Sensors and Actuators B 249 (2017) 478–485

Contents lists available at ScienceDirect

Sensors and Actuators B: Chemical

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

A highly sensitive EDTA-based senor for detection of disease biomarker and drug

Haowen Huang*, Yuan Zhou, Qian Zhao, Lingyang Zhang, Lanfang Liu, Xiaodong Xia, Shoujun Yi

Key Laboratory of Theoretical Organic Chemistry and Function Molecule, Ministry of Education, Hunan Provincial University Key Laboratory of QSAR/QSPR, School of Chemistry and Chemical Engineering, Hunan University of Science and Technology, Xian, China

ARTICLE INFO

Article history:
Received 9 December 2016
Received in revised form 18 April 2017
Accepted 20 April 2017
Available online 23 April 2017

Keywords:
EDTA
Peroxidase-like
Colorimetric nanosensor
Cancer antigen 15-3
Methamphetamine

ABSTRACT

In this study, a fascinating photocatalytic property of EDTA under light irradiation was observed, much the same as that of natural horseradish peroxidase. The small EDTA molecules enable it easily attaches to amine terminal on antibody surface, an ELISA-like assay may be fabricated through combining with gold nanoparticles (AuNPs) in suspensions can exhibit various colors. The labeled EDTA on the proteins which may catalyze decomposition of hydrogen peroxide and in situ mediate the growth of hydrogen peroxide-induced formation of AuNPs, displaying various color nanoparticle dispersion as a read-out means. A straightforward plasmonic sensor was accordingly designed to enable naked-eye observation and quantitative determination of ultra-trace target analytes based on peroxidase-like EDTA. The highly sensitive assay was utilized to determine cancer antigen 15-3 (CA15-3, a breast cancer biomarker) and methamphetamine, and their limit of detection CA15-3 and methamphetamine are 7.5×10^{-15} U/mL and 2.8×10^{-20} mg/mL by naked eyes, respectively. Furthermore, practical applications for detection of CA15-3 and MA in human blood samples were also carried out. The results of detecting CA15-3 of breast cancer serum samples are close agreement with those given by hospital, implying this sensor with high accuracy. This assay has a great potential to be developed into a platform to quantitatively determine analytes as long as the specific antibodies against them were available. Its versatile features will broaden the applicability toward the ultrasensitive detection of target molecules in clinical diagnosis, environmental monitoring, and food quality control only using naked eyes.

1. Introduction

Enzyme-linked immunosorbent assay (ELISA) is often used nowadays for qualitative and quantitative determinations of target molecules, which is extensively used in clinical diagnosis, environmental monitoring and food quality control [1–5]. ELISA works on the natural enzymatic reactions which require additional introduction and conjugation of enzyme(s). However, natural enzymes have some intrinsic drawbacks, such as low stability, high costs in preparation and purification as well as catalytic activity subjected to environmental conditions. Thereby, the use of mimetic enzyme substitutes for natural enzymes has markedly increased in recent years [6–9]. Metal and semiconductor nanoparticles, especially noble metal nanoparticles, have attracted great interests [10–14]. These enzyme-like particles might also be used to fabricate ELISA-like sensors [15–18]. Whereas, some uncertain factors resulted from the size of nanoparticles are not neglectable. The size of enzyme-like nanoparticle is close to or larger than that of conjugated proteins, which maybe affect the conformation and function of the protein conjugated. Thus, small enzyme-like molecules are expected to develop more effective ELISA-like immunoassay.

Ethylene diamine tetraacetic acid (EDTA) is such a powerful chelating agent that it can form stable complexes with most metal ions [19,20]. The six lone-pair electrons attributed to six atoms of EDTA not only facilitate to coordinate with most of metal ions but also serve as hole scavenger in the photocatalytic process [21,22]. The specific structure of EDTA favors the activation of H_2O_2 to produce hydroxyl radicals. For example, EDTA facilitates to generate hydroxyl radical in the Fenton reaction [23]. Recently, EDTA was introduced to semiconductor materials to attend the photocatalytic process, leading to significantly enhancing degradation rate of these particles to some organic dyes and organic pollutions [24–26]. Our further exploration on EDTA shows it exhibits a fas-

* Corresponding author.
E-mail address: hhwn09@163.com (H. Huang).

http://dx.doi.org/10.1016/j.snb.2017.04.122
0925-4005/

cinating photocatalytic property under light irradiation, much the same as that of natural horseradish peroxidase.

As the previous works reported [15,27], gold nanoparticles (AuNPs) in suspensions can exhibit various colors, giving very sensitive detection or even allowing naked-eye readout. In this study, the significant discovery of peroxidase-like EDTA was explored to an attractive assay by combing color AuNPs. In this strategy, analyte-recognizable antibody labeled with EDTA induces competitive (decomposition of H_2O_2 and reduction $HAuCl_4$ to form AuNPs) reactions involved in H_2O_2, which results in the formation of AuNPs with color variation. A colorimetric and highly sensitive assay was accordingly developed, allowing naked-eye determination of targets. Furthermore, this assay was used to detect cancer antigen 15-3 (CA15-3, a breast cancer biomarker) and small molecular drug, such as methamphetamine, with high accuracy. The cost-effective EDTA-based assay provides promising applications in environmental monitoring and food quality and life science.

2. Material and methods

2.1. Materials and reagents

Hydrogen peroxide (H_2O_2), chloroauric acid ($HAuCl_4{\cdot}3H_2O$), Trisodium citrate, 3,3,5,5-tetramethylbenzidine (TMB), N-hydroxysuccinimide (NHS), ethylcarbodiimide hydrochloride (EDC), 2- (N-morpholino) ethanesulphonic acid (MES), bovine serum albumin (BSA) were purchased from Aladdin Industrial Corporation (Shanghai, China). Breast cancer antigen and rabbit polyclonal breast cancer antibody were received from Solarbio Science and Technology Co., Ltd (Beijing, China). Rat MA antibody was obtained from Shanghai Ji Ning Industrial Co., Ltd (Shanghai, China). All of the chemicals, unless mentioned otherwise, were of analytical reagent grade and obtained from the commercial source. Breast cancer antigen, breast cancer antibody, rat MA antibody and BSA were all diluted by 0.01 mol/L phosphate buffered saline (PBS) solution. The H_2O_2 were dissolved by MES solution.

2.2. Modification of protein with EDTA

The modification of antibodies, such as CA15-3 antibody and rat MA antibody, with EDTA was performed by covalently attaching the EDTA to protein via the $-NH_2$ of amino acid residues. Briefly, EDTA mixed with 75 mM EDC and 15 mM NHS and incubated for 30 min at room temperature. Then 0.02 mg/mL antibody diluted in PBS buffer solution was added to the activated EDTA 1 h. After that, the mixture solution was dialysis against water for 24 h and transferred the solution into a flask.

2.3. Photocatalytic activity of EDTA

The photocatalytic activity of EDTA was assessed through colorimetric display of TMB. 50 μL of 5 mM TMB, 50 μL of 100 mM EDTA and 50 μL of 10 mM H_2O_2 were added to 350 μL of water. Under the irradiation of room visible light (~0.48 Mw/cm^{-2}) for 30 min, an occurrence of colored solution appeared and the intensity of the absorbance peak at 652 nm was measured by an UV–vis spectrophotometer. As a control, the identical mixture solution of TMB, EDTA and H_2O_2 was kept in dark.

2.4. Detection of CA15-3 and MA

96-well polystyrene plates were modified with CA15-3 antibody solution (300 μL, 0.004 mg/mL) at 4 °C overnight. Then, plates were washed three times with wash buffer (0.01 mol/L PBS buffer solution). After that, the plates immobilized with CA15-3 were blocked using 1 mg/mL BSA solution for 1 h at room temperature. Subsequently, three times wash were carried out again on the blocked plates, and then 300 μL CA15-3 (7.5×10^{-9}–7.5×10^{-15} U/mL) was added. After 3 h, the plates underwent the same washing procedures before the addition of CA15-3 antibody labeled with EDTA, which were then kept for 1 h reaction time at room temperature.

For the visual read-out, the above plates were washed three times with PBS buffer solution, then, trisodium citrate (100 μL, 4 mM) and hydrogen peroxide (100 μL, 320 μmol/L) in MES buffer (1 mM, pH 6.5) was added to each well of the plate, respectively. After 30 min, 100 μL of 2 mM freshly prepared $HAuCl_4$ was added to each well. The control experiment without CA15-3 in the well was carried out at the same time. The photographs were taken after 30 min and the absorbance at 550 nm was recorded with an UV–vis spectrophotometer. Similar procedure was performed to detect MA.

2.5. Application to human serum samples analysis

The breast cancer serum samples were collected from Hunan Provincial Tumor Hospital and stored at 4 °C until use. All experimental procedures were performed in compliance with the relevant laws and institutional guidelines. The proposed colorimetric assay was applied to the determination of CA15-3 and MA in human serum samples. For detection of CA15-3, the blood sample was diluted 1:10^{10} in PBS buffer solution. The blood samples of MA were obtained by adding the MA into the healthy people blood.

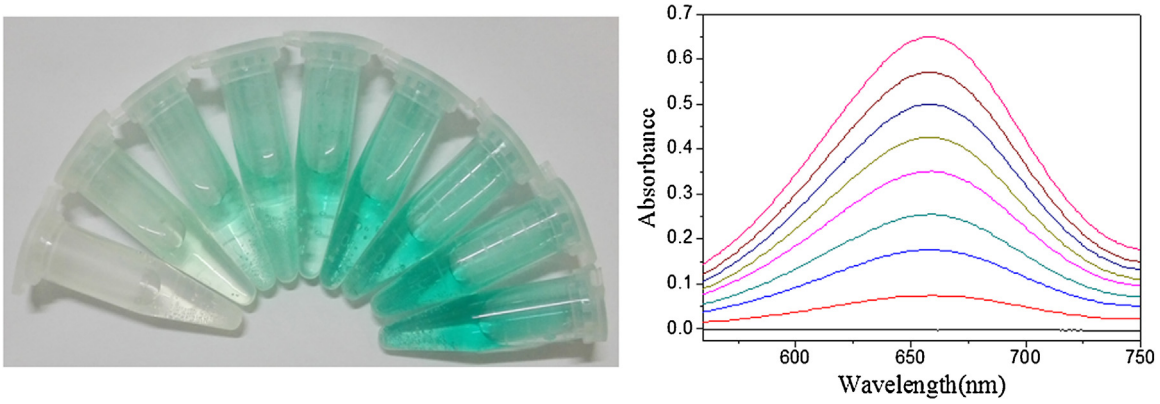

Fig. 1. *a:* Photographs for various concentrations of H_2O_2 (from left to right: 0, 3.4×10^{-7}, 1.7×10^{-6}, 3.4×10^{-6}, 1.7×10^{-5}, 3.4×10^{-5}, 9.5×10^{-4}, 3.2×10^{-4}, 9.7×10^{-3}, M) and TMB reaction solution catalyzed by EDTA. *b:* Typical absorption spectra of the produced TMB corresponding to (a).