main additives[a]	Found[b] ($\mu g\ mL^{-1}$)	Recovery (%)	RSD (%, n=3)
0.51	A (Ca^{2+}, K^{+}, Mg^{2+}, Na^{+}, Zn^{2+}, Cd^{2+})	0.56	109.8	1.7
5.11		4.70	92.0	0.9
0.51	B (Glucose, L-tyrosine, BSA)	0.48	94.1	1.2
5.11		5.83	114.1	1.5

[a] The concentrations of $CaCl_2$, KCl, $MgCl_2$, NaCl, $Zn(NO_3)_2$ and $CdCl_2$ are 100 $\mu g\ mL^{-1}$. The concentrations of glucose, L-tyrosine and BSA are 10 $\mu g\ mL^{-1}$.

[b] Mean of three replicates.

the quenched PL intensity of Mn:ZnSe d-dots-DOX system was directly proportional to the hsDNA concentration, which can be best described by a Stern-Volmer equation:

$$I_0/I = 1 + K_{SV}[Q] \quad (2)$$

where I_0 and I are the PL intensity of Mn:ZnSe d-dots-DOX system in the absence and presence of DNA respectively, [Q] is the hsDNA concentration, and K_{SV} is the Stern-Volmer quenching constant. From the slope of the Stern-Volmer plot, K_{SV} is calculated to be 1.20×10^5 L mg^{-1}. The calibration plot of I_0/I versus concentration of hsDNA is linear in the range from 0.006 $\mu g\ mL^{-1}$ to 6.4 $\mu g\ mL^{-1}$ (See the inset in Fig. 4). The regression equation is $I_0/I=1.015+1.206\times10^5C$ ($\mu g\ mL^{-1}$) and the corresponding regression coefficient is 0.999, which indicates that there is a good linear relationship between I_0/I and hsDNA concentration. The detection limit (S/N=3) for hsDNA is 0.5 ng mL^{-1}. According to the bimolecular quenching equation:

$$K_{SV} = K_q\tau_0 \quad (3)$$

where K_q is bimolecular rate constant and τ_0 is the fluorescence lifetime of the Mn:ZnSe d-dots-DOX complexes without quencher, which was determined to be 2.86 ns (Fig. 7). Therefore, K_q is about 1.45×10^{12} L mol^{-1} S^{-1}. Generally, the maximum scatter collision quenching constant of various quenchers with the biological macromolecules is 2×10^{10} L mol^{-1} S^{-1}. Thus, K_q is about two orders of magnitude higher than 2×10^{10}. It can reasonably be inferred that the quenching process in this study is mainly static and initiated through the formation of a new complex.

To distinguish between static and dynamic mechanisms, their difference in temperature dependence should be addressed. If K_{SV} decreases with increasing temperature, it may be concluded that the quenching process is static rather than dynamic. Figure 8 shows the Stern-Volmer plots of the quenching system of Mn:ZnSe d-dots-DOX-DNA at different temperatures. The K_{SV} in 283 K is 1.46×10^5 L mg^{-1} while the K_{SV} in 298 K is 1.20×10^5 L mg^{-1}. The results show that the Stern–Volmer quenching constant is inversely proportional to temperature, which suggests that the PL quenching of the Mn:ZnSe d-dots-DOX system by DNA is initiated by the formation of ground-state complex [14]. The result simultaneously indicated the PL of the Mn:ZnSe d-dots-DOX system quenched by DNA with static quenching mechanism.

In order to further study the quenching process, the fluorescence quenching results of Mn:ZnSe d-dots by DOX were analyzed to obtain their binding constants with DOX, the binding constant K_{LB} was calculated by the following Lineweaver-Burk double-reciprocal equation [15, 16]:

$$(I_0 - I)^{-1} = (I_0)^{-1} + K_{LB}{}^{-1}(I_0)^{-1}[Q]^{-1} \quad (4)$$

where K_{LB} is the binding constant with the unit of L mol^{-1}. I_0 and I are the PL intensity of Mn:ZnSe d-dots in the absence and presence of DOX. [Q] is the concentration of DOX. From the intercept and the slope of the Lineweaver-Burk double-reciprocal equation, we can calculate that K_{LB}(Mn:ZnSe)=2.19×10^4 L mol^{-1}. According to a previous report [17], we obtained that the binding constant of DOX with DNA was ~10^5 L mol^{-1}. The results showed the binding constant of Mn:ZnSe d-dots with DOX was similar to that of DNA with DOX. Because of the strong binding constant of Mn:ZnSe d-dots with DOX, the addition of DNA can not remove DOX from the surface of Mn:ZnSe d-

Table 3 Comparison of different fluorescent probes for determination of DNA

Fluorescence probes	Species of DNA	Linear range ($\mu g\ mL^{-1}$)	LOD (ng mL^{-1})	References
Ethidium bromide (EB)	DNA	Not mentioned	10	[18]
Al^{3+}-8-hydroxyquinoline	fsDNA/ctDNA[a]	0.25–3.5/0.25–3.0	130/240	[19]
Cysteine-capped ZnS	fsDNA/ctDNA	100–600/100–1200	24.6/32.9	[20]
9-Anthracenecarboxylic acid –CTMAB	fsDNA/ctDNA	80–1000	19/20	[21]
CdS/poly N-(Hydroxymethyl) -acrylamide	hsDNA/ctDNA	0.05–35.0/0.08–30.0	2.2/1.5	[22]
Norfloxacin-Tb^{3+}	fsDNA/ctDNA	0.005–1.0	0.9/0.6	[23]
Mn:ZnSe d-dots-DOX	hsDNA	0.006–6.4	0.5	This work

[a] fsDNA refers to fish sperm DNA and ctDNA refers to calf thymus DNA.

dots, it can only bind with DOX. So the fluorescence of Mn:ZnSe d-dots would continue to be quenched by the addition of DNA, which suggested that DNA and Mn:ZnSe d-dots-DOX complexes form the ternary complexes. Therefore, the DNA concentration-dependent PL quenching of Mn:ZnSe d-dots-DOX system can be utilized for the determination of DNA.

Interference Study

The detection selectivity of the proposed fluorescence quenching method for DNA detection was further evaluated with various coexistence substances added. Table 1 shows the interference of metal ions, sugar and protein on the PL intensity of Mn:ZnSe d-dots-DOX-DNA system in DNA detection. I_0 and I are the fluorescence intensities of Mn:ZnSe d-dots-DOX-DNA system in absence and presence of interfering species. Tolerable concentrations, defined as the concentrations of foreign species causing less than ±5% relative error, were examined. The results showed that the tolerable concentration ratios of coexisting substances to 5.0 μg mL^{-1} hsDNA was over 1000-fold for Na^+, K^+, and Cl^-, 250-fold Mg^{2+} $Zn^{2+}$and NO_3^-, 100-fold Ca^{2+} and Cd^{2+}, 50-fold glucose, L-tyrosine and BSA.

Determination of hsDNA in Synthetic Samples

To examine the applicability and accuracy of proposed fluorescence quenching method for DNA detection, two synthetic samples were analyzed according to the above assay procedure and the results were shown in Table 2. From Table 2, it can be seen that the recoveries of the two synthetic samples were found to be in the range 92–115%. The RSDs are less than 2.0%. The results obtained in the synthetic samples indicate that the determination of hsDNA based on the Mn:ZnSe d-dots-DOX-DNA ternary complexes is sensitive, simple and satisfactory.

A comparison between this method and other reported methods for DNA determination in detection limit and linear range was summed up in Table 3. It could be seen from Table 3 that the sensitivity of this method was better than most of the well-known methods including the ethidium bromide (EB) probe. With respect to provide a bioassay platform for high sensitivity detection of DNA, our system offered a competitive sensitivity.

Conclusion

A simple, rapid, sensitive method has been developed for the detection of DNA based on the Mn:ZnSe d-dots-DOX-DNA ternary complexes. DOX can effectively quench the PL intensity of Mn:ZnSe d-dots, and the addition of DNA can continue to quench the PL intensity of Mn:ZnSe d-dots-DOX system. The quenched PL intensity is linearly proportional to the concentration of DNA. The mechanism of the interaction between Mn:ZnSe d-dots-DOX system and DNA has been studied in detail. The proposed method was applied to the determination of DNA in synthetic samples with satisfactory results. Mn:ZnSe d-dots as a new class of non-cadmium doped QDs demonstrated almost no damage for DNA molecules, which have great potentials as fluorescent labels in the applications of biomedical assays, imaging of cells and tissues, even in vivo investigations.

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