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Short communication

Cadmium ion-doped magnetic poly(styrene-acrylic acid) nanospheres for sensitive electrochemical immunoassay

Bing Zhang, Yuling Cui, Bingqian Liu, Huafeng Chen, Guonan Chen, Dianping Tang*

Key Laboratory of Analysis and Detection for Food Safety (Ministry of Education & Fujian Province), Department of Chemistry, Fuzhou University, Fuzhou 350108, PR China

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ABSTRACT

A novel class of molecular tags, cadmium ion-doped magnetic poly(styrene-acrylic acid) nanospheres (Cd-MPSA), was first synthesized and functionalized with polyclonal rabbit anti-human luteinizing hormone antibodies (PAb_2) for highly efficient electrochemical immunoassay of luteinizing hormone (LH). Transmission electron microscope (TEM) and Fourier transform infrared spectroscope (FTIR) were employed to characterize the prepared Cd-MPSA. By using Cd-MPSA-labeled PAb_2 as molecular tags, a novel sandwich-type immunoassay protocol was built for determination of LH on monoclonal mouse anti-human luteinizing hormone antibody (MAb_1)-functionalized gold electrode. The assay was carried out in pH 5.3 HAc-NaAc buffer solution by square wave voltammetry (SWV). The signal was obtained by the reduction of the doped cadmium ions in the Cd-MPSA. Under optimal conditions, the currents increased with the increasing LH level in the sample, and exhibited a linear range from 0.25 to 240 mIU mL^{-1} with a detection limit of 0.08 mIU mL^{-1} LH at $3s_B$. The precision, reproducibility, and specificity were acceptable. No obvious difference was encountered in the analysis of spiking LH samples into newborn calf serum with the referenced values.

1. Introduction

Luteinizing hormone (LH), as a hormone produced by the anterior pituitary gland, is responsible for ovulation induction in women and controls testosterone production in men. The level of luteinizing hormone in the blood or urine has an important effect on the regulation of the menstrual cycle, female egg production and male sperm production (Xu et al., 2009). Moreover, many diseases are inspected by testing the level of LH, such as menstrual problems, precocious puberty, delayed puberty, or response to medicines given to stimulate ovulation (Laks Dan, 2010). Therefore, a LH assay is now also recommended as a screening tool for disease diagnosis.

Immunoassay, the measurement of antibody or antigen concentrations based on biospecific recognition interactions, has been considered as a major analytical method used in clinical diagnosis (Tang et al., 2010a; Liu et al., 2011). Various immunoassays have been reported for determination of LH comprising radioimmunoassay (Beijerink et al., 2007), chemiluminescent enzyme immunoassay (Xiao et al., 2009), electrochemical enzyme immunoassay (Qu et al., 1998), and enzyme-linked immunosorbent assay (Valares et al., 2007). Among these methods, electrochemical immunoassays have gained increasing attention due to their inherent advantages of high sensitivity, low cost, low power requirement, and high compatibility with advanced micromachining technologies (Tang et al., 2011a).

To achieve a high sensitivity in the electrochemical immunoassays, routine approaches are usually adopted by enzyme labels or nanolabels (Akanda et al., 2011; Tang et al., 2008). In the past, our group reported an electrochemical immunosensor for detection of biomarkers by using horseradish peroxidase-labeled secondary antibodies (Tang and Ren, 2008). Unexpectedly, we later found that one of the problems commonly associated with enzyme labels was to decrease their bioactivity when the biomolecules were exposed to reactive groups and harsh reaction conditions. The rapidly emerging research field of hybrid nanomaterials, and the processes used to generate, manipulate and deploy nanomaterials, provides excitingly new possibilities for advanced development of new analytical tools for bioanalytical applications. Sekhar et al. (2008) employed metal-decorated silica nanowires as an active surface-enhanced Raman substrate for biomarker detection. Wang and Tian (1998) constructed a potentiometric immunosensor by using metal ions as labels. Cadmium is an essential element in many forms of a new class of semiconductor known as quantum dots. These advanced materials show promise in the areas of electronics, photo-voltaics and medical imaging. Cadmium ions with

* Corresponding author at: Key Laboratory of Analysis and Detection for Food Safety (Ministry of Education), Department of Chemistry, Fuzhou University, Fuzhou 350108, PR China. Tel.: +86 591 2286 6125; fax: +86 591 2286 6135.
E-mail address: dianping.tang@fzu.edu.cn (D. Tang).

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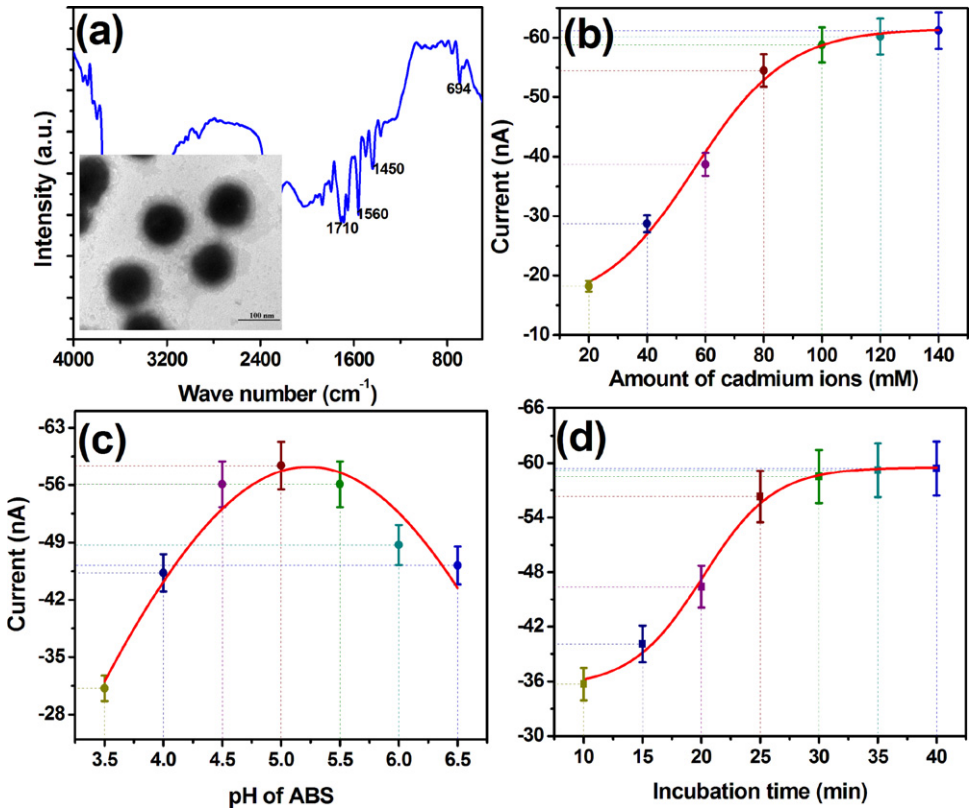

Fig. 1. (a) FTIR spectra of the Cd-MPAS nanospheres (inset: TEM image), and the effects of (b) Cd^{2+} concentration, (c) pH of ABS, and (d) incubation time on the current of the electrochemical immunoassay (25 mIU mL^{-1} used here).

favorable electron conductivity, low toxicity and chemical stability have been used for determination of biomolecules (Yin et al., 2010; Xiang et al., 2011). Typically, cadmium ions were doped into the nanoparticles during the synthesis. However, the doped amount was limited, and sometimes the nanocomposites were difficultly purified.

Herein, we designed a new method for the synthesis of cadmium ions-doped poly(styrene-acrylic acid) nanoshells by using magnetic beads (MB) as seeds. Then the prepared nanostructures were used as molecular tags for the label of secondary antibodies with a sandwich-type immunoassay format (anti-luteinizing hormone antibody, anti-LH, used in this case). The hybrid nanostructures showed good adsorption properties toward anti-LH antibodies, and analytical performance for detection of luteinizing hormone (LH). The unique merit of this methodology is that the signal amplification does not require the routine and cumbersome process such as enzyme-labeled secondary antibody.

2. Experimental

2.1. Chemicals

MAb_1 antibodies and LH standards with 0, 7.5, 25, 60, 120 and 240 mIU mL^{-1} were purchased from Biocell Biotech. Co. Ltd. (Zhengzhou, China). PAb_2 antibodies were achieved from Abcam (Hongkong, China). 16-nm gold colloids and 10-nm magnetic beads were synthesized and described in our previous reports, respectively (Tang et al., 2010b, 2011b). N-(3-Dimethylamminopropyl)-N′-ethyl-carbodiimide hydrochloride (EDC), N-hydroxysulfosuccinimid sodiumsalt (NHS), styrene, acrylic acid, $HAuCl_4{\cdot}4H_2O$, and L-cysteine were purchased from Alfa. All other reagents were of analytical grade and used without further purification. Ultrapure water was obtained from a Millipore water purification system (≥18 MΩ, Milli-Q, Millipore), and used in all runs. 0.1 M acetic acid-buffered saline (ABS) solutions with various pHs were prepared by mixing 0.1 M HAc and 0.1 M NaAc, and 0.1 M KCl was added as the supporting electrolyte.

2.2. Synthesis of cadmium-doped magnetic poly(styrene-acrylic acid) nanospheres (Cd-MPSA)

The Cd-MPSA nanospheres were synthesized in a 250 mL three-necked round-bottom flask equipped with a condenser, a nitrogen inlet and a thermometry. The experiment was carried out with the protection of nitrogen. Initially, magnetic beads (5 mg) and $CdCl_2$ (0.1 mol) were added into 150 mL deionized water, and the mixture was stirred for 60 min at room temperature (RT). During this process, partial cadmium ions were adsorbed on the magnetic beads through the negatively charged magnetic beads. Following that, styrene (5 mL) and acrylic acid (0.1 mL) were injected into the mixture with stirring, and heated to 70 °C. Upon addition of $K_2S_2O_8$ into the flask (0.1 g, 5 mL), cadmium-doped poly(styrene-acrylic acid) nanoshells were gradually formed on the magnetic beads. The final Cd-MPSA nanospheres were obtained after stirring for 8 h at 70 °C. The as-prepared Cd-MPSA nanospheres were separated and purified using an external magnet.

Next, Cd-MPSA nanospheres were used for the label of PAb_2. Briefly, Cd-MPSA nanospheres (20 mg) was initially dispersed into 2.0 mL deionized water, and then PAb_2 (500 μL, 1.0 mg mL^{-1}) was added into the mixture. The mixture was incubated for 12 h at 4 °C with gentle stirring. Finally, the Cd-MPSA-labeled PAb_2 was separated with an external magnet, and stored at 4 °C until use.

2.3. Immunosensor fabrication and measurement protocol

A cleaned gold electrode (2 nm in diameter) was initially dipped into L-cysteine aqueous solution for 4 h to make L-cysteine