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Multivalent glycosylated Cu:CdS quantum dots as a platform for rapid bacterial discrimination and detection

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ABSTRACT

In this work, a rapid bacterial discrimination and detection system based on multivalent glycosylated Cu:CdS quantum dots (QDs) was presented. Cu:CdS QDs with excellent fluorescent properties were synthesized via a facile one-step method, then three natural carbohydrates, glucose, stachyose and raffinose, were coupled onto the surface of Cu:CdS QDs to construct glycosylated platforms. This is the first time that glucose, stachyose and raffinose were used for construction bacterial detection and identification platform. Bacterial discrimination was realized by analyzing the difference of binding strength between glycosylated Cu:CdS QDs and bacterial cell walls. Linear discriminant analysis was used to discriminate the response patterns, and six different bacterial samples could be identified within 30 min. The greatest strengths of the bacterial discrimination platform based on multivalent glycosylated Cu:CdS QDs were rapid, simple and low-cost, there was no need for expensive reagents and instruments.

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1. Introduction

Advances in biology continue to reveal that carbohydrate-carbohydrate and carbohydrate-protein interactions are integral to numerous cellular processes such as cell growth, adhesion, differentiation and infections. Studies of carbohydrate based interactions are of special interest to mimic cellular processes for the development of cell binding systems [1–4]. Recently, bacterial recognition platforms using carbohydrates as receptors with superior sensitivity and selectivity has been constructed [5–7]. Compared with antibodies used in immunoassays that are often fragile and unstable, the structure and activity of carbohydrates could be properly maintained during synthesis and fabrication processes.

In developing carbohydrate based biosensors, carbohydrate receptors were preferred to be used by immobilization on certain substrate over as free form. That was because that the monomeric carbohydrate bindings were of low affinity and specificity [8–10], and immobilized receptors could mimic the multivalent binding interaction of natural cellular process [11–13]. To mimic the multivalent binding interactions, considerable effort has focus on design of multivalent glycosylated systems. Due to their outstanding structural, electronic, magnetic, thermal and optical properties, nanoparticles was considered particularly attractive for carbohydrates modification [14–16]. Thus, application of multivalent glycosylated nanomaterials has gained considerable attention in recent years.

In this work, we presented a rapid bacterial discrimination and detection system based on multivalent glycosylated Cu:CdS quantum dots (QDs). Firstly, carboxyl-capped Cu:CdS QDs with excellent fluorescent properties were employed as carbohydrates carriers for their excellent structural property, water-solubility, and chemical-stability. Next, three natural carbohydrates, glucose, stachyose and raffinose, were coupled onto the surface of Cu:CdS QDs to construct glycosylated platforms. Most research in development of carbohydrate based biosensors for bacterial detection employed synthetic gangliosides, but bacterial recognition systems using natural carbohydrates were rarely reported. Finally, rapid bacterial detection and discrimination were realized by analyzing the interactions between glycosylated Cu:CdS QDs and bacterial cell walls. The response signals towards various bacteria were highly repeatable and could be distinguished by linear discriminant analysis (LDA). Based on the response matrix from LDA, the proposed glycosylated Cu:CdS QDs could identify and detect six different bacterial samples within 30 min.

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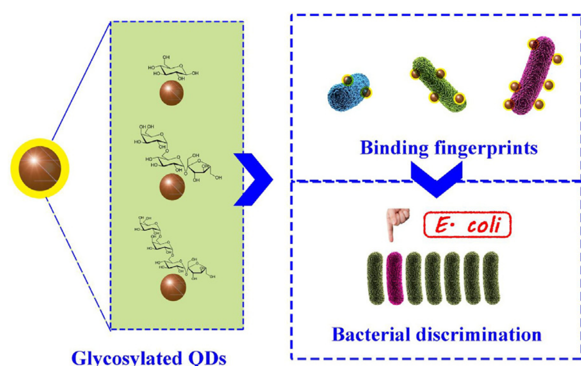

Scheme 1. Schematic description of the bacterial detection and discrimination platform based on multivalent glycosylated Cu:CdS QDs.

2. Experimental section

2.1. Preparation of Cu:CdS QDs as carbohydrates carriers

Water-soluble Cu:CdS QDs were synthesized through a facile one-pot method. In the optimal synthesis condition process, 5.0 mmol $CdSO_4$, 5.0 mmol thiourea and 0.1 mmol $CuCl_2$ were dissolved in 50 mL water, and then 12.5 mmol 3-mercaptopropionic acid was added to the mixture. The pH of the mixed solution was adjusted to 11.0 carefully with 2.0 M NaOH. Afterwards, the reaction solution was heated to 120 °C and refluxed for 120 min under strong stirring.

2.2. Preparation of glycosylated Cu:CdS QDs

Glycosylated Cu:CdS QDs were prepared with a typical NHS/EDC reaction. Briefly, 0.001 mol carbohydrate ligand (glucose, stachyose and raffinose) was added to 20 mL 4 mg mL^{-1} Cu:CdS QDs solution. Then, 0.02 mol NHS and 0.024 mol EDC was added to the mixture under stirring with N_2 protection, the mixed solution was allowed to react for 12 h at room temperature. Finally, obtained glycosylated Cu:CdS QDs were washed and re-dispersed in 20 mL PBS.

2.3. Bacterial detection and identification

P. aeruginosa, *M. luteus*, *E. coli*, *V. alginolyticus*, *S. algae* and *D. desulfuricans* were washed and diluted to the desired concentration or optical density (10^7 cfu mL^{-1} or OD_{600} = 0.3) with PBS. Then 200 μL of the bacterial suspensions were mixed with 200 μL glycosylated Cu:CdS QDs in centrifuge tubes and incubated for 30 min at 4 °C. After centrifugation at 6000 rpm for 5 min, the supernatants, containing unconjugated glycosylated Cu:CdS QDs, were discarded with a pipette, and the deposits, bacterial cells with glycosylated Cu:CdS QDs conjugated, were washed and redispersed in 400 μL PBS for fluorescence measurement. For each kind of bacteria, these processes were repeated to generate four replicates to get the train data, then the data matrix was processed with LDA in SPSS statistics 13.0. The detection and discrimination processes based on multivalent glycosylated Cu:CdS QDs were illustrated in Scheme 1.

3. Results and discussion

3.1. Constructions of multivalent glycosylated platforms

The synthetic conditions, reaction pH and time, were studied and optimized to obtain high fluorescent Cu:CdS QDs for further bacterial detection and identification process. The influence of pH on the fluorescence response of Cu:CdS QDs was shown in Fig. S1, the highest fluorescence intensity was obtained at pH 11. When the pH of reaction solution was lower than 11, Cu:CdS QDs were not successfully synthesized. And solutions with a pH value greater than 13 made Cu:CdS QDs quickly precipitated from solution rather than forming stable and homogeneous solution. So, 11 was selected as the optimum pH for the synthesis of fluorescent Cu:CdS QDs. Besides, as showed in Fig. S2, the fluorescence responses of obtained Cu:CdS QDs increased with the prolongation of reaction time and reached the maximum response at 2 h, after that the fluorescence intensity decreased as the increase of reaction time. So, the optimal reaction time for Cu:CdS QDs synthesis was selected as 2 h.

The optical and structural properties of synthesized Cu:CdS QDs were characterized. UV–vis absorption response study was shown in Fig. 1A, UV–vis spectrum of the synthesized Cu:CdS QDs exhibited strong absorption in ultra-violet region with the main absorption peak at 360 nm. As shown in Fig. 1B, there are two excitation peaks around 325 and 380 nm. When excited at 380 nm, the obtained Cu:CdS QDs exhibited strong fluorescence emission centered at 540 nm. Besides, compared with the non-doped CdS QDs, the Cu doping did not change the excitation and emission wavelength, but it improved the fluorescence intensity greatly. Using the slopes of absorbance at 380 nm and the area of emission spectra excited at 380 nm for varied Cu:CdS concentrations, the fluorescence quantum efficiency was calculated to be 24.7% compared with rhodamine 6G with a quantum efficiency of 95%. Next, the storage stability of Cu:CdS QDs was studied by measuring once a day over a period of 8 days. The fluorescence responses of Cu:CdS QDs retained almost 100% of the initial signal within 8 days (shown in Fig. S3), indicating the Cu:CdS QDs held a good stability. The structural properties of Cu:CdS QDs were characterized with TEM and HRTEM. As shown in Fig. 1C, the as-prepared Cu:CdS QDs exhibited quasi-spherical geometry and high monodispersity. The

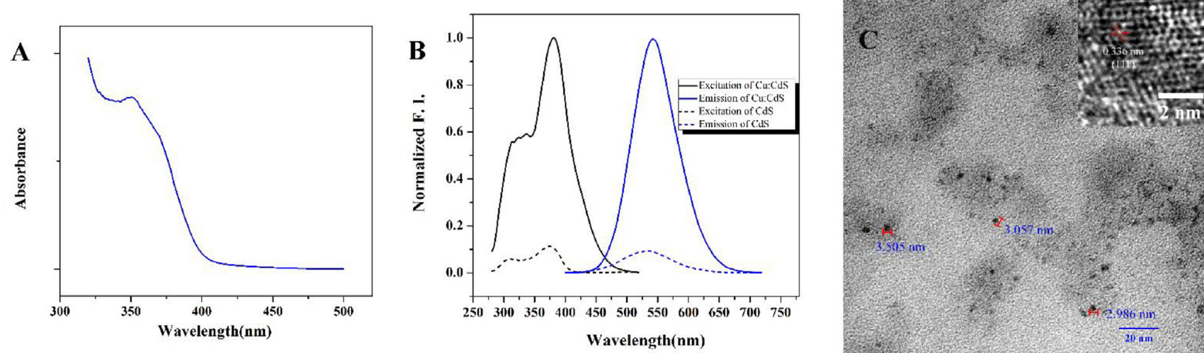

Fig. 1. UV–vis spectrum (A), excitation and emission spectra (B), and TEM image and HRTEM (inert) images (C) of Cu:CdS QDs.

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