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A novel ultrasensitive carboxymethyl chitosan-quantum dot-based fluorescence "turn on–off" nanosensor for lysozyme detection

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ABSTRACT

In this work, we developed an ultrasensitive "turn on–off" fluorescence nanosensor for lysozyme (Lyz) detection. The novel nanosensor was constructed with the carboxymethyl chitosan modified CdTe quantum dots (CMCS-QDs). Firstly, the CMCS-QDs were fabricated via the electrostatic interaction between amino groups in CMCS polymeric chains and carboxyl groups on the surface of QDs. In the fluorescence "turn-on" step, the strong binding ability between Zn^{2+} and CMCS on the surface of QDs can enhance the photoluminescence intensity (PL) of QDs. In the following fluorescence "turn-off" step, the N-acetyl-glucosamine (NAG) section along the CMCS chains was hydrolyzed by Lyz. As a result, Zn^{2+} was released from the surface of QDs, and the Lyz–QDs complexes were formed to quench the QDs PL. Under the optimal conditions, there was a good linear relationship between the PL of QDs and the Lyz concentration (0.1–1.2 ng/mL) with the detection limit of 0.031 ng/mL. The developed method was ultrasensitive, highly selective and fast. It has been successfully employed in the detection of Lyz in the serum with satisfactory results.

1. Introduction

As a kind of ideal nanomaterial, luminescent quantum dots (QDs) have attracted great attention in the past decades due to their unique properties (Hanif et al., 2002; Pradhan et al., 2005; Yang et al., 2006), including narrow, symmetrical and size-tunable emission spectrum, broad excitation spectrum and so on. QDs have been employed widely in biosensing and biolabeling applications (Wu et al., 2008) to detect multifarious substances (e.g. small molecules and proteins) (Wang et al., 2013). QD-based nanosensors are becoming the most important nanomaterials for biological and medical applications (Vannoy et al., 2010). However, there are still some concerned problems with how to prepare QD-based sensing systems for high sensitive and selective analysis. Therefore, various techniques have been employed in the assembled strategies of QDs-based sensors. These assembled strategies not only effected the efficiency and stability of sensors, but also determined the cost and practicability of application. Currently, more and more surface modified reagents have been employed to render diverse affinity and specificity of QDs towards different targets. As an ideal functional material, the natural polysaccharide—carboxymethyl chitosan (CMCS) which composed of N-acetyl-glucosamine (NAG) have many attractive physical and biological properties such as hydrophilicity, good biocompatibility, biodegradability, low toxicity, and remarkable affinity for metal ions (Gaberc and Menart , 2001; Lee et al., 2009; Zhou et al., 2006). These properties made CMCS become a promising modification material (Chen et al., 2002; Zhu and Fang, 2005; Chenn et al., 2006). The amino (–NH_2), carboxyl (–COOH) and hydroxyl (–OH) groups on CMCS chains can serve as electrostatic interaction and coordination sites (Zeng and Ruckenstein 1999). So, CMCS had strong binding ability towards metal ions, especially with Zn^{2+} (Shen et al., 2012; Upadhyaya et al., 2013). Furthermore, we found a strong photo-luminescence-activation effect after Zn^{2+} binding with CMCS-QDs (Ma and Lin et al., 2014).

Lyz (Mw: 14.3 kDa) is a relatively small single chain protein with only 129 amino acids. Lyz can only recognize the NAG section and hydrolyze the similar glycan backbone, such as chitin, partially deacetylated chitosan (Kurita et al., 2000, Amano and Ito, 1978). As a tool enzyme, Lyz has been used in gene and cell engineering, which exists in body tissues and secretions (e.g. serum, urine and tears). Because the increased Lyz concentration in urine and serum are potential indicators for leukemia and meningitis (Klockars et al., 1978), the reliable and sensitive methods for the analysis of Lyz are required. To date, a variety of strategies for the Lyz detection have been reported, such as surface plasmon resonance (SPR)

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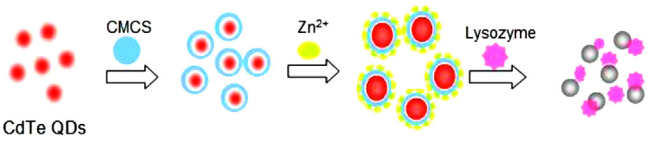

Scheme 1. The schematic illustration of the novel Zn^{2+}–CMCS-QDs nanosensor for Lyz detection.

(Vasilescu et al., 2013), Resonance Rayleigh-scattering (Cai et al., 2011), quartz crystal microbalance (QCM) (Senera et al., 2010), fluorescence anisotropic sensing (Zou et al., 2012), colorimetric & fluorometric dual-readout sensor (Zheng et al., 2013) and so on. However, many methods suffered from some drawbacks including interfering substances, unsatisfactory detection limit and time consuming.

In present study, we prepared CMCS-QDs to establish a fluorescence "turn on–off" sensing system, as shown in Scheme 1. Firstly, CMCS-QDs can absorb Zn^{2+} to enhance the PL of QDs in the fluorescence "turn on" step. In the next fluorescence "turn off" step, Lyz was added into Zn^{2+}–CMCS-QDs system. CMCS was degraded quickly to small molecule by hydrolyzing of Lyz, which can release Zn^{2+} from the QDs surface and form Lyz–QDs complexes. As a result, the QDs PL was quenched. The established fluorescence "turn on–off" sensing system has high selectivity and ultrasensitivity for Lyz detection, and this sensing system has been successfully applied to detect Lyz in the human serum samples with good precision and accuracy.

2. Experiment section

2.1. Materials and apparatus

3-Mercaptopropyl acid (MPA) (99%) was purchased from J&K Chemical Co.. And tellurium powder (~200 mesh, 99.8%), $CdCl_2$ (99%) and $NaBH_4$ (99%) were purchased from Aldrich Chemical Co. Carboxymethyl chitosan, NaH_2PO_4, Na_2HPO_4, NaCl and $ZnCl_2$ were purchased from Beijing Dingguo Chemicals Co. (Beijing, China). All chemicals used were of analytical grade. The water used in the study had a resistivity higher than 18 MΩ cm. Lyz were purchased from Genview. The human serum was obtained as a gift from the university hospital.

The fluorescence measurements were performed on a Shimadzu RF-5301 PC spectrofluorophotometer (Shimadzu Co., Kyoto, Japan) equipped with a xenon lamp using right-angle geometry and a 1 cm path-length quartz cell. All pH measurements were taken with a PHS-3C pH meter (Tuopu Co., Hangzhou, China).

2.2. Synthesis of CMCS-QDs

QDs used in our work were synthesized by refluxing routes as the method described in our previous papers (Ma et al., 2011). In brief, $CdCl_2$ solution (1.25 mM) with MPA as stabilizer was added into a 250 mL three-necked flask under N_2 atmosphere. NaHTe was produced in an aqueous solution by the reaction of tellurium powder with $NaBH_4$ at a molar ratio of 1:2. Later, the fresh NaHTe was added into the $CdCl_2$ solution. The molar ratio of Cd^{2+}/MPA/NaHTe was at 1:1.5:0.2. The solution was subjected to a reflux at 100 °C under open-air conditions to obtain water-compatible MPA-capped QDs. The fluorescence emission wavelength of the QDs used in present experiments was 579 nm. Then, 3.56 nM QDs was added into 10 mL CMCS stock solution (5 g/L). The reaction mixtures were sonicated for 5 min, stirried and vibrated overnight at room temperature in dark. The resultant mixture was transferred into ethanol and centrifuged to remove the excessive CMCS. The collected CMCS-QDs were washed with ethanol/water and stored with PBS (pH 7.4) in dark. The final concentration was 3.56 nM QDs/0.05 g CMCS.

2.3. Preparation of Zn^{2+}–CMCS-QDs

In the fluorescence "turn on" step, 1 mL CMCS-QDs (3.56 nM QDs/0.05 g CMCS) were mixed with a series of different concentrations of Zn^{2+} solution. In this process, all measurements were performed under the same condition. The slit widths of the excitation and emission were 5 nm and 10 nm. The excitation wavelength was set at 380 nm. And the fluorescence spectra were recorded in the 500–700 nm emission wavelength range.

2.4. Lyz detection

In the fluorescence "turn off" step, a series of different concentrations of Lyz and the Zn^{2+}–CMCS-QDs complexes in phosphate-buffered saline solutions (PBS, 2 mM, pH 7.4) were mixed in 2.0 mL calibrated test tubes. Subsequently, the mixtures were gently shaken for 30 min at room temperature. The human blood samples were segregated by centrifugation at 10,000 rpm for 10 min after adding CH_3COOH in samples (CH_3COOH:serum 1.5:1). Finally, all supernatant serum samples were subjected to a 1000-fold dilution with PBS before analysis, and the different concentrations of Lyz were added to prepare the spiked samples.

3. Results and discussion

3.1. Spectra characteristics of CMCS-QDs.

The fluorescence spectra of QDs and CMCS-QDs were shown in Fig. 1A. After coated with CMCS, the slight blue shifts of the QDs PL peaks (from 579 nm to 570 nm) can be observed (Fig. 1A b and d). It was due to the photo-induced surface reconstruction of QDs surface atoms (Ma et al., 2014). Then, the enhancement effect of Zn^{2+} on the PL of QDs and CMCS-QDs was studied. It can be seen that the PL of CMCS-QDs was significantly enhanced to about 130% with the increase of Zn^{2+} concentration (Fig. 1A d and e). The fluorescence quantum yield of CMCS-QDs and Zn^{2+}–CMCS-QDs were 26.31% and 35.02%, respectively. It also proved that Zn^{2+} had the ability to enhance the PL of CMCS-QDs. By comparison, the PL of QDs without CMCS did not change (Fig. 1A a and b). It indicated that CMCS played a significant role in enhancing the PL of QDs. CMCS was bound on the surface of QDs via the electrostatic interaction between amino groups in its polymeric chains and carboxyl groups on the QDs surface. The Zn^{2+} ions can be captured by CMCS-QDs due to the high affinity of CMCS to Zn^{2+} ions. Zn^{2+} can prevent the surface nonradiative relaxation of QDs. As a result, the fluorescence signal of CMCS-QDs was "turned on". With the addition of Lyz, the PL of Zn^{2+}–QDs (c) and Zn^{2+}–CMCS-QDs (f) were "turned off". The PL quenching effect of Lyz on Zn^{2+}–CMCS-QDs (from 130% to 90%) was stronger than that of Zn^{2+}–QDs (from 101% to 92%), and there was no significant shift of QDs PL peak after the addition of Lyz. It indicated that Lyz can quench the PL of both Zn^{2+}–QDs and Zn^{2+}–CMCS-QDs systems. In the Zn^{2+}–QDs system, Lyz can bind with QDs to form Lyz–QDs complexes (Wu et al., 2008). The complexes can quench the PL of Zn^{2+}–QDs system. In the Zn^{2+}–CMCS-QDs system, Lyz can recognize the NAG section along the CMCS chains which act as a hydrolyzing site. So, CMCS was hydrolyzed to small molecules by Lyz, and the captured Zn^{2+} was released from the surface of CMCS-QDs firstly. After CMCS was hydrolyzed, the formation of Lyz–QDs complexes can further quench the PL of Zn^{2+}–CMCS-QDs. From Fig. S1a, the size of QDs (3–5 nm) were well-distributed