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A study on the interaction between CdTe quantum dots and chymotrypsin using optical spectroscopy

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ABSTRACT

Thioglycolic acid (TGA) capped CdTe quantum dots (QDs) with the diameter of 2–3 nm were synthesized. The interaction between CdTe QDs and chymotrypsin (chy) was investigated by ultraviolet–visible (UV–vis) absorption, fluorescence and resonance Rayleigh scattering (RRS) spectroscopy. Under pH 7.2, CdTe QDs effectively quenched the intrinsic fluorescence of chy via static quenching. The binding constants for the formation of a complex between CdTe QDs and chy were 2.85, 2.58, 2.48 × 103 M^{-1} at 293, 298 and 303 K, respectively. ΔS° (49.72 kJ mol^{-1}) and ΔH° (−4.61 kJ mol^{-1}) indicated that electrostatic attraction was the dominant intermolecular forces in stabilizing the complex. The interaction between CdTe QDs and chy lead to the remarkable enhancement of RRS and the enchantments were in proportional to the concentration of chy in a certain range. The reasons for the enhancement of RRS were discussed. The experimental results showed that chy molecules have a relatively high affinity with CdTe QDs.

1. Introduction

Quantum dots (QDs), a brand new class of fluorescent nanoprobes, are of advantage over fluorescent dyes because of their tunable emission color, high quantum yield and long-term photostability. Moreover, the emission of QDs is narrow, symmetric, and independent of the excitation wavelength [1,2]. QDs had been tested as luminescent probes for labeling of cells and tissues [3], targeting the image of surface proteins [4] and immunostaining of membrane proteins [5]. QDs and their molecular conjugates are becoming increasingly important for a wide range of applications in biotechnology and medicine [6–12]. Up to now, several analytical methods have been used to investigate the interaction of QDs with biomolecules, such as surface plasmon resonance analysis, electrochemical analysis, fluorescence resonance energy transfer/quenching, and so forth [13–20]. However, there were a few papers to investigate the interaction of QDs with biomolecules by ultraviolet–visible (UV–vis) absorption, resonance Rayleigh scattering (RRS) and fluorescence spectroscopy simultaneously.

The fluorescence emission from proteins is frequently heterogeneous. This heterogeneity can arise from different fluorophores like tyrosine, tryptophan and phenylalanine residues, which are identical but located in different environments within the protein matrix [21]. The fluorescence from the protein chymotrypsin (chy) arises uniquely from its tryptophan residues when selectively excited at 280 nm. Yang et al. [22] have successfully built the fluorescence resonance energy transfer (FRET) assembles between luminescent quantum dots (QDs) and chy, QDs and gold nanoparticles (NPs) in one system. Chy was linked to CdTe QDs after chy being activated by N-hydroxysulfosuccinimide sodium (NHS), yet other parameters like mode of interaction and binding mechanism are also important. The parameters may provide major theoretical information for understanding the mechanisms involved in the biocompatibility of fluorescent QDs, but the studies have been rather limited.

In this paper, the interaction between CdTe QDs and chy was investigated by UV–vis absorption, fluorescence and RRS spectroscopy. It was found that the UV–vis absorption spectrum of CdTe QDs and chy obviously changed, showing that CdTe QDs could associate with chy to form a new complex. The influences of some factors on the interaction were discussed in detail. CdTe QDs effectively quenched the intrinsic fluorescence of chy, the fluorescence quenching mechanism, binding constant, and thermodynamics parameters during the binding process were reported in the paper. After CdTe QDs interacted with chy, the intensities of RRS enhanced and the enhancements were in proportion to the concentration of chy. Based on this, it is possible to build a method for the determination of chy using CdTe QDs as probes. The reasons for the enhancement of RRS intensity were also discussed.

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2. Materials and methods

2.1. Materials

A Hitachi F-2500 spectrofluorophotometer (Hitachi Company, Japan) was used to record the RRS and fluorescence spectra and measure the intensities of fluorescence and RRS. A UV-8500 spectrophotometer (Tianmei Corporation, Shanghai) was applied to record the absorption spectra. TECNAI-10 transmission electron microscopy (TEM) (Philips Company, Holland) was used to observe the appearance and the size of nanoparticles. A PHS-3C pH meter (Leici, Shanghai) was used to adjust pH.

$CdCl_2{\cdot}2.5H_2O$ (Shanghai Chemicals Reagent Co., Shanghai), Te powder (Sinopharm chemical Reagent Co., Shanghai), Thioglycolic acid (TGA, Sinopharm chemical Reagent Co., Shanghai), $NaBH_4$ (Tianjin Huanwei Fine Chemical Co., Tianjin), Chymotrypsin (Sinopharm chemical Reagent Co., Shanghai), Britton–Robinson buffer solution was used to control the acidity of the aqueous medium. All reagents were analytical grade without further purification and deionized water was used throughout.

2.2. Methods

Aqueous colloids of CdTe QDs solution was prepared according to previously published methods [23]. Under N_2 atmosphere, deionized water (5 mL) was added dropwise to tellurium (0.0151 g) and excessive sodium borohydride under magnetic stirring at room temperature, the colorless solution of NaHTe was prepared.

0.0553 g $CdCl_2{\cdot}2.5H_2O$ was dissolved in 150 mL of deionized water and 0.04 mL of TGA stabilizer was added under stirring, followed by adjusting the pH to the 11.5 by dropwising addition of 1 mol L^{-1} NaOH solution. The solution was placed in a three-necked flask and deaerated by N_2 bubbling for about 30 min.

Under stirring, H_2Te gas generated by the reaction of the solution of NaHTe with diluted H_2SO_4 (50 mmol L^{-1}) was passed through the oxygen-free original solution together with a slow nitrogen flow for 30 min. CdTe precursors were formed at this stage. The molar ratio of Cd^{2+}/TGA/HTe^- was fixed at 1:2.4:0.5. Then the resulting mixture was subjected to reflux at 369 K for 4 h under open-air condition with condenser. Then emitting-green quantum dots were obtained. The concentration of TGA-CdTe QDs was dependent on the Te^{2-} concentration. [24] 1.0 mL above prepared CdTe QDs, buffer solution and an appropriate amount of chy was added into a 10 mL volumetric flask, then diluted with deionized water to the mark and mixed thoroughly with gentle shake. After incubation for 10 min, the fluorescence, RRS spectra of solution were examined.

3. Results and discussion

3.1. TEM images

The shape and diameter of the CdTe QDs was observed by TEM (Fig. 1). The water-soluble, TGA functionalized CdTe QDs were successfully synthesized according to the protocol described in Section 2.2. The average size of the particle was about 2–3 nm analyzed by TEM. The TEM image shows clearly that these QDs are monodisperse.

3.2. UV–vis spectra studies

UV–vis absorption measurement is a very simple method and applicable to explore the structural change and to know the complex formation. The interaction between CdTe QDs and chy was investigated by UV–vis absorption spectroscopy. As shown in Fig. 2, absorption peak in 280 and 490 nm are assigned for the characteristic absorption band of chy and CdTe QDs. A new band was not being observed (Fig. 2D) when the CdTe QDs was added into the chy solution. The results indicated that CdTe QDs may not affect the structure of chy. In order to study the change of absorption spectrum intensity on the interaction between CdTe QDs and chy, the difference of the absorption spectrum intensity of the mixture (CdTe QDs and chy) and the absorption spectrum intensity of CdTe QDs is shown in Fig. 2B. The difference of absorption spectrum intensity of Fig. 2A and B is obviously changed, which indicates that there are strong interaction between CdTe QDs and chy.

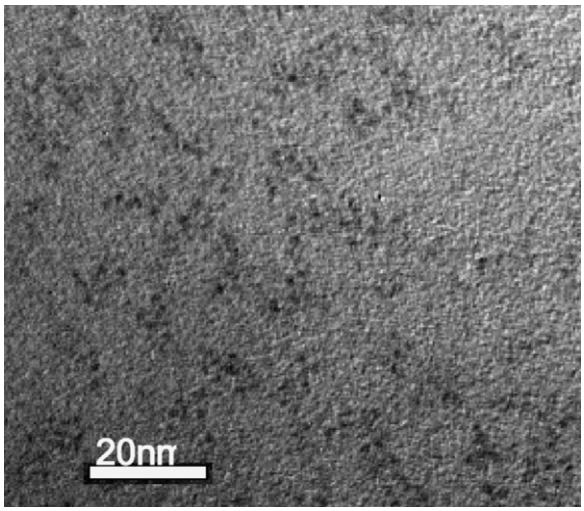

Fig. 1. TEM image of CdTe QDs.

3.3. Fluorescence quenching mechanism and binding constant

The fluorescence intensity of a compound can be decreased by a variety of molecular interactions, such as excited-state reactions, molecular rearrangements, energy transfer, ground–state complex formation and collision quenching [25]. Such a decrease in intensity is called fluorescence quenching. Collisional or dynamic quenching refers to a process that the fluorophore and the quencher come into contact during the lifetime of the excited-state, whereas

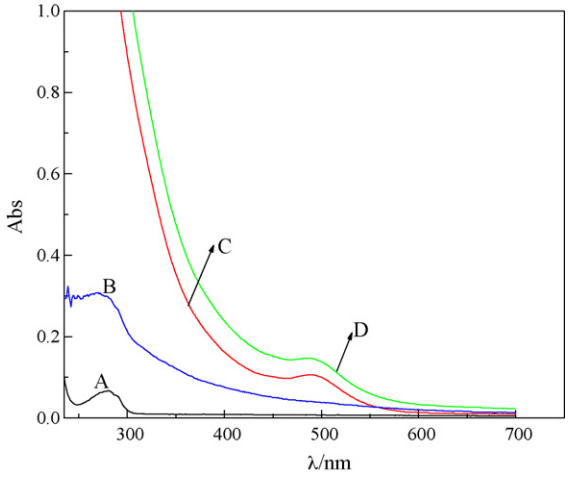

Fig. 2. The UV–vis absorption spectra of (A) the absorption spectrum of chy, (B) the difference of the absorption spectrum intensity of the mixture (CdTe QDs and chy) between the absorption spectrum intensity of CdTe QDs, (C) the absorption spectrum of CdTe QDs, and (D) the absorption spectrum of the mixture (CdTe QDs and chy). The concentration of CdTe QDs, chy were 2.0×10^{-4} mol L^{-1}, 40 μg mL^{-1}.