Fluorescence biosensor based on CdTe quantum dots for specific detection of H5N1 avian influenza virus

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Abstract
This report highlights the fabrication of fluorescence biosensors based on CdTe quantum dots (QDs) for specific detection of H5N1 avian influenza virus. The core biosensor was composed of (i) the highly luminescent CdTe/CdS QDs, (ii) chromatophores extracted from bacteria Rhodospirillum rubrum, and (iii) the antibody of β-subunit. This core part was linked to the peripheral part of the biosensor via a biotin–streptavidin–biotin bridge and finally connected to the H5N1 antibody to make it ready for detecting H5N1 avian influenza virus. Detailed studies of each constituent were performed showing the image of QDs-labeled chromatophores under optical microscope, proper photoluminescence (PL) spectra of CdTe/CdS QDs, chromatophores and the H5N1 avian influenza viruses.

Keywords: photoluminescence, fluorescence labeling, virus detection

Classification numbers: 4.01, 4.03, 5.04

1. Introduction

One of the most highlighted applications of semiconductor quantum dots (QDs) in life sciences is their use as novel fluorescent probes in bio-medical labeling [1–17]. As fluorescent labels, QDs possess remarkable optical characteristics compared to conventional organic dyes in terms of having size-tunable absorption and photoluminescence (PL) spectra, and having broad/non-selective absorption and bright/narrow emission. One should classify clearly different types of biological labels corresponding to their sophisticated structure and sensitivity. For example, the simplest label is just to bind luminescent QDs on the surface or inside the cells (e.g. yeast cells) for enough time and then observe their photoluminescence under microscope. This is usually used for counting cells without any specific identification. If a specific labeling is needed, a much more complicated label must be designed in that an antibody attached with the luminescent QDs would specifically recognize its corresponding antigen. The most highly sensitive and selective detection of antigens (e.g. H5N1 virus or pesticide) could be realized by some biosensor structures in which the corresponding antibody must be a constituent to promote the specific antibody–antigen interaction. Such interaction could then result in the change in the PL intensity or electrochemical signal [1, 6–17]. In fact, the dependence of the PL intensity from QDs on the pH of the medium surrounding has been exploited to fabricate biosensors characteristic to pesticides or viruses [6–19]. For antigen recognition by specific antibody bound on chromatophores, the antigen–antibody reaction could change the proton flux, consequently the PL intensity of QDs attached on the chromatophores. The proton flux, which is generated during the synthesis of adenosine triphosphate (ATP) with the catalysis of the F0F1–ATPase universal enzyme, is influenced by the virus load and then
demonstrated as the change in PL of QDs. This fluorescence biosensor has been proposed for detecting some diseases caused by viruses in the early-stage [10, 13, 20].

In the past two decades, significant advances have been made toward the synthesis of colloidal semiconductor QDs, particularly II–VI compound such as CdSe, CdS and CdTe [1–5]. Among II–VI semiconductor compounds, CdTe has attracted much interest because it is a direct gap material with a bandgap energy of 1.52 eV which is suitable for emitting in the visible spectral range by controlling the size correspondingly with the quantum confinement of charge carriers. We have synthesized in aqueous phase CdTe QDs in different sizes that all emit strong luminescence in the green-red spectral region [2, 9]. Using CdTe/CdS core/shell QDs at different sizes bound specifically with different antibodies, it is quite possible to distinguish different kinds of antigen at the same test [17, 22].

In this paper we present the results on the fabrication of fluorescence biosensors based on CdTe/CdS QDs for specific detection of H5N1 avian influenza virus. The biosensor consists of the core part that is essentially the same for detection of any antigen, and the peripheral part that is specific to each kind of antigens. The core part was made from the high-luminescence CdTe/CdS QDs, chromatophores extracted from bacteria Rhodospirillum rubrum, and the antibody of β-subunit. The peripheral part of the biosensor consisted of the antibody of H5N1 virus and H5N1 avian influenza virus. A biotin–streptavidin–biotin bridge was finally used to connect the core part with the peripheral part to realize the virus detection. Different characterization techniques were used to study the morphology of QDs-labeled chromatophores, the PL properties of CdTe/CdS QDs, chromatophores, and of H5N1 avian influenza virus as well.

2. Experimental

2.1. Synthesis of CdTe/CdS QDs

CdTe QDs were synthesized in aqueous solution following the procedure described elsewhere [2, 9]. Briefly, the freshly prepared NaHTe solution was quickly injected into N2-saturated CdBr2 solution at room temperature in the presence of mercaptopropionic acid (MPA) as a stabilizing agent to create the CdTe seeds. MPA surfactant plays an important role to make CdTe QDs existent in colloidal form. The molar ratio of Cd:Te:MPA was fixed at 2:1:3 and the pH of this solution was adjusted in the range of 7–12 by adding the 1.0 M NaOH stock solution. Then, CdTe QDs were grown up in an autoclave at 120 °C for different growth times between a few minutes to several hours that would enable us to obtain the high quality CdTe QDs with different sizes.

To passivate surface dangling bonds and stabilize the photophysical characteristics of CdTe QDs, the CdS shell was made over the CdTe core by adding an excessive amount of thiourea (for the S precursor) into the solution containing CdTe QDs core (with the available excessive Cd²⁺ ions). Subsequently, the mixture was annealed at 120 °C for certain periods of time depending on the required sizes of CdTe/CdS QDs. This shelling for a few minutes to a few hours not only formed the CdS shell but also developed the CdTe core to be bigger, giving the emission at longer wavelengths. All the CdTe/CdS QDs synthesized in aqueous phase can be used directly in fluorescence bio-labeling.

2.2. Preparation of biomaterial-QDs radicals and biosensors

We have fabricated biosensors based on the measurement of the PL intensity of CdTe/CdS QDs as a function of pH that correspondingly reflects the H5N1 virus content. The pH is supposed to be locally changed with the proton generated during the synthesis of ATP from ADP. Figure 1 demonstrates a typical representation of such a kind of biosensor [13]. With the normal size of chromatophore of 70 nm diameter, many CdTe/CdS QDs could bind on the surface of one chromatophore just by the electrostatic force. In the biosensor structure, one can see clearly that the core part is composed of CdTe/CdS QDs (1) bound on the surface of chromatophores (2) and Fn–F1−ATPase antibody of β-subunit (3). This core part in essence is designed the same for detecting various kinds of antigens that influence the proton flux pumped out of chromatophores. A biotin–streptavidin–biotin bridge (4) is necessary to conjugate the antibody against H5N1 avian influenza virus (5) to detect specifically H5N1 avian influenza virus (6). The peripheral part consists of (5) and (6) can be flexible, meaning that other kinds of viruses could be detected just by replacing the antibody specific to the determined target virus.

To realize the biosensor, each constituent must be prepared. The necessary hemagglutinin (HA) antigen was produced from the NIBRG-14 vaccine strain (National Institute for Biological Standards and Control NIBSC, UK). This recombinant strain has been created by reverse genetic technique originated from Vietnamese strain A/Vietnam/1194/2004(H1N1). The purified antigen was used for anti-HA antibodies production by rabbit immunization. The antibodies from rabbit serum were precipitated with ammonium sulfate 45% saturated and purified with protein A-sepharose affinity column. The purified antibodies were conjugated with biotin to obtain anti-HA antibody–biotin conjugates. The gene coding for Fn–F1−ATPase β-subunit was amplified by polymerase chain

Figure 1. A typical fluorescence biosensor for virus detection. The core part consisted of CdTe/CdS QDs (1), chromatophores with Fn–F1−ATPase (2), and the antibody of β-subunit (3). The biotin–streptavidin–biotin bridge (4) linked the core part with the peripheral part consisted of the antibody of H5N1 avian influenza virus (5) and H5N1 avian influenza virus (6).
reaction (PCR) using the specific primer pairs: \(\beta\)-sub-F: 5'-GGAATTCCATATGACATCGTGCGTTTG-3' and \(\beta\)-sub-R: 5'-GGAATCTCGAGGAACCCATCGCTTTCG-3'. To facilitate the expression vector construction, two restriction sites of NdeI and XhoI were hung into the 5' ends of the primers \(\beta\)-sub-F and \(\beta\)-sub-R, respectively. The PCR product was ligated directly to the pCR2.1 cloning vector and sequenced by automated DNA sequencer. The gene coding for \(\beta\)-subunit was cut from the cloning vector and ligated into the pET21a(+) expression vector. Recombinant \(\beta\)-subunit was purified with probond™ nickel-chelating resin affinity column as per the procedure reported in [25]. Chromatophores were extracted from bacteria \textit{R. rubrum} [26, 27]. The strain \textit{R. rubrum} were inoculated in the DSMZ-27 medium (Deutsche Sammlung von Mikroorganismen und Zellkulturen DSMZ or German Collection of Microorganisms and Cell Cultures in English), then the chromatophores were extracted and purified using an ultracentrifugal machine (Sorvall Ultra Pro80). The antibody against the \(\beta\)-subunit of \(F_oF_1\)-ATPase was prepared according to [28] and purified by precipitation with 33% (NH_4)_2SO_4 followed by protein A-Sepharose and stored at \(-20^\circ\text{C}\) until use. Anti-H5N1 antibody was produced by rabbit immunization using purified H5N1 antigen as described in [10].

2.3. Optical characterizations

The absorption and PL spectra were obtained using a Varian Cary 5000 UV–Vis–NIR spectrophotometer and iHR550 (Horiba) spectrometer equipped with a thermolectrically cooled Si-CCD camera (Synapse), respectively. In the PL measurement, a 405 nm diode laser was used as the excitation source.

3. Results and discussion

Before discussing the realization of a fluorescence biosensor for detecting H5N1 avian influenza virus, we are presenting the following results on the PL studies of each part of the whole biosensor, namely the CdTe/CdS QDs and chromatophores with \(F_oF_1\)-ATPase \(\beta\)-subunit. Also, it is necessary to check the proper PL of H5N1 avian influenza virus.

High-quality CdTe QDs have been synthesized with different sizes by changing the growth temperature (100 or 120°C) and/or duration time (several minutes to hours) to emit luminescence in the spectral range of 490–700 nm corresponding to the mean size of 2.5–5 nm. The optimal conditions for producing the highest quality CdTe QDs were determined to be pH 7–8 and the Cd : Te : MPA molar ratio of 2 : 1 : 3. Without the CdS shell these CdTe QDs could emit at a desired wavelength but not very efficiently. After growing the CdS shell on the CdTe core QDs the luminescence quantum yield (LQY) of CdTe/CdS core/shell QDs was significantly increased (reaching 80%) because of the passivation of dangling bonds on the surface of CdTe core QDs. We also observed a red-shift of the emission peak, evidencing the successful shell growth. Figure 2 shows the absorption and PL spectra of typical CdTe/CdS QDs synthesized with the Cd : Te : MPA molar ratio equal to 2 : 1 : 3, pH 8 at 120°C, and the growth/shelling time of 10 min. Figure 2 shows the PL spectra of CdTe/CdS QDs as a function of pH. The inset shows the PL intensities decrease with increasing pH.

Because we are going to fabricate fluorescence biosensors which are based on the measurement of the PL intensity of CdTe/CdS QDs as a function of pH, we need first to study the detailed pH-dependent PL of CdTe/CdS QDs. The colloidal CdTe/CdS QDs used in our study have been capped with MPA ligands (due to the aqueous phase synthesis or from the ligand exchange of CdSe QDs [29]), in which the thiol (SH⁻) and carboxylic (COOH⁻) groups exist on the surface; they have therefore a negatively charged surface and are very sensitive to pH of the medium surrounding. Figure 3 shows the PL spectra of MPA-capped CdTe/CdS QDs as a function of pH in the range of 6.0, 6.5, 7.0, 8.0 and 9.0. The...
pH values were controlled by adding 1 M HCl or 1 M NaOH into tricine buffer solution (pH 6.5). The inset shows that the PL intensity of CdTe/CdS QDs decreases greatly with the pH. This result supports well the fabrication of pH-sensitive fluorescent biosensors from CdTe/CdS QDs.

By electrostatic force, the negatively charged MPA-capped CdTe/CdS QDs could easily be bound to the positively charged chromatophores. The resultant QDs–chromatophore conjugates were stable as a colloidal solution. Figure 4 shows the TEM image of purified chromatophores produced from bacteria *R. rubrum*. With the mean size of a chromatophore of 70 nm diameter, many CdTe/CdS QDs could bind on the surface of one chromatophore and then appeared to luminesce strongly under the optical microscope (epifluorescence microscope Olympus IX71). In the designed biosensor, the PL intensity from CdTe/CdS QDs reflects the H5N1 avian influenza virus content because H5N1 avian influenza viruses influence the photon flux pumping out of the chromatophores during the synthesis of ATP. In fact, chromatophore and H5N1 avian influenza virus are protein in nature, consequently they can emit strong luminescence under UV light excitation. Therefore, we need to measure the PL spectrum of not only the CdTe/CdS QDs but also all the chromatophores and H5N1 avian influenza virus. This is the important procedure for analyzing the final PL signal obtained from the whole biosensor.

Figure 5 presents the PL spectra taken from CdTe/CdS QDs, chromatophores, H5N1 avian influenza virus, and from the whole biosensor prepared. The overall PL spectrum (curve (d) of figure 5) shows clearly the superposition of the PL spectrum from CdTe/CdS QDs (peaking at 527 nm), from H5N1 avian influenza virus (with the peaks at 523, 618 and 681 nm), and chromatophores (with the peaks at 597, 636 and 705 nm). It is clearly seen that the PL of each constituent does not influence the PL of CdTe/CdS QDs while the proper PL spectra from each of them could be observed and distinguished. For the first time the PL spectrum of H5N1 avian influenza virus is presented (curve (b) of figure 5); this is important for identifying the contribution of each PL signal in the whole PL obtained from the biosensor. In the present study, the used CdTe/CdS QDs emit PL at the same peak wavelength of H5N1 avian influenza virus but we can distinguish the right PL signal for specific detection of H5N1 avian influenza virus upon the proper spectrum of each component in the whole biosensor.

We now discuss a practical measurement of the fluorescence biosensor for detecting H5N1 avian influenza virus. In a concrete biosensor the amount of CdTe/CdS QDs and chromatophores were fixed in quantity. After adding ADP to promote the proton pumping from chromatophores during the synthesis of ATP, the PL spectra were recorded using the 405-nm laser light excitation. It was clearly observed that the whole biosensor could give the strong PL that come from (i) MPA-capped CdTe/CdS QDs, (ii) H5N1 avian influenza viruses, and (iii) chromatophores. Among the constituents mentioned above, the PL from chromatophores could have no change with increasing the H5N1 avian influenza virus content because the amount of chromatophores was fixed in the designed biosensor, while the PL from the other two left components must increase with increasing an amount of H5N1 detected avian influenza viruses. Figure 6 shows the relationship between the PL intensity (taken at the peak wavelength around 525 nm) of H5N1 avian influenza viruses and of the biosensor composed of CdTe/CdS QDs and chromatophores and the virus contents. The increase of the PL intensity proper to H5N1 avian influenza virus directly resulted from the increase of the virus amount (curve (a) of figure 6). In comparison, the increase of the PL from CdTe/CdS QDs-based biosensor was caused by lowering pH due to the proton (H+) flux which pumped out of the chromatophores during the synthesis of ATP catalysed by the $F_o F_1$–ATPase enzymes. The proton flux was increased with increasing H5N1 avian influenza virus loaded into the biosensor because the antibody–antigen reaction enhances the activity of the $F_o F_1$–ATPase enzymes. As the H5N1
avian influenza virus increased, the overall PL intensity was increased by both the PL from H5N1 avian influenza virus itself and from CdTe/CdS QDs at lower pH values. This adding effect was clearly observed and demonstrated in curve (b) of figure 6. Note that in the literature [10], the integral PL signal has been presented instead of the proper spectrum from H5N1 avian influenza viruses and that from the CdTe/CdS QDs-based biosensor. Although at this stage we have not yet exactly determined the virus concentration (number of viruses per volume unit), we have obtained the linear dependence between the PL recorded from biosensor and the amount of H5N1 avian influenza virus in the range of 3–50 ng µl⁻¹ with a detection limit of 3 ng µl⁻¹.

4. Conclusion

In conclusion, we have studied the optical properties of CdTe/CdS QDs labeled on the chromatophore’s surface where pH could be locally changed by the proton flux driven by the synthesis of ATP. We observed the increase of the PL intensity from CdTe/CdS QDs with decreasing pH. Such PL change has been used as the basis to fabricate fluorescence biosensors for detection of any kind of viruses just by replacing the antibody specific to the kind of viruses just by replacing the antibody specific to H5N1 avian influenza virus and H5N1 avian influenza virus. In the present study we have not yet exactly determined the H5N1 avian influenza virus concentration, but the fabricated biosensor could be used to detect the amount of H5N1 avian influenza virus as low as 3 ng µl⁻¹.

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