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An aptamer-based immunoassay in microchannels of a portable analyzer for detection of microcystin-leucine-arginine

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ABSTRACT

The rapid detection of microcystin-leucine-arginine (MC-LR), the most highly toxic among MCs, is significantly important to environmental and human health protection and prevention of MC-LR from being used as a bioweapon. Although aptamers offer higher affinity, specificity, and stability with MC-LR than antibodies in the immunodetection of MC-LR due to steric hindrance between two antibodies and limited epitopes of MC-LR for use in a sandwich immunoassay, no sandwich immunoassay using an aptmer has been developed for MC-LR detection. This study is aimed at developing an aptamer-antibody immunoassay (AAIA) to detect MC-LR using a portable analyzer. The aptamers were immobilized onto the glass surface of a microchamber to capture MC-LR. MC-LR and horseradish peroxidase (HRP)-labeled antibody were pulled into the microchamber to react with the immobilized aptamer. The chemiluminescence (CL) catalyzed by HRP was tested by a photodiode-based portable analyzer. MC-LR at 0.5–4.0 μg/L was detected quantitatively by the AAIA, with a CL signal sensitivity of 0.3 μg/L. The assay took less than 35 min for a single sample and demonstrated a high specificity, detecting only MC-LR, but not MC-LA, MC-YR, or nodularin-R. The recovery of two spiked real environmental samples calculated as 94.5–112.7%. Therefore, this AAIA was proved to be a rapid and simple method to detect MC-LR in the field by a single analyst.

1. Introduction

Cyanobacteria hepatotoxins-microcystins (MCs), a group of 800- to 1000-Da cyclic peptides with five non-protein and two protein amino acids, is mainly produced by *Microcystis aeruginosa* [1]. The widespread bloom-forming microcystis [2] is an increasing concern in regard to water contamination worldwide [3] due to the eutrophication of water and global warming [4]. MCs have been recognized as a contributory factor to human health problems [5–7] and a serious threat in biological warfare [8].

Microcystin-leucine-arginine (MC-LR), the most common and most toxic MC, contains protein amino acids leucine and arginine [9] shown in Fig. 1. Acute poisoning by MC-LR can cause skin irritation, vomiting, diarrhea, and functional and structural disturbances and damage to the liver [10]. Even a low level of MC-LR in the human body long-term causes primary liver cancer [11]. Poisoning with MC-LR and other MCs can occur through drinking or having skin contact with contaminated water [12] and consuming poisoned aquatic or agricultural products [12,13]. More importantly, MC-LR will pose a serious threat if it is used as a biological warfare agent [8]. Recent studies have demonstrated that even trace amounts of MCs (1 μg/L, 1.0×10^{-9} mol/L) in water significantly interrupt cellular process [14], and the World Health Organization (WHO) recommends a limit of 1.0 μg/L MC-LR in drinking water [15]. Therefore, an effective method for detecting

Abbreviations: AAIA, aptamer-antibody immunoassay; HRP, horseradish peroxidase; APTES, 3-Aminopropyl triethoxysilane; MCs, microcystins; CL, chemiluminescence; MC-LR, microcystin-leucine-arginine; ELISA, sandwich enzyme-linked immunosorbent assay; RLU, relative light units

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MC-LR is extremely important to prevent MCs from being used as bioweapons and to environmental and human health protection.

Although some methods can be used to detect MCs in water, including high-performance liquid chromatography and mass spectrometry [16] for known or novel MCs in the laboratory [17], it has not been reported a simple and rapid portable analyzer for MC-LR detection in the field [18–20]. Recently, some miniature immunoassays for MCs [21,22] based on the binding affinity between antibody and antigen [23] such as immusensors [24] have been developed for compact and portable analysis [25]. However, the selection of a proper antibody/antigen complex and the stability of antibody binding to MCs have prevented effective application of these immunoassays, because there are only a limited number of specific antigenic epitopes for MCs. Furthermore, the steric hindrance of MCs poses a difficulty in developing a sandwich enzyme-linked immunosorbent assay (ELISA) to capture and detect the small MC cyclic peptides. Thus, although many competitive immunoassays using a single antibody have been used for MC-LR detection [26–29], the large biomolecules such horseradish peroxidase (HRP) used to label antibodies to MC-LR may interfere with the affinity and specificity to the small size targets. In addition, antibodies to MC-LR, such as MC8C10, exhibit lower cross-reactivity with other four-arginine MCs but a high cross-reactivity with nodularin-arginine [30].

An aptamer is a small ssDNA/RNA that recognizes various targets molecules, including cells, proteins, peptides, and amino acids [31]. Compared to antibodies, aptamers offer higher affinity, specificity and stability with targets under a variety of conditions and thus conjugate with some biological molecules more easily [32]. Recently, RNA [32] or ssDNA [33,34] aptamers for the detection of MC-LR, such as RC4, RC6, RC22, and HC1, have shown a similar weaker or increased affinity to other MCs [33]. These aptamers have two or more selectivities for binding at positions 2 and 4 to MCs congeners' seven-cyclic peptide structure. Therefore, aptamers are advantageous over antibodies for immunodetection of MC-LR.

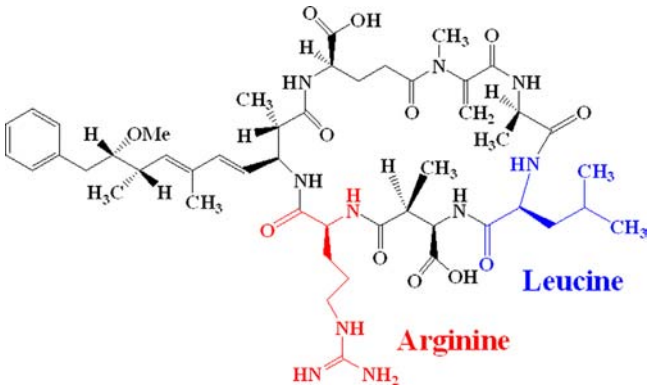

Fig. 1. Chemical structure of microcystin-LR (MC-LR). The two specific L-amino acids of MC-LRare represented with Blue (leucine, L) and Red (arginine, R).

In this study, we attempted to establish a sandwich ELISA using an aptamer to recognize distinct epitopes of MC-LR.

2. Materials and methods

2.1. Materials and reagents

Monoclonal antibodies to microcystin-LR (MC8C10), microcystins (MC-LR, -YR, and -LA), and nodularin-R were purchased from Beijing Puhuashi Technology Development Co., Ltd (Beijing, China). Super signal ELISA femto maximum sensitivity luminol substrates were obtained from Thermo Scientific (Pierce, Rockford, IL, USA). The HOOK HRP PLUS labeling kit for tagging monoclonal antibodies to MC-LR with HRP was purchased from Sangon (Shanghai, China). 3-Aminopropyl triethoxysilane (APTES), glutaraldehyde, and bovine serum albumin (BSA) were obtained from Sigma-Aldrich (St Louis, MO, USA). Polythene tubing was obtained from Shenzhen Woer Heat-shrinkable material co., LTD (Shenzhen, China). Glass capillaries (length: 12.0 cm; inner diameter: 1.5 mm) were obtained from Sichuan University, West China Center of Medical Sciences (Chengdu, China). All other reagents were analytical grade, and all solutions were prepared with Ultra-pure water (resistance > 18.2 MΩ cm, Milli-Q, Billerica, MA, USA).

2.2. Aptamer preparation

To obtain an appropriate aptamer that can bind MC-LR with antibody, several ssDNA aptamers specific to MC-LR [35,36] (Table 1) were immobilized onto glass capillary surfaces with $-NH_2$ groups, and a linker $(-CH_2-)_6$ was added between the amino group and the aptamer. Aptamer buffer solution consisted of 50 mM Tris bases, pH 7.5, 75 mM NaCl, and 10 mM $MgCl_2$. The ssDNA aptamer sequences were synthesized by Sangon (Shanghai, China). The secondary structure of the ssDNA aptamers sequences were predicted using the Mfold Web Server [37] at web site: http://www.bioinfo.rpi.edu/ applications/mfold under true reaction conditions at 25 °C, 15.0 mmol/L $[Na^+]$, and 2 mmol/L $[Mg^{++}]$. The estimation of folding free energy change (ΔG) presents the trends of the predicted secondary structures.

2.3. Conjugation of aptamer to glass microchannels

The ssDNA aptamers were grafted onto the surface of glass capillaries through an amidation reaction in the following steps. The glass capillaries were first treated with 5% HNO_3 to remove organic contamination and to allow metal ions to desorb under ultrasonic treatment for 2 h and washed with triple distilled water. The glass capillaries were dipped into 4 mol/L NaOH solution in 95% ethanol to obtain a sufficient amount of SiOH groups under ultrasonic treatment at 60 °C for 2 h and treated at 200 °C for 2 h to remove the physically adsorbed water to expose the SiOH groups. Then the glass capillaries were soaked in a mixture of 25 ml methylbenzene and 5 ml APTES at 60 °C for 2 h and washed with methanol to remove unreacted APTES. The capillaries were

Table 1
Sequences and modification of ssDNA aptamers used in this study.

Aptamer name	ssDNA Sequences and modification, 5′-3′
Ap1[34]	NH2-$(CH_2)_6$-TTTTTGGGTCCCGGGGTAGGGATGGGAGGTATGGAGGGGTCCTTGTTTCCCTCTTG
Ap2[33]	NH_2-$(CH_2)_6$-GGCGCCAAACAGGACCACCATGACAATTACCCATACCACCTCATTATGCCCCATCTCCGC
Ap3[33]	NH_2-$(CH_2)_6$-CACGCAACAACACAACATGCCCAGCGCCTGGAACATATCCTATGAGTTAGTCCGCCCACA
Ap4[33]	NH_2-$(CH_2)_6$-CACGCACAGAAGACACCTACAGGGCCAGATCACAATCGGTTAGTGAACTCGTACGGCGCG
Negative control	NH_2-$(CH_2)_6$-AAAGCGAAGTTTGAGAAGTAGCCGTAGGGATGCAAAGTGTCGTACTGGGTGATATCTCCG

further dried at 80 °C for 2 h to remove SiOH-bonding water and fixed in phosphate-buffered saline (1.37 mol/L PBS; 27 mmol/L NaCl, 43 mM KCl, 14 mmol/L $Na_2HPO_4 \cdot 12H_2O$ and KH_2PO_4, pH 7.4) containing 5.0% (ν/ν) glutaraldehyde for 0.5 h. The glass capillaries were soaked in 50 μL aptamer PBS (pH 7.4), incubated at 4 °C overnight, rinsed with PBS, and incubated in saline sodium citrate (SSC, 15 mmol/L NaCl, 15 mmol/L sodium citrate, pH 7.4) containing 1 mol/L glycine at 65 °C for 0.5 h to block the unreacted aldehyde groups. The final prepared glass capillaries were washed and subpackaged in sealed packets at 4 °C.

2.4. Apparatus

The experimental procedure is illustrated in Fig. 2. A glass capillary, 1.5 mm in diameter that was immobilized with the aptamer (Fig. 2(I)). A combined microchannel was used as the reaction chamber (Fig. 2(II)). The inner region of the microchannel was a polythene tube stretched tightly over the glass capillary by thermal contraction was used as a protective cover layer. A silicon photodiode (S1226-8 K, spectral response range λ: 320–1000 nm, Hamamatsu Photonics, Japan) based portable capillary chemiluminescence analyzer BioLay (width: 8.0 cm; length: 19 cm; depth: 4.5 cm; weight: 0.3 kg, Fig. 2(III)) that we developed in collaboration with Xi'an Tianlong Technology Co. Ltd., Xian, China was used to convert the CL signal into an electrical signal in the range of 0–9999 relative light units (RLUs) with a repeatability > 98%.

2.5. Measuring procedures

After mixing with 4 μL 5 × binding buffer (0.25 mol/L Tris, 75 mmol/L NaCl, 10 m mol/L $MgCl_2$, pH 7.5) and 8 μL HRP-antibody, 8 μL MC-LR was diluted to 0.1–50 μg/L. All of the 20 μL mixture was pulled into the reaction chamber coated with aptamer at 25 °C for 5–60 min. After the reaction mixture was squeezed out, 0.5 mL wash buffer (50 mmol/L Tris, 15 mmol/L NaCl, and 2 mmol/L $MgCl_2$, pH7.5) was pulled into the chamber using a micropump or gently sucked and squeezed repeatedly using a bulb to remove nonspecifically bound materials. The 50 μL of substrate mixture was pulled into the microchamber, and the CL signal that developed within 15 s was measured using the portable analyzer.

2.6. Analysis of spiked water samples by AAIA

To verify the suitability of the AAIA for MC-LR in environmental samples, some water samples from Xingqing Lake and Qujiang

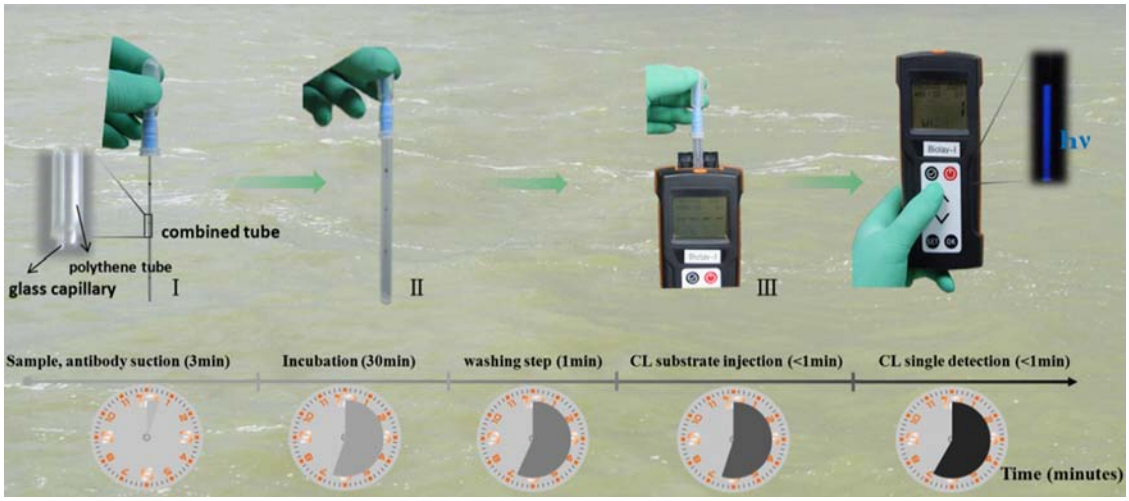

Fig. 2. Procedure of MC-LR detection in a microchannel of a portable analyzer. (I) Microchannel within a glass capillary serves as the reaction chamber. A polythene tube is used as a cover layer, and an air pressure bulb is used for manual suction. (II) A transparent polystyrene tube is nested on the lower edge of the bulb to keep liquid samples in the reaction chamber. (III) CL signal was quantitatively measured using a photodiode-based portable analyzer.

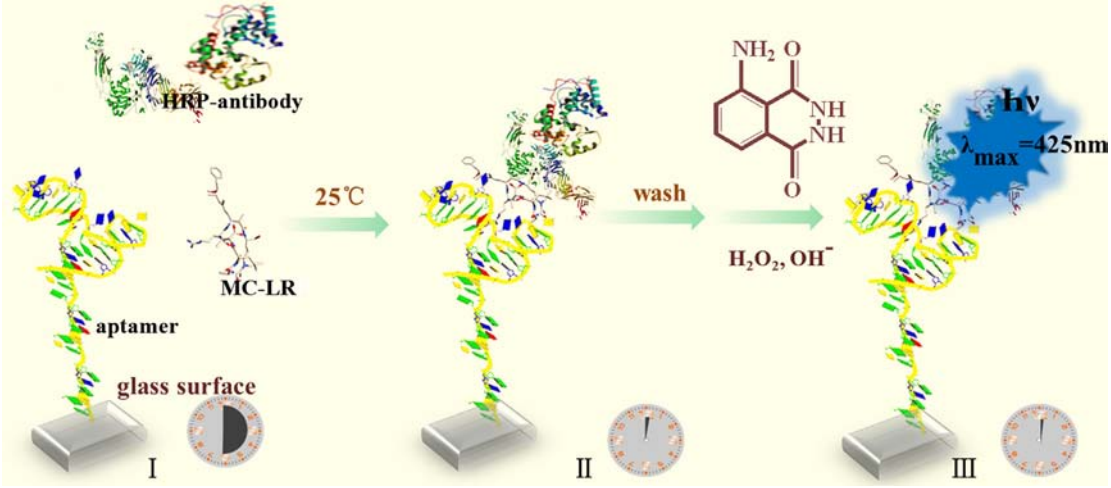

Fig. 3. Schematic of aptamer-based immunoassay for MC-LR detection in microchannel. (I) Formation of complex between immobilized aptamer, MC-LR, and HRP-labeled antibody; (II) aptamer-MC-LR-antibody complex, and (III) enhanced CL signal from HRP.

Pond, both near the city of Xi'an, Southwest of China were filtered with filters (aperture 0.45 μm, Xingya Purification Material Factory, Shanghai, China) and spiked with various concentrations of MC-LR. The next experimental procedures were performed as described above.

2.7. Statistical analysis

Data are expressed as the mean ± standard deviation (SD). The difference between two groups was analyzed by Student's *t*-test using Origin (version 8.1; OriginLab, Northampton, MA, USA). A *p* value of less than 0.05 was considered statistically significant.

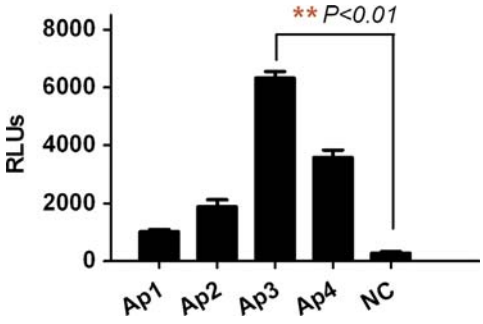

Fig. 4. Binding affinities of different ssDNA aptamers in AAIA. Data are expressed as the means ± SD of CL signals from three separate experiments.

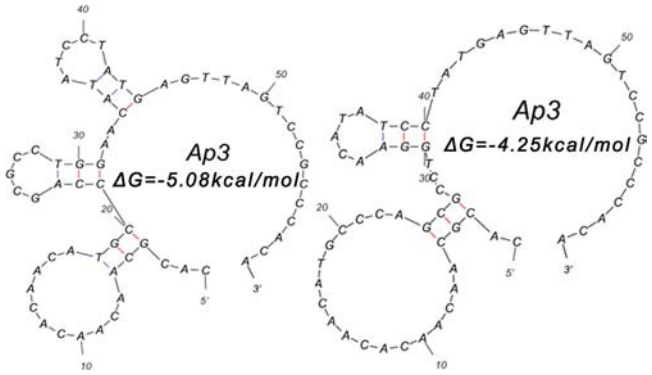

Fig. 5. The predicted secondary structure of the ssDNA aptamer AP3. The secondary structures were predicted using the mfold web server (http://www.bioinfo.rpi.edu/ applications/mfold.) at 25 °C, [Na^+]=15.0 mM, and [Mg^{++}] = 2.0 mM. The predicted lowest free energy structure ($\Delta G = -5.08$ kcal/mol) and the suboptimal secondary structure ($\Delta G = -4.25$ kcal/mol) were shown with Base pairs (AT and CG) indicated in different colors.

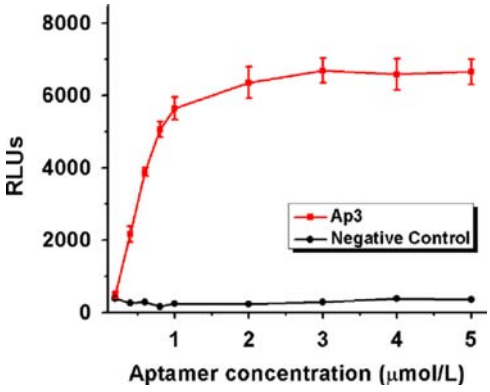

Fig. 6. Influence of different concentrations of aptamer Ap3 on CL signal. Different concentrations (0.2–5.0 μM) of aptamer Ap3 were immobilized on capillaries for detecting the CL signal. Data are expressed as the means ± SD of CL signals from three separate experiments.

3. Results

3.1. Principle of aptamer-antibody sandwich immunoassay

The mechanism of the assay is described in Fig. 3. We used a glass capillary as a reaction chamber in which the ssDNA aptamer was immobilized (Fig. 3(I)). The MC-LR was captured by aptamer and reacted with HRP-labeled antibody against another epitope to form an aptamer-MC-LR-antibody complex (Fig. 3(II)). After reacting with the chemical luminous substrate of HRP, the CL signal (Fig. 3(III)) was converted into an electrical signal using a silicon photodiode-based portable analyzer.

3.2. Aptamer selection

To choose an appropriate aptamer for the assay, 2 μmol/L of four representative ssDNA aptamers were incubated with 2 μg/L MC-LR and HRP-labeled antibody (diluted 1:6000) in microchannels at 25 °C for 30 min. The CL signals of these ssDNA aptamers have different affinities with MC-LR (Fig. 4). The CL signal from aptamer Ap3 (6322 ± 390.6 RLUs) was significantly greater ($P < 0.01$) than that of the negative control (NC, 255.8 ± 88.2 RLUs). The CL signals of aptamers Ap4 and Ap2 were 3574 ± 442 RLUs and 1980 ± 414 RLUs, respectively. Therefore, aptamer Ap3 was selected for use as the linker in the microreaction chamber in subsequent experiments.

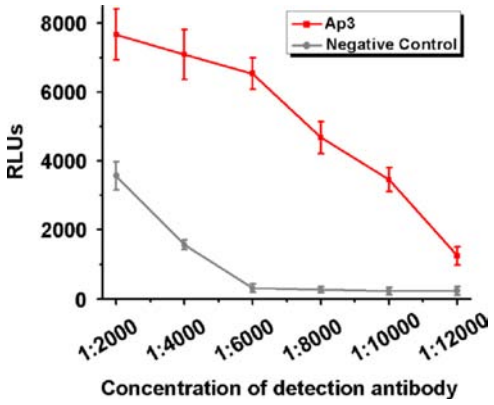

Fig. 7. Influence of different concentrations of HRP-labeled antibody on CL signal. The capillaries were coated with 2.0 μmol/L aptamer Ap3 and loaded with 2.0 μg/L MC-LR and various dilutions of detection antibody from 1:2000 to 1:12000. Data are expressed as the means ± SD of CL signals from three separate experiments.

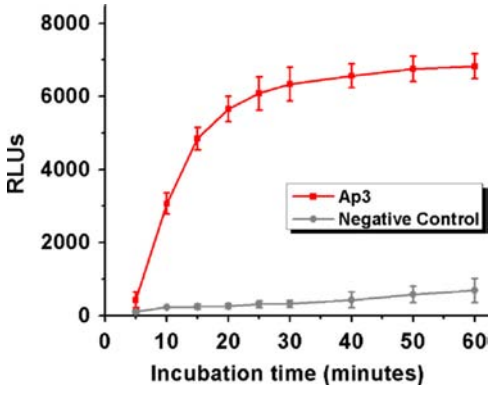

Fig. 8. Influence of incubation time periods on CL signal. The capillaries were coated with 2.0 μM aptamer Ap3 and reacted with 2.0 μg/L MC-LR mixed with detection antibody diluted at 1:6000 for the indicated time periods to detect the CL signal. Data are expressed as the means ± SD of CL signals from three separate experiments.

To analyze the relationship between binding affinity and secondary structure of aptamers, the secondary structure of the aptamers were predicted using m-fold software under true reaction conditions (25 °C, 15 mm Na^+, 2 mm Mg^{++}). Aptamer Ap3 folded into multiple secondary structures at the same temperature and positive ion concentration. The predicted lowest free energy structure ($\Delta G = -5.08$ kcal/mol) and the suboptimal secondary structure ($\Delta G = -4.25$ kcal/mol) of aptamer Ap3 and other aptamers were shown with Base pairs (AT and CG) indicated in different colors in Fig. 5 and Figure S1.

3.3. Optimization of measuring procedures

To optimize the processes of the AAIA for MC-LR, the effects of various aptamer Ap3 concentrations (0.2–5.0 μmol/L) on CL signals were studied. The intensity of CL signals increased with increasing aptamer concentration from 0.2–2.0 μmol/L and plateaued at 2.0 μmol/L Ap3 (Fig. 6).

The optimal dilution of HRP-labeled anti-MC-LR antibody was determined by measuring CL signals in the AAIA with 2.0 μg/L MC-LR. The CL signals increased with increasing dilutions of anti-MC-LR antibody from 2000 to 12000 (Fig. 7). The best CL signal was obtained at positive to noise ratio of 21.35 with an anti-MC-LR antibody dilution of 1:6000 (Fig. S2).

We examined reaction periods from 5–60 min and found that CL signal reached a peak with 2.0 μg/L MC-LR at 25-30 min (Fig. 8). We washed the microreaction chamber by gently squeezing the bulb for 15–90 s and quickly extracting the wash buffer remaining in the capillary (Fig. 9). A high ratio of positive to negative (positive/noise) CL signal occurred with a washing time of 60 s (Figure S3). The assay time for a single sample was less than 33 min, including 30 min for incubation, 1 min for washing, and 15–45 s for CL signal detection (CL signal was read once every 15 s).

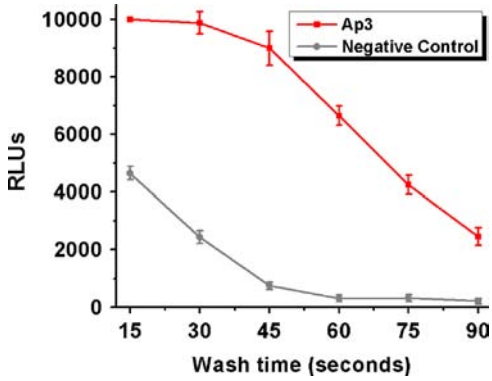

Fig. 9. Influence of washing procedure on CL signal. The microreaction chamber was coated with 2.0 μM aptamer Ap3 and reacted with 2.0 μg/L MC-LR mixed with detection antibody diluted at 1:6000 for 30 min, followed by washing according to the indicated washing procedures for 15–90 s to detect CL signal. Data are expressed as the means ± SD of CL signals from three separate experiments.

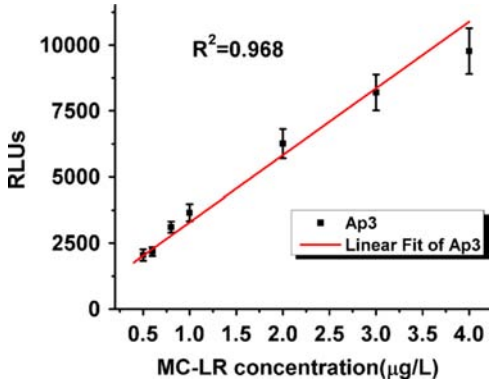

Fig. 10. MC-LR dose-dependent CL signal. The microreaction chamber was coated with 2.0 μmol/L aptamer Ap3 and reacted with different concentrations (0.5–4.0 μg/L) of MC-LR and detection antibody diluted at 1:6000 for 30 min, followed by washing to detect CL signal. A linear correlation between CL signal and MC-LR concentration (0.5–4.0 μg/L; $R^2 = 0.968$) was found. Data are expressed as the means ± SD of CL signals from three separate experiments.

3.4. MC-LR measurement

CL signals with different concentrations of MC-LR from 0.1–5.0 μg/L were measured with an optimal concentration of aptamer Ap3 at 2 μmol/L and HRP-labeled detection antibody dilution of 1:6000. The strengths of CL signals were linearly correlated with the concentrations of MC-LR from 0.5–4.0 μg/L ($Y = 2288.9$ (± 162.7) $X + 1072.8$ (± 343), $R^2 = 0.968$, Fig. 10). When the concentration of MC-LR reached 5.0 μg/L, the CL signals exceeded the maximum detect limitation (9999 RLUs) of the portable analyzer. Therefore, to obtain accurate results, samples with MC-LR concentrations greater than the upper limit of 4.0 μg/L should be diluted appropriately (10-fold or more) prior to detection. The precision of the AAIA was tested in a series of MC-LR-spiked water samples with different MC-LR concentrations. The results indicated that the sensitivity of the assay was 0.3 μg/mL, with a cut-off value similar to the sum of the mean of negative controls plus 3 × SD with RLUs of 520.4 (Table 2). More importantly, the AAIA did not detect 2.0 μg/L MC-LA, MC-YR, or nodularin-R ($P < 0.05$;

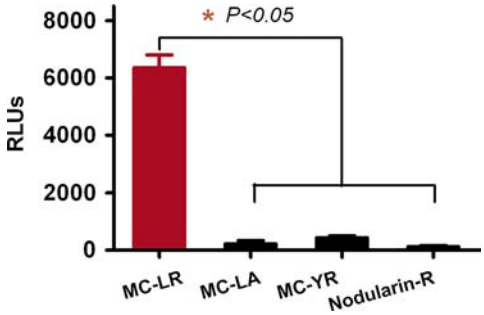

Fig. 11. Specificity of aptamer-based sandwich immunoassay. The microreaction chambers were coated with MC-LR, MC-LA, MC-YR, or nodularin-R mixed with detection antibody diluted at 1:6000 to detect CL signal. Data are expressed as the means ± SD of CL signals from three separate experiments.

Table 2
Precise measurements of the AAIA.

CL signal	MC-LR (μg/L)					Cut-off value
(RLUs)	2.0	0.8	0.4	0.3	NC[a]	
Mean	6360	3226	1346	956	255.8	520.4 (mean+3 SD)
SD	452.2	217.4	132.2	98.8	88.20	
Coefficient of variation (CV, %)	7.5	8.5	9.8	10.3	34.5	

[a] NC: Negative control.

Table 3
Recovery measurements of AAIA with MC-LR in water samples ($n=5$).

Samples	Added MC-LR (μg/L)	Found MC-LR (μg/L)	Recover (%)	CV [a] (%)
Xingqing lake water	2.0	2.22	110.9	9.2
	1.0	1.13	112.7	7.5
Qujiang pond water	2.0	1.96	98.2	6.6
	1.0	1.09	109.1	8.3
Tap water	2.0	2.15	107.5	7.8
	1.0	0.95	94.5	9.8

[a] CV: Coefficient of variation.

Table 4
Performance of different detection methods for MCs.

Name of the method	Linearity	LOD[a]	Reaction time	Properties
Immunosensor based on the relaxation of magnetic nanoparticles [39]	1–18 ng/g	0.6 ng/g	3 h	NMR determination
A label-free, aptamer-based electrochemical impedance biosensor [36]	1.0×10^{-7}–5.0×10^{-11} mol/L[b]	1.8×10^{-11} mol/L	2 h	Electrochemical analyzer
Liquid chromatography coupled with tandem mass spectrometry (LC–MS/MS)[40]		1.2–5.4 ng/g[c]	hours	Multiple detection for microcystins
Microfluidic immunoassay [18]	0–5.0 μg/L	0.02 μg/L	30 min	Competitive immunoreaction and detected by fluorescence microscopy
Chemiluminescence multichannel immunosensor [25]		0.2 μg/L	13 min	A portable immunosensor (11 kg)
Lateral flow immunoassay (ImmunoStrip) [19]		0.5 μg/L	35 min	Lateral flow immunoassay
AAIA	0.5–4.0 μg/L	0.3 μg/L	35 min	A portable analyzer (0.3 kg)

[a] LOD: limit of detection;
[b] 1 mg/L MC-LR equivalent to 1.0×10^{-9} mol/L.
[c] The World Health Organization (WHO) suggested a tolerable daily intake value (0.04 ng/g body weight) for microcystin-LR.

Fig. 11). These results indicate that this assay has high specificity for MC-LR detection. Finally, to investigate the suitability of this AAIA in environmental samples, two water samples (Xingqing Lake water and Qujiang pond water) were spiked with two concentrations of MC-LR (1.0 μg/L and 2.0 μg/L) had been studied. As shown in Table 3, the recovery was calculated as 94.5–112.7%. Therefore, the AAIA might be preliminarily applied for the determination of MC-LR in environmental sample.

4. Discussion

An aptamer-MC-LR-antibody sandwich immunoassay based on distinct epitopes of MC-LR was developed to rapidly detect MC-LR in water using a portable analyzer. This AAIA required less than 35 min to detect MC-LR at concentrations ranging from 0.5–4.0 μg/L with sensitivity of 0.3 μg/L and without cross reactivity to MC-LA, MC-YR, or nodularin-R.

In this study, the ssDNA aptamers Ap3, Ap2, and Ap4 had affinities to MC-LR and other MCs, such as MC-LA and MC-YR [35], indicating that, unlike antibodies, these aptamers have a flexible spatial structure but are not limited by steric hindrance in targeting MCs. In this aptamer-antibody assay, its specificity not only depends on the these ssDNA aptamers but also on the monoclonal antibody to microcystin-LR used in this assay (MC8C10), which exhibit lower cross-reactivity with other four-arginine MCs. Instead, the MC-LR antibody (MC8C10) exhibit high cross-reactivity with nodularin-arginine [30], which does not cross-reactivity with the ssDNA aptamer AP3 used in this study [35]. So aptamer Ap3 binding to MC-LR did not present steric hindrance affecting the binding of the detection antibody (MC8C10) to the target, leading to high sensitivity and specificity without cross reactivity with MC-LA or nodularin-R. Additionally, this assay also had decreased cross reactivity to MC-YR.

We compared the AAIA with other MC testing methods listed in Table 4. The AAIA is simple, reliable, and practical, and is thus valuable as a generalized field test. Although the sensitivity, efficiency and the accuracy of detection are not as desirable as those precision instruments such as sensors [25,36], nuclear magnetic resonance (NMR) [38], surface plasmon resonance (SPR) [39] and liquid chromatography–tandem mass spectrometry (LC–MS/MS) [40], this assay met the recommended guideline of 1.0 μg/L from the WHO and reached the guideline limit of 0.5 μg/L set up by some European countries for MC-LR testing in drinking water.

More importantly, the AAIA was combined with a photodiode-based portable analyzer and the economical synthetic antibody (aptamer) [32], and is thus suitable for quick testing in field by a single analyst. This is a clear advantage over precision instruments, such as a competitive immunoreaction followed by detection using fluorescence microscopy [18] or chemiluminescence immunosensor [25]. Additionally, the AAIA is a more sensitive and quantitative method than other point-of-care tests, such as the ImmunoStrip, which is based on lateral flow immunoassay [19].

In conclusion, we have developed an aptamer-antibody sandwich immunoassay within a portable optic sensor device for simple and rapid detection of MC-LR with high specificity. Our device can potentially be used for on-site monitoring of MC-LR pollutants in environmental water.

Conflict of interest

The authors of this manuscript declare no personal or professional relationships which may pose a conflict of interest.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.talanta.2014.07.008.

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