Talanta 181 (2018) 258–264

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Fluorescence ELISA based on glucose oxidase-mediated fluorescence quenching of quantum dots for highly sensitive detection of Hepatitis B

Yunqing Wu[a], Lifeng Zeng[b], Ying Xiong[a], Yuankui Leng[a], Hui Wang[a,*], Yonghua Xiong[a,c,**]

[a] State Key Laboratory of Food Science and Technology, Nanchang University, 235 Nanjing East Road, Nanchang 330047, PR China
[b] The People's Hospital in Jiangxi Province, Nanchang 330006, PR China
[c] Jiangxi-OAI Joint Research Institute, Nanchang University, Nanchang 330047, PR China

ARTICLE INFO

Keywords:
Fluorescence ELISA
H_2O_2 sensitive quantum dots
Glucose oxidase
Hepatitis B virus surface antigen

ABSTRACT

Herein, we present a novel sandwich fluorescence enzyme linked immunosorbent assay (ELISA) for highly sensitive detection of Hepatitis B virus surface antigen (HBsAg) based on glucose oxidase (GOx)-induced fluorescence quenching of mercaptopropionic acid-modified CdTe quantum dots (MPA-QDs). In this system, hydrogen peroxide (H_2O_2) sensitive MPA-QDs was used as a signal output, and glucose oxidase (GOx) was used as label which can generate H_2O_2 via catalytic oxidation of glucose. The proposed method showed dynamic linear detection of HBsAg both in the range of 47 pg mL^{-1} ~ 380 pg mL^{-1} and 0.75 ng mL^{-1} ~ 12.12 ng mL^{-1}. The detection limit of the proposed fluorescence ELISA was 1.16 pg mL^{-1}, which was approximately 430-fold lower than that of horseradish peroxidase (HRP)-based conventional ELISA. The average recoveries for HBsAg-spiked serum samples ranged from 98.0% to 126.8% with the relative standard derivation below 10%, thus indicating acceptable precision and high reproducibility of the proposed fluorescence ELISA for HBsAg detection. Additionally, the developed method showed no false positive results analyzing 35 real HBsAg-negative serum samples, and exhibited excellent agreement ($R^2 = 0.9907$) with a commercial time-resolved fluorescence immunoassay (TRFIA) kit for detecting 31 HBsAg-positive serum samples. In summary, the proposed method based on fluorescence quenching of H_2O_2 sensitive QDs is considerably to be an excellent biodetection platform with ultrahigh sensitivity, good accuracy and excellent reliability.

1. Introduction

Hepatitis B virus surface antigen (HBsAg) is one of the most important serological biomarkers used in the diagnosis of HBV infection [1]. It is the primary cause of liver cancer and the most dominating reason for liver transplantation, and is generally spread by transfusing blood or blood products [2]. The concentration of HBsAg in serum is used to monitor disease progression and to evaluate the efficacy of treatment [1]. Therefore, a quantitative method with a high level of accuracy and sensitivity is very important for the diagnosis of HBV infection and the prevention of HBV transmitted disease.

Various improved methods have been developed for HBsAg detection, such as radioimmunoassay [3], chemiluminescence assay [4], electrophoresis -chemiluminescence immunoassay [5], amperometric immunoassay, enzyme-linked immunosorbent assay (ELISA) [6] and so on. Among all these methods, ELISA is the most popular technique due to its high throughput, low cost and straightforward readouts [6]. While conventional ELISA using horseradish peroxidase (HRP) catalyzed tetramethylbenzidine (TMB) as a signal output confronts a relative low sensitivity problem with detection limit of ng mL^{-1}, thus makes it insatiable for the increasing need in the clinical practice.

Novel signal transducers converting molecular recognition events into detectable outputs have been widely used to enhance the detection sensitivity of conventional ELISA, and thus various techniques including fluorescent ELISA [7], chemiluminescent ELISA, electrochemical ELISA [8], Raman ELISA [9] and plasmonic ELISA [10] have been developed. The use of fluorescence signals to replace absorbance is a convenient and promising method where a relatively small amount of fluorescent molecules are needed to generate measurable fluorescence signal thus providing enhanced sensitivity of ELISA [11,12]. Never-

* Correspondence author.
** Corresponding author at: State Key Laboratory of Food Science and Technology, and Jiangxi-OAI Joint Research Institute, Nanchang University, 235 Nanjing East Road, Nanchang 330047, PR China.
E-mail addresses: wanghui00072@aliyun.com (H. Wang), yhxiongchen@163.com (Y. Xiong).

https://doi.org/10.1016/j.talanta.2018.01.026
Received 16 November 2017; Received in revised form 9 January 2018; Accepted 10 January 2018
Available online 11 January 2018

theless, the commonly used traditional organic fluorophores possess relatively low fluorescence intensity and are vulnerable to photobleaching thus would limit the sensitivity and reproducibility of fluorescence ELISA [11–13]. Therefore, luminescent nanomaterials with enhanced fluorescence intensity and photostability have been widely used as signal output to overcome these problems [14,15]. Among these materials, quantum dots (QDs) have attracted considerable interest and been regarded as optimum fluorescent labels due to their excellent fluorescent properties including broad excitation, narrow emission spectra, large stocks shifts, high photostability, and size- and shape-dependent fluorescence emissions [13,16]. Generally, QDs are conjugated with reporter molecules (e.g., antibodies) [17] and thus can serve as fluorescence labels. However, nanoparticles conjugation techniques have not yet been perfected. Antibodies immobilized on the surface of QDs always suffer from inevitable loss of antibody activity because of their random orientation, thus reducing detection sensitivity of the methodology [18,19]. Meanwhile, the purification of antibody-QD conjugates present technological challenge that limits their application in immunoassay. Therefore, there is strong need to develop simple and sensitive signal transition strategies for QDs deprived of conjugation of antibodies.

Various methods based on the fluorescence switch of QDs have been developed. Among them, the strategy based on hydrogen peroxide (H_2O_2) induced fluorescence quenching of mercaptopropionic acid-modified QDs (MPA-QDs) is versatile because numerous oxidases generate H_2O_2 as a product [20,21]. Previous studies have confirmed that the fluorescence intensity of mercaptopropionic acid-modified CdTe QDs (MPA-QDs) is extremely vulnerable to low concentration hydrogen peroxide (H_2O_2) in a solution due to the detachment of thiolate molecules from QD surface and the oxidation of tellurium in CdTe QDs [22–25]. Glucose oxidase (GOx) can catalyze glucose to produce gluconic acid and H_2O_2 [26,27], thereby quenching the fluorescence of MPA-QDs. In this study, a novel fluorescent ELISA was developed for the sensitive detection of HBsAg by using GOx-induced fluorescence quenching of MPA-QDs as a fluorescent signal output, where GOx was introduced as label via biotin-streptavidin system (Scheme 1). The proposed method showed high detection sensitivity of HBsAg with limit of detection as low as 1.16 pg mL^{-1}, which is approximately 430-folds lower than that of HRP-based conventional ELISA (0.5 ng mL^{-1}). Moreover, the developed method was comparable with the commercial TRFIA in reliability and also demonstrated good accuracy, precision and reproducibility.

2. Experimental

2.1. Materials

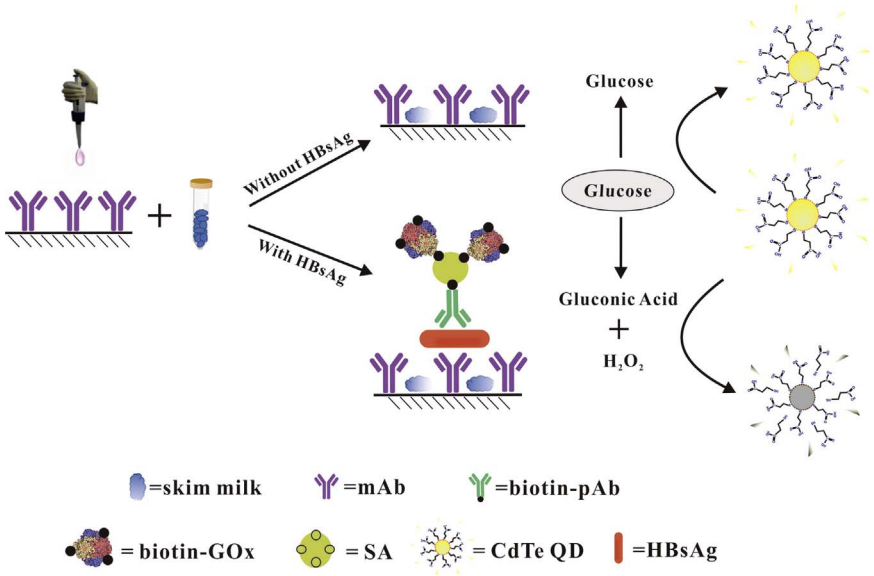
Deactivated hepatitis B surface antigen (HBsAg, 2.5 mg mL^{-1}), mouse anti-HBsAg monoclonal antibodies (anti-HBsAg mAb, 7.2 mg mL^{-1}) and goat anti-HBsAg polyclonal antibodies (anti-HBsAg pAb, 6.8 mg mL^{-1}) were kindly supplied by Jinyuan Jiahe Biotechnology Co. Ltd. (Beijing, China). Biotin-3-sulfo-N-hydroxysuccinimide ester sodium salt and streptavidin were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). D-(+)-glucose and glucose oxidase (GOx) were purchased from Sigma-Aldrich Chemical Co. (St. Louis, Mo, USA). Mercaptopropionic acid (MPA) was procured from Afla Aesar. Tellurium powder, cadmium nitrate, and sodium borohydride were purchased from Institute of Tianjin linke Fine Chemicals. The 96-well plates were obtained from Corning Inc. (New York, USA). All other chemicals reagents were of analytical grade purchased from Sinopharm Chemical Corp. (Shanghai, China). All of the reagents were used without further purifications.

2.2. Preparation of biotinylated anti-HBsAg pAb and Biotin-GOx

To introduce GOx as a label for the sandwich immunoassay, a biotinylated detection antibody (biotinylated anti-HBsAg pAb, biotin-pAb) and biotinylated GOx (biotin-GOx) were synthesized via amide bonds between anti-HBsAg pAb (or GOx) and biotin-3-sulfo-N-hydroxysuccinimide ester. In brief for biotin-pAb preparation, anti-HBsAg pAb was mixed with biotin-3-sulfo-N-hydroxysuccinimide ester in 1 mL PBS (0.01 M, pH 7.4) at a mole ratio of **1:40**. After reaction under gentle magnetic stir for 3 h at room temperature, the conjugates were dialyzed in 2 L PBS buffer solution (0.01 M, pH 7.4) for 72 h by using a dialysis bag. The final solution was stored in refrigerator at −20 °C for fluorescence ELISA analysis. The biotin-GOx conjugate was prepared using a similar procedure with desired feeding mole ratio of biotin to GOx, where three different ratios of 5:1, 10:1 and 20:1 were studied. The resulting biotin-GOx solutions were also stored in refrigerator at −20 °C for further use.

2.3. Synthesis of water-soluble CdTe QDs

The hydrophilic MPA-capped CdTe QDs were prepared according to a previously reported method [28]. Briefly, a freshly prepared oxygen-



Scheme 1. Schematic of fluorescence ELISA based on GOx-induced fluorescence quenching of MPA-QDs for HBsAg detection.