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Development of novel portable and reusable fiber optical chemiluminescent biosensor and its application for sensitive detection of microcystin-LR

Rong Yang[a], Dan Song[a], Shunyan Fang[a], Yanping Liu[a], Xiaohong Zhou[b], Feng Long[a,*], Anna Zhu[c,d,**]

[a] School of Environment and Natural Resources, Renmin University of China, Beijing 100872, China
[b] State Key Joint Laboratory of ESPC, School of Environment, Tsinghua University, Beijing 100084, China
[c] Research Institute of Chemical Defense, Academy of Military Sciences PLA China, Beijing 102205, China
[d] State Key Laboratory of NBC Protection FOR Civilian, Beijing 102205, China

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ABSTRACT

A novel portable fiber optical chemiluminescent biosensor (FOCB) system was successfully constructed using fiber optic bio-probe as biorecognition element as well as transducer and a Si-based photodiode detector (PD-3000). The compact design of FOCB with free magnetic beads and photomultiplier allows it to be portable and suitable for on-site and automatic detection of targets, as well as to be cost-effective and small sample volume (only 40.0 μL). The sensitivity of 5 fW could be obtained for the FOCB due to the high performance of PD-3000 and no background light signal. Next, the FOCB was applied for the CL-based detection of microcystin-LR (MC-LR) in water using hapten-carrier protein conjugates modified fiber optic bio-probe as biorecognition element. Based on indirect competitive sandwich-like CL immunoassay principle, the highly sensitive detection of MC-LR was achieved using FOCB system. Under optimal conditions, the limit of detection for MC-LR is 0.03 μg/L, which is comparable with that of most reported MC-LR immunoassay methods. The robustness of the hapten–carrier protein-modified biosensor surface allowed multiple MC-LR immunoassays without any significant loss of performance, which ensured accurate and cost-effective detection results. The proposed strategy demonstrated good recovery, precision, and accuracy through the evaluation of the spiked water samples. The FOCB system can be readily extended toward the on-site real-time sensitive detection of other targets in the field of environment, food, and medical diagnosis.

1. Introduction

Cyanobacterial blooms, one of the most pressing global environmental problems, represent a great threat to public health and the water environment due to the release of cyanotoxins. Microcystins (MCs), the most widespread cyanotoxins with a group of bioactive cyclic heptapeptide, are serious hepatotoxins which can strongly suppress the activity of protein phosphatases and result in liver damage and necrosis (Campos and Vasconcelos, 2010). Many cases of animal-poisoning and human health diseases, some of which caused liver cancer and even death, have been reported (Zhang et al., 2011, 2017). Several studies demonstrated that long periods of exposure to microcystins, even at very low concentration, promoted the risk of tumor formation (Amé et al., 2010; Campos and Vasconcelos, 2010; Qileng et al., 2017). Nowadays, more than 90 known microcystin congeners have been found, and most of them have a lethal dose of about 50–600 μg/kg (Welker et al., 2004). Among them, microcystin-LR (MC-LR), containing leucine (L) in position 2 and arginine (R) in positions 4, is one of the most common microcystin congeners. To minimize public exposure to MCs, the World Health Organization (WHO) has set a guideline value of 1.0 μg/L for MC-LR in drinking water. Several detection methods, including high-performance liquid chromatography (HPLC) (Rivasseau et al., 1998), protein phosphatase inhibition assays (PPIA) (Rivasseau et al., 1999), and enzyme-linked immunosorbent assay (ELISA) (Lindner et al., 2004; Sheng et al., 2006; Campàs and Marty, 2007), have been applied for MC-LR detection. HPLC is more precise than the other methods and can distinguish microcystin congeners, while it requires expensive equipment, complex procedure, long analysis time, and trained personnel (Tsuji et al., 1994). ELISA and PPIA are sensitive and effective detection methods but they cannot be used for routine

* Corresponding author.
** Corresponding author at: Research Institute of Chemical Defense, Academy of Military Sciences PLA China, Beijing 102205, China.
E-mail addresses: longf04@ruc.edu.cn (F. Long), zhuanna00@mails.thu.edu.cn (A. Zhu).

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screening and may yield false positives if the enzyme is inhibited by other unrelated environmental pollutants. To ensure human health and environmental safety, it is still essential to develop novel techniques for the real-time on-site detection of MC-LR with high sensitivity and specificity due to its low concentration level and large scale analogues.

Chemiluminescence (CL), the luminescence produced from a chemical reaction, has been proved to be a powerful technique for the detection of trace analytes due to its high sensitivity, wide linear range, and low background noise (Williams et al., 2006). Through integrating molecular biotechnology with different transducers, various CL biosensors have successfully applied for medical diagnosis, environmental monitoring, and food analysis (Amstislavski et al., 2012; Zhang et al., 2013; Timofeeva et al., 2017). Because of the absence of an excitation light source and a spectral resolving system, CL biosensor are very suitable for developing a portable detection instrument for the on-site real-time detection of environmental pollutants. However, the conventional CL instruments used magnetic-beads coated with antibodies to capture targets to form immune complex, which needed to be separated from the original solution using magnetic field to eliminate the matrix effect and reduce the background interference. This resulted in the complex structure of the conventional CL instrument and multiple detection steps (Amstislavski et al., 2012; Iranifam, 2013). Moreover, the immune magnetic-beads were not generally reused, leading to high detection cost and inaccurate results. Because most of the CL based reactions have low quantum efficiencies and produce a weak luminescence, the photomultiplier (PMT) are applied for the CL intensity detection (Timofeeva et al., 2017). However, the use of PMT makes CL instrument difficult to be miniature and portable because of its large volume, high price, high polarizing voltage, and sensitive to electromagnetic field interference (Amstislavski et al., 2012; Iranifam, 2013). A chemiluminescence multichannel immunosensor based on a capillary immunoassay technique was used to detect MC-LR, but it employed PMT to detect CL intensity and was semi-quantitative (Lindner et al., 2009).

Herein, a novel portable and reusable fiber optic CL biosensor (FOCB) has been developed for the sensitive on-site detection of MC-LR in water samples. To avoid the deficiency of immune magnetic-beads, coating-antigen modified fiber optical probe in the FOCB system was used as both biorecognition elements and CL transducer. Our previous studies demonstrated that the coating-antigen modified fiber optic bioprobe had a good regeneration performance and could be reused more than 100 times without any loss of activity (Lou et al., 2017; Hao et al., 2015). Meanwhile, the fiber optical probe as a transducer could effectively improve the collection efficiency of CL signal, resulting in a higher sensitivity of CL biosensor. Instead of using PMT, a high sensitive and low noise photodiode (PD) detector was applied for the detection of CL intensity. Compared to PMT, PD detector has advantages of small volume, cost-effective, broad spectral response, and large dynamic range (Geng et al., 2018). Through integrating microfluidic cell and automatic sampling system, the proposed CL biosensor is very suitable for the real-time on-site detection of MC-LR due to its high sensitivity, specificity, portability, and reusability.

2. Experimental

2.1. Immunoreagents and chemicals

Monoclonal anti-MC-LR antibody and coating-antigen MC-LR-OVA were produced by our research group (Long et al., 2009). Goat anti-mouse-HRP secondary antibody was purchased from Thermo Fisher Scientific (Beijing, China). Bovine serum albumin (BSA), 3-mercaptopropyl-trimethoxysilane (MTS), N-(4-maleimidobutyryloxy) succinimide (GMBS) were purchased from Sigma-Aldrich (Steinheim, Germany), the CL substrate (HRP-H_2O_2-luminol system) was purchased from Yesen Bio-Technology Limited (Shanghai, China). Methanol, acetone, acetonitrile, H_2SO_4, H_2O_2, and all the other reagents, unless specified, were supplied by the Beijing Chemical Agents (Beijing, China). These reagents were all of analytical grade and used without further purification. Distilled deionized water was used throughout the investigation. Buffers used in this experiment were 0.01 mol/L phosphate buffered saline (PBS, including 137 mmol/L NaCl, 2.7 mmol/L KCl, 4.3 mmol/L Na_2HPO_4 and 1.4 mmol/L KH_2PO_4, pH = 7.4) and antibody dilution buffer (1.0 g BSA, 0.02 g NaN_3 and 100 mL 0.01 mol/L PBS). MC-LR stock solutions were prepared with PBS and stored at 4 °C. Standard concentrations of the analyte were prepared from the stock solution by serial dilutions in PBS. And all the antibody solutions were diluted by antibody dilution buffer. 0.5% sodium dodecyl sulfate (SDS, pH = 1.9) was used as the regenerated solution.

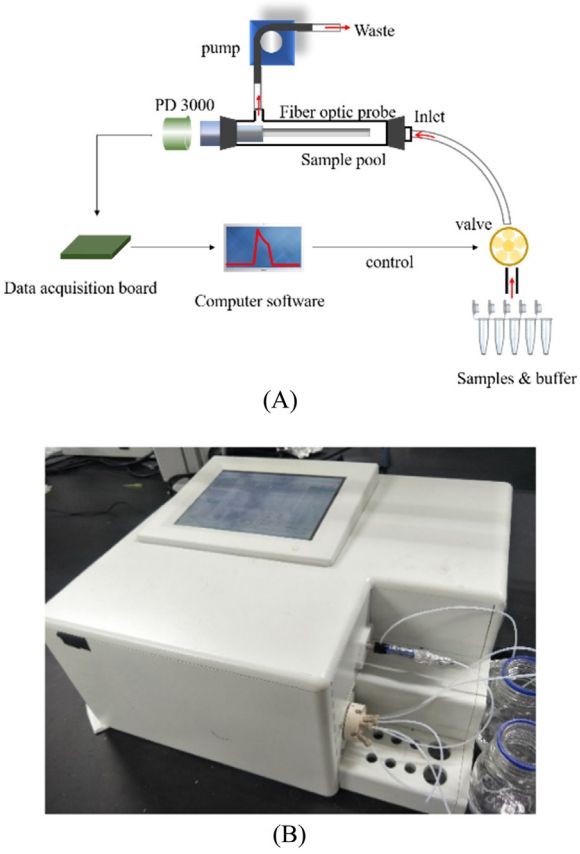

Fig. 1. (A) Schematic of portable fiber optic CL biosensor; (B) picture of the FOCB.

2.2. Design of FOCB

The Scheme of FOCB system for the CL detection is shown in Fig. 1A, and the picture of the FOCB (29.5 × 23.2 × 15.8 cm) were shown in Fig. 1B. In this system, a fiber optical probe (600 μm, NA = 0.22) modified by coating-antigen conjugates (e.g. MC-LR-OVA) was regarded as biorecognition element and embedded in a black cylindrical microfluidic cell (ϕ1.5 mm, 35 mm length, effective volume ~ 60 μL) to block environmental light. Meanwhile, the fiber optic bioprobe was also used as a transducer to collect the CL signal. To simplify the system structure and improve the detection efficiency, a new Si-based photodiode detector PD-3000 developed by our group was directly placed in the end of fiber optical probe to detect the CL signal without any other optical separated elements. The flow sampling system was significant for achieving automated measurement. This system consisted of a peristaltic pump, a six-way injection valve, and a microfluidic cell. Various solutions (e.g. buffer, sample, and regeneration solution) were successively introduced into the microfluidic cell.