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A label-free fluorescence biosensor for highly sensitive detection of lectin based on carboxymethyl chitosan-quantum dots and gold nanoparticles

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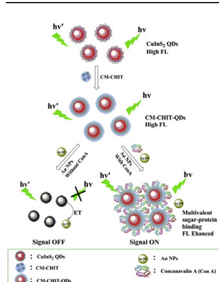
HIGHLIGHTS

- A label-free near-infrared fluorescence "turn off-on" biosensor for detection of lectin was established.
- The highly sensitive biosensor was based on the inner filter effect of Au NPs on CM-CHIT-QDs.
- Lectin could bind exclusively to CM-CHIT-QDs via multivalent carbohydrate-protein interactions, and "turn on" the fluorescence.
- The biosensor sensor showed good selectivity for the detection of lectin with a low detection limit of 0.07 nM.

GRAPHICAL ABSTRACT



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ABSTRACT

In this work, we report a novel label-free fluorescence "turn off-on" biosensor for lectin detection. The highly sensitive and selective sensing system is based on the integration of carboxymethyl chitosan (CM-CHIT), $CuInS_2$ quantum dots (QDs) and Au nanoparticles (NPs). Firstly, $CuInS_2$ QDs featuring carboxyl groups were directly synthesized via a hydrothermal synthesis method. Then, the carboxyl groups on the $CuInS_2$ QDs surface were interacted with the amino groups ($-NH_2$), carboxyl groups ($-COOH$) and hydroxyl groups ($-OH$) within CM-CHIT polymeric chains via electrostatic interactions and hydrogen bonding to form CM-CHIT-QDs assemblies. Introduction of Au NPs could quench the fluorescence of CM-CHIT-QDs through electron and energy transfer. In the presence of lectin, lectin could bind exclusively with CM-CHIT-QDs by means of specific multivalent carbohydrate-protein interaction. Thus, the electron and energy transfer process between CM-CHIT-QDs and Au NPs was inhibited, and as a result, the fluorescence of CM-CHIT-QDs was effectively "turned on". Under the optimum conditions, there was a good linear relationship between the fluorescence intensity ratio I/I_0 (I and I_0 were the fluorescence intensity of CM-CHIT-QDs-Au NPs in the presence and absence of lectin, respectively) and lectin concentration in the range of 0.2–192.5 nmol L^{-1}, And the detection limit could be down to 0.08 nmol L^{-1}. Furthermore, the proposed biosensor was employed for the determination of lectin in fetal bovine serum samples with satisfactory results.

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1. Introduction

Lectins are proteins or glycoproteins of nonimmune origin that come from plants, animals or microorganisms. They are capable of binding ability to mono/oligosaccharides as well as sugar residues in cell walls/membranes with a high degree of stereospecificity [1,2]. The specific carbohydrate-protein interactions mediate many important physiological and patho-physiological processes in living organism, such as bacterial and viral infections, cancer metastasis, inflammation, and immune surveillance [3]. Therefore, their study has attracted great attention due to their significance in understanding the important biological processes and in developing biosensors for clinical diagnostics and drug development.

Up to now, there are many assays for lectins quantification and/or detection because of their important functions including cell-cell interactions, homing of leukocytes, host pathogen interactions, biosynthesis and quality control of glycoproteins, malignancy and metastasis [4]. They also find applications in bioanalytical chemistry and molecular biology [5]. Assays for lectins based on the principle of carbohydrate-protein interaction are routinely realized by agglutination inhibition assays [6], enzyme-linked immunosorbent assay (ELISA) [7], colorimetry [8,9] or surface plasmon resonance (SPR) [10,11]. But most of these analytical methods are time-consuming, expensive, and must be manipulated by trained personnel. Moreover, the conventional approaches relying on the monovalent carbohydrate-protein interaction are generally weak and involve low sensitivity [12]. So far, great efforts have been devoted to overcome such shortcomings.

Recently, there are exciting findings that the weak carbohydrate-protein interactions can be effectively strengthened by presenting multiple ligands to their respective binding proteins [13,14]. Several works have proved that the resulting polyvalent interactions between the multiple ligands and proteins are much stronger than the corresponding monovalent carbohydrate-protein interactions [15,16]. However, tedious procedures for preparing the derivatives of carbohydrate, complicated detection facilities, controlled storing conditions are involved in these methods, which severely restricted their extensive applications for sensitive and rapid lectin detection. In addition, many sensing systems required labeling procedures, which are usually laborious, cumbersome, and error-prone [17]. Thus far, developing fast, facile, label-free, cost-effective glycotechnologies and biosensors with high sensitivity for lectins detection still remains a great challenge.

For high-throughput and easy operation homogeneous detection of proteins, QDs-based assays are promising alternatives to circumvent some of the functional limitations encountered by protein arrays and suspension arrays due to their unique electro-optical properties and photophysical features, like high quantum yields, long fluorescence lifetimes, higher brightness and stability against photobleaching, broad absorption spectra coupled with narrow photoluminescent emission spectra [18,19]. Currently, QDs integrated with biomolecules have attracted great interest for advanced biological applications in biosensing, medical diagnostics, cell imaging, and gene delivery [20,21]. But the applications of QDs to the clinical field have been hampered owing to their high toxicity. Regular QDs-based reports are focused on the cadmium-based QDs that are toxic to biological systems, chemical and photochemical disturbances, and eventually would cause serious environmental problems due to the leakage of cadmium [22].

With regard to this, two main approaches have been employed to reduce the toxicity of QDs. One is to cover non-toxic substance, such as silica shell, and the other is to develop novel QDs without heavy metal ions. In recent years, the newly emerging I-III-VI $CuInS_2$ QDs are particularly impressive because they do not contain any toxic class A elements (Cd, Pb, and Hg) or class B elements (Se and As) [23,24]. Moreover, $CuInS_2$ QDs are near-infrared region (NIR) QDs (generally wavelengths > 650 nm), and can operate in the NIR and avoid interference from biological media, mainly tissue autofluorescence and scattering light [25]. Despite of great explorations of NIR fluorescence sensors in the field of biological imaging, the NIR fluorescence sensors for the determination of complex analytes, such as protein molecules, are still a highly desirable research objective [26].

Herein, we report a facile near-infrared label-free fluorescence “turn off-on” biosensor for lectin detection based on the integration of CM-CHIT, $CuInS_2$ QDs and Au NPs. Concanavalin A (Con A), widely exploited for studying carbohydrate-lectins interactions, is chosen as a model lectin to demonstrate proof-of-concept of the methodology. Con A exists as a tetramer at neutral pH and binds exclusively with α-glucose, α-mannose and their derivatives containing mannose and glucose residues [27,28]. CM-CHIT, a water soluble chitosan derivative, is composed of *N*-acetyl-glucosamine that can specifically recognize Con A by means of specific carbohydrate-ConA interaction. Noteworthy, the amino groups ($-NH_2$), carboxyl groups ($-COOH$) and hydroxyl groups ($-OH$) within CM-CHIT chains can serve as electrostatic interaction and hydrogen bonding sites [29]. In addition, CM-CHIT also possesses many attractive physical and biological characteristics, such as high hydrophilic, low toxicity, good biocompatibility and biodegradability, which facilitate CM-CHIT a promising modification material [30,31]. Integration of Au NPs can significantly quench the fluorescence of CM-CHIT-QDs through electron transfer and energy transfer between CM-CHIT-QDs and Au NPs. Electron transfer and energy transfer can be inhibited by specific protein-carbohydrate interaction between CM-CHIT-QDs and ConA, and the quenched florescence of CM-CHIT-QDs is greatly recovered in the presence of ConA. To the best of our knowledge, this label-free and Au NPs quenching-based lectins assay using CM-CHIT-QDs was developed for the first time.

2. Experimental section

2.1. Materials and apparatus

All chemicals and reagents were of at least analytical reagent grade and used as-received without any further purification. Copper (II) chloride dehydrate ($CuCl_2 \cdot 2H_2O$), indium (III) chloride tetrahydrate ($InCl_3 \cdot 4H_2O$), sulfourea ($CS(NH_2)_2$), mercaptopropionic acid (MPA), Con A, acid phosphatase (ACP), adenosine-5′-triphosphate (ATP), trypsin (Try) and carboxypeptidase Y (CPY) were purchased from Sigma-Aldrich Corporation. Chloroauric acid ($HAuCl_4$) was purchased from Acros Organics. Bovine serum albumin (BSA), pepsin (Pep) and human serum albumin (HSA) were obtained from Sino-American Biotechnology Co. Ltd. Lysozyme (Lyz) was purchased from Genview. Heparinase (Hep) was purchased from Si Qing Yuan Biotechnology (Beijing). CM-CHIT, sodium hydroxide (NaOH), sodium dehydrogenized phosphate (NaH_2PO_4), disodium hydrogen phosphate (Na_2HPO_4), sodium phosphate (Na_3PO_4) and the other chemicals used were all purchased from Beijing Dingguo Changsheng Biotechnology Co. Ltd. The water used in all experiments had a resistivity higher than 18 $M\Omega\ cm^{-1}$.

All 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. A 1 cm path-length quartz cuvette was used in all experiments. The hydro-thermal synthesis experiments were accomplished in a JCZ-JL intelligent digital display drum wind drying oven. The ultrasonic processes was proceeded on a KQ2200 ultrasonic cleaner

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