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A highly specific immunoassay for microcystin-LR detection based on a monoclonal antibody

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ABSTRACT

Microcystins (MC) are cyanobacterial hepatotoxins responsible for animal-poisoning and human health incidents. Immunoassays provide a sensitive and fast means to detect these toxins, but cross-reactivity (CR) characteristic of different antibodies was variable. Here, we have produced and characterized a monoclonal antibody (Clone MC8C10) with highly specificity against the most frequent and most toxic variant of microcystins, MC-LR. MC8C10 is more specific against MC-LR among the reported antibodies before. The immunogen was synthesized from the modified MC-LR and bovine serum albumin (BSA). An indirect competitive enzyme-linked immunosorbent assay (ic-ELISA) with MC8C10 was established to detect the MCs in waters, which showed highly specificity with MC-LR, and have a detection limit for MC-LR 0.1 $\mu g L^{-1}$, the 50% inhibition concentration (IC_{50}) for MC-LR was $1.8 \pm 0.1\ \mu g L^{-1}$ and the quantitative detection range was from 0.3 to 10 $\mu g L^{-1}$. The [4-arginine] microcystins and the nodularin-R showed lower cross-reactivates (CR < 10%), and other MCs such as MC-LF and MC-LW are not recognized ($CR < 10^{-4}$). The analysis results of real water samples with ic-ELISA showed that all the coefficients of variation were less than 15%, and the recovery was (100.3 ± 5.9)%. So the highly specific ic-ELISA will commendably suit for sensitive analysis for MC-LR in surface water as well as drinking water.

1. Introduction

Microcystins (MCs) are a group of closely related toxic cyclic heptapeptides produced by common cyanobacteria. Several cyanobacterial genera, including *Microcystis*, *Anabaena*, *Planktothrix*, *Nostoc* and *Anabaenopsis*, can produce MCs, and the *Nodularia spumigena* produces related toxic cyclic pentapeptides termed nodularins [1]. Over 80 structural variants of MCs are described [2] with the general structure cyclo-(D-alanine^1-*X*^{2}-D-MeAsp^3-*Z*^{4}-Adda^5-D-glutamate^6-Mdha^7), where Adda is the unique β-C20 amino acid, 3-amino-9-methoxy-2,6,8-trimethyl-10-phenyldeca-4,6-dienoic acid, and *X* and *Z* represent two variable L-amino acids [3]. Nodularin has the structure of cyclo-(D-Masp^1-Arg^2-Adda^3-D-Glu^4-Mdhb^5) [4]. Microcystin-leucine-arginine (MC-LR) is the most frequent and most toxic microcystin congeners. MCs inhibit several protein phosphatases [5,6] and can act as tumor promoters implicated as causative agents of primary liver cancer after exposure to MCs in drinking water and surface water [7–10]. MCs have also been responsible for animal-poisonings and other human health incidents [11,12].

Increased awareness and detection of these toxins is necessary for the protection of water quality and human health. Several rapid detection methods are currently in use, including invertebrate bioassays involving mouse bioassay [13–14] and brine shrimp assay [15], protein phosphatase inhibition assays

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Table 1 – Comparison of biological detection methods for microcystins [26]

Method	Sensitivity (IC_{50})[a]	Specificity	Cross-reactivity
Mouse bioassay[b]	LD_{50}[c]: 25–150 μg kg^{-1}	Non-specific	All microcystins
Brine shrimp assay	LD_{50}: 5–10 mg L^{-1}	Non-specific	All microcystins
Protein phosphatase	Radiometric: 0.1 μg L^{-1}, colorimetric: 0.25–2.5 μg L^{-1}, fluorometric: 0.1 μg L^{-1}	Non-specific	All microcystins
Polyclonal antibodies	Anti-MC-LR: 2.5 μg L^{-1}, Anti-adda: 0.6 μg L^{-1}	Specific	Variable[d], below 1 μg L^{-1} for tested variants
Monoclonal antibodies	Anti-MC-LR: 0.06 μg L^{-1} (4-arginine specific)	Very specific	Only detects 4-arginine MCs
Recombinant antibody fragments	4 μg L^{-1}	Specific	Variable
Molecularly imprinted polymers	Approx. 0.2 μg L^{-1}	Very specific	Specific for MC-LR

[a] IC50 determined for MC-LR.
[b] Toxicity of MC-LR by intraperitoneal injection (μg kg^{-1} body weight).
[c] LD_{50}: dose required to kill 50% of animals.
[d] Variable: cross-reactivity is uncertain, because the reproducibility of antibody is not so good.

(PPIA) [16–18], immunoassays involving both polyclonal and monoclonal antibodies [19–21], and recently, new technologies employing recombinant antibodies [22] and molecularly imprinted polymers [23,24] have been exploited to develop bioassays and biosensors for microcystins. All these biological detection methods for microcystins are compared in Table 1. The mouse bioassay was used in most laboratories to determine the presence of hepatotoxins in a sample. However, the assay is lack of sensitivity and specificity, and has suffered from increasing public opposition to the use of animals in toxicity testing. The use of immunoassays is becoming increasingly attractive due to the need for monitoring microcystin levels at lower concentrations. Immunoassays offer the potential for the detection of microcystins at or below the WHO drinking water guideline values (1 μg L^{-1}) by simple procedures using minimal facilities and without the need for prior sample concentration steps.

The specificity of antibody is very important for immunoassay, there showed one "very specific" methods in Table 1, one is the monoclonal antibody MC10E7 [25], and which was very specific to all [4-arginine] microcystins, whereas other MCs such as MC-LF, MC-LA, are not recognized. The other 'antibody' is the molecularly imprinted polymers (MIP), which belong to high-molecular synthetic compounds, not the real antibody.

In this paper, we have produced and characterized a monoclonal antibody (Clone MC8C10) with highly specificity against the most frequent and most toxic variant, MC-LR. The indirect competitive enzyme-linked immunosorbent assay (ic-ELISA) incorporating MC8C10 was established to analyse environmental samples from wild surface waters. The results showed that the highly specific ic-ELISA would well suit for sensitive analysis for MC-LR in wild surface water as well as drinking water samples.

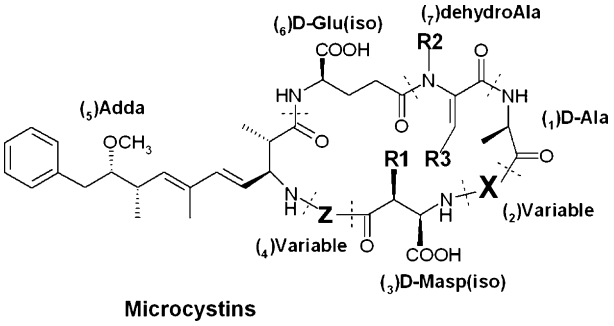

Fig. 1 – Molecular structure of microcystins. A characteristic of MCs and related cyanobacterial toxins (such as nodularin) is the hydrophobic amino acid Adda (2S, 3S, 8S, 9S-3-amino-9-methoxy-2,6,8-trimethyl-10-phenyl-4E,6E-decadienoic acid) which contains two conjugated double bonds in position 5. Numbers represent the position of the amino acid residue.

2. Materials and methods

2.1. *Microcystins and nodularin*

Structures and abbreviations of MCs used in the described experiments are shown in Fig. 1. Some MCs and nodularin

Table 2 – The structures of several MCs and nodularin. Nodularin has an arginine and adda amino acid residue at its 3rd and 4th positions, respectively

Microcystin derivatives	X (2)	Y (4)	Molecular weight	Production no.
Microcystin-LR, MC-LR	Leu	Arg	995.2	ALX-350-012
Microcystin-RR, MC-RR	Arg	Arg	1038.2	ALX-350-043
Microcystin-YR, MC-YR	Tyr	Arg	1045.2	ALX-350-044
Microcystin-LW, MC-LW	Leu	Trp	1025.2	ALX-350-080
Microcystin-LF, MC-LF	Leu	Phe	986.2	ALX-350-081
Nodularin	(3) Arg		825.0	ALX-350-061