Biosensors and Bioelectronics 92 (2017) 68–73

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

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

The preparation of dual-functional hybrid nanoflower and its application in the ultrasensitive detection of disease-related biomarker

Yucheng Liu, Jinyang Chen, Mingyuan Du, Xinxin Wang, Xinghu Ji, Zhike He*

Key Laboratory of Analytical Chemistry for Biology and Medicine (Ministry of Education), College of Chemistry and Molecular Sciences, Wuhan University, Wuhan 430072, China

ARTICLE INFO

Keywords:
Hybrid nanoflower
Colorimetric sensor
Disease-related biomarker

ABSTRACT

In this work, dual-functional streptavidin (SA)-horseradish peroxidase (HRP) hybrid nanoflowers, integrating the functions of biological recognition and signal amplification, were prepared through facile one-pot green synthesis method. The prepared SA-HRP-$Cu_3(PO_4)_2$ hybrid nanoflowers loaded abundant HRP and simultaneously exhibited enhanced catalytic activity, stability, and durability compared with free enzyme, which fits greatly well with the requirement of signal tag for bioassay. Besides, due to the general SA-biotin linking interaction, the SA-HRP-$Cu_3(PO_4)_2$ hybrid nanoflowers possess universal capture ability to the biotinylated antibody. Hence, combined with enzyme-linked immunosorbent assay (ELISA), the dual-functional hybrid nanoflowers were used to construct a colorimetric sensor for the ultrasensitive detection of alpha-fetoprotein (AFP). The detection limit is 78 pg/mL, which is far superior to commercial ELISA kits. This presented approach holds great promise to develop on-demand hybrid system for a variety of applications ranging from biosensor and biomedicine to biocatalytic process.

1. Introduction

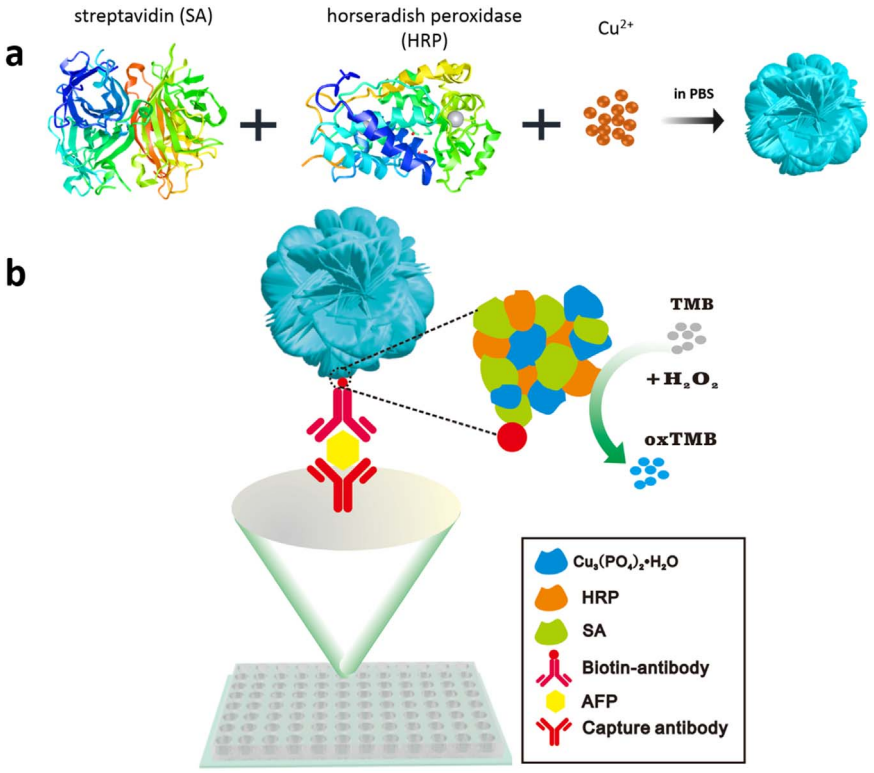
Colorimetric sensors, with the advantages of low-cost, simplicity and ease of measurement, have been extensively used to detect variety of targets including metal ions (Liu et al., 2011), small molecules (Liu et al., 2011; Liu and Lu, 2006), nucleic acids (Mao et al., 2016; Wu et al., 2015, 2016), proteins (Wei et al., 2007) and especially for the detection of disease-related biomarkers (Chen et al., 2015). However, those clinically important protein biomarkers are often at a very low concentration, which is a challenge for their assay. To address this challenge, vast endeavors have been attempted to improve the sensitivity of colorimetric sensors through constructing various signal amplification strategies (Xu et al., 2009; Zhao et al., 2015). Among them, enzyme-based signal amplification has been the most commonly used technique for bioassay, for instance enzyme linked immunosorbent assay (ELISA) (Ye et al., 2014). And the key is to load a large amount of enzymes and simultaneously maintain their activity.

Recently, a new type of organic-inorganic hybrid nanoflowers made of protein and $Cu_3(PO_4)_2$ have received significant investigative interests due to their facile preparation (one-step coprecipitation method) and large surface-to-volume ratio (Ge et al., 2012; Zeng and Xia, 2012). Notably, when an enzyme was used as the protein component, the nanoflowers exhibited significantly enhanced catalytic activity, stability and durability (Cui et al., 2016; Yin et al., 2015; Zhang et al., 2015). This strategy of constructing protein-inorganic nanoflowers has provided a blueprint for other hybrid system. For example, the horseradish peroxidase (HRP)-$Cu_3(PO_4)_2$ hybrid nanoflower has been employed as a colorimetric platform for visual detection of hydrogen peroxide and phenol (Lin et al., 2014), and the glucose oxidase (GOx)-$Cu_3(PO_4)_2$ hybrid nanoflower has been applied to degrade organic pollutants (Huang et al., 2015). According to recent development, multi-protein co-embedded nanoflowers have attracted much attention for the combination of different proteins to achieve comprehensive functions. GOx-HRP-$Cu_3(PO_4)_2$ hybrid nanoflowers, for example, were prepared to achieve one-step two-enzyme cascade catalytic reaction (Sun et al., 2014). Additionally, concanavalin A (Con A)-GOx-$Cu_3(PO_4)_2$ hybrid nanoflowers were successfully used for on-site detection of food pathogen (Ye et al., 2016). Based on these achievements, protein-inorganic nanoflower would be a promising tool in biomedical fields and bioanalytical process (Hua et al., 2016; Lee et al., 2015; Wei et al., 2016; Yu et al., 2015). However, the previously designed nanoflowers are limited in the material universality. Therefore, hybrid nanoflowers with improved versatility should be designed.

In this work, we prepared the streptavidin (SA) and HRP-$Cu_3(PO_4)_2$ hybrid nanoflower through facile one-pot green synthesis strategy, simply adding SA and HRP to a copper ion solution in PBS

* Corresponding author.
E-mail address: zhkhe@whu.edu.cn (Z. He).

http://dx.doi.org/10.1016/j.bios.2017.02.004
Received 24 November 2016; Received in revised form 31 January 2017; Accepted 2 February 2017
Available online 04 February 2017



Scheme 1. (a) Synthesis process of SA-HRP hybrid nanoflowers; (b) Schematic illustration of ultrasensitive ELISA for AFP detection based on the dual-functional hybrid nanoflowesrs.

buffer for certain time at room temperature, as illustrated by Scheme 1. The flower-like structures were formed mainly for the coordination between amine group of the protein and copper ions (Zeng and Xia, 2012). It is worth noting that this synthetic approach does not involve any toxic elements, extreme harsh conditions and complex synthesis procedure. Therefore, the biological substances (SA and HRP) employed in the synthesis suffer from less manipulation compared with other conventional methods to maintain the activity of the immobilized protein. Moreover, in the SA-HRP-$Cu_3(PO_4)_2$ hybrid nanoflower, SA was selected as the recognition unit due to its high affinity to biotin, which is a classic system generally used in ELISA. Meanwhile, HRP, which serves as the signal amplification unit, can effectively catalyze the oxidation of 3, 3′, 5, 5′-tetramethylbenzidine (TMB) to a blue-colored product in the presence of H_2O_2. Therefore, the introduction of SA and HRP brings the nanoflower a dual function: the specific capture ability to biotinylated antibody, and enhanced enzymatic activity and stability for producing signal amplification. The prepared SA-HRP nanoflower was further used in ELISA to construct a simple but powerful colorimetric sensor for AFP assay.

2. Experimental

2.1. Reagents and materials

Human alpha-fetoprotein (AFP), anti-human AFP monoclonal antibody (capture Ab), biotin-labeled AFP antibody (biotin-Ab), AFP ELISA kit were purchased from Linc-Bio Science Co. Ltd. (Shanghai, China). Horseradish peroxidase (HRP), copper (II) sulfate pentahydrate ($CuSO_4{\cdot}5H_2O$), 3, 3′, 5, 5′-tetramethylbenzidine (TMB) liquid substrate system for ELISA, phosphate-buffered saline (0.1 M phosphate buffer, 0.15 M sodium chloride, pH 7.4, at 25 °C), Tween 20, and potassium chloride were purchased from Sigma–Aldrich (St. Louis, MO, USA). Bovine serum albumin (BSA) was obtained from Roche (Los Angeles, CA, USA). Streptavidin (SA) was purchased from Amresco (Solon, OH, USA), and 96-well high-binding ELISA strip plates were purchased from Thermo Fisher Scientific (Waltham, MA, USA). Human serum sample was supplied by Zhongnan Hospital of Wuhan University (Wuhan, China). All of the other reagents were of analytical grade. Ultrapure water from a Millipore water purification system (Billerica, MA, USA) was used in all of the assays. All oligonucleotide with different sequences were synthesized and HPLC purified by Sangon Biotechnology Co., Ltd. (Shanghai, China). The sequences of the oligonucleotide used in this work are listed as followed:

B-DNA-F: 5′-biotin-AAAAAA-FAM-3′

DNA-F: 5′-AAAAAA-FAM-3′

2.2. Synthesis and characterization of SA-$Cu_3(PO_4)_2$, HRP-$Cu_3(PO_4)_2$, and SA-HRP-$Cu_3(PO_4)_2$ hybrid nanoflowers

Synthesis of SA-$Cu_3(PO_4)_2$ hybrid nanoflowers (SA nanoflowers): typically, 20 μL of aqueous $CuSO_4$ solution (120 mM) was added to 3 mL of PBS (0.1 mM, pH 7.4) containing different concentrations of SA, followed by incubation at 25 °C for 18 h. The prepared nanoflower precipitate was collected through centrifugation (10,000 rmp for 5 min) and washed with ultrapure water three times.

Synthesis of HRP-$Cu_3(PO4)_2$ hybrid nanoflowers (HRP nanoflowers): typically, 20 μL of aqueous $CuSO_4$ solution (120 mM) was added to 3 mL of PBS (0.1 mM, pH7.4) containing different concentrations of SA, followed by incubation at 25 °C for 18 h. The prepared nanoflower precipitate was collected through centrifugation (10,000 rmp for 5 min) and washed with ultrapure water three times.

Synthesis of SA-HRP-$Cu_3(PO_4)_2$ hybrid nanoflowers (SA-HRP nanoflowers): typically, 20 μL of aqueous $CuSO_4$ solution (120 mM) was added to 3 mL of PBS (0.1 mM, pH 7.4) containing different concentrations of SA and HRP at a ratio of 1:10, followed by incubation at 25 °C for 18 h. The prepared nanoflower precipitate was collected through centrifugation (10,000 rmp for 5 min) and washed with ultrapure water three times.

2.2.1. . Characterization

The size and morphology of prepared nanoflowers were characterized by Scanning Electron Microscope (SEM, HITACHI S-4800),