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ORIGINAL PAPER

Interaction among Cadmium Sulfide Nanoparticles, Acridine Orange, and Deoxyribonucleic Acid in Fluorescence Spectra and a Method for Deoxyribonucleic Acid Determination

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Abstract Some studies on quantum dots (QD) as donors that enhance the fluorescence of a dye as an acceptor through fluorescence resonance energy transfer (FRET) have been reported. However, in the present work we discovered that CdS quantum dots sharply quenched the fluorescence of acridine orange (AO). Also, DNA enhanced the fluorescent signals of AO quenched by CdS. The extents of enhancement were in good proportion to the DNA concentrations. Based on this, a sensitive method was employed to determine DNA with both good selectivity and sensitivity. The calibration curve was linear over 60–4,000 ng mL^{-1} and the determination limit (3σ) was 4.39 ng mL^{-1}.

Keywords CdS · Acridine orange · Fluorescence · DNA

Introduction

Semiconductor QDs have been intensively investigated during the last decade [1–5]. Their optical, electronic, and catalytic properties can be basically attributed to two factors, i.e. a large surface-to-volume ratio and a size quantization effect, both leading to alterations of the semiconductor properties. Nowadays, compatibility with living biological systems presents significant synthetic and interfacial chemical challenges. QD must be coated with hydrophilic molecules to render them water soluble, and ultimately biocompatible. The kind of hydrophilic molecules is of great importance [6], since it affects both the chemical and the physical properties of the semiconductor from stability to solubility to light emission.

Fluorescent reagents have been widely used as DNA probes. The use of AO as a fluorescence probe to study the interaction of AO with DNA has been reported [7, 8]. Many methods have been developed for the determination of trace amounts of DNA. Among these simple and rapid methods, some applied fluorescence resonance energy transfer (FRET) [8], and some applied resonance light scattering (RLS), and so on. However, most methods that exhibited relative good result were by RLS [9–11].

AO is often used as an important fluorescence probe of DNA due to its intercalative binding ability [12], and also a well-known intercalator of DNA in studies of molecular dynamics and photophysical processes in DNA [13, 14]. To our knowledge, QDs are excellent FRET donors with proximal organic dyes[15–22]. However, we found that QD did not enhance the fluorescence of AO, but quenched it sharply, and the addition of calf thymus DNA restored some of the AO fluorescence, in proportion to the amount of added DNA. In this paper, we report on the interactions among CdS, AO, and DNA in fluorescence spectra and the determination of DNA. This method using water-soluble CdS as an effective fluorescence quencher of AO to investigate DNA has not been reported so far.

Experimental

Reagents and chemicals

The concentration of DNA (ctDNA; Beitai Co., Beijing, China) was determined according to the absorbance value at 260 nm with εDNA=6,600 L mol^{-1} cm^{-1} after establishing

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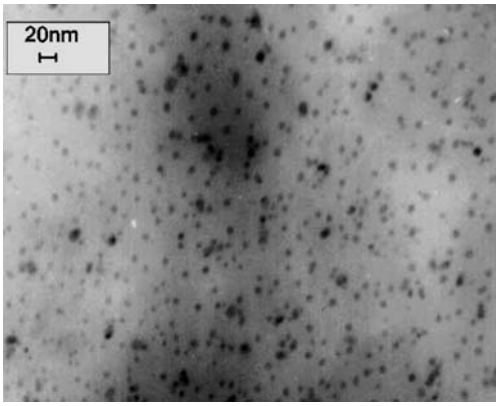

Fig. 1 Transmission electron micrograph of the CdS

that the absorbance ratio, A260/A280, was in the range 1.80–1.90 for DNA [23]. Tris–HCl buffer was prepared by adjusting a 0.1 mol L^{-1} Tris solution to pH 8.00 with 0.1 mol L^{-1} HCl.

AO (Shanghai Chemical Reagents Co., Shanghai, China). Cadmium chloride, sodium sulfide (Tianjin Fucheng Chemical Reagent Co., Tianjin, China), Mercaptoacetic acid (MAA, $HOOCHCH_2SH$, Beijing 89942 chemical reagent co., Beijing, China). All reagents were of analytical grade, or the best grade commercially available, and were put into use without further purification. Doubly distilled water was used throughout.

Apparatus

The fluorescence spectrum was measured with an RF-540 spectrofluorometer (Shimadzu, Japan). A TEM (transmission electron micrograph) image was obtained using an H-600 transmission electron microscope (HITACHI, Japan). The absorption spectra were observed by a UV-Cary100 spectrophotometer (Varian, Australia). A pH-10C digital pH meter was utilized to measure the pH values of aqueous solutions.

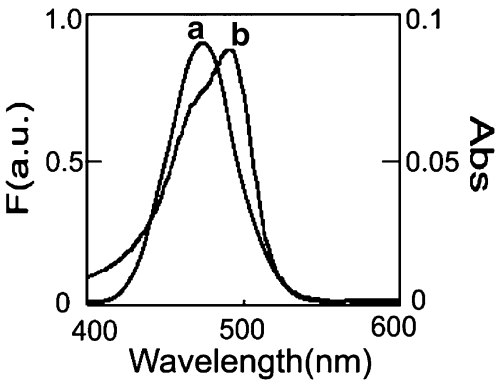

Fig. 2 Fluorescence spectrum of CdS (**a**) and absorbance spectrum of AO (**b**)

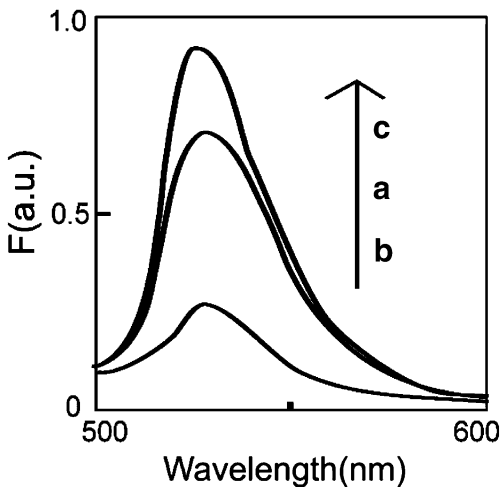

Fig. 3 Fluorescence spectra of AO (**a**), AO–CdS (**b**), AO–CdS–ctDNA (**c**). Concentration: AO, 1.0×10^{-6} mol L^{-1}; C_{CdS}, 4.466×10^{-5} mol L^{-1}; C_{DNA}, 1.0 μg mL^{-1}. λ_{ex}=490 nm

Procedure

As a standard case, MAA-modified CdS was synthesized as follow [24]: 7.08 μL MAA was added to 100 mL of 1.0 mM $CdCl_2$. The pH was adjusted by the dropwise addition of concentrated 0.5 M NaOH to pH 11. Under a N_2 atmosphere, 50 mL of a 1.34 mM Na_2S solution was added to this solution with rapid stirring. The reaction mixture was stirred over night. The concentration of the CdS solution was 4.466×10^{-4} mol L^{-1}. The intensity of the fluorescence of CdS was measured at 480 nm (λ_{ex}= 360 nm) in a 1 cm quartz cell with a slit width of 10 nm for both excitation and emission.

To a 10.0 mL colorimetric tube, the solutions were added in the following order: 1.0 mL Tris–HCl buffer (pH 8.00), 1.0 mL 1.0×10^{-5} mol L^{-1} AO solution, 1.0 mL of a 4.466×10^{-4} mol L^{-1} CdS solution, and an appropriate volume of sample or working solution of DNA. The mixture was diluted to 10.0 mL with water, and was allowed to stand for

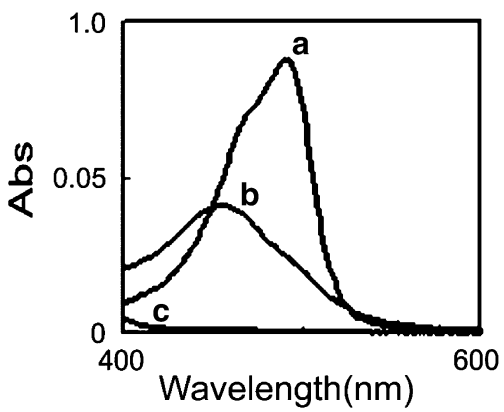

Fig. 4 Absorbance spectra of AO(**a**), AO–CdS (**b**) and CdS (**c**)

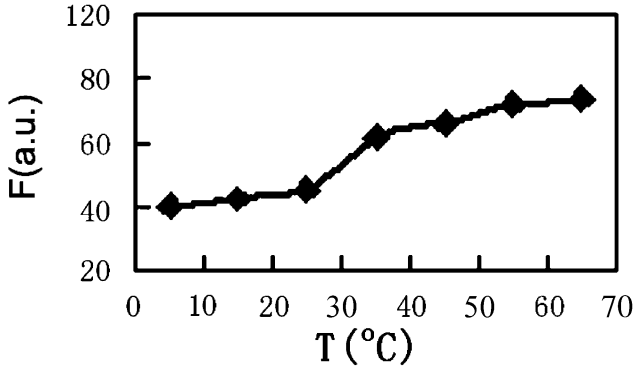

Fig. 5 Fluorescence intensity of AO in the presence of CdS at different temperatures

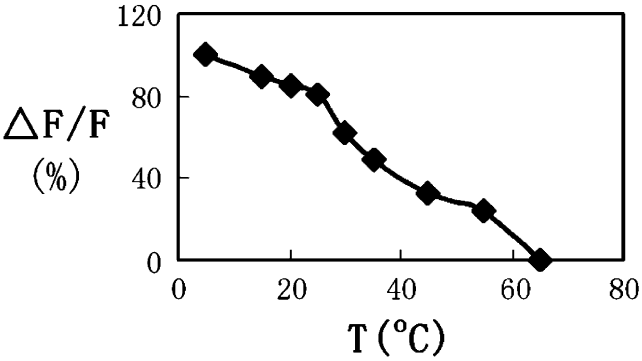

Fig. 7 Influence of temperature on the extent of a fluorescence intensity increase upon the addition of ctDNA. Other conditions are the same as those in Fig. 6

10 min at 25 °C before a fluorescence measurement was made. The intensity of the fluorescence was measured at 523 nm (λ_{ex}=490 nm) in a 1 cm quartz cell with a slit width of 10 nm for both excitation and emission.

Results and discussion

Spectroscopy

A micrograph of water-soluble CdS is shown in Fig. 1. The small dark regions indicate CdS dispersed in water. The dark regions indicate particle diameters of 8 nm on the average. The quantum yield of CdS was determined using Rhodamine 6G as a reference; the quantum yield was up to 30%.

The fluorescence spectrum of CdS and the absorbance spectrum of AO are shown in Fig. 2. It clearly shows a significant overlap between the emission and the absorption spectrum of the CdS and AO, respectively. For that reason, FRET between CdS and AO was highly possibility. Correspondingly, as an acceptor of FRET, the fluorescence intensity of AO should have increased. However, CdS did not enhance the AO's fluorescence intensity, but quenched it. It can be seen from Fig. 3 that the fluorescence intensities exhibited different features at 523 nm. The intensity of AO–CdS was much less than that of AO, i.e. CdS quenched the fluorescence of AO. The intensity of AO–CdS–DNA was much stronger than that of AO–CdS. Further, the enhanced extents were in good proportion to the DNA concentrations. We speculated that the fluorescence quenching of AO by CdS was attributed to an inner filter effect. CdS and AO formed a ground-state complex compound that caused the absorbance spectrum of AO to change (Fig. 4) [25]. The compound absorbed a portion of the excitation energy, leading to the fluorescence quenching of AO. Also, a temperature raise could render the ground-state complex compound unstable, i.e. the fluorescence intensity of AO, quenched by CdS, increased as the temperature increased (Fig. 5), which confirmed the formation of the ground-state complex compound.

Consequently, CdS quenched the fluorescence of AO. With the addition of DNA, the DNA and AO interacted and

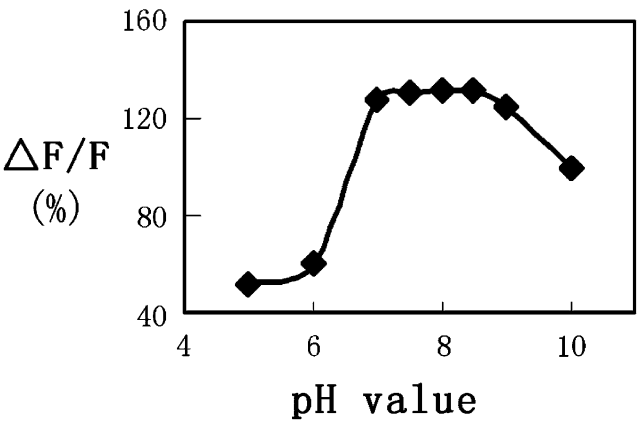

Fig. 6 Influence of the pH on the extent of the fluorescence intensity increase upon the addition of ctDNA. AO, 1.0×10^{-6} mol L^{-1}; C_{CdS}, 4.466×10^{-5} mol L^{-1}; C_{DNA}, 1.0 μg mL^{-1}. *F*, the fluorescence intensity of AO in the absence of CdS. Δ*F*, the fluorescence intensity increase of AO in the presence of CdS in addition of ctDNA

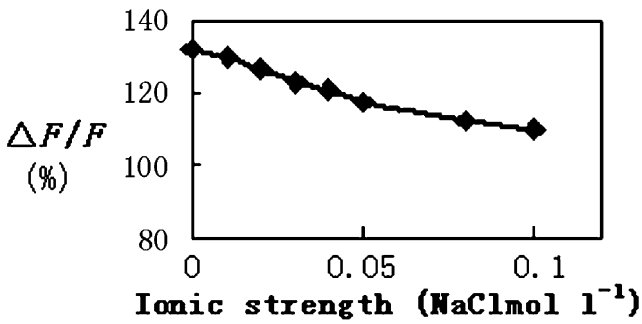

Fig. 8 Influence of the ionic strength on the extent of fluorescence intensity increase upon the addition of ctDNA. Other conditions are the same as those in Fig. 6

Table 1 Interference of coexisting substances

Co-existing substance	Co-existing concentration ($\times 10^{-6}$ mol L^{-1})	Change in intensity of fluorescence (%)
K^+, iodide	10.0	−4.45
Ca^{2+}, chloride	25.0	3.87
Na^+, chloride	23.0	2.30
Mg^{2+},chloride	4.1	−1.32
Fe^{3+}, sulfate	3.1	1.10
Zn^{2+}, acetate	15	−3.87
Pb^{2+}, nitrate	4.0	−3.25
Co^{3+},chloride	1.8	−1.40
Ba^{2+}, nitrate	3.0	−1.30
L-Cysteine	1.9	2.50
Glucose	2.3	2.1

Conditions: CdS, 4.466×10^{-5} mol L^{-1}; AO, 1×10^{-6} mol L^{-1}; ctDNA, 300 ng mL^{-1}, pH 8.0

Table 2 Analytical parameters for the determination of DNA

Sample	Linear range (ng mL^{-1})	Linear regression equation (c, ng mL^{-1})	Detection limit (3σ, ng L^{-1})	Correlation coefficient
ctDNA	60–4,000	$\Delta F=0.3753c+27.514$	4.39	0.9995

Table 3 Comparison of the probe of detection limit of ctDNA

Probe	Methods	LOD (ng mL^{-1})	Incubating time	Ref.
Acridine Orange, Safranine T	FRET	1.9×10^{4}	10 min	[8]
Acridine Orange	RLS	5.8	15 min	[9]
Acridine Red	RLS	1.28	20 min	[10]
Janus Green B	RLS	8.7	15 min	[11]
QD,Cy3, Alexa647	FRET	a	7–8 h	[22]
QD, Ethidium Bromide	FRET	a	3–4 h	[22]
HCl	RLS	18.0	b	[26]
H_2SO_4	RLS	17.8	b	[26]
HNO_3	RLS	18.4	b	[26]
Azur A	RLS	19.9	b	[27]
This system	This method	4.39	10 min	

a No specific LOD, b No specific incubating time

Table 4 Results of the sample analysis

ctDNA concentration (ng mL^{-1})	Main interference	Mean found (ng mL^{-1}; n=7)	Recovery range (%)	RSD (%)
600	K^+, Ca^{2+},Pb^{2+},Ba^{2+}, L-Cysteine	607	99.0–104.3	1.8
500	K^+,Fe^{3+},Zn^{2+},Co^{3+}, L-Cysteine	492	97.3–102.1	2.2

Concentration of main interference: K^+, 5×10^{-5}; Ca^{2+}, 7.5×10^{-5}; Fe^{3+}, 1.5×10^{-5}; Zn^{2+}, 1.5×10^{-4}; Co^{3+}, 5×10^{-6}; Pb^{2+}, 2.0×10^{-5}; Ba^{2+}, 1.5×10^{-5}; L-Cysteine, 1.5×10^{-5} mol L^{-1}

readily formed a binary ion-association of AO-DNA [9]. Since CdS a took negative charge, a negatively charged oxygen atom of the phosphate group in the DNA backbone prevented AO from the CdS atmosphere because of an electrostatic repulsion, and the fluorescence efficiency of AO was sensitive to DNA [7], leading to a sharp increase of the AO fluorescence. It was interesting that the enhanced extents of the fluorescence were in good proportion to the concentrations of DNA, indicating that an assay of DNA in this way to be good practice.

DNA determination

Effect of CdS and AO concentrations. Both the concentrations of CdS and AO influenced the DNA determination. If the concentration ratio of AO to CdS was too high, the sensitivity for the detection of ctDNA by the AO–CdS system would be decreased. On the contrary, the linear range would become narrow if the ratio was too low. For a balance between the sensitivity and the linear range, the optimum concentrations of CdS and AO for the DNA determination were 4.466×10^{-5} and 1.0×10^{-6} mol L^{-1}, respectively.

Effect of the pH. The effect of pH on the fluorescence intensity in the system is shown in Fig. 6. It can be seen that $\Delta F/F$ remains maximum when the pH is in the range of 7.0 to 8.5. Also, pH 8.0 was chosen as the optimum value.

Effect of temperature. Under a temperature series, the difference of $\Delta F/F$ is shown in Fig. 7. It can be seen that the optimum temperature was at 5 °C, but considering the convenience and maneuverability, 25 °C was chosen. Besides, the result at 25 °C was satisfying, though not as good as that at 5 °C.

Effect of ionic strength. From Fig. 8, the media ionic strength had a great influence on this system. The higher was the ionic strength, the lower was the sensitivity. The reason was that high ionic strength weakened the intercalations of AO into ctDNA. Therefore, a fixed amount of buffer solution was added into the system to maintain a constant ionic strength.

Effect of time. Under the optimum conditions, the effect of time on the DNA determination was studied at room temperature. The fluorescence intensity remained stable from 5 min to 5 h. After all reagents had been added they were left standing for 10 min ahead of a measurement. In this study, 10 min was set as the standard for all fluorescence intensity measurements.

Tolerance of foreign substances. The effect of substances, including common metal ions and amino acid et al., was examined for interference. The results are summarized in Table 1. It contained most metal ions in biological systems, such as K^+, Ca^{2+}, $Zn^{2+}$and Fe^{3+} et al.

Calibration curve and detection limit

Under the optimum conditions, the relationship between ΔF (the enhancement of fluorescence intensity of AO+CdS upon the addition of varying concentrations of ctDNA) and the concentration of ctDNA was obtained. The analytical parameters are given in Table 2, which demonstrates a linear relationship between ΔF and the concentrations of ctDNA over a wide range, and the limit of detection reaching the ng mL^{-1} level.

A comparison of this method with other methods for ctDNA determination in sensitivity is summarized in Table 3. It can be seen that the sensitivity of this method was higher than that of most other RLS methods. Compared with the other methods, this one possessed an advantage, a rapid incubation time (Table 3). Therefore, this method was sensitive and rapid, and should be a valuable tool for studying the biological properties of nucleic acids.

Sample determination

Samples of ctDNA containing common metal ions, amino acid, etc. as co-existing species were analyzed. The results are given in Table 4. It can be seen that they were determined with satisfactory results.

Conclusions

The interactions among CdS, AO, and DNA were investigated by using various techniques, including fluorescence, absorbance spectra, and TEM imaging. In our research, we found that CdS quenched the fluorescence of AO with an inner filter effect. Furthermore, DNA enhanced the fluorescence of AO, which was quenched by CdS prior to DNA addition. The enhanced extents were in good proportion to the concentration of DNA over a curtain range. Further, this new method showed satisfactory results for ctDNA determination.

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