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Chikashi Nakamura [a] , Teruaki Kobayashi [a b] , Masato Miyake [a] , Makoto Shirai [b] & Jun Miyakea [a]

[a] National Institute for Advanced Interdisciplinary Research, AIST, MITI, Tsukuba, Ibaraki, 305-8562, Japan

[b] Division of Biotechnology, School of Agriculture, Ibaraki University, Ami, Ibaraki, 300-0331, Japan

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Usage of a DNA Aptamer as a Ligand Targeting Microcystin

CHIKASHI NAKAMURA[a], TERUAKI KOBAYASHI[a,b], MASATO MIYAKE[a], MAKOTO SHIRAI[b] and JUN MIYAKEa*

[a]*National Institute for Advanced Interdisciplinary Research, AIST, MITI, Tsukuba, Ibaraki, 305-8562, Japan and* [b]*Division of Biotechnology, School of Agriculture, Ibaraki University, Ami, Ibaraki 300-0331, Japan*

Microcystin is a bacterial hepatotoxin generated from the cyanobacteria causing waterblooms. A DNA aptamer binding to microcystin was screened by the *in vitro* selection method of twelve rounds in order to obtain a sorbent suitable for a microcystin detection system. The obtained DNA aptamer showed specific binding to microcystin. The direct detection range using an immobilized aptamer and a surface plasmon resonance biosensor was 50 μg/ml to 1000 μg/ml.

Keywords DNA aptamer; *in vitro* selection; microcystin; surface plasmon resonance

INTRODUCTION

New sensor technology which can realize high-sensitivity, fast, simple and cost-effective performance is required to detect environmental toxins. Although antibodies and receptors have been used as material monitoring targets, there are some problems, e.g. stability, productivity and renewability due to the characteristics of proteins. Therefore, we

*Address for correspondence, e-mail; miyake @nair.go.jp, Fax +81-298-61-3009.

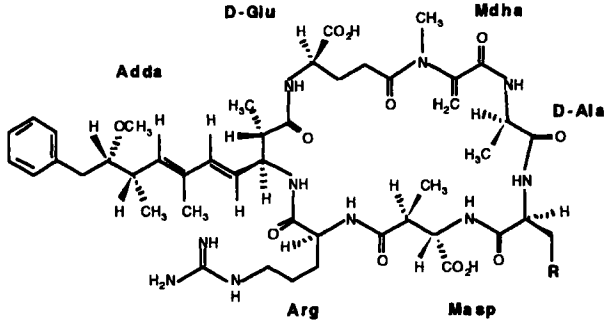
need alternative binding materials which conquer the defects of the proteins. A DNA aptamer obtained by *in vitro* selection is an appropriate candidate binding target [1,2], as it has the great advantage of being simply amplified by PCR. The potential of a DNA aptamer in the sensor system was demonstrated in the thrombin-detection biosensor [3].

In this study we tried to obtain an aptamer targeting microcystin which is a bacterial hepatotoxin produced by cyanobacteria such as *Microcystis* which cause waterblooms in fresh and brackish waters [4]. Some high-sensitivity detection methods for microcystin (not animal tests) have been reported using antibodies [5] or protein phosphatase [6,7]. Of course there are defects with protein usage in such methods, as mentioned above. The microcystin-bound DNA aptamers were isolated and the potential of the isolated aptamer as a sensing molecule was estimated using a surface plasmon resonance (SPR) biosensor.

EXPERIMENTAL

Reagents

The microcystins were purchased from Wako Ltd. (Osaka, Japan). Template, (5'-AAGAGAGGCTGGGAATGCTA$(N)_{40}$TGACTTGACCG CCGTATTTA), sense primer (5'-AAGAGAGGCTGGGAATGCTA) and biotinylated antisense primer (5'-TAAATACGGCGGTCAAGTCA) of oligonucleotides were synthesized by Greiner Labortechnik Co. Ltd. (Tokyo, Japan). Microcystin-immobilized gel, Microcystin-Sepharose™ was purchased from Upstate Biotechnology Inc. (Lake Placid, USA). The other reagents used in this study were analytical grade.



Microcystin LR R = $CH(CH_3)_2$, (leucine)
Microcystin RR R = $CH_2CH_2NHC(NH_2)=NH$, (arginine)
Microcystin YR R = C_6H_4-p-OH, (tyrosine)

FIGURE 1 Structure of microcystins used in this study. The three microcystins have the same cyclic heptapeptide skeleton.

In Vitro Selection

DNA fragments were amplified by PCR and then the sense strand was harvested and purified by affinity isolation using an avidin-immobilized affinity column. The single strand DNA solution at concentration of 10 μg/ml (50 μg/ml at the first round) was mixed with 40 μl of microcystin-LR-immobilized gel matrix in a binding buffer containing 50 mM TrisHCl (pH 7.5), 150 mM NaCl, 2mM $MgCl_2$ for 5 min. Subsequently, the gel was washed with 15 ml of the binding buffer. Finally bound DNA fragments were released by addition of microcystin LR (final conc. 2 mM). The obtained DNA was amplified by PCR again. This cycle was repeated twelve times and the obtained fragments were cloned into a plasmid and their nucleotide sequences were analyzed.

Preliminary Affinity Estimation

Each aptamer sample was mixed with the microcystin-LR-immobilized gel and incubated for 5 min. The supernatant was then harvested and subjected to polyacrylamide gel electrophoresis (PAGE). The amount of absorbed DNA on the microcystin-LR-immobilized gel was estimated by image analysis of the DNA band of the PAGE.

SPR Measurement

SPR apparatus, BIACORE X (Biacore International AB, Uppsala, Sweden) was used to detect the microcystins in this study. A biotinylated aptamer was immobilized on the sensor chip, SA (Biacore International AB) which is streptoavidin-immobilized. On the reference flow cell, a thyroxin DNA aptamer of 14 bases [2] was immobilized as a control. Microcystins were dissolved in the binding buffer at various concentrations and injected at a flow rate of 20 μl/min at 25 degrees C.

RESULTS AND DISCUSSION

Characteristics of Selected Aptamers

After twelve-round selection, thirty DNA fragments were cloned and the nucleotide sequences determined. Subsequently, no consensus sequence or structural motif was found in the thirty clones. Compared to the control of random sequence DNA, all clones showed higher affinity with the microcystin-LR-gel in the preliminary estimation. Clone 6 showed the strongest binding to the microcystin-LR-gel and was subjected to further analysis.

Binding Specificity

Clone 6 of 5'-biotinylated was immobilized on the sensor chip SA. SPR signals observed from three kinds of microcystin or a control heptapeptide are represented in Fig. 2. While no obvious SPR signal was observed in the control, clear signals appeared readily after the injections of each microcystin. The signal of microcystin YR was higher than LR (the original target). The microcystins differ by only one amino acid residue: leucine for LR, arginine for RR and tyrosine for YR (see Fig. 1). This implies that the aromatic ring of tyrosine (YR) contributes to binding more than the alkyl chain of leucine (LR). In any case, from this result, the DNA aptamer, clone 6 was utilized for microcystin-specific detection as a binding molecule.

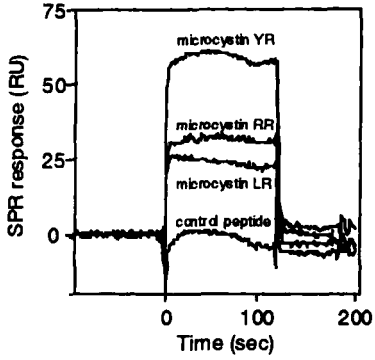


FIGURE 2 Specific binding of the clone 6 to microcystins. The concentrations of each microcystin sample and control peptide were 1 mg/ml. The amino acid sequence of control peptide is HASYSAC.

Binding Sensitivity

The calibration graph representing the correlation between microcystin LR concentrations and SPR responses is shown in Fig. 3. The detection range was 50 μg/ml to 1 mg/ml. The variation coefficient was within 10-

20 %. The binding constant obtained from the SPR measurement was low, approx. 10^3 M^{-1}, due to the screening conditions in this study. The sensitivity and precision of microcystin detection in this system was not as high compared with the methods reported previously [5,7]. A simple direct detection was demonstrated in this SPR measurement whereas previous methods needed complicated competitive-reactions for sensitization. In this study we demostrated the possibility of using a DNA aptamer as a binding molecule in a detection system. In further work, detection sensitivity would be improved using an aptamer of higher affinity.

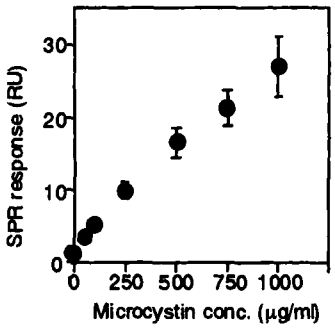


FIGURE 3 Correlation between microcystin concentration SPR signal response.

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