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An ultrasensitive competitive immunosensor for impedimetric detection of microcystin-LR via antibody-conjugated enzymatic biocatalytic precipitation

Li Hou, Yunhua Ding, Lili Zhang, Yuan Guo, Minsheng Li, Zhen Chen, Xiaoping Wu *

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

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ABSTRACT

Herein we design a sensitive impedimetric immunosensor for quantitative detection of microcystin-LR (MC-LR) by combining antibody-conjugated enzymatic biocatalytic precipitation reaction and a competitive format. MC-LR-bovine serum albumin (MC-LR-BSA), which conjugated onto the nanogold-modified glassy carbon electrode through cysteamine and glutaraldehyde, was introduced as an analyte competitor. Competitive assays were performed by incubating the target MC-LR and the analyte competitor with horseradish peroxidase-labeled monoclonal anti-MC-LR antibodies (HRP-mAb). After the competitive reaction, HRP were introduced as the signal labels onto the immunosensor, accelerating the biocatalytic precipitation reaction of substrate 4-chloro-1-naphthol with the aid of H_2O_2. The enzymatically generated insoluble precipitate formed an insulting layer on the electrode surface and efficiently restricted the electron transfer of redox probe $Fe(CN)_6^{4-/3-}$, resulting in an enhanced amplification of impedimetric signal. The impedimetric signal decreased with the increase of concentration of MC-LR, and exhibited a dynamic linear range from 0.01 to 100 $\mu g\,L^{-1}$ with a detection limit of 0.004 $\mu g\,L^{-1}$. The proposed immunosensing method can be applied for rapid detection of MC-LR in real water samples with accuracy comparable to the high-performance liquid chromatography. The platform shows good precision, high sensitivity and specificity, which opening a promising horizon for monitoring of cyanobacterial pollution in environmental matrices.

1. Introduction

Microcystins (MCs), the most widespread lethal cyanotoxins produced by blooming cyanobacteria in fresh and brackish waters, represent a tremendous threat to the aquatic ecosystem and human health. The cyclic polypeptide structure makes MCs stable enough to persist in environment for many years and accumulate in aquatic animals [1]. Exposure to the hepatotoxic MCs can lead to serious damage of the liver because it can dramatically inhibit the activity of protein phosphatases 1 and 2A [2,3], and act as a latent tumor promoter [4]. Among 80 MCs variants, microcystin-LR (MC-LR) that contains leucin (L) and arginine (R) is the most prevalent and abundant toxin, accounting for 46.0–99.8% of the total concentration of MCs in the toxic cyanobacterial blooms [5–7]. Recently an increase of incidents of poisoning was reported, due to the presence of toxic MC-LR in the contaminated water supply. Based on the strong toxicity and ubiquity, a guideline value 1.0 $\mu g\,L^{-1}$ for MC-LR in drinking water has been set by World Health Organization (WHO) [8]. The development of a rapid, robust and sensitive detection method for monitoring low level of MC-LR is urgently necessary to manage the risk associated with drinking water safety.

Numerous methods have been reported for analysis of MC-LR, including high-performance liquid chromatography (HPLC) [9], enzyme-linked immunosorbent assay (ELISA) [10], immunochromatographic assay [11], protein phosphatase inhibition assay (PPIA) [12], and electrochemical immunoassay [13]. HPLC is the most commonly used and accepted method for identification and quantification of MC-LR. However, several shortages, such as expensive instrumentation, complicated pre-treatment procedure,

Abbreviations: 4-CN, 4-chloro-1-naphthol; BCP, enzymatic biocatalytic precipitation; FRET, fluorescence resonance energy transfer; HRP, horseradish peroxidase; HRP-mAb, horseradish peroxidase-labeled monoclonal anti-MC-LR antibodies; MCs, microcystins; MC-LR, microcystin-LR; MC-RR, microcystin-RR; MC-LR-BSA, MC-LR-bovine serum albumin; AuNPs, nanogold particles; PPIA, protein phosphatase inhibition assay; QDs, quantum dots; QCM, quartz crystal microbalance.

* Corresponding author.
E-mail addresses: wapple@fzu.edu.cn, w-apple@sohu.com (X. Wu).

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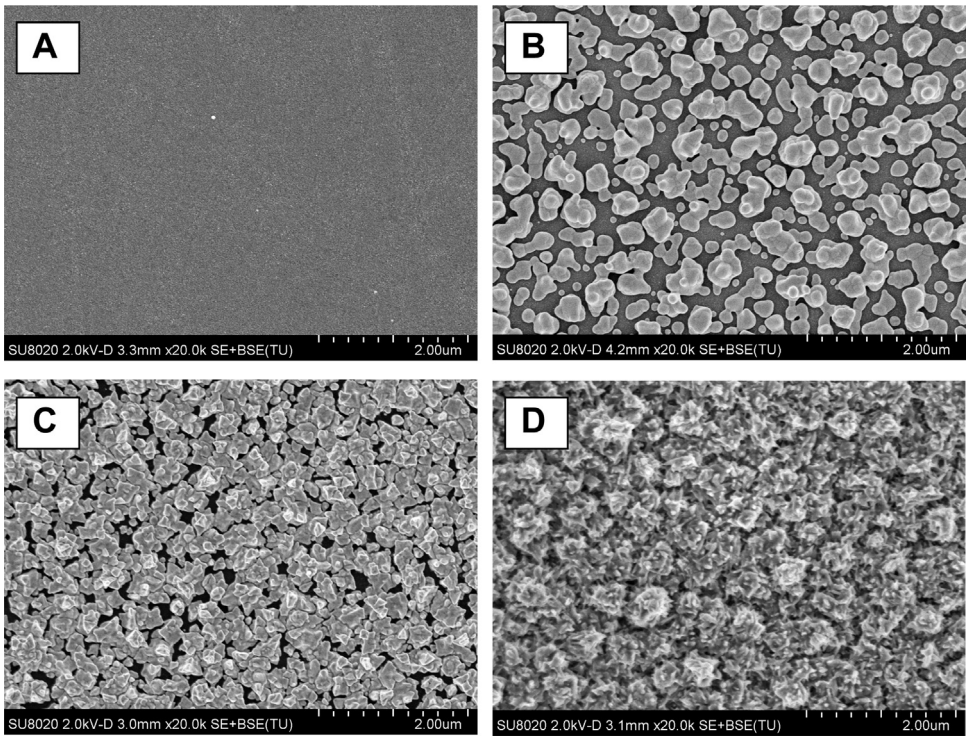

Fig. 1. SEM images of (A) GCE, (B) AuNPs/GCE, (C) the newly prepared immunosensor after conjugation with HRP-mAb, and (D) the electrode '*c*' after BCP deposition.

and skilled personnel [14], make it not suitable for rapid screening and detection of multiple samples. Most of the alternative methods for MC-LR screening are immunoassays based on the affinity recognition between antibody and antigen, which are very sensitive and ease of use. Among various immunoassays, electrochemical immunoassay is becoming increasingly attractive because of its simplicity, portability, and low cost. Much effort has been spent on exploring new approaches that could improve the sensitivity and simplicity of immunosensor.

In recent years, impedimetric immunosensors received particular attention as an enhanced signal transducer in electrochemical biosensors, because of the fact that impedimetric measurement is less affected by electric interference [15,16]. Owing to the characters of accurate, stable and reproducible, they have been widely applied in studying antigen-antibody interaction and showed extensive linear range and low detection limits. Typically, the existed immune affinity between antigens and antibodies can inhibit the electron transfer of redox probe on the electrode surface, which will cause the changes in charge transfer resistance (R_{et}) to some extent [17]. However, measuring the impedance signal caused by the antibody-antigen interaction in competitive format is usually not easy for small molecules, because such interfacial changes are very minor. A major difficulty is still encountered in the development of impedimetric immunosensor which is capable of signal amplification for low level of small molecules.

Background reduction and amplification of measurable product play essential roles in enhancing the detectable signal of impedimetric immunosensors. Many works have been focused on signal amplification using nanomaterials modification to improve response properties of electrochemical transducers, such as background capacity, rate of electron transfer, and signal to noise ratio [18]. In addition, nanomaterials have been employed for increasing the loading amount of antibody or antigen by simply modifying the substrates [19–21]. Recently, gold nanoparticles have been recognized as an effective alternative of conventional nanomaterials for sensing the interface biological recognition events, due to their large surface area, high electrical conductivity, biocompatibility, and ease of assembly with very simple method like electro-deposition [22].

Another concern on signal amplification of impedimetric immunosensors is to employ enzymatic reaction to form measurable product on the sensing interface [23,24]. Enhanced amplification through enzymatic catalyzing the substrate to yield an insoluble precipitate on the electrode surface, known as enzymatic biocatalytic precipitation (BCP), has been utilized for improving the detection limits. Several characterization techniques, including surface plasmon resonance (SPR), quartz crystal microbalance (QCM), photoelectrochemical detection and electrochemical detection method, have been applied in this strategy [6,25–30]. Ebersole and Ward firstly prepared a chip-based QCM sensor for sandwich enzyme-linked immunoassay [26], on which the antibodies-immobilized enzymes catalyzed the substrates to form a mass of precipitates and cause a significant mass enhancement, thus resulting in the improvement of sensitivity and the extension of detection limit. Apart from mass effect, utilizing BCP on a conductive support can also alter the interfacial electron transfer feature due to the formation of an insoluble product. Willner's group applied faradaic impedance spectroscopy and cyclic voltammetry as detection methods to probe the insulating layer formed by BCP on the electrode surface, and developed a sensing technique for glucose [31]. More recently, Yang et al. designed a novel sandwich-type electrochemical immunosensor for detection of α-fetoprotein by using functionalized single-walled carbon nanohorns as labels and BCP [32]. Horseradish peroxidase (HRP) and glucose oxidase that linked to functionalized SWCNTs could accelerate the oxidation of 4-chloro-1-naphthol (4-CN) by H_2O_2 to produce the insulating precipitates on the electrode surface. Due to the mass loading of precipitates, the resistance value on the electrode surface can be significantly enlarged.