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A Computational Approach for Selection of Suitable Monomer for the Detection of Cyanobacterial Hepatotoxin

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Cyanotoxin-Microcystin and Nodularin, Monomer, Molecular Imprinted Polymer

ABSTRACT:
The molecularly imprinted polymers (MIPs) development is an efficient system that can be used to create binding sites for template molecules that take into account the test molecules' size, shape, and functional groups. Presently, knowledge of the interaction of MIPs as Systems for recognizing hosts-guests is limited. In the present study, we screened out different monomers IA, MAA, and AMPSA and their Insilco interaction with cyanotoxin to evaluate the possibility of developing MIP-based biosensors. By using Insilco computational tools monomers were designed and screened against toxins. To examine their interaction with the template, the highest affinity monomers were chosen and employed in a simulated annealing procedure. The generation of MIPs and their attributes can both be demonstrated using this low-cost computational method.

Introduction

Cyanobacteria are photosynthetic bacteria; some of them grow in the form of algal bloom. Few bloom produced toxins which are known as Cyanotoxins. Most frequently detected cyanotoxins are Microcystin, Nodularin, Neurotoxins and Cylindrospermopsin. Microcystin and Nodularin both are hepatotoxic cyclic peptide produced by harmful bloom of cyanobacteria (blue-green algae) (1,2,3,4).

The building block of Microcystin is cyclic hepta-peptide and their sequence are cyclo(D-Ala-X-D-MeAsp-Z-Adda-D-Glu-Mdha), here Mdha is *N*-methyldehydroalanine, D-MeAsp is D-erythromethylaspartic acid and Adda is (2*S*, 3*S*, 8*S*, 9*S*)-3-amino-9-methoxy-2,6,8-trimethyl-10-phenyldeca-4,6-dienoic acid. There are two variables of amino acids X and Z which classify the Microcystin as (LR) -leu- and -arg-, (YR) -tyr- and -arg-, two-arg- (RR). The chemical structure of Nodularin is cyclic penta-peptide and their building blocks are cyclo (-D-MeAsp (iso-linkage)-L-Z-Adda-DGlu-2-(methylamino)-2(*Z*)-dehydrobutyric acid). There is one variable Z which classifies Nodularin as (R) L-Arg (5,6,7). The cyclic backbone is saddle shaped with Arg above saddle, Adda behind the saddle, Mdha at the top, front of saddle and negatively charged carboxyl group located at underneath the saddle (8,9). The backbone has root-mean square deviation (RMSD) of 0.65 A°. In cyclic peptides the salt-bridge or H-bond involves Arg96 and this Arg96 have favorable interaction and contribution to complex formation. The cyclic and folded nature of backbone has high affinity. So, cyclic part is more important in binding. Because the chemical contains the amino acid (Adda), microcystins and nodularins are poisonous (10, 11, 12, 13).

The main methods used to analyses these poisons at the moment are bioassay, liquid chromatography (HPLC), or immunoassay (14). Synthetic receptors for toxins are now being developed due to the challenges associated with producing antibodies against toxins also, the ongoing trend to utilize fewer animals in the process. In recent years, the concept of molecular imprinting regarded as one of the easiest, most direct and

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economical ways to create synthetic receptors for harmful chemical substances (15, 16, 17).

There are currently a number of MIP preparation techniques available. The most widely used technique; however, is the development of MIPs from monomers in the presence of a target/template. First, the target develops a brief connection with the template molecule and engages in intermolecular interactions with the monomers like hydrogen bonds. Second, a MIP is created from the association of chemical or electrochemical polymerization. Thirdly, the target molecule is rebound, causing the template to be later washed out to leaving behind a cavity, leading to the formation of NIP (non-imprinted polymer) (18, 19, 20).

The best monomer candidates for imprinting have been found using combinatorial screening methods and thermodynamic simulations. The job proved challenging as the monomer libraries grew in size and now contained hundreds of polymerizable chemicals. Using molecular modelling tools and standard search strategies used in drug creation could help solve this issue. Numerous scientific fields, including biosensors, solid-phase extraction, chromatography, and drug delivery, are covered in studies on the use of MIPs (21, 22).

We focused our efforts on the initial step of this procedure, which involves choosing monomers that can form solid complexes with the template through molecular modelling. In fact, this study is the first attempt to synthesise a molecularly imprinted polymer (MIP) specifically for microcystin and nodularin utilising this computational approach and aim of study, to find out the bound conformation of the monomers with the cyanotoxin that possibly to develop MIP based sensor for onsite detection of cyanotoxin. Presently, onsite cyanotoxin detection technique in the market is not available.

Material and Methods:

In few steps computational deign were completed using various computational tools.

***In-Silico* Generation of Ligands**

Chosen from the virtual library are the functional monomers and these monomers were comparable to the template using molecular modeling tools. The monomers utilized to investigate their interaction with the template were chosen based on the least binding energy during a simulated annealing procedure. Additionally, docking of the chosen monomers against the cyanotoxin protein (Microcystin and Nodularin) was done. Cyanotoxin protein sequences were acquired from the Protein Data Bank (PDB). It was investigated how the chosen discovered monomers bound to the target proteins (23, 24, 25).

Monomers structure

Designing 3-D chemical structures, such as ligands, polymers, organics, and chemical symbols, is done using molecular modelling software. The software, according to Li (2004), was utilised for the 3D spectral processing. Understanding the chemical structure in 2- or 3-dimensional formats facilitates comprehension of the functional group and chemical bond. Tools used to convert file format (26, 27,28, 29).

Monomers draw in chemsketch are shown in (Figure-1) IA (Itaconic acid), MAA (Methacrylic acid), AMPSA (2-acrylamido-2-methyl-1-propanesulfonic acid)

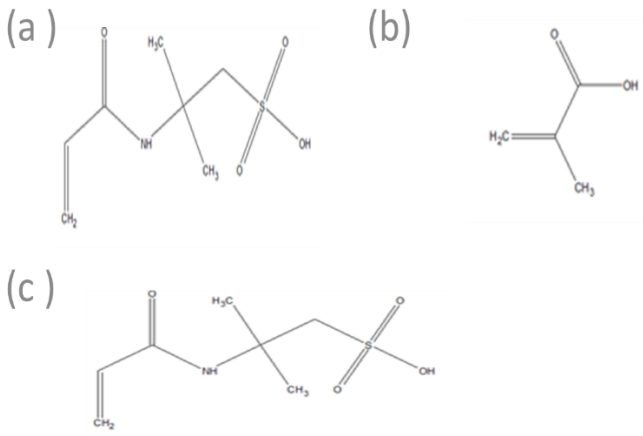

Figure 1. Draw the structure of monomers (a) IA (b) MAA (c) AMPSA

Protein Optimization

The Protein Data Bank was used to get a molecular model of the toxin (template). In the study, two proteins were optimized for docking purpose namely 1fjm, 3e7a from microcystin and nodularin respectively (30,31). The protein 1fjm and 3e7a download from pdb shown in Figure-2.

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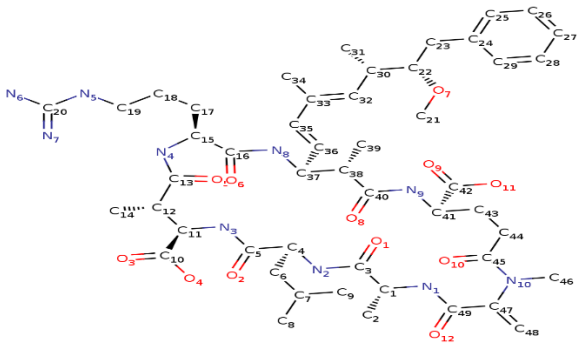
a) Microcystin:



b) Nodularin:

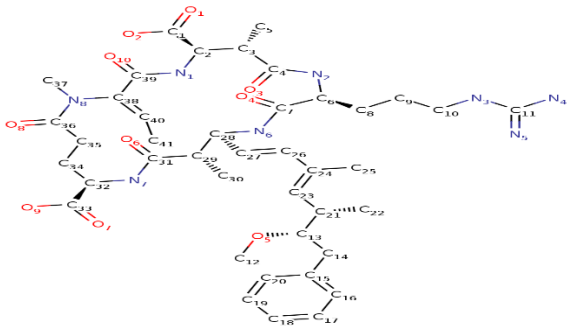


Figure 2- a) Microcystin b) Nodularin

Energy minimization and docking analysis are performed using automated homology modelling in the following stage. Docking used as screening tool for prediction of interaction between ligand and target also for better atomic resolution of interactions (32,33). At the conclusion, the docking algorithm was used for the selection of the best molecule with minimum binding energy and hydrogen bond analysis was used for visualization and analysis of results. Blue/orange color is an indicator for the active sites of the protein (34).

Result& Discussion

Commercial software is often used in drug design. In the present study, computational modeling of the monomer-template interaction was carried out for the development of MIP Based sensor for the detection of Cyanotoxins (microcystin and nodularin). The virtual libraries contain a wide range of monomers in terms of functions, including the ability to establish ionic bonds, hydrogen bonds, and van der Waals interactions with the template.

Prior to doing in-vitro experiments, we conducted an in-silico analysis to choose the most appropriate monomer. UCSF Chimera and Auto Dock (32,34) software used for Hydrogen bond analysis and docking between monomer and protein interactions. As we know that hydrogen bonding is a type of inter-molecule interaction that involves a hydrogen atom binding to a highly electronegative atom and a second electronegative atom in hydrogen bonding. In this study, Three monomers (AMPSA, IA, and MAA) were selected which interact with the template (microcystin and nodularin). These three monomers are acidic and interacted with the basic amino acid arginine, which is accessible in the microcystin and nodularin complex. In the contest of molecule docking hydrogen bonding plays a crucial role in stability between the monomer and protein molecule it reveals the strength of interaction between the molecules (35,36).

During the docking process identify the most favorable binding mode, h-bond analysis as such the H-bond interaction toward between the specific atom in the monomer and protein residue. This analysis involve in the way H-bond donor and acceptors in the monomer and protein residue.

Monomer MAA and IA used for the current study contain carboxylic acid and AMPSA have sulfonic group (37) which involves hydrogen bonding with the selected protein moiety (microcystin and nodularin). In microcystin and nodularin, the sulfonic acid of AMPSA made H-bond with Arg96 and Tyr 134 whereas carboxylic acid made a hydrogen bond with Arg96, Tyr 272, Gly 67, 135. These bonding show that these monomers are stable for molecular modeling or docking. The AMPSA made a minimum hydrogen bond with microcystin and nodularin followed by IA and MAA. The distance and geometry between the atoms are evaluated to determine the stability of the H bond formation angle and length. H bond angle was close to the ideal bond angle of 20^0 A° with a distance of 6.0 A° (38). Figure 3a,b-8a,b shows the final complex's structure as anticipated by computer modeling. The blue and orange line shows water molecule interaction with protein. The water molecule includes during the docking. Hydrated docking predicts the observed conformation (34). The modeling outcome shows that

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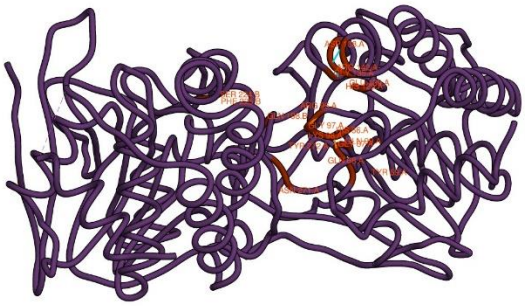
even in a polar environment, the chosen monomer and template have a strong complexation, which is crucial for the effective imprinting of a water-soluble template like microcystin and nodularin.

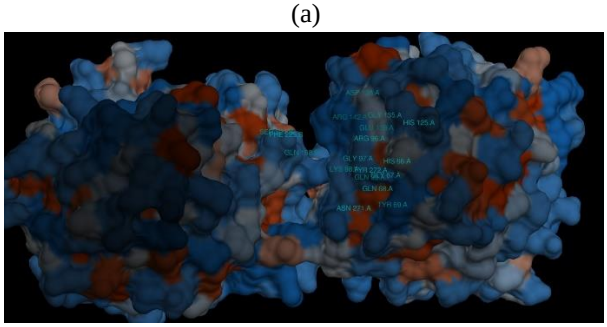
Sr no.	Monomers	$M_{m.e}$	$N_{m.e}$	M_r	N_r
1	IA	-1.30	+0.99	8	10
2	MAA	-2.89	-2.52	4	7
3	AMPSA	-0.66	+1.12	9	2

Table-1. Minimum binding energy of Microcystin and Nodularin with Monomers ($M_{m.e}$= Microcysin minimum binding energy (Kcal/mol); $N_{m.e}$= Nodularin minimum binding energy; M_r= run of Microcystin; N_r= run of Nodularin).

Table 1 showed that out of all three monomers (IA, MAA and AMPSA) the MAA having minimum binding energy with both cyanotoxins (Microcystin and Nodularin) and also have high affinity (Figure 3a,b-8a,b) results are comparable as previous reports where MAA potentially employed as an active component for MIP based biosensors for the detection of D- glucose (22). Therefore, MAA monomer for MIP development for the cyanotoxin (microcystin and nodularin) may be used as it can be easily washed or removed to form cavity which will be further study as a biosensor.



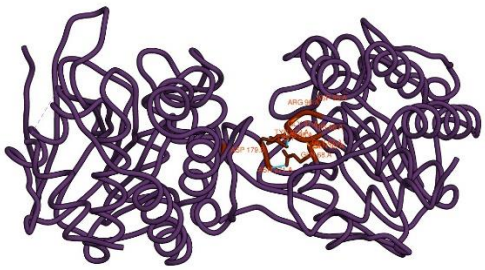
(a)



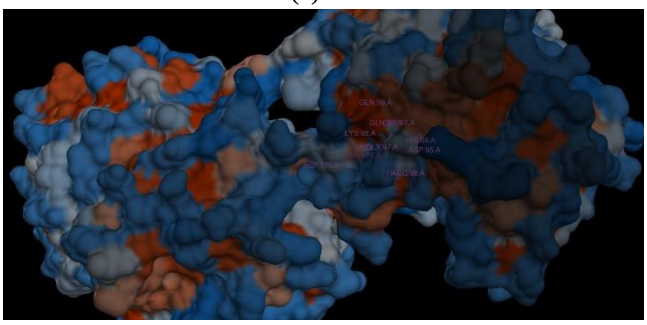
(b)

Figure3- Microcystin with IA- The blue and orange line shows water molecule interaction with protein.

a) H-binding b) Hydrophobicity

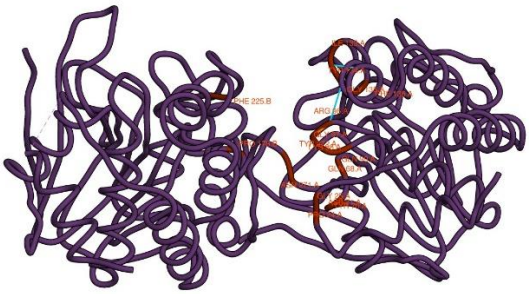


(a)



(b)

Figure 4- Microcystin with MAA The blue and orange line shows water molecule interaction with protein.a) H-binding b) Hydrophobicity



(a)

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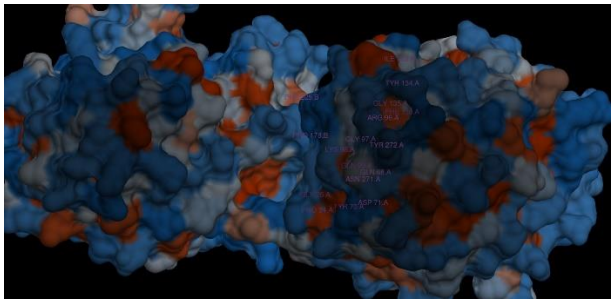
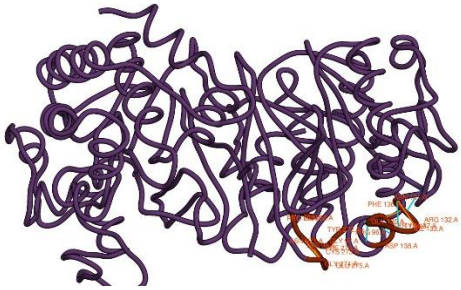
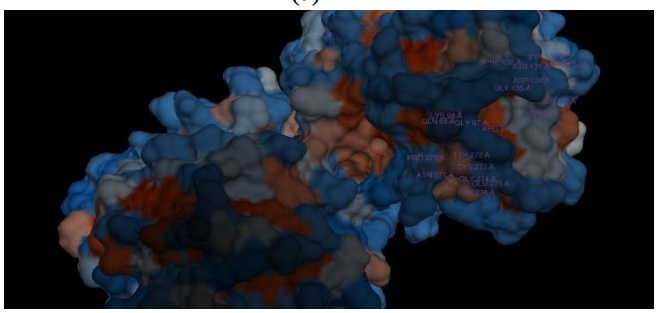


(b)

Figure5- Microcystin with 2AMPSA.The blue and orange line shows water molecule interaction with protein a) H-binding b) Hydrophobicity

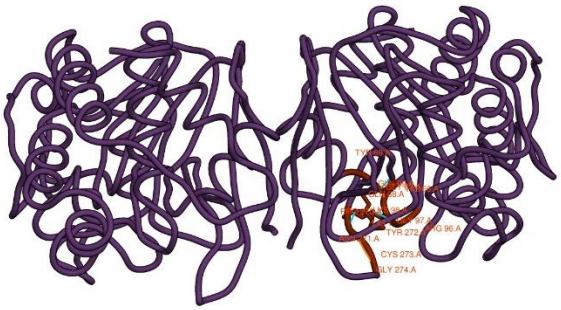


(a)

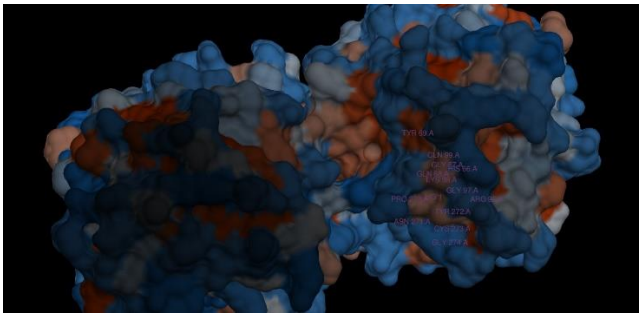


(b)

Figure6-Nodularin with IAThe blue and orange line shows water molecule interaction with protein.
a) H-binding b) Hydrophobicity.



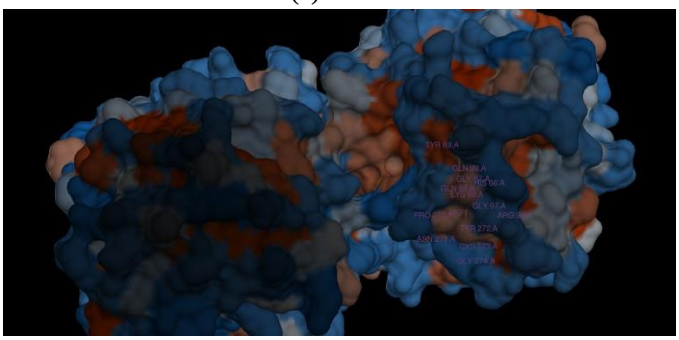
(a)



(b)

Figure7-Nodularin with MAA The blue and orange line shows water molecule interaction with protein.
a) H-binding b) Hydrophobicity

(a)



(b)

Figure8- Nodularin with AMPSA. The blue and orange line shows water molecule interaction with protein. a) H-binding b) Hydrophobicity.

Conclusion:

From this study, it was concluded that there is a physical interaction exist between monomers (IA, MAA, and AMPSA) and cyanotoxin (microcystin and nodularin). Out of these monomers MAA has high affinity following by IA and AMPSA. Therefore, MAA most suitable monomer for the development of a

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potential MIP based biosensor and it can be also used for the extraction of cyanotoxin based on the solid phase extraction principle. This work proposed effective study for the development of MIP based biosensor for the detection of cyanotoxin. Additionally, the newfound understandings will aid in the discovery of novel super molecular systems for the detection of tiny biomolecules employing piezoelectric and electrochemical MIP-based sensors.

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