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Determination of microcystin-LR in water by a label-free aptamer based electrochemical impedance biosensor

Zhenyu Lin *, Huiming Huang, Yixiang Xu, Xiaoyao Gao, Bin Qiu, Xi Chen, Guonan Chen *

Ministry of Education Key Laboratory of Analysis and Detection for Food Safety, Fujian Provincial Key Laboratory of Analysis and Detection for Food Safety, Fuzhou University, Fuzhou, Fujian 350002, China

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ABSTRACT

In this study, an electrochemical impedance biosensor for cyanobacterial toxin microcystin-LR (MC-LR) detection has been developed. MC-LR aptamers were immobilized on the gold electrode through Au–S interaction, in the presence of target (MC-LR); the binding of MC-LR and aptamers probe led to a complex formation change on the electrode surface and resulted in the impedance decreasing. The decrease rate had a linear relationship with logarithm of the MC-LR concentration in the range of 1.0×10^{-7}–5.0×10^{-11} mol/L, with a detection limit of 1.8×10^{-11} mol/L. The sensor has good selectivity and stability, it has been applied to detect MC-LR in three kinds of real water samples with satisfying results.

1. Introduction

Cyanobacteria can form blooms in water and become a global environmental issue, which causes a threat to the ecological, recreational and esthetic values of water sources. Cyanotoxins are a group of cyclic polypeptides produced by different species of cyanobacteria cells. In the situation of cell rupture, a mass of toxins were released into the water, causing a series of public health and environment problems. The basic molecule structure of microcystins is D-Ala-L-X-erythro-β-methyl-D-iso-Asp-L-Y-Adda-D-iso-Glu-N-methyldehydro-Ala [1]. Micro-cystin-LR, which contains leucine (X represented) and arginine (Y represented) in the main variant positions, is the most frequent and toxic variant among the more than 80 microcystins identified to date [2,3]. Meanwhile, it is also the most extensively studied one.

It had been confirmed that microcystins were responsible for some poisonings of animals and humans where water sources contained toxic cyanobacteria blooms [4]. Fujiki and co-workers [5] had reported that microcystins were potent and specific in inhibiting protein phosphatases 1 and 2A (PPl, PP2A). Acute or prolonged exposure to microcystins would cause liver damage, followed by a massive intrahepatic hemorrhage and probably leading to death. Therefore, in 1998, WHO had given a provisional guideline value for total microcystin-LR (MC-LR) in drinking water of 1 μg/L (equivalent to 1.0×10^{-9} mol/L). Thus, development of a sensitive method for microcystin-LR detection and qualification is quite necessary.

Methods developed for MC-LR detection had been reported massively [6–10]. Conventional methods for microcystin analysis involve high performance liquid chromatography (HLPC) or enzyme-linked immunosorbent assay (ELISA). Pyo et al. [11] had compared the HPLC method and immunochromatography method for the analysis of cyanobacterial toxins with the low detection limit of 0.5 μg/mL for HPLC and 200 pg/mL for the fluorescence immunochromatographic strip, respectively. Lei and co-workers [12] developed an ELISA-like method, time-resolved fluorescence immunoassay, based on anti-microcystin-LR monoclonal antibodies and europium-labeled antimouse IgG conjugate for microcystin detection. It exhibited a typical sigmoidal response for MC-LR in the range of 0.005–50 ng/mL, with a wide quantitative range between 0.01 and 10 ng/mL. These methods showed a good limit of detection (LOD), but the procedures were complex and needed time-consuming sample preparation.

Aptamers are selected in vitro by the SELEX process from large random-sequence oligomers [13,14]. Aptamer-based biosensors had revealed a bright prospect because of their excellent features. They can recognize a wide range of targets, such as small molecules, proteins, viruses and even cells, with high affinity and specificity. In addition, aptamers are easy to manipulate and synthesize. These characters facilitate the application of aptamers in developing sensing platform.

Electrochemical impedance spectroscopy (EIS) is one of the frequently used methods in the development of electrochemical aptasensor for monitoring the interaction between aptamers and

* Corresponding authors. Tel./fax: +86 591 22866135.
E-mail addresses: zylin@fzu.edu.cn (Z. Lin), gnchen@fzu.edu.cn (G. Chen).

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biomolecules at electrode surface, which owns excellent advantages, such as high sensitivity, ease of performance and use of simple equipment [15]. For example, Chen and co-workers [16] applied EIS technique in a three-dimensional ordered macroporous gold film modified electrode for C-reactive protein (CRP) detection. The CRP concentration was measured through the increase of impedance values in the corresponding specific binding of CRP antigen and CRP antibody. Recently, Nguyen and co-workers [17] had employed the EIS technique to investigate physical parameter changes of a nanobiosensor during virus capture.

In this study, combining the merits of high selectivity of aptamers and high sensitivity of impedance method, a novel, simple and rapid detection electrochemical impedance spectroscopy biosensor for MC-LR has been developed. The proposed biosensor has been applied to detect the MC-LR in real water samples with satisfying results.

2. Experimental

2.1. Materials and reagents

The single-strand DNA (ssDNA) aptamer for MC-LR used in this study was chosen and designed according to the prior reported literature [18]. In order to modify the aptamer onto the electrode surface, a thiol had been added at its 5′ end. This functionalized aptamer was synthesized by Sangon (Shanghai, China), purified by high-performance liquid chromatography, and used as received. The sequences were shown as follows:

5′-SH-TTT TTG GGT CCC GGG GTA GGG ATG GGA GGT ATG GAG GGG TCC TTG TTT CCC TCT TG-3′

Microcystin-LR, sodium monohydrogen phosphate, sodium dihydrogen phosphate, potassium ferricyanide, potassium ferrocyanide were purchased from Sigma (St. Louis, MO). All chemicals were of analytical grade and used as received. Standard microcystin-LR samples had been dissolved by 100 mmol/L Tris–HCl buffer (pH 7.4, containing 200 mmol/L NaCl) to get various concentrations of microcystin-LR. DNA solution was prepared by dissolving DNA into 0.05 mol/L (pH 7.4) phosphate buffer solutions (PBS). Deionized water (18.4 MΩ) purified by a milli-QTM system (Millipore) was used throughout the experiment.

2.2. Sensor preparation

The gold electrodes (3 mm in diameter) were polished firstly with aqueous slurries of 1.0 μm, 0.3 μm and 0.05 μm α-Al_2O_3 powders on a polishing microcloth, followed by an ultrasonic bath for 5 min to prevent the invisible alumina particles from adsorbing over the electrode surface. Then, the electrode was washed with Milli-Q water for 5 min and dried in a nitrogen stream to obtain clean gold electrode surface, and then they were further cleaned and activated in a fresh 0.5 mol/L H_2SO_4 solution through cyclic voltammetry (CV) scanning in the range of $0\sim+1.65$ V until ideal CV grams were reached. Finally, the solution containing 10 μmol/L thiolated aptamer probes was dropped on the electrode surface and covered by a closed container for 2 h at 37 °C to form an aptamer-immobilized electrode. Then the modified electrodes were incubated with different concentrations of MC-LR at room temperature for 4 h. Then the electrode was rinsed again with Milli-Q water for 5 min to remove the excess of material that were not immobilized over the electrode surface.

2.3. Measurement

All electrochemical measurements were carried out on a CHI660D electrochemical analyzer (Chenhua Instruments, Shanghai, China). Electrochemical impedance spectroscopy (EIS) studies were performed in 10 mmol/L $[Fe(CN)_6]^{3-}$/$Fe[(CN)_6]^{4-}$ solution containing 0.1 mol/L KCl with a three-electrode system; a gold electrode was used as the working electrode after pre-immobilization with aptamers, an Ag/AgCl electrode and a Pt wire had been used as reference electrode and counter electrode, respectively. Impedance measurements were recorded between 0.1 MHz and 1 Hz at a sinusoidal voltage perturbation of 5 mV amplitude.

3. Results and discussion

3.1. The principle of the biosensor

The mechanism of the electrochemical impedance biosensor is described in Fig. 1. An alkanethiol moiety was modified to the

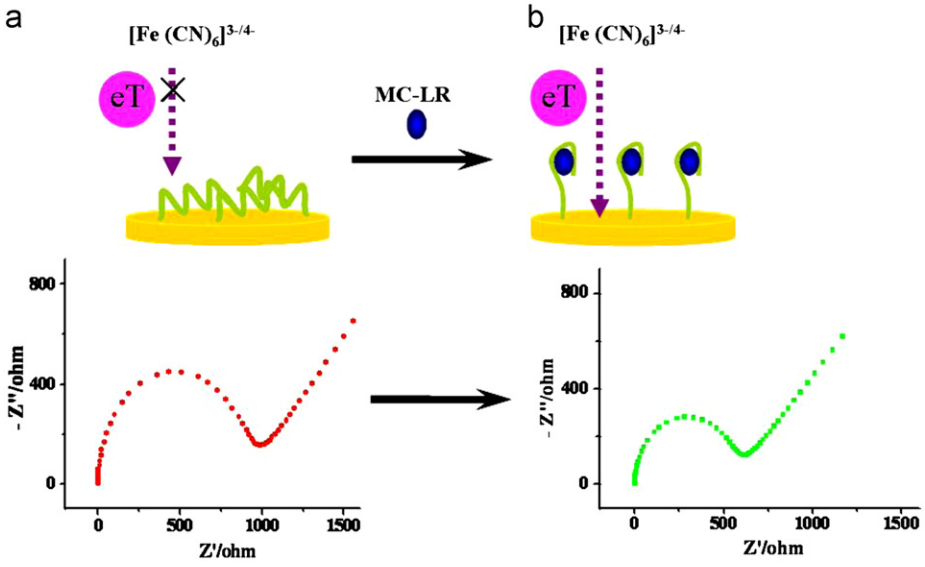

Fig. 1. Mechanism of the electrochemical impedance biosensor for MC-LR detection.

5′-end of the 56-mer DNA aptamer; hence, aptamers would be well immobilized on the gold electrode surface through Au–S covalent bonds and a monolayer on the electrode surface formed. This keeps the external redox mediator, $[Fe(CN)_6]^{3-/4-}$, from penetrating to the gold electrode surface and a bigger electrochemical impedance can be detected. A Randles equivalent circuit has been used to fit the obtained impedance spectra, which contains the electrolyte solution resistance R_s, the surface electron transfer resistance R_{ct} that reflects the surface property of the electrode, the Warburg impedance Z_w and the constant phase element related to double layer capacitance $C_{dl.}$

The modified electrode was then incubated into the target solution (MC-LR). MC-LR would bind with the aptamer and was captured on the electrode surface, which changed the structure of the aptamers. This change made the $[Fe(CN)_6]^{3-/4-}$ reach the electrode surface more freely, resulting in a decrease in R_{ct} [19,20]. And the decrease of the R_{ct} (impedance) had a relationship with the MC-LR concentration. Thus, the concentration of MC-LR could be detected indirectly by the electrochemical impedance changes.

3.2. Effect of aptamer-immobilization time and sensor incubation time

The aptamer-immobilization time had a great effect on the performance of the proposed sensor. As shown in Fig. 2A, the impedance increases with the increase of time. When it reached 2 h, the impedance had reached a plateau, this meant the surface of the electrode had been full covered by the modified aptamer or reached equilibrium. Hence, 2 h was the optimal time to modify the aptamer onto the electrodes surface.

The effect of sensor incubation time on the impedance detection was investigated. The electrochemical impedance of the proposed biosensors at different incubation times in 1.0×10^{-9} mol/L MC-LR solution under 37 °C has been shown in Fig. 2B. The results showed that the impedance decreased rapidly from 1 to 4 h, and then achieved equilibrium at 4 h. Hence, 4 h was chosen as the optimal incubation time for the following experiment.

3.3. Calibration curve and reproducibility

The impedance decrease rate is calculated by the following equation:

$$\theta(\%) = 1-\left(\frac{R_{ct(after)}}{R_{ct(before)}}\right)$$

where $\theta(\%)$ is the impedance decrease rate, $R_{ct(before)}$ is the impedance of electrode before incubation with MC-LR, and $R_{ct(after)}$ is the impedance of electrode after incubation with MC-LR. As shown in Fig. 3, the impedance decreases with the increasing of MC-LR concentration, and the decrease rate $\theta(\%)$ has a linear relationship with the logarithm of the MC-LR concentration in the range of 1.0×10^{-7}–5.0×10^{-11} mol/L. The linear regression equation is

$$\theta(\%) = 9.9724\ \lg C + 111.24,\quad R = 0.9972$$

where C represents the concentration of MC-LR and R is the correlation coefficient. The LOD is calculated to be 1.8×10^{-11} mol/L (defined as S/N=3), which is one order lower than the enzyme-linked immunosorbent assay (ELISA) reported previously [12].

The reproducibility of the proposed aptasensor was also examined by detecting the impedance spectra of the different functionalized electrodes at the same concentration (1.0×10^{-9} mol/L) of MC-LR. The relative standard deviation (RSD) of the resulting impedances was 3.52% ($n=5$). It is indicated that the proposed sensing platform has good reproducibility. Furthermore, the developed biosensor was tested after 4 weeks of storage (soaked in PBS solution) at room temperature, and the EIS response did not change greatly (R_{ct} reaches 95% of the freshly prepared one), which indicated that the proposed method has good stability.

3.4. Interference study and sample analysis

The specificity of the proposed biosensor had been studied by choosing microcystin-RR (MC-RR, one of the microcystins mutations, where both X and Y represented arginine) as interference. As shown in Fig. 4, with the increasing of MC-RR concentration, the interferences will become big. However, the impedance changing rate at 1.0×10^{-4} mol/L MC-RR was only 28% of that with 1.0×10^{-9} mol/L MC-LR and the same concentration of MC-RR caused only about 10% impedance changing rate. The results showed that high concentration of MC-RR may cause some interference. Since the concentration of MC-RR in real samples was much lower than MC-LR, little MC-RR interference would be found in real sample detection for proposed sensor.

To investigate the application of the proposed sensor in real samples, the MC-LR in three water samples (lake water, river water and tap water) had been studied. The lake water was from Fuzhou University and river water from Minjiang River in Fuzhou. As shown in Table 1, concentrations of MC-LR in the water samples were 2.94×10^{-10}, 1.57×10^{-10}, 0 mol/L, respectively. Furthermore, to evaluate the reliability of our sensor system, the

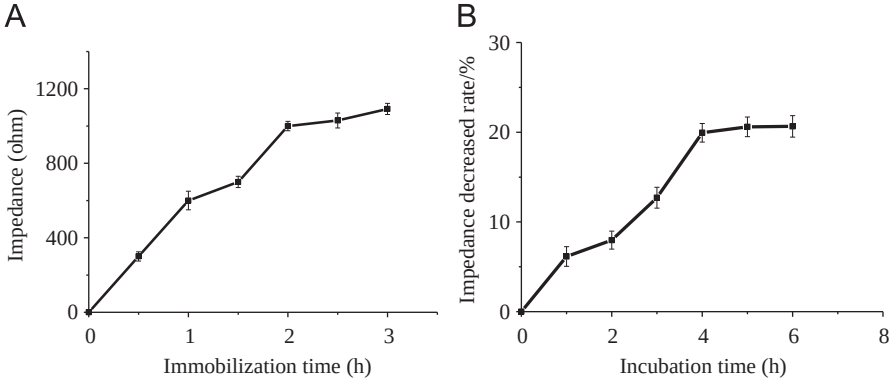

Fig. 2. (A) The change of impedance at different aptamer-immobilization times. The aptamer was immobilized on the electrode surface by dropping a droplet of 10 μmol/L thiolated aptamer probes and covering by a closed container under 37 °C. (B) The change of impedance at different incubation times. The modified Au electrode was incubated with 1×10^{-9} mol/L MC-LR solution at room temperature. All impedances were recorded in a 10 mmol/L $[Fe(CN)_6]^{3-/4-}$ solution containing 0.1 mol/L KCl, using a frequency range of 1 Hz–1 MHz, a bias potential of +0.225 V and an AC amplitude of 5 mV.

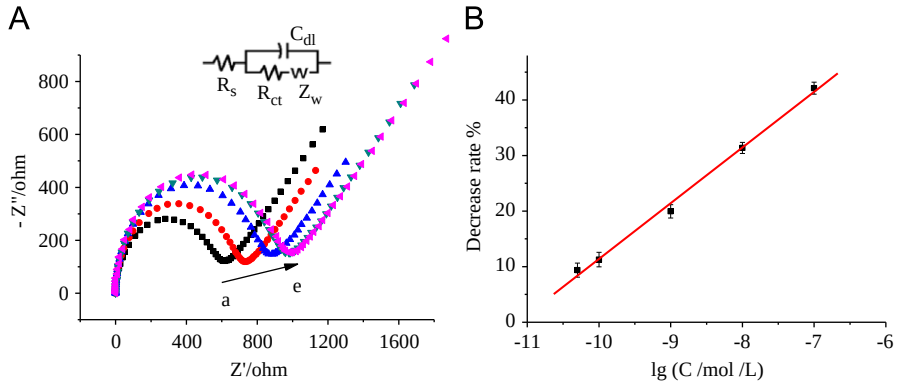

Fig. 3. (A) Impedance spectra (Nyquist plots) of electrodes incubated with different concentrations of MC-LR: (a) 1.0×10^{-7} mol/L; (b) 1.0×10^{-8} mol/L; (c) 1.0×10^{-9} mol/L; (d) 1.0×10^{-10} mol/L; and (e) 5.0×10^{-11} mol/L. (B) The linear relationship between the impedance decreased rate (θ) and logarithm of the MC-LR concentration.

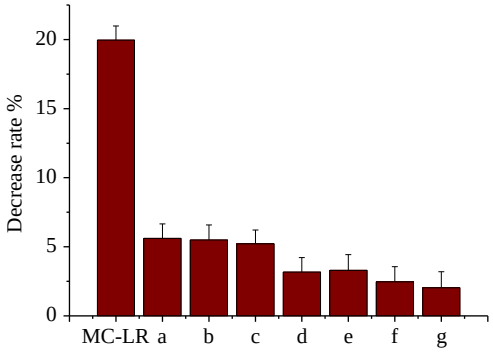

Fig. 4. Impedance decrease at 1.0×10^{-9} mol/L MC-LR and different MC-RR concentrations: (a) 1.0×10^{-4} mol/L; (b) 1.0×10^{-5} mol/L; (c) 1.0×10^{-6} mol/L; (d) 1.0×10^{-7} mol/L; (e) 1.0×10^{-8} mol/L; (f) 1.0×10^{-9} mol/L; and (g) 1.0×10^{-10} mol/L.

Table 1
Determination and recoveries of MC-LR in different water samples ($n=3$).

Samples	Content (mol/L)	Added amount (mol/L)	Found amount (mol/L)	Recovery (%)	RSD (%)
Lake water	2.94×10^{-10}	1.0×10^{-10}	1.02×10^{-10}	102.2	3.7
		2.0×10^{-10}	1.82×10^{-10}	91.2	4.2
		3.0×10^{-10}	2.93×10^{-10}	97.7	3.9
River water	1.57×10^{-10}	1.0×10^{-10}	0.94×10^{-10}	94.2	4.1
		2.0×10^{-10}	2.21×10^{-10}	110.5	4.0
		3.0×10^{-10}	3.41×10^{-10}	113.7	3.9
Tap water	–	1.0×10^{-10}	1.08×10^{-10}	107.9	3.0
		2.0×10^{-10}	1.99×10^{-10}	99.5	3.3
		3.0×10^{-10}	3.07×10^{-10}	102.3	2.6

"–" means not detected.

recovery was investigated by adding two different amounts of MC-LR into all the three samples, and the recovery was calculated as 91.2–113.7%. Therefore, the developed sensor might be preliminarily applied for the determination of MC-LR in water sample.

4. Conclusion

A label-free EIS biosensor for MC-LR based on ssDNA-aptamer had been developed. DNA aptamers modified on the gold electrode can capture the MC-LR which resulted in the decreasing of impedance. The reason may lie in the fact that the conformational changes of the aptamer on the electrode surface made $[Fe(CN)_6]^{3-/4-}$ reach the electrode surface more freely. The sensor exhibited high sensitivity and specificity. It showed a good linear relationship with the logarithm of the MC-LR concentration in the range of 1.0×10^{-7}–5.0×10^{-11} mol/L. And the LOD was calculated to be 1.8×10^{-11} mol/L, which was much better than that in most of the detecting methods to our knowledge. The proposed method had been applied to detect MC-LR in water sample with satisfying results.

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References

[1] C. MacKintosh, K.A. Beattie, S. Klumpp, P. Cohen, G.A. Codd, FEBS Lett. 264 (1990) 187–192.
[2] E. Dittmann, C. Wiegand, Mol. Nutr. Food Res. 50 (2006) 7–17.
[3] Y. Tanabe, K. Kaya, M.M. Watanabe, J. Mol. Evol. 58 (2004) 633–641.
[4] J.E. Eriksson, G.I. Paatero, J.A. Meriluoto, G.A. Codd, G.E. Kass, P. Nicotera, S. Orrenius, Exp. Cell Res. 185 (1989) 86–100.
[5] S. Yoshizawa, R. Matsushima, M.F. Watanabe, K. Harada, A. Ichihara, W.W. Carmichael, H. Fujiki, J. Cancer Res. Clin. Oncol. 116 (1990) 609–614.
[6] P.B. William, A.C. Geoffrey, Environ. Technol. Lett. 9 (1988) 1343–1348.
[7] J. An, W.W. Carmichael, Toxicon 32 (1994) 1495–1507.
[8] K. Kaya, T. Sano, Anal. Chim. Acta 386 (1999) 107–112.
[9] J.L. Ott, W.W. Carmichael, Toxicon 47 (2006) 734–741.
[10] K. Tsuji, H. Masui, H. Uemura, Y. Mori, K. Harada, Toxicon 39 (2001) 687–692.
[11] D. Pyo, G. Park, J. Choi, C. Oh, Bull. Korean Chem. Soc. 26 (2005) 268–272.
[12] L.M. Lei, Y.S. Wu, N.Q. Gan, L.R. Song, Clin. Chim. Acta 348 (2004) 177–180.
[13] A.D. Ellington, J.W. Szostak, Nature 346 (1990) 818–822.
[14] C. Tuerk, L. Gold, Science 249 (1990) 505–510.
[15] M. Zayats, Y. Huang, R.C.A. Gill, Ma, I. Willner, J. Am. Chem. Soc. 128 (2006) 13666–13667.
[16] X. Chen, Y. Wang, J. Zhou, W. Yan, X. Li, J.J. Zhu, Anal. Chem. 80 (2008) 2133–2140.
[17] B.T. Nguyen, A.E. Peh, C.Y. Chee, K. Fink, V.T. Chow, M.M. Ng, C.S. Toh, Bioelectrochemistry 88 (2012) 15–21.
[18] K.D. Gu, M. Famulok, Chin. J. Prev. Med. 38 (2004) 369–373.
[19] M. Labib, A.S. Zamay, D. Muharemagic, A.V. Chechik, J.C. Bell, M.V. Berezovski, Anal. Chem. 84 (2012) 1813–1816.
[20] J. Liu, Z. Cao, Y. Lu, Chem. Rev. 109 (2009) 1948–1998.