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Recognition of DNA based on changes in the fluorescence intensity of CdSe/CD QDs–phenanthroline systems

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ABSTRACT

The CdSe quantum dots (QDs) modified by mercapto-β-cyclodextrin (CD) were synthesized and characterized by transmission electron microscopy, powder X-ray diffraction, excitation and emission spectra, and fluorescence lifetime. When λ_{ex} = 370 nm, the fluorescence peak of CdSe/CD QDs is at 525 nm. Phenanthroline (Phen) is able to quench their fluorescence, which can be recovered by the addition of DNA. The quenching and restoration of fluorescence intensity were found to be linearly proportional to the amount of Phen and DNA, respectively. The variation of the fluorescence intensity of the CdSe/CD QDs–Phen system was studied, and it was demonstrated to result from a static mechanism due to the formation of a Phen inclusion complex with the CdSe QDs modified by mercapto-β-cyclodextrin. The fluorescence recovery was due to the binding of DNA with Phen in the inclusion complex, leading to the freeing of the CdSe/CD QDs. The binding constants and sizes of the binding sites of the Phen–DNA interaction were calculated to be 1.33×10^7 mol^{-1} L and 10.79 bp.

1. Introduction

In recent years, semiconductor quantum dots (QDs) have been an active subject of research in nanoscience and nanotechnology. Because of their unique optical properties, such as broad excitation spectra, narrow and symmetric emission peaks, enhanced photobleaching resistance, and tuneable spectra, quantum dots have been applied in a variety of research areas [1–3].

The water solubility and biocompatibility of QDs were investigated in order that they could be better used as fluorescent biological labels [4,5]. Fang and coworkers [6] probed lectin and sperm with carbohydrate-modified QDs; the Ersöz and coworkers [7] recognized DNA with QDs nanocrystals having guanosine-imprinted nanoshells; the Xu and coworkers [8] studied protein detection and cell fluorescence imaging with glutathione-capped CdS QDs. The studies mentioned above all utilized synthesized QDs to detect biological materials using a single site.

Recently, the Raymo and coworkers [9] added the quencher 1-methyl-1′-(3-(5-(2-oxohexahydrothieno [3,4-d]imidazol-6-yl)-pentanoxy)-propyl)-4,4′-bisiodide into CdSe-ZnS core–shell quantum dots coated with tri-n-octylphosphine oxide ligands, and observed a quenching of fluorescence by surface adsorption as a result of electrostatic attraction. Fluorescence was restored by adding streptavidin. Wang and coworkers [10] studied the interaction of an anticancer drug and DNA, using mitoxantrone (MTX) as a model drug. MTX is adsorbed on the surface of the mercaptosuccinic acid modified CdTe QDs through electrostatic action, leading to quenching of the fluorescence of the QDs, the intensity of which is recovered by the addition of DNA. Chan and coworkers [11] found, that by adding a Ru complex into thioglycolic acid capped CdTe QDs, the QDs fluorescence was quenched as a result of the ionic conjugate formation of a positively charged Ru complex with the negatively charged QDs. The addition of DNA brings about the recovery of the QDs' fluorescence intensity.

β-Cyclodextrins (CDs) are cyclic amylose-derived oligomers composed of seven α-1-4-linked glucose units. They can envelop some inorganic, organic or chiral molecules to form host-guest complexes or supramolecular complexes because of their hydrophobic cavities. In this paper, CdSe QDs modified by mercapto-β-cyclodextrin were synthesized. A DNA recognition method was established based on the fact that phenanthroline (Phen) can quench their fluorescence, whereas DNA can bring about the recovery of the fluorescence intensity of CdSe/CD QDs. The related mechanism is shown in Scheme 1. The mechanism of fluorescence quenching differs from the reported methods [9–11] which used a quencher adsorbed on the surface of the QDs through electrostatic association. CdSe QDs modified by mercapto-β-cyclodextrin have high sensitivity and selectivity due to β-cyclodextrin enveloped Phen through a host–guest interaction.

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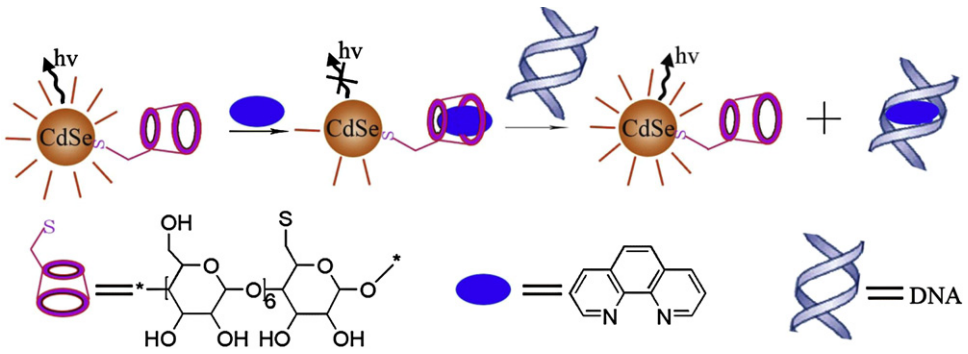

Scheme 1. Proposed mechanism for the variation of the fluorescent intensity of CdSe/CD QDs.

2. Experimental

2.1. Apparatus

Fluorescence measurements were performed using an F-2500 spectrofluorometer (Hitachi, Japan). UV–vis absorption spectra were recorded with a UV–vis 1700 spectrophotometer (Techcomp, Shanghai, China). Fluorescence lifetime was measured using a model FLS-920 Combined fluorescence lifetime and steady state spectrometer (Edinburgh, Scotland). X-ray diffraction patterns were recorded using a model Y-2000 powder X-ray diffractometer (Dandong Aolong Radiative Instrument Co., Ltd., Liaoning, China). pH values were measured with a model PHS-3C pH meter (Leici Analytical Instrument Factory, Shanghai, China). Transmission electron micrographs were recorded by a FEI-Tecnai 12 analytical transmission electron microscopy (FEI, Holand). Temperature was controlled by a model HZ-8802S constant temperature bath (Guoling Instrument Co., Ltd., Guangzhou, China).

2.2. Reagents

All chemicals used were of analytical grade or of the highest purity available. All solutions were prepared with double-distilled water. β-Cyclodextrin, DNA, Se powder, $NaBH_4$, NaOH were purchased from Shanghai Reagent Company (China) and used as received. 0.1 mol L^{-1} physiological NaCl, 500 μg mL^{-1} physiological DNA sodium chloride solution was prepared by adding some DNA in 0.005 mol L^{-1} physiological NaCl solution and stored at 4 °C. Tris–HCl buffer solution (pH 7.6) was prepared.

2.3. Synthesis of CdSe/CD quantum dots

6-Mercapto-β-cyclodextrin was synthesized as a Ref. [12]. NaHSe was prepared following the method reported previously [13]. 0.0274 g of $CdCl_2{\cdot}2.5H_2O$ was dissolved in 120 mL of water and 0.0696 g of 6-SH-β-CD. The solution pH was adjusted to 10.20 by dropwise addition of 1.0 mol L^{-1} NaOH solution. The solution was placed in a three-necked flask fitted with valves, and deaerated N_2 by bubbling through it for about 30 min. While stirring, 90 μL of freshly prepared, oxygen-free NaHSe solution (1.0 mol L^{-1}) was quickly added to the solution. The resulting mixture was refluxed at 75 °C for 15 min and was used in the experiment to promote the growth of CdSe/CD QDs. The final concentration of CdSe/CD QDs, as measured by the Se^{2-} concentration, was 7.5×10^{-4} mol L^{-1}.

2.4. Fluorescence determination

1.0 mL of 7.5×10^{-4} mol L^{-1} CdSe/CD QDs solution mentioned above, 1.0 mL Tris–HCl buffer (pH 7.6), and varying amounts of Phen, or fixed the amounts of Phen, and varying amounts of DNA were successively placed into a 10 mL calibrated test tube, then diluted to the mark with water and mixed thoroughly. At an excitation wavelength of 370 nm, the fluorescence spectra of the CdSe/CD QDs–Phen system and CdSe/CD QDs–Phen–DNA system were determined in the 400–700 nm emission wavelength range. The fluorescence intensity was recorded at 525 nm. 700 V is selected as sensitivity.

3. Results and discussion

3.1. Characterization of CdSe/CD QDs

3.1.1. TEM analysis of CdSe/CD QDs

The particle size and morphology of CdSe/CD QDs were determined by TEM. From Fig. 1a, it is observed that the CdSe/CD QDs are well-dispersed, and spherical, with uniform particle sizes of about 2 nm.

3.1.2. Powder X-ray diffraction spectra of CdSe/CD QDs

Powder X-ray diffraction spectra of CdSe/CD QDs were analyzed. As shown in Fig. 1b, their diffraction peaks are broad and weak, but the main peaks are centered at approximately $2\theta = 22°$, 43° and 49°, which basically conform with those of CdSe, centered at $2\theta = 25.4°$, 42.0° and 49.7°, respectively [14].

3.1.3. Excitation and emission spectra of CdSe/CD QDs

Fig. 1c shows excitation and emission spectra of CdSe/CD QDs. The excitation spectrum is broad, while the emission spectrum is narrow and symmetric, coinciding with the basic nature of QDs.

3.1.4. Fluorescence lifetime of CdSe/CD QDs

Fluorescence lifetime of CdSe/CD QDs is illustrated in Fig. 1d. Single exponential fitting of the decay curve yielded a τ_0 value of 13.82 ns.

3.2. Quenching effect of Phen on the fluorescence intensity of CdSe/CD QDs

As shown in Fig. 2a, when the excitation of CdSe/CD QDs is performed at 370 nm, the fluorescence peak is centered at 525 nm. The addition of Phen gradually decreases the fluorescence intensity of the system, but the intrinsic spectral peak wavelength of CdSe/CD QDs remains constant. It was found that 1.0 μmol·L^{-1} Phen resulted in a quenching effect of 15.8%, 10.0 μmol L^{-1} Phen yielded 64.1%, 15.0 μmol L^{-1} Phen gave 73.7% and 50.0 μmol L^{-1} Phen reduces the fluorescence intensity of CdSe/CD QDs to an intrinsic value of 5.2%.

Fig. 2b illustrates that the relative fluorescence intensity, F/F_0 (F and F_0 are the fluorescence intensity of CdSe/CD QDs in the presence/absence of Phen, respectively) exhibits a linear relationship with the concentration of Phen in the range from 0.0 μmol L^{-1}