Rational Design of a Polymer Specific for Microcystin-LR Using a Computational Approach

ARTICLE *in* ANALYTICAL CHEMISTRY · APRIL 2002

Impact Factor: 5.64 · DOI: 10.1021/ac010840b · Source: PubMed

7 AUTHORS, INCLUDING:

Iva Chianella
Cranfield University
50 PUBLICATIONS **1,820** CITATIONS
SEE PROFILE

Ibtisam E Tothill
Cranfield University
80 PUBLICATIONS **2,411** CITATIONS
SEE PROFILE

Beining Chen
The University of Sheffield
170 PUBLICATIONS **2,123** CITATIONS
SEE PROFILE

Anthony P F Turner
Linköping University
374 PUBLICATIONS **12,234** CITATIONS
SEE PROFILE

Anal. Chem. **2002,** *74,* 1288−1293

Rational Design of a Polymer Specific for Microcystin-LR Using a Computational Approach

Iva Chianella,* Manuela Lotierzo, Sergey A. Piletsky, Ibtisam E. Tothill, Beining Chen, Khalku Karim, and Anthony P. F. Turner

Institute of BioScience and Technology, Cranfield University, Silsoe, Bedfordshire, MK45 4DT, U.K.

A computational approach for the design of a molecularly imprinted polymer (MIP) specific for Cyanobacterial toxin microcystin-LR is presented. By using molecular modeling software, a virtual library of functional monomers was designed and screened against the target toxin, employed as a template. The monomers giving the highest binding energy were selected and used in a simulated annealing (molecular dynamics) process to investigate their interaction with the template. The stoichiometric ratio observed from the simulated annealing study was used in MIP preparation for microcystin-LR. The monomers were copolymerized with a cross-linker in the presence of the template. A control (blank) polymer was prepared under the same conditions but in the absence of template. A competitive assay with microcystin−horseradish peroxidase conjugate was optimized and used to evaluate the affinity and cross-reactivity of the polymer. The performance of the artificial receptor was compared to the performance of monoclonal and polyclonal antibodies raised against the toxin. The results indicate that imprinted polymer has affinity and sensitivity comparable to those of polyclonal antibodies (the detection limit for microcystin-LR using the MIP-based assay was found to be 0.1 μg L^{-1}), while superior chemical and thermal stabilities were obtained. Moreover, cross-reactivity to other toxin analogues was very low for the imprinted polymer, in contrast to the results achieved for antibodies. It is anticipated that the polymer designed could be used in assays, sensors, and solid-phase extraction.

Cyanobacteria such as *Microcystis*, *Anabaena*, *Nodularia*, *Nostoc*, and *Oscillatoria* are aquatic microorganisms, often known as "blue-green algae", that produce toxic cyclic heptapeptides (microcystins) and pentapeptide (nodularin) during the period of bloom formation.[1] Toxins from Cyanobacteria are responsible for intermittent but repeated cases of sickness and death in aquaculture species, livestock, wildlife, and humans.[2,3] At present, analysis of these toxins is largely achieved by bioassay, liquid chromatography (HPLC), or immunoassay.[4] The difficulty of raising antibodies against toxins and the continuing trend to reduce the use of animals for antibody production has stimulated research and development of synthetic receptors for toxins. In the past few years, molecular imprinting has been considered as one of the simplest, most straightforward, and cost-effective methods to develop artificial receptors for toxic organic compounds.

The technology of molecular imprinting was developed in 1972[5] and has been advancing with a wide range of applications appearing in the literature.[6−11] The basic concept of imprinting involves three stages: (i) selection of components (functional monomers, analyte as a template, solvent, cross-linker, and initiator); (ii) formation of a functional monomer−template complex in solution; and (iii) polymerization process. The polymer obtained is then washed to remove the template and cavities with shape, and functionalities complementary to the target analyte are left behind. A crucial element for the success of the imprinting procedure is the creation of a strong monomer−template complex, whose complex should be preserved during the entire polymerization step. However, due to the exothermic nature of the polymerization reaction and to conformational changes of the monomers and template, it is inevitable that some percentage of the preformed complexes will be destroyed or changed in the resulting polymer. Since these changes can be sufficiently minimized when a strong interaction between template and monomers is achieved, the choice of monomers is of utmost importance. Thermodynamic calculations and combinatorial screening approaches have been successfully used to identify the best monomer candidates for imprinting.[12−15] The work became difficult

* Corresponding author. Fax: 44 1525 863001. E-mail: I.Chianella.1998@Cranfield.ac.uk.

(1) Carmichael, W. W. *J. Appl. Bacteriol.* **1992**, *72*, 445−459.
(2) Codd, G. A.; Ward, C. J.; Beattie K. A.; Bell, S. G. In *The Phototrophic Prokaryotes*; Peschek,G. A., Loffelhardt, W., Schmetterer, G., Eds.; Kluwer Academic/Plenum Publishers: New York, 1999; pp 623−632.
(3) Carmichael, W. W. *Adv. Bot. Res.* **1997**, *27*, 211−256.
(4) Chu, F. S.; Huang, X.; Wei, R. D. *J. Assoc. Off. Anal. Chem.* **1990**, *73* (3), 451−456.
(5) Wulff, G.; Sarhan, A. *Angew. Chem., Int. Ed. Engl.* **1972**, *11*, 341−344.
(6) Vlatakis, G.; Andersson, L. I.; Müller, R.; Mosbach, K. *Nature (London)* **1993**, *361*, 645−657.
(7) Andersson, L. I. *Anal. Chem.* **1996**, *68*, 111−117.
(8) Chen, G. H.; Guan, Z. B.; Chen, C. T.; Fu, L. T.; Sundaresan, V.; Arnold, F. H. *Nat. Biotechnol.* **1997**, *15*, 354−357.
(9) Matsui, J.; Jenkins, A. L.; Uy, O. M.; Murray, G. M. *Anal. Chem.* **1999**, *71*, 373−378.
(10) Alexander, C.; Smith, C. R.; Whitcombe, M. J.; Vulfson, E. *N. J. Am. Chem. Soc.* **1999**, *121*, 6640−6651.
(11) Piletsky, S. A.; Matuschewski, H.; Schedler, U.; Wilpert, A.; Piletska, E. V.; Thiele, T. A.; Ulbricht, M. *Macromolecules* **2000**, *33*, 3092−3098.
(12) Nicholls, I. *A. Chem. Lett.* **1995**, 1035−1036.
(13) Whitcombe, M. J.; Martin, L.; Vulfson, E. N. *Chromatographia* **1998**, *47*, 457−464.
(14) Takeuchi, T.; Fukuma, D.; Matsui, J. *Anal. Chem.* **1999**, *71*, 285−290.
(15) Lanza, F.; Sellergren, B. *Anal. Chem.* **1999**, *71*, 2092−2096.

10.1021/ac010840b CCC: $22.00
Published on Web 02/16/2002

with increase in size of the monomer libraries, which include now thousands of polymerizable compounds. A possibility of solving this problem is to use molecular modeling software and searching algorithms traditionally applied in drug design.[16] The capacity of modern computational approaches is inadequate to model all processes involved in MIP preparation, especially the polymerization step. Therefore, we concentrated our efforts on the first stage of this process, which includes the selection by molecular modeling of monomers able to form strong complexes with the template.[17] In fact, this work shows the first attempt of using this computational approach in the synthesis of a molecularly imprinted polymer (MIP) specific for microcystin-LR. Affinity, specificity, cross-reactivity, and stability of the computationally designed MIP were studied using an enzyme-linked competitive assay[18] and compared with those of monoclonal and polyclonal antibodies raised for microcystin-LR.

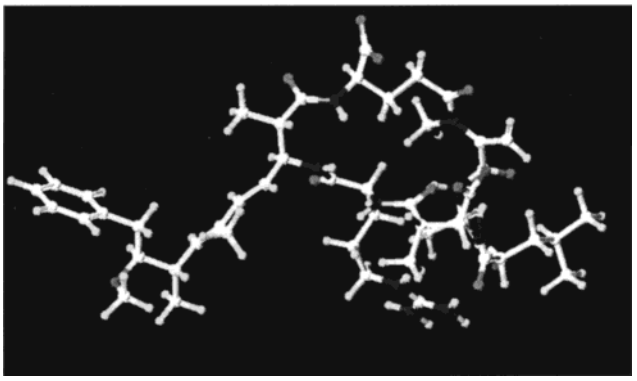

Figure 1. Microcystin-LR structure in a minimum energy conformation that interacts with serine/threonine phosphatases 1 and 2A. The picture was downloaded from file lfjm.pdb of the Protein Data Bank (PDB).

EXPERIMENTAL SECTION

Materials. Microcystin-LR and its analogues, microcystin-YR, microcystin-RR, and nodularin, were purchased from Alexis Corp. Ltd. (Nottingham, U.K.). Methacrylic acid (MAA), 2-acrylamido-2-methyl-1-propanesulfonic acid (AMPSA), methylenesuccinic acid (itaconic acid, IA), imidazole-4-acrylic acid (urocanic acid, UA), ethylene glycol dimethacrylate (EGDMA), 1,1′-azobis(cyclohexanecarbonitrile), and tetramethylbenzidine (TMB) were purchased from Aldrich (Poole, Dorset, U.K.). Imidazole-4-acrylic acid ethyl ester (urocanic acid ethyl ester, UAEE) was obtained from the Institute of Molecular Biology and Genetics (Kiev, Ukraine). Microcystin−horseradish peroxidase (HRP) conjugate was from Strategic Diagnostics Inc. (SDI), (Newark, DE). Monoclonal antibodies were obtained from Wako Pure Chemicals Industries GmbH (Neuss, Germany).[19] Polyclonal antibodies were provided by Prof. Hennion from the Department of Environmental and Analytical Chemistry in Paris.[20] The Multiscreen System Resist Vacuum Manifold was from Millipore (Watford, U.K.). All solvents were of analytical or HPLC grade and were used as received.

Computer Simulation. The workstation used to simulate monomer−template interactions was a Silicon Graphics Octane running IRIX 6.6 operating system. The workstation was configured with two 195-MHz reduced instruction set processors, 712-MB memory, and a 12-GB fixed drive. This system was used to execute the software package SYBYL 6.7 (Tripos Inc., St. Louis, MO). The computational design was performed in three steps. In the first step, since microcystin-LR is a potent inhibitor of the protein serine/threonine phosphatases 1 and 2A, a molecular model of the toxin (template), in a minimum energy conformation interacting with protein phosphatase, was chosen and downloaded from the Protein Data Bank (PDB, file: lfjm.pdb) (Figure 1). Moreover, a virtual library of 20 commonly used monomers was designed (Figure 2). All the monomer structures were charged using the Gasteiger−Hückel computational method and refined using the molecular mechanics method by applying an energy minimization with the MAXIMIN2 command.[21] In the second step, the Leapfrog algorithm was applied to screen each single functional monomer of the library for its possible interaction with the template. Leapfrog is an algorithm that allows the evaluations of new ligand structures mainly on the basis of their binding score. This is calculated with electrostatic screening by trying repeatedly different ligands (one each time) in different positions of the template and then either keeping or discarding the results.[22] The program was activated for different lengths of runs (10 000, 30 000, and 40 000 steps). The results from each run were examined by evaluating the empirical binding scores. The monomers giving the highest binding score (Table 1) and capable of forming the strongest complexes with the template were selected as candidates for the polymer preparation. In the third step, microcystin-LR and multiple copies of the best four monomers (AMPSA, IA, UA, and UAEE) were assembled in a virtual box (precomputed solvent box) and the energy of the system was minimized. A simulated annealing process was then applied to optimize the arrangement of functional monomers around the template. Annealing conditions were fixed as 1000−300K sweeping in 32 000 consequent steps. At each different temperature, dynamic equilibrium was reached in 2000 fs. At the end of the program, the number and the position of functional monomers were examined. The type and quantity of the monomers participating in the complex determined the ratio of template and monomers used for the polymer preparation.

Polymer Synthesis. Microcystin-LR (1×10^{-6} mol, 1 mg) was dissolved in 300 μL of DMSO and mixed with 1×10^{-6} mol of AMPSA, 6×10^{-6} mol of UAEE, cross-linker EGDMA, and initiator 1,1′-azobis(cyclohexanecarbonitrile) in a 2-mL glass bottle. This solution was purged with nitrogen to remove the oxygen, sealed, and kept at 80 °C for 24 h. The synthesized bulk polymer was then ground with a mechanical mortar. The resulting powder was washed extensively with 100 mM HCl in 50% methanol, 100 mM NaOH in 50% methanol, distilled water in 50% methanol, and pure methanol. After washing, the polymer particles were wet- sieved in acetone through two sieves with aperture sizes of 63 and 45 μm. Finally, the computationally designed polymer was collected from the two sieves and then dried overnight in an oven

(16) Böhm, H. J. *J. Comput.-Aided Mol. Des.* **1992**, *6*, 61−78.
(17) Subrahmanyam, S.; Piletsky, S. A.; Piletska, E. V.; Karim, K.; Chen, B.; Day, R.; Turner, A. P. F. *Biosens. Bioelectron.* **2001**, *16*, 631−637.
(18) Piletsky, S. A.; Piletska, E. V.; Chen, B.; Karim, K.; Weston, D.; Barrett, G.; Lowe, P.; Turner, A. P. F. *Anal. Chem.* **2000**, *72*, 4381−4385.
(19) Nagata, S.; Soutome, H.; Tsutsumi, T.; Hasegawa, A.; Sekijima, M.; Sugamata M.; Harada, K.; Suganuma, M.; Ueno, Y. *Nat. Toxins* **1995**, *3*, 78−86.
(20) Rivasseau, C.; Hennion, M.-C. *Anal. Chim. Acta* **1999**, *399*, 75−87.
(21) Labanowski, J.; Motoc, I.; Naylor, C. B.; Mayer, D.; Dammkoehler, R. A. *Quant. Struct.-Act. Relat.* **1986**, *5*(4), 138−152.
(22) Tripos Associates, Inc., St. Louis, MO.

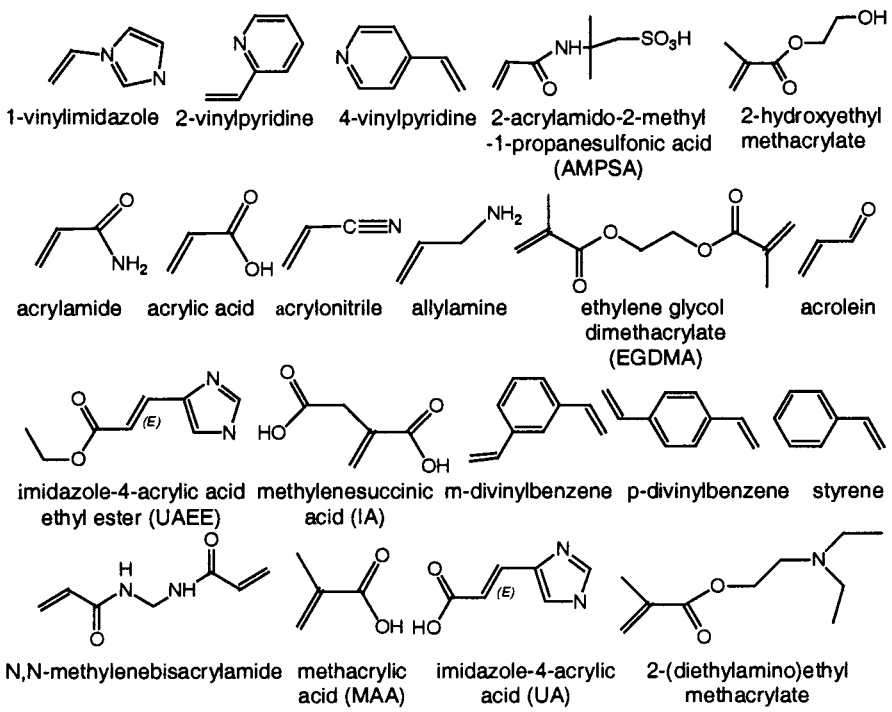

Figure 2. Virtual library containing the most commonly used functional monomers. The virtual library was used as a database for computational design.

Table 1. Leapfrog Algorithm Result: Binding Score of the Interactions of Microcystin-LR with the Five Best Monomers and Methacrylic Acid Selected from the Virtual Library

monomers	binding score (kJ mol^{-1})
AMPSA	−213.01
IA	−169.03
UA	−167.44
UAEE	−163.80
bisacrylamide	−155.31
MAA	−112.51

at 60 °C. A blank polymer was also synthesized as described above but in the absence of template.

Another imprinted polymer was synthesized using a more traditional approach where methacrylic acid is the functional monomer (MAA-MIP). A methacrylic acid blank polymer was also prepared in the absence of template. The full compositions of the polymerization mixtures are presented in Table 2.

In Table 2 are also listed the results of a Brunauer−Emmett−Teller (BET)[23] analysis performed by Micromeritics U.K. (Dunstable, Bedfordshire, U.K.) using the nitrogen adsorption method. The surface area and the total pore volume for the computationally designed MIP, MAA-MIP and blank polymers are reported.

Competitive Assay. A suspension of polymer (50 μL, 0.5 g L^{-1} in 50 mM phosphate buffer, pH 7.0) was dispensed into a 96-well filtration microplate (Multiscreen Resist, Millipore). To each well, 50 μL of a mixture of microcystin-HRP conjugate (0.1 mg L^{-1} in 50 mM phosphate buffer, pH 7.0) and different dilutions of free microcystin-LR (0.1−0.8 μg L^{-1} in 50 mM phosphate buffer, pH 7.0) were added. After 1 h of incubation, the mixture was filtered under vacuum and the filtrate collected in a microtiter 96-well plate. From each well, 10 μL of filtrate was transferred to a new microtiter plate and mixed with 90 μL of TMB substrate. After 20-min incubation at room temperature, the absorbance of the solution was measured at 650 nm using an MRX microplate reader (Dynex Technologies Inc., Billingshurst, U.K.).

A direct competitive ELISA test with polyclonal and monoclonal antibodies against microcystin-LR was performed as described in the literature.[4,24] Monoclonal antibodies were used as received and polyclonal antibodies were diluted 1:15 000. The dissociation constants were calculated from the competitive assays using a double-reciprocal plot.[25]

Cross-Reactivity Study. Cross-reactivity of MIPs for microcystin-LR analogues such as microcystin-RR, microcystin-YR, and nodularin was evaluated[26] using an enzyme-linked competitive assay. Samples of each analogue was added to the polymer suspension and tested as already described for microcystin-LR. The results were then compared with the cross-reactivity values of polyclonal and monoclonal antibodies anti-microcystin-LR, which were obtained by the ELISA test.

Analysis of Receptor Stability. Stability tests were performed by comparing the affinity of MIPs and antibodies to microcystin-LR before and after 2 h of incubation under predefined experimental conditions. Antibody solution (100 μL) and MIP suspension (0.5 g L^{-1}, 100 μL) were added to the same volume of each of the following solutions: 80% dimethylformamide in phosphate-buffered saline (PBS, 10 mM, pH 7.4), 10 mM HCl at pH 2.0, 50 mM NaOH at pH 11.0, and CuSO4, 0.1 g L^{-1}. In addition, 100 μL of antibody

(23) Hiemenz, P. C.; Rajagopalan, R. *Principles of Colloid and Surface Chemistry*; Marcel Dekker Inc.: New York, 1997; pp 425−439.

(24) Nagata, S.; Tsutsumi, T.; Hasegawa, A.; Yoshida, F.; Ueno, Y. J. *AOAC Int.* **1997**, *80* (2), 408−417.

(25) Sergeyeva, T. A.; Piletsky, S. A.; Brovko, A. A.; Slinchenko, E. A.; Sergeeva, L. M.; El'skaya, A. V. *Anal. Chim. Acta* **1999**, *392*, 105−111.

(26) Metcalf, J. S.; Beattie, K. A.; Pflugmacher, S.; Codd, G. A. *FEMS Microbiol. Lett.* **2000**, *189*, 155−158.

Table 2. Composition of Monomer Mixtures in MIP Preparation and Some Physical Properties of the Synthesized Polymers

polymer	MC-LR[a] (μmol)	AMPSA (μmol)	MAA (μmol)	UAEE (μmol)	EGDMA (μmol)	surface area (m^2/g)	total pore volume (cm^3/g)
comp MIP	1	1		6	1500	443.31 ± 3.75	0.533
comp blank	–	1		6	1500	444.72 ± 3.01	0.571
MAA-MIP	1		10		1500	414.69 ± 2.99	0.513
MAA-blank			10		1500	437.24 ± 2.61	0.600

[a] MC-LR, microcystin-LR.

solutions and polymer suspension were mixed with 100 μL of PBS buffer and the resultant mixture maintained at temperature of 80 °C. After 2 h of incubation under these conditions, polymers and antibodies were reconditioned by adding 200 μL of 100 mM sodium phosphate buffer, pH 7.0. The activity was then evaluated using the competitive assay as described previously. Untreated polymers and antibodies were used as positive controls. The results of the tests were reported as a percentage of reduction in binding affinity compared to the untreated receptors.

RESULTS AND DISCUSSION

Computer Simulation. The modeling of monomer−template interaction was performed using commercial software traditionally applied in drug design. Nevertheless, in the present work a virtual library of polymerizable functional monomers was created rather than using, for example, amino acids as commonly applied in drug design. The monomers included in the virtual library contain a broad variety of functionalities, capable of forming ionic bonds, hydrogen bonds, and van der Waals interactions with the template (Figure 2). In the Leapfrog results, the top three monomers interacting with the template were acids, AMPSA, IA, and UA (Table 1), and probably this is due to the presence of a basic amino acid like arginine, sterically available in the microcystin-LR structure.[27] The three monomers and UAEE were selected for the simulated annealing process. The structure of the final complex, predicted by computer modeling, is presented in Figure 3. This complex contains one molecule of microcystin-LR, one molecule of AMPSA, and six molecules of UAEE. The same molar ratio predicted by the modeling program was then used in the synthesis of the polymer. Two of the four monomers selected for the annealing process were unable to establish direct binding with the toxin, probably due to sterical factors and internal competition (the monomers preferred to interact between each other than with microcystin-LR).

The presence of solvent and cross-linker can influence the binding between monomers and template. This effect was evaluated by comparing the total energy (E_T) of the monomers−template complex, obtained by computer modeling, with and without the cross-linker (EGDMA) and the solvent (DMSO). The calculated E_T of the monomer−template complex was −479.386 kJ mol^{-1}. Dynamic simulation performed in the presence of saturated quantities of cross-linker molecules showed small disruption of the complex with E_T = −457.504 kJ mol^{-1}. As expected, the effect of polar solvent molecules was much more pronounced. The calculated total energy of the complex in the presence of saturated quantities of DMSO molecules was E_T = −355.109 kJ mol^{-1}. In the latter, the electrostatic component of the total energy was the most affected (−397.706 kJ mol^{-1} without solvent and −225.421 kJ mol^{-1} with solvent). The modeling result indicates that the complexation between the selected monomer and template is strong even in a polar environment, which is important for successful imprinting of a water-soluble template such as microcystin-LR.

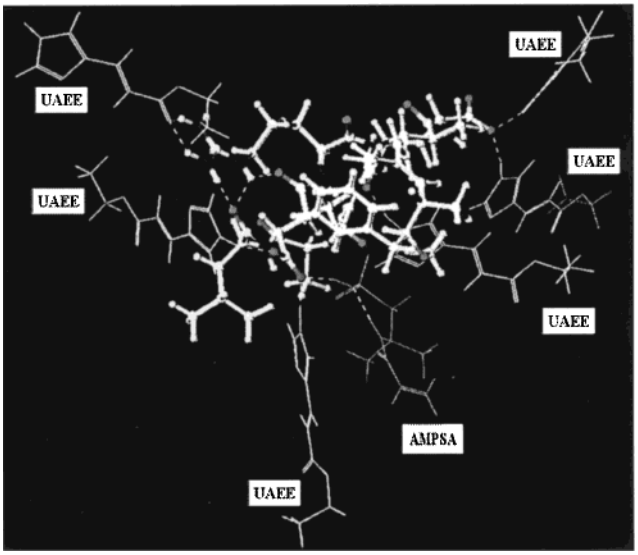

Figure 3. Final result of computer modeling. Interactions between microcystin-LR and monomers. Microcystin-LR, in balls and sticks in the center of the picture, interacts with six molecules of urocanic acid ethyl ester (UAEE) and 1 molecule of 2-acrylamido-2-methyl-1-propanesulfonic acid (AMPSA).

Polymer Synthesis and Competitive Assay. The computationally designed MIP was synthesized as described in the Experimental Section. An ELISA kit for microcystin-LR detection was used to confirm the removal of the template from the synthesized polymer. The results indicate that 92% of the template was successfully removed from the polymer.

The computationally designed MIP was then tested for its affinity for the target toxin by using an enzyme-linked competitive assay with microcystin−HRP conjugate. The concentration of the conjugate was optimized for the use of MIP as affinity receptor. The result of the optimization experiments is shown in Figure 4. A relevant specific signal was detected using 0.1 mg L^{-1} conjugate whereas the detection limit achieved with the computational MIP for free microcystin was 0.1 μg L^{-1} (Figure 4).

Purely for comparison purposes, a molecular imprinted polymer (MAA-MIP) was also synthesized using methacrylic acid,

(27) Trogen, G.-B.; Edlund, U.; Larsson, G.; Sethson, I. *Eur. J. Biochem.* **1998**, *258*, 301−312.

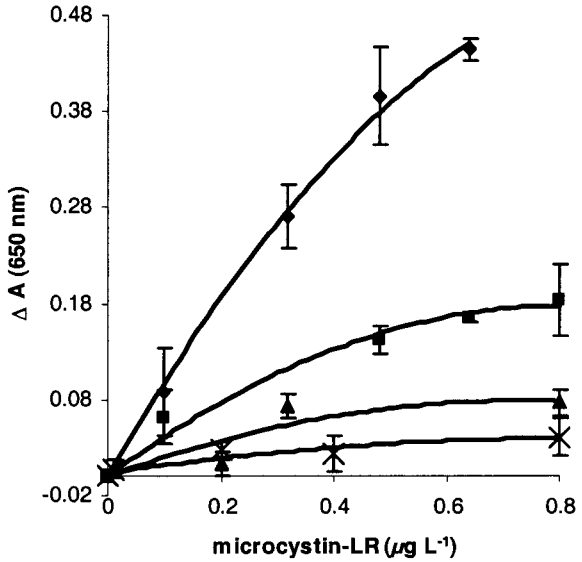

Figure 4. Optimization of microcystin−HRP conjugate concentration. Three different conjugate concentrations are used: (◆) 0.1, (■) 0.05, and (×) 0.0125 mg L^{-1}. The signal obtained using blank polymer (▲) and the best concentration of microcystin−HRP conjugate (0.1 mg L^{-1}) is reported.

Table 3. Affinity and Sensitivity Range of MIPs and Antibodies for Microcystin-LR Evaluated by Competitive Assay

receptor	K_d (nM)	sensitivity range (μg L^{-1})
computational MIP	0.3 ± 0.08	0.1−100
MAA-MIP	0.9 ± 0.1	0.8−100
monoclonal antibody	0.03 ± 0.004	0.025−5
polyclonal antibody	0.5 ± 0.07	0.05−10

which is one of the most commonly used monomers for imprinting of peptides and positively charged templates. The ratio between monomer and template was 10:1 as is traditionally used in noncovalent imprinting for large molecules.[28] The performance of MAA-MIP was then examined using the enzyme competitive assay and compared in affinity, sensitivity, and cross-reactivity with that of the computationally designed MIP.

Dissociation constants for polymer−template complexes were obtained from the competitive assay experiments, using a double-reciprocal plot.[25] These values were compared to those obtained for polyclonal and monoclonal antibodies raised for microcystin-LR (Table 3). The affinity of the computationally designed MIP for microcystin-LR was found to be at least 3 times higher than the affinity of MAA-MIP. The affinity of the computationally designed MIP was even slightly better than that of the polyclonal antibodies but lower than the affinity of the monoclonal antibodies.

The detection range of microcystin-LR in a competitive assay was broader for MIPs compared to the tested antibodies (Table 3). This indicates that the polymers, in contrast to the monoclonal antibodies, have a distribution of high- and low-affinity binding sites,[29] broader than even the polyclonal antibodies.

A BET analysis, performed for all polymers, has shown that MIPs and blank polymers have similar surface area and total pore volume (Table 2). This result confirms that these physical characteristics are not the cause for the higher sensitivity of computationally designed MIP for microcystin-LR.

Table 4. Cross-Reactivity of MIPs and Antibodies[a]

receptor	MC-LR[a] (%)	MC-RR[b] (%)	MC-YR[c] (%)	nodularin (%)
computational Mip	100	21 ± 0.9	27 ± 2	22 ± 2
MAA-MIP	100	19 ± 0.8	30 ± 3	36 ± 0.5
monoclonal antibody[e]	100	106 ± 0.3	44 ± 2	18 ± 0.8
polyclonal antibody	100	92 ± 2	142 ± 0.8	73 ± 1

[a] The receptor binding affinity for the analogues, evaluated by competitive assay, are calculated as a percentage of the affinity for microcystin-LR (100%). [b] MC-LR, microcystin-LR. [c] MC−RR, microcystin-RR. [d] MC−YR, microcystin-YR. [e] The calculated cross-reactivity values of monoclonal antibodies for the analogues are in complete agreement with those reported in the literature.[19]

Cross-Reactivity Study. Table 4 shows the cross-reactivity of antibodies and polymers for the analogues of microcystin-LR: microcystin-RR, microcystin-YR, and nodularin. Both imprinted polymers showed very low cross-reactivity for any of the analogues in contrast to polyclonal and even monoclonal antibodies. The cross-reactivity of the polymers for some of the analogues (microcystin-RR and microcystin-YR) was 5 times lower than that of antibodies. This result, together with the similarity of the cross-reactivity values, can suggest the presence of a nonspecific binding between the synthetic receptors and all the tested analogues.

Analysis of Receptor Stability. The stability of the synthetic receptors (MIPs) and natural receptors (antibodies) was examined. MIPs; monoclonal and polyclonal antibodies were exposed to harsh treatment with acid, alkali, organic solvent, heavy metals, and high temperature. Table 5 shows the stability of the MIPs and antibodies under these conditions, calculated as a percentage of the remaining affinity. MIPs are materials well known for their superior chemical, mechanical, and thermal stability and also in this work they demonstrated better robustness than antibodies. Although computationally designed MIP and MAA-MIP both have shown excellent stability in acidic and basic conditions, their performance at high temperature and in the presence of heavy metals was markedly different. In contrast to MAA-MIP, the affinity of computationally designed MIP was decreased by almost 50% after 2 h of heating in water at 80 °C and after exposure to heavy metals. High sensitivity toward high temperature could be explained by the ability of SO_3^- to catalyze the hydrolysis of the polymer. The results from elemental analysis of the polymer before and after treatment confirmed that a possible hydrolysis had occurred. In fact, a significant decline in sulfur content by up to 50% was observed following the treatment in boiling water. The high sensitivity of the computationally designed MIP toward heavy metals might be explained by the ability of the imidazole ring of UAEE to form a strong complex with transition metals. A conclusion from this stability study is that further progress toward more successful mimicking of natural receptors could improve some characteristics (such as affinity and specificity) but would likely decrease other features (such as polymer stability).

CONCLUSIONS

A synthetic receptor for microcystin-LR was designed using a new computational approach combined with molecular imprinting.

(28) Rachkov, A.; Minoura, N. J. *Chromatogr., A* **2000**, *889*, 111−118.
(29) Andersson, H. S.; Nicholls, I. A *Bioorg. Chem.* **1997**, *25*, 203−211.

Table 5. Stability of MIPs and Antibodies under Harsh Conditions[a]

receptor	80 °C (%)	80% DMF (%)	100 mM HCl, pH 2 (%)	100 mM NaOH, pH 11 (%)	10 mM $CuSO_4$ (%)
MAA-MIP	88.8 ± 1	102.3 ± 2	99.5 ± 3	102.4 ± 3	98.1± 1
computational MIP	52.1 ± 2	96.9 ± 2	102.0 ± 2	117.6 ± 5	55.6 ± 2
monoclonal antibody	16.8 ± 5	19.2 ± 4	16.3 ± 0.8	17.7 ± 5	24.2 ± 1
polyclonal antibody	9.9 ± 0.2	17.6 ± 0.9	93.7 ± 2	44.4 ± 7	16.8 ± 1.22

[a] The remaining affinity of receptors was evaluated by competitive assay as a percentage of the affinity under optimum conditions.

The affinity of the computationally designed MIP, studied using an enzyme-linked competitive assay, was found to be comparable to polyclonal antibodies, while the MIP had superior stability over natural receptors. The computationally designed MIP also showed higher affinity in comparison with the MAA-MIP. It was also found that MIPs had much lower cross-reactivity for microcystin-LR analogues than both polyclonal and monoclonal antibodies; thus, MIPs offer the advantage of specifically recognizing the target analyte microcystin-LR instead of the entire family of toxins. It is anticipated that the polymer designed could be used in assays, sensors, and solid-phase extraction.

ACKNOWLEDGMENT

We gratefully acknowledge the European Union, Environmental and Climate Program (ENV4-CT98-0784) for the financial support and Prof. Hennion (from the Department of Environmental and Analytical Chemistry in Paris, France) for providing the polyclonal antibodies.

Received for review July 24, 2001. Accepted December 8, 2001.

AC010840B