Biosensors and Bioelectronics 87 (2017) 122–128

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

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

A universal multicolor immunosensor for semiquantitative visual detection of biomarkers with the naked eyes

Xiaoming Ma [a,b], Yue Lin [a,b], Longhua Guo [a,b,*], Bin Qiu [a,b], Guonan Chen [a,b], Huang-hao Yang [a,b], Zhenyu Lin [a,b,*]

[a] State Key Laboratory of Photocatalysis on Energy and Environment, College of Chemistry, Fuzhou University, Fuzhou, Fujian, 350116 China
[b] MOE Key Laboratory of Analysis and Detection for Food Safety, Fujian Provincial Key Laboratory of Analysis and Detection Technology for Food Safety, Institute of Nanomedicine and Nanobiosensing, College of Chemistry, Fuzhou University, Fuzhou, Fujian, 350116 China

ARTICLE INFO

Article history:
Received 27 May 2016
Received in revised form
7 August 2016
Accepted 8 August 2016
Available online 8 August 2016

Keywords:
Immunoassay
Gold nanorods
Colorimetric detection
Carcinoembryonic antigen
ELISA

ABSTRACT

3, 3′, 5, 5′-tetramethylbenzidine (TMB) has been widely used as a chromogenic substrate for colorimetric immunoassays. Normally, the colorless TMB is oxidized into yellow TMB^{2+} (in acidic solution) to indicate the presence of the target molecules. However, this kind of monochromic intensity changes seriously confine the accuracy of visual inspection. Herein, we demonstrate for the first time that TMB^{2+} can quantitatively and efficiently etch gold nanorods (AuNRs). The addition of AuNRs into a solution containing different amount of TMB^{2+} generates vivid color responses as colorful as a rainbow, and the etching process can be finished within 90 s. As a result, the exact concentration of TMB^{2+} can be easily distinguished with the naked eyes by the corresponding solution color. Based on this finding, we incorporate AuNRs into the well-developed, commercially available horseradish peroxidase (HRP)-TMB immunoassay system, so that it can be utilized for semiquantitative detection of a broad range of disease biomarkers with the naked eyes (termed 'NEQ-IA'). Carcinoembryonic antigen (CEA) and Prostate specific antigen (PSA) had been chosen as example targets to test the feasibility of the proposed biosensor. The results showed good accordance with the conventional methods. Because no sophisticated apparatus but human eyes are used as the readout, the proposed NEQ-IA could be a good supplementary to current state-of-the-art immunoassay methods for those applications that require the use of portable and affordable devices, for example, for the detection of disease biomarkers at home and in the field.

© 2016 Elsevier B.V. All rights reserved.

1. Introduction

Immunoassays have been widely used in the hospital for the detection of all kinds of disease biomarkers. It shows high specificity via antibody-antigen recognition and high sensitivity via enzyme-triggered signal amplification (Brennan et al., 2010; Ito et al., 2014). Normally, immunoassay is conducted in the hospital with an automatic microplate reader. It is worth to note that the expensive and bulky microplate reader seriously confines the utility of conventional immunoassay approaches for point-of-care (POC) diagnostics and in-home personal healthcare (Qu et al., 2011; Yan et al., 2013). In order to meet these challenges, an accessible and affordable detection device should be developed to replace the sophisticated readout (Tram et al., 2014; Zhu et al., 2015). In this regard, naked-eye inspection could be a good choice because it does not involve the use of any analytical devices but human eyes for the detection (Liu et al., 2013; Xia et al., 2010; Zhou et al., 2008, 2010).

Although colorimetric immunoassay kits that enable naked-eye inspection are already commercially available, most of them can be only used for qualitative detection of disease biomarkers due to the limited colors displayed in response with different concentrations of targets. For example, the widely used horseradish peroxidase (HRP)-3, 3′, 5, 5′-tetramethylbenzidine (TMB) immunoassay system shows monochromic (yellow) intensity change in response to the variations of target concentrations (John Goka and Farthing, 1987). Recently, Stevens and coworkers (de la Rica and Stevens, 2012, 2013) reported a plasmonic immunoassay approach for sensitive determination of disease biomarkers that generated dual color response (red and blue) in the presence and absence of the analytes. It was reported that this kind of dual color response was easier to be distinguished by human eyes than the conventional single color immunoassay. Even though, color

* Corresponding authors at: State Key Laboratory of Photocatalysis on Energy and Environment, College of Chemistry, Fuzhou University, Fuzhou, Fujian 350116, China.
E-mail addresses: guolh@fzu.edu.cn (L. Guo), zylin@fzu.edu.cn (Z. Lin).

http://dx.doi.org/10.1016/j.bios.2016.08.021
0956-5663/© 2016 Elsevier B.V. All rights reserved.

transformation between red and blue was still not accurate enough for quantifying the concentration of the target molecules without the aid of a spectrometer (de la Rica and Stevens, 2012).

Experts estimated that human eyes can distinguish as many as 10 million of different colors (Wyszecki, 2006). Therefore, we believe that the accuracy of visual inspection can be greatly improved as long as plenty of colors are presented in the presence of different amount of target molecules. In order to develop such a multicolor immunoassay, the searching of an appropriate chromogenic substrate that shows vivid color variations upon the addition of targets is important. Noble metal nanostructures could be potential candidates because the solution color of noble metal nanoparticles is highly dependent on their size, shape and composition (Anker et al., 2008; Meinzer et al., 2014). In fact, in recent years, noble metal nanostructures have been widely explored for the construction of colorimetric sensors (Elghanian et al., 1997; Guo et al., 2015, 2013; Swierczewska et al., 2012; Zhou et al., 2015). Among diverse metal nanostructures, Au nanorods (AuNRs) could be an idea chromogenic substrate for multicolor display because the longitudinal plasmon bands of AuNRs are easily to be tuned by adjusting their aspect ratios (Huang et al., 2006; Kabashin et al., 2009). Various aspect ratios of AuNRs display a range of different color as colorful as a rainbow (Tsung et al., 2006; Vigderman et al., 2012; Ni et al., 2008). These vivid colors can be easily distinguished by naked eyes. Most recently, we demonstrated that AuNRs were ideal colorful chromogenic substrates for colorimetric immunoassays, and the proposed approach was utilized for semiquantitative detection of nucleic acids, proteins and small molecules with the naked eye (Ma et al., 2016). However, this method required labeling the antibody with catalase. Thus it cannot well accommodate conventional immunoassay platforms (Xianyu et al., 2014).

In this work, we disclose for the first time that the product (TMB^{2+}) of HRP catalyzed oxidation of TMB can quantitatively etch AuNRs. Based on this finding, we develop a novel immunoassay scheme that utilizes HRP as the enzyme label and TMB-AuNRs mixture as the chromogenic substrate. Carcinoembryonic antigen (CEA) and Prostate specific antigen (PSA) were widely investigated as disease biomarkers during the cancer diagnosis, therefore, we choose them as model targets to verify the performance of our scheme (Choi et al., 2013; Gao et al., 2015; Han et al., 2016; Zhai et al., 2015, 2016). By using this novel immunoassay scheme, the solutions display vivid colors in response to concentration variations of disease biomarker so that visual quantification of the analyte is achieved. We define this novel approach as naked-eye semiquantitative immunoassay (NEQ-IA) in order to distinguish it from those conventional immunoassay methods that detected with sophisticated readouts. Since HRP has been widely used as enzyme labels in commercial available immunoassay kits, the proposed NEQ-IA is easily to accommodate conventional immunoassays for the detection of a large number of disease biomarkers.

2. Experimental section

2.1. Reagents

CEA ELISA test kits and control solutions were purchased from Biocell Biotechnol. Co., Ltd. (Zhengzhou, China). The ELISA kit comprised a microtiter plate coated with anti-CEA (monoclonal), anti-CEA-HRP conjugate (polyclonal), urea peroxide (0.05%), TMB (1.25 g/mL), 2 M sulfuric acid, a surfactant wash buffer (WB), and a series of CEA standards. Human PSA-total ELISA Kit was obtained from Sigma Aldrich Chemical Co. (USA). TMB Substrate A and B were obtained from Aladdin Chemistry Co., Ltd (Shanghai, China). Lyophilized Horseradish peroxidase (HRP, EC 1.11.1.7, A > 300 U/mg) was purchased from Sangon Biotech Co. Ltd. (Shanghai, China). Gold (III) chloride trihydrate (99.9%) ($HAuCl_4 \cdot 4H_2O$), cetyltrimethyl ammonium bromide (CTAB), sodium borohydride ($NaBH_4$), silver nitrate ($AgNO_3^-$) and ascorbic acid were purchased from Sinopharm Chem. Re. Co., Ltd. (Shanghai, China). All other chemicals were of extra pure analytical grade and used without further purification. All solutions were prepared with deionized water obtained from a Milli-Q water purifying system (≥ 18 MΩ, Milli-Q, Millipore). Clinical serum samples were made available by Fujian Provincial Hospital, China. The serum samples had already been processed as required and were therefore ready for analysis.

2.2. Instruments

Extinction spectra of AuNRs solutions were measured using a Microplate Spectrophotometer (Multiskan GO, Thermo Scientific, USA) at room temperature. Transmission electron microscopy (TEM, Tecnai G2 F20 S-TWIN, FEI, USA) was used to study the different oxidation stage of the AuNRs. The photographs of the reaction wells were taken with a Canon digital camera.

2.3. Synthesis of the starting gold nanorods

AuNRs were synthesized by a silver ion-assisted seed mediated method (Nikoobakht et al., 2003; Ye et al., 2013). In brief, the seed solution was prepared by adding a freshly prepared, ice-cold $NaBH_4$ solution (0.01 M, 0.6 mL) into a solution containing $HAuCl_4$ (0.005 M, 5 mL) and CTAB (0.2 M, 5 mL) in a 15 mL glass tube under vigorous stirring (1200 rpm). After vigorous stirring for 2 min, the color of the seed solution turned from yellow to brownish yellow. Then the stirring was turned off and the seed solution was kept at room temperature for at least 30 min before use. To prepare the growth solution, ascorbic acid (5.5 mL, 0.1 M) was added to a well-mixed solution containing $HAuCl_4$ (5 mL, 0.01 M), $AgNO_3$ (0.60 mL, 0.01 M), and CTAB (50 mL, 0.2 M). The mixture solution was diluted to 100 mL with deionized water. Finally, 200 μL of the seed solution was injected into the growth solution. The resultant mixture was vigorously stirred for 30 s and then left undisturbed at 30 °C for 24 h. The as-synthesized AuNRs was centrifuged at 11,000 rpm for 15 min followed by removal of the supernatant. Then the precipitate was redispersed into 0.06 M CTAB solution with the same volume. The same separation procedures were repeated twice to obtain a final solution containing AuNRs and 0.06 M CTAB. This solution was utilized as chromogenic substrate for color display in the subsequent experiments. The concentration of this AuNRs stock solution was ~0.24 nM, as calculated according to an extinction coefficient of AuNRs at 744 nm of $4.13 \pm 0.5 \times 10^9\ M^{-1}\ cm^{-1}$(Orendorff and Murphy, 2006).

2.4. TMB^{2+} titration experiments

The TMB^{2+} stock solution was synthesized via the ultraviolet radiation of TMB. Briefly, 2 mL TMB solution was irradiated under 337 nm laser irradiation for 10 min and then 1 M HCl solution (1 mL) was added. The concentration of TMB^{2+} in the final solution was 167 μM, calculated by the extinction coefficient of the diimine, $\varepsilon = 5.9 \times 10^4\ M^{-1}\ cm^{-1}$ (Josephy et al., 1982). Different concentration of TMB^{2+} solutions were prepared by dilution of the TMB^{2+} stock solution with distilled water. 100 μL AuNRs solution (concentration of Au[0] was $\sim 0.69 \pm 0.01$ mM as measured by ICP-MS) was added into each prepared TMB^{2+} solution and the mixture solutions were allowed to incubate for 2 min before spectral measurements.

Download English Version:

https://daneshyari.com/en/article/866168

Download Persian Version:

https://daneshyari.com/article/866168

Daneshyari.com