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Study on the interaction between CdSe quantum dots and hemoglobin

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Abstract

The interaction between CdSe quantum dots (QDs) and hemoglobin (Hb) was investigated by ultraviolet and visible (UV–vis) absorption spectroscopy, Fourier transform infrared (FTIR) spectroscopy, and fluorescence (FL) spectroscopy. The intensity of UV–vis absorption spectrum of a mixture of CdSe QDs and Hb was obviously changed at the wavelength of 406 nm at pH 7.0, indicating that CdSe QDs could bind with Hb. The influences of some factors on the interactions between CdSe QDs and Hb were studied in detail. The binding molar ratio of CdSe QDs and Hb was 12:1 by a mole-ratio method. The mechanism of the interaction between CdSe QDs and Hb was also discussed.

Keywords: CdSe quantum dots; Hemoglobin; Interaction

1. Introduction

Quantum dots (QDs) have gained increasing attention of scientists and engineers of various disciplines in the past decade, which have been widely used in biology, such as biosensing, biolabeling, bioimaging, and so on [1–6]. Therefore, it is very important to study the interaction of QDs with biomolecules. 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 [7–16]. Medintz et al. have studied the interaction between QDs and *Escherichia coli* maltose-binding protein system by fluorescence resonance energy transfer [15]. Liu et al. have investigated the interaction of PVP-capped CdS QDs and hemoglobin (Hb) with electrochemical method, showing that PVP-capped CdS QDs have good biocompatibility to Hb [16]. Based on the fluorescence quenching of CdSe/CdS nanocrystals, Ma et al. have developed a novel method for the determination of cytochrome *c*, Hb, and myoglobin [17]. However, the number of binding sites and the mechanism of interaction between CdSe QDs and protein are not clear. This suggests a variety of future experiments.

Hb is an iron-based complex that serves biochemical roles involving electron transfer, oxygen transfer and storage, and metabolism. It is contained in red blood cells, consisting of four subunits (two identical-chains of 141 amino acids each, and two identical-chains of 146 amino acids each). The four subunits are held together at their contacting sites by hydrophobic bonds and salt bridges [18]. Study on the interaction between QDs and Hb will not only make novel procedure for the label of QDs with proteins, but also develop new method for the determination of Hb. Spectroscopic methods are well known for their high accuracy and low dissipation properties. However, to the best of our knowledge, so far it is scarce to study the interaction of QDs and Hb by spectroscopic methods.

In this paper, the interaction between CdSe QDs and Hb was investigated by ultraviolet and visible (UV–vis) absorption spectroscopy, Fourier transform infrared (FTIR) spectroscopy, and fluorescence (FL) spectroscopy. It was found that the UV–vis absorption spectrum of CdSe QDs and Hb obviously changed at the wavelength of 406 nm, showing that CdSe QDs could associate with Hb to form a new complex. The influences of some factors on the interactions were discussed in detail. Furthermore, the FTIR spectra indicated that CdSe QDs and Hb did not form a new chemical bond and CdSe QDs were possible directly adsorbed onto Hb through the electrostatic attraction and surface bound complexation equilibrium attraction. The FL spectra showed that slight fluorescence enhancement was observed with increasing the concentration of Hb from 0.0 to 4.0×10^{-7} mol l^{-1}. The fluorescence quenching of CdSe QDs was seen at relatively higher concentration of Hb, which indicated that new surface states were not formed and the electron transfer process occurred between CdSe QDs and Hb.

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2. Experimental

2.1. Reagents and chemicals

Hb was bought from Shanghai Bio Life Science & Technology Co., Ltd. (China). $CdCl_2{\cdot}2.5H_2O$, thiolglycolic acid, Selenium powder (200 mesh) were purchased from Shanghai Reagent Factory. All other chemicals used were of analytical grade and were used without further purification. The CdSe QDs were prepared as described elsewhere [19]. The UV–vis absorption spectrum of CdSe QDs revealed that the absorption band had a maximum at 376 nm. The average size of as-prepared CdSe QDs was 1.3 nm, which was calculated according to a well-resolved absorption maximum of the first electronic transition of UV–vis absorption spectrum [20]. All the solutions used in this study were prepared with doubly deionized water and filtered through a 0.2 μm filter membrane.

2.2. Apparatus

The UV–vis absorption spectra were acquired on a Thermo Nicolet Corporation Model evolution 300 UV–vis spectrometer. The FTIR spectra were performed on a Thermo Nicolet Corporation Model avatar 330 spectrometer. The FL spectra were acquired with Perkin Elmer Model LS-55 luminescence spectrometer equipped with a 20 kW xenon discharge lamp as a light source. The excitation wavelength was 390 nm. The pH measurements were made with a Model pHS-3C meter (Shanghai Leici Equipment Factory, China).

2.3. Procedure

The UV–vis absorption spectra were recorded between 350 and 500 nm. The A_0 was the sum of the absorption spectrum intensity of CdSe QDs and the absorption spectrum intensity of Hb. The A was the absorption spectrum intensity of the mixture of CdSe QDs and Hb. The ΔA was the difference between A_0 and A.

The FTIR spectra were performed with a spectral range of 500–4000 cm^{-1}. The KBr disks were prepared by well blending KBr with dried Hb, vacuum-dried CdSe QDs or the mixture. The plate was placed into the measuring chamber. During each measurement the chamber was flushed with dry air.

The FL spectra were acquired by adding different concentrations of Hb solution into the CdSe QDs solution, which were diluted with Phosphate Buffered Saline (PBS) buffer and stirred for 1 h.

3. Results and discussion

3.1. The UV–vis absorption spectra of the interaction between CdSe QDs and Hb

The interaction between CdSe QDs and Hb was investigated by UV–vis absorption spectroscopy. As shown in Fig. 1, absorption peak in 376 and 406 nm are assigned for the characteristic absorption band of CdSe QDs and Hb. A new band was not being observed (Fig. 1b) when the CdSe QDs was added into the Hb solution. The results indicated that CdSe QDs may not affect the structure of Hb. In order to study the change of absorption spectrum intensity on the interaction between CdSe QDs and Hb, the sum of the absorption spectrum intensity of Hb (Fig. 1c) and the absorption spectrum intensity of CdSe QDs (Fig. 1d) is shown in Fig. 1a. The difference of absorption spectrum intensity of Fig. 1a and b is obviously changed, which indicates that there existes strong interaction between CdSe QDs and Hb.

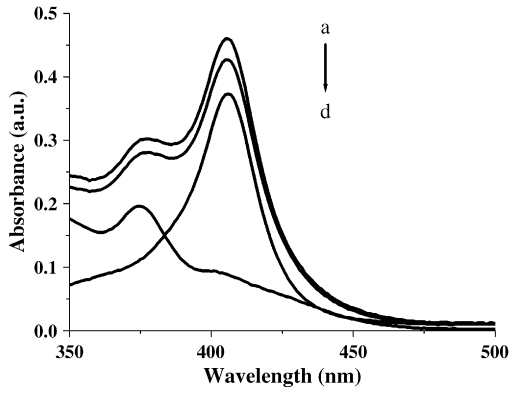

Fig. 1. The UV–vis absorption spectra of (a) the sum of the absorption spectrum intensity of QDs and the absorption spectrum intensity of Hb, (b) the absorption spectrum of the mixture (CdSe QDs and Hb), (c) the absorption spectrum of Hb, and (d) the absorption spectrum of CdSe QDs. The concentration of CdSe QDs and Hb were 6.0×10^{-6} and 1.0×10^{-6} mol l^{-1}, respectively.

3.2. The effect of pH on the interaction of CdSe with Hb

Fig. 2 shows the effect of pH on the interaction between CdSe QDs and Hb. The concentrations of CdSe QDs and Hb were kept at 6.0×10^{-6} and 1.0×10^{-6} mol l^{-1}, respectively. The absorption spectrum intensity of the mixture of CdSe QDs and Hb (A) were acquired by adding CdSe QDs and Hb into the PBS buffer with different pH value. The A_0 was the sum of the absorption spectrum intensity of CdSe QDs and the absorption spectrum intensity of Hb at different pH value. The ΔA was the difference between A_0 and A at different pH value. In the present study, the effect of pH was investigated in the range of 3.0–10.0 using PBS as buffer. It was found that the maximum change of

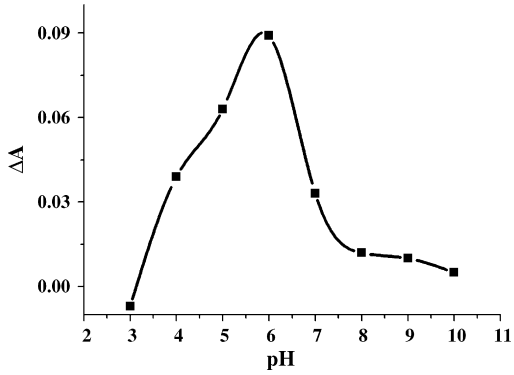

Fig. 2. The effect of pH on the interaction of CdSe with Hb. The concentrations of CdSe QDs and Hb were kept at 6.0×10^{-6} and 1.0×10^{-6} mol l^{-1}, respectively.

ΔA occurred at pH 6.0. So, PBS buffer of pH 6.0 was chosen in the following experiments.

3.3. The effect of reactive time on the interaction of CdSe QDs with Hb

The interaction of CdSe QDs and Hb was monitored at different time scales at room temperature. The absorption spectrum intensity of mixture had a rapid decrease with the increase of the time until 1 h, which indicated that certain time is needed to complete the interaction. However, the absorption spectrum intensity of CdSe QDs and the absorption spectrum intensity of Hb did not change with the time extension. The results showed that the interaction process reached equilibrium after CdSe QDs reacting with Hb for 1 h, which was chosen in the following experiments.

3.4. The effect of concentration of Hb on the interaction of CdSe QDs with Hb

Fig. 3 elucidates the effect of concentration of Hb on the interaction of CdSe QDs and Hb. First, Hb solution was diluted by PBS buffer two times and five times alternately, which was added to 10 ml tubes, and then 0.5 ml 6.0×10^{-5} mol l^{-1} CdSe QDs solution was added to the tubes. The mixture was diluted to 5 ml with PBS buffer. The UV–vis absorption spectra were measured at the wavelength of 406 nm using PBS buffer as the blank. ΔA of this system increased at the 406 nm with the increasing of Hb concentration, but higher Hb concentration resulted in no more changes of the UV–vis absorption spectra, due to completely binding of CdSe QDs and Hb. The number of binding sites was acquired by a mole-ratio method [21]. The stochiometry of CdSe QDs and Hb can be found by extrapolating the straight-line portions of the graph, which is to say that the point at which these lines intersect corresponds directly to the ratio of of CdSe QDs and Hb (point D in Fig. 3). As shown in Fig. 3, the concentration of Hb is 12 that of CdSe QDs at the stoichiometric ratio, indicating that the stochiometry of CdSe QDs and Hb is 12:1.

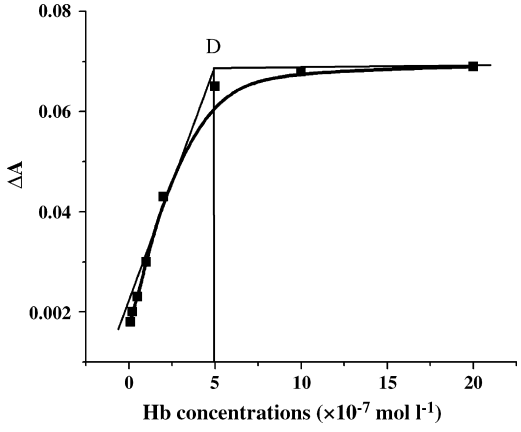

Fig. 3. The effect of concentration of Hb on the interaction of CdSe QDs and Hb in 0.1 mol l^{-1} PBS buffer at pH 6.0. The concentration of CdSe QDs was 6.0×10^{-6} mol l^{-1}.

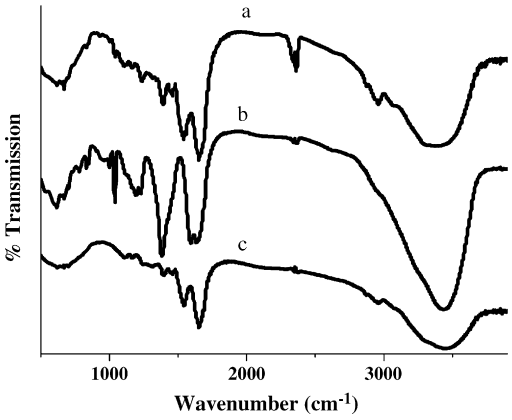

Fig. 4. FTIR spectra of CdSe QDs and Hb. (a) CdSe QDs + Hb; (b) CdSe QDs; (c) Hb.

3.5. The FTIR spectra of CdSe QDs with Hb

FTIR spectroscopy has been frequently used for determining the protein's secondary structure [22]. Absorbance at the amide I and amide II region are due to the amide C=O bond stretching vibration and the in-plane NH bending (or the CN stretching) modes, which are sensitive to the geometry and characteristic hydrogen bonding patterns of the secondary structure. In this study, we have applied the FTIR spectroscopy to study the binding mechanism of CdSe QDs and Hb. As shown in Fig. 4, native Hb appeares two absorption bands located at 1655 and 1543 cm^{-1} for amide I and amide II (Fig. 4c) [23]. For the mixture of CdSe QDs and Hb, the bands are located at 1659 and 1541 cm^{-1} for amide I and amide II (Fig. 4a). However, the FTIR spectrum of CdSe QDs does not appear a band in those two places (Fig. 4b). It can be seen that the amide I band and the amide II band of the mixture do not change obviously compared with the native Hb. Therefore, it can be concluded that CdSe QDs and Hb do not form a new chemical bond, and the CdSe QDs are possible directly adsorbed onto Hb through the electrostatic attraction and surface bound complexation equilibrium attraction, which is in agreement with the results of UV–vis absorption spectra.

3.6. The FL spectra of CdSe QDs with Hb

The fluorescence emission spectra were further used to study the interaction of CdSe QDs and Hb. The FL spectra were acquired by adding different concentrations of Hb solution into 1.2×10^{-5} mol l^{-1} CdSe QDs solution, which were diluted with PBS buffer and stirred for 1 h. As shown in Fig. 5, the position of the fluorescence peak of CdSe QDs does not change in the presence of Hb, however, the slight fluorescence enhancement is observed with the increasing of Hb concentration from 0.0 to 4.0×10^{-7} mol l^{-1} and the fluorescence quenching is observed at relatively higher concentration of Hb. Fig. 6 indicates absorption intensity of the mixture vs Hb concentration in the 538 nm. Two distinctively different trends are evident with increasing the concentration of Hb from 0.0 to 4.0×10^{-7} mol l^{-1} and from 4.0×10^{-7} to 1.1×10^{-6} mol l^{-1}. With increasing the

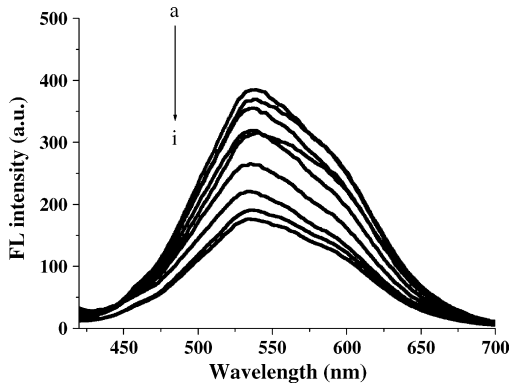

Fig. 5. The effect of Hb on the fluorescence emission spectra of CdSe QDs in 0.1 mol l^{-1} PBS buffer at pH 6.0. The Hb concentrations were follows: (a) 1.3×10^{-7}; (b) 3.3×10^{-8}; (c) 2.6×10^{-7}; (d) 4.0×10^{-7}; (e) 0; (f) 5.3×10^{-7}; (g) 6.6×10^{-7}; (h) 8.3×10^{-7}; (i) 1.0×10^{-6} mol l^{-1}. The concentration of CdSe QDs was 1.2×10^{-5} mol l^{-1}. $\lambda_{ex} = 390$ nm.

concentration of Hb from 0.0 to 4.0×10^{-7} mol l^{-1}, slight fluorescence enhancement is observed and reaches to the maximum at 1.3×10^{-7} mol l^{-1}. However, the CdSe QDs fluorescence quenching is seen with the increase of Hb concentration sequentially, but higher Hb concentration resultes in no more changes of the FL spectra, due to completely binding of CdSe QDs and Hb. In Fig. 6, it is possible to distinguish two linear trends intersecting at the stoichiometric ratio (point B), showing that the stochiometry of CdSe QDs and Hb is 12:1. The results obtained from FL spectra are in agreement with those obtained from UV–vis absorption spectra.

3.7. The possible interaction mechanism

Several mechanisms for the interaction between CdSe QDs and protein are possible, including electrostatic attraction, surface bound complexation equilibrium attraction, hydrophobic attraction, and so on [24]. One hemoglobin molecule has a diameter of 6.4 nm [25], and CdSe QDs, capped with thioglycolic acid, have a diameter of 1.3 nm [22]. CdSe QDs and Hb possibly attractes each other through the electrostatic attraction and surface bound complexation equilibrium attraction.

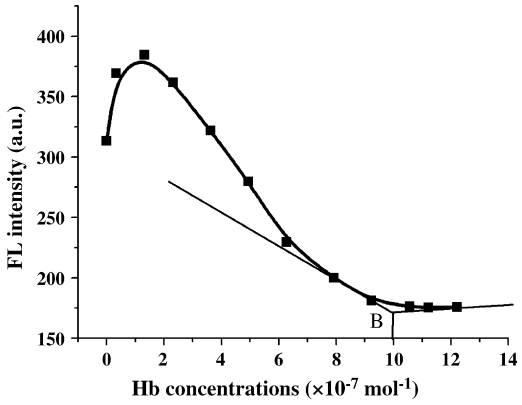

Fig. 6. The effect of Hb on the fluorescence emission spectra of CdSe QDs in 0.1 mol l^{-1} PBS buffer at pH 6.0. The concentration of CdSe QDs was 1.2×10^{-5} mol l^{-1}. $\lambda_{ex} = 390$ nm.

CdSe QDs are electron-transfer complexes and Hb is an electron-accept complex [16]. UV–vis absorption spectroscopy is usually applied to study on the interaction between electron-transfer complexes and electron-accept complexes [26]. The net charge of CdSe QDs and Hb changes with various pH. If there exists electrostatic interactions, the binding amount of CdSe QDs should change when we gradually alter the pH of the solution [27]. As shown in Fig. 2, the maximum change of absorption intensities occurrs at pH 6.0, which suggests CdSe QDs have the highest binding amount at pH 6.0. The results of UV–vis absorption spectra shows that CdSe QDs could form a complex with Hb by electrostatic attraction.

The FTIR spectra showed that the amide I band and the amide II band of the mixture were actually the same as those of the native Hb, which suggested that CdSe QDs and Hb did not form a new chemical bond. Therefore, it can be concluded that CdSe QDs are possible directly adsorbed onto Hb through the electrostatic attraction and surface bound complexation equilibrium attraction, which is in agreement with the results of UV–vis absorption spectra.

Hb enhances the fluorescence of CdSe QDs at lower concentration, which is similar to the interaction between ethylenediamine and CdSe QDs reported by Liang et al. [28]. According to their mechanisms, Hb at low concentration passivates the surface and suppresses nonradiative recombination at surface vacancies rules out formation of new surface states. However, Hb quenches the fluorescence of CdSe QDs at higher concentration, which is similar to the interaction between CdSe QDs and cytochrome *c*, Hb, myoglobin, and *o*-phenylenediamine [16,28]. The mechanism of interaction between CdSe QDs and Hb can be explained using the electrostatic attraction and surface bound complexation equilibrium attraction model and a photo-induced electron transfer process.

The quenching mechanism is proposed as follows:

$$\text{CdSe} + \text{[Fe(III)-Heme]} \leftrightarrow \{\text{CdSe}\cdots\text{[Fe(III)-Heme]}\} \quad (1)$$

$$\{\text{CdSe}\cdots\text{[Fe(III)-Heme]}\} + h\nu \rightarrow \{\text{CdSe}^*\cdots\text{[Fe(III)-Heme]}\} \quad (2)$$

$$\{\text{CdSe}^*\cdots\text{[Fe(III)-Heme]}\} \rightarrow \{\text{CdSe}\cdots\text{[Fe(II)-Heme]}\} \quad (3)$$

CdSe QDs and Hb form a complex by electrostatic attraction and surface bound complexation equilibrium attraction. The electrons within the CdSe QDs are firstly excited to the excited state under photo-irradiation, [Fe(III)-Heme] can directly intercept one of the charge carriers and is reduced to [Fe(II)-Heme], which can disrupt the radiative recombination of the holes and the excited electrons and quench the fluorescence of the CdSe QDs.

4. Conclusion

The interaction of CdSe QDs with Hb has been investigated by several spectroscopic methods. The results showed

that twelve CdSe QDs interacted with one Hb molecule. The obtained results suggest that the CdSe QDs have bound with Hb possibly by electrostatic attraction and surface bound complexation equilibrium attraction. It is potentially possible to study the interactions of nanoparticles with biomolecules based on present method.

Acknowledgements

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