Detection Methods of Microcystins

Subjects: Others

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Cyanobacterial harmful algal blooms (CyanoHABs) are globally on the increase in both frequency and intensity as a result of eutrophication and climate change. The most frequently reported CyanoHABs toxins are cyclic heptapeptide hepatotoxins microcystins (MCs) which have attracted worldwide studies. MCs most often found in water and to a lesser extent in desert environments are primarily produced by cyanobacteria species of the genera Microcystis, Anabaena, Aphanizomenon, Nostoc, Cylindrospermopsis, and Planktothrix.

detection microcystins biosensor

1. Introduction

The cyclic heptapeptide hepatotoxins are relatively stable in natural environments and resistant to chemical and physical factors including extreme temperatures, pH changes, sunlight and degradation via non-specific enzymes owing to their cyclic structure [1][2][3]. The common structure of MCs is cyclo-(-D-Ala-L-X-DisoMeAsp-L-Z-Adda-D-isoGlu-Mdha), where X and Z are highly variable amino acids, D-MeAsp is D-erythro-b-methylaspartic acid, Mdha is N-methyldehydroalanine, and Adda is (2S, 3S, 8S, 9S)3-amino-9 methoxy-2,6,8-trimethyl-10-phenyldeca-4, 6-dienoic acid [4][5][6]. More than 270 MC variants have been isolated from CyanoHABs [7]. On the basis of toxicity microcystin-LR (MC-LR) is by far the most potent hepatotoxin among the different variants of MC and has become a global focus [8][9][10]. The International Agency for Research on Cancer classified this toxin as a group 2B carcinogen [11], and the World Health Organization (WHO) recommended a provisional 1 µg/L MC-LR guidelines for drinking water quality [12].

In recent years, MCs production has been reported from all continents especially from tropical and subtropical areas under an extensive variety of environmental conditions [13][14][15][16][17][18][19][20][21]. Human and animal health problems are prone to be associated with chronic exposure of MCs concentration primarily through ingestion and body contact [10]. MCs are potent and specific inhibitors of protein phosphatases 1 (PP1) and protein phosphatases 2A (PP2A) from both mammals and higher plants [22]. This may alter the expression levels of miRNA, induce cytoskeleton disruption, DNA destruction, inflammation, autophagy and apoptosis [9][23][24][25]. Exposure to MCs may severely damage mammalian organs including the liver, intestines, brain, heart, lungs, kidney and reproductive system. In addition, through the accumulation of these toxins, plants growth and yield may be threatened [26][10][27]. This may further exhibit moderate or high human health risk and intoxicate other organisms through food transfer.

To effectively manage and control MCs, as well as prevent or minimize their health risks, sensitive, fast and reliable screening methods capable of detecting these toxins are urgently required. Early detection of MCs can help to counteract these deadly toxins, to avoid further posing ecosystem and human health threat. An important consideration in analyzing water samples for MCs is to determine the differences between intracellular and extracellular toxins [28]. To successfully determine the toxins level, there should be cell lysis to release intracellular toxins, mostly by freeze-thawing and ultrasonication bath [29][30][31].

2. Biosensor Methods to Detect Microcystins

While these analytical techniques are precise and sensitive, expensive instrumentation, well-trained personnel and time-consuming procedures are involved. This suggests that their applications may primarily be limited to well-resourced and centralized laboratory facilities. Consequently, development of low-cost and ultrasensitive measuring method would help limit exposure by enabling early detection and continuous monitoring of MCs.

In recent times, the robust, simple, specific, sensitive, portable, easy to utilize and rapid biosensor method which functions as an enhanced monitoring tool for MCs, particularly to analyze low MCs concentrations and manage the risk associated with health, is gradually gaining global focus. Biosensor is an analytical tool made up of a biological recognition element termed as bioreceptor, in direct contact with a transducer. This analytical tool can be classified either by their biological recognition element or signal transduction methods. A bioreceptor in biosensor is often combined with a suitable transduction method to generate a signal following interaction with the target molecule of interest [32][33][34][35]. It is worthwhile noting that various natural and artificial biological elements including whole cells, enzymes, antibodies, molecularly imprinted polymers (MIPs) and nucleic acids are employed in biosensors [32][34][35][36].

At present, enzyme based biosensors (including optical and electrochemical biosensors), immunosensors (including electrochemical, piezoelectric, NMR-based and optical immunosensors such as Surface Plasmon Resonance (SPR) immunosensors, Evanescent Wave Fiber-optic immunosensors, Luminescent immunosensors, Fluorescent immunosensors, and Immunoarray biosensors) and nucleic acid biosensors (including electrochemical DNA and SPR-DNA biosensor) have been developed for successful MCs determination [32][33][34][37]. A rapid and sensitive SPR biosensor, which incorporates commercial Adda-group antibody (Ab) and has the capacity for broader recognition of various MC variants than earlier developed sensors for BGA supplements was established and validated. This technique is capable to further observe BGA products to aid risk assessment, ascertain regulatory guidance levels and respond to potential consumer complaints linked to BGA products [36]. The constructed electrochemical biosensor that possesses good stability against other components in natural water sample, prepared via physically immobilizing calf thymus DNA (ctDNA) on gold electrode after characterizing the deleterious effect of MC-LR to ctDNA, was utilized to detect MC-LR in local water bodies. The technique indicated linear range of 4–512 ng/L, LOD of 1.4 ng/L (perceived to be 700-fold lower than WHO's suggested guideline value) and recoveries were 95.1% to 107.6% [37]. A novel fiber optical chemiluminescent biosensor (FOCB) system was successfully generated utilizing fiber optic bio-probe as biorecognition element as well as transducer and a Si-based photodiode detector (PD-3000). The FOCB system is robust, portable, cost-effective, utilizes small sample

volume, is suitable for on-site and automatically detects targets. A highly sensitive MC-LR determination was attained with LOD of 0.03 µg/L under optimal conditions and recoveries were in the range of 80% to 120% [32]. A Surface-Enhanced Raman Scattering (SERS) spectroscopic immunosensor with outstanding sensitivity, selectivity and robustness was established to detect and quantify MC-LR in aquatic settings. The established SERS sensor could reach LOD (0.014 µg/L) at least 1 order of magnitude lower, exhibited a linear dynamic detection range (0.01 µg/L to 100 µg/L) 2 orders of magnitude wider in comparison to the conventional techniques, and recoveries were 100% to 107%. Further, the SERS immunosensor enabled monitoring of the dynamic production of MC-LR from a *Microcystis aeruginosa* culture [35]. The developed phosphorescent immunosensor, which acquired antigens and antibodies as recognition units and employed Mn-ZnS RTP QDs as sensing materials to specifically bind with MC-LR, also demonstrated rapid and sensitive MC-LR detection with linear ranges of 0.2–1.5 µg/L and 1.5–20 µg/L, LOD up to 0.024 µg/L, and recoveries were 93.1% to 105%. Interestingly, no significant obstruction was observed from coexisting MC-LR pollutants in water during the toxin's determination [33]. Further, a novel Cu/Au/Pt trimetallic nanoparticles (Cu/Au/Pt TNs)-encapsulated DNA hydrogel prepared for colorimetric detection of MC-LR also detected the toxin with a linear range of 4.0–10.000 ng/L, LOD of 3.0 ng/L, and recoveries of fresh crucian carp tissue were in the range of 95.34% to 107.07% while the recoveries of water ranged from 93.96% to 105.33% [38].

Aptasensors are biosensors that employ aptamer as recognition element. In developing aptasensors, nanomaterials that are regarded as potential agents are mostly considered because of their physico-chemical properties including small size, disposability and high surface area [39]. Various highly specific and sensitive aptasensors-based optical (such as colorimetric, fluorescent, SERS, Electrochemiluminescence (ECL) aptasensors) and electrochemical-based aptasensors currently exist for the determination of MC-LR [39][40][41]. A novel aptasensor based on SERS where MC-LR aptamer and its corresponding complementary DNA fragments (cDNA) were conjugated to gold nanoparticles (AuNPs) and magnetic nanoparticles (MNPs), respectively, used as signal and capture probes (aptamer-AuNPs and cDNA-MNPs conjugates) was constructed and applied for highly sensitive MC-LR detection. The technique revealed a linear range from 0.01 to 200 ng/mL, LOD of 0.002 ng/mL, and the recovery values ranged from 88.84% to 105.72% [41]. A sensitive and selective electrochemical aptasensor that exhibited a linear range of 0.005–30 nM, LOD of 0.002 nM and recovery rates from 95% to 106% for MC-LR determination was developed based on a dual signal amplification system comprising of a novel ternary composite (prepared via depositing AuNPs on molybdenum disulfide (MoS_2) covered TiO_2 nanobeads) and horseradish peroxidase (HRP) [40]. Further, a novel dual-mode aptasensor based on MoS_2-PtPd NPs and zeolitic imidazolate framework (ZIF)-8-thionine (Thi)-Au (ZIF-8-Thi-Au) (as signal material) was established and demonstrated ultra-sensitive and quick MC-LR detection. The aptasensor indicated a liner range from 0.01 to 50 ng/mL, lowest LOD at 0.006 ng/mL, and recovery was from 95.5% to 109.6% [42]. The collective effects of these methods were evaluated using the recovery rate. The findings demonstrate that the biosensors recoveries ranged from 88.84% to 109%. The good recovery rates exhibited indicate that the biosensors possess good stability against other components (matrix effect) in water and fish samples.

The ability to assess health status, disease onset and progression, and monitor treatment outcome is the primarily objective in health care promotion and delivery. Biosensors and point-of-care devices have the potential to improve delivery of healthcare. The latest development in biosensor technologies can deliver point-of-care diagnostics that

match or exceed conventional standards in terms of cost, time and accuracy [43]. However, the practical application of biosensors in medical diagnosis and treatment is still advancing. Since the development of the first glucose electrochemical sensor, substantial efforts to construct implantable biosensors have been made. Although the devices may be challenged with matrix effects and sample preparation, they can be used to monitor patients, improve the management of patient health and quality of life, enable drug treatments to be administered at specified times, increase survival rates and reduce health care costs and the number of invasive interventions required [43]. A precise diagnostic for a disease is essential for a successful treatment and recovery of patients suffering from it. Diagnostics methods must be simple, sensitive, detect multiple biomarkers, perform multiplex analysis and assimilate different functions. With successful biosensor integration, biomarkers can be monitored in samples such as saliva, sputum, blood, stool, swab, skin and interstitial fluid [43]. The electrochemical and optic based biosensors are mainly used for routine evaluation of blood parameters like urea, creatinine, glucose and lactate, as well as point-of-care testing of glucose in clinical chemistry laboratories. Moreover, for high sensitivity and faster analysis in near-patient testing for cardiac and few cancer markers, immunosensors are preferred [43]. Most studies concerning MCs detection have been based on water and biological samples. Although biosensors have been used to detect MC-LR, their application in the context of medical diagnosis and the associated matrix effects regarding MC intoxication are yet to be determined. Further studies are therefore recommended.

It is of interest that biosensors are considered as catalytic (enzymes and whole cells) or affinity (antibodies and nucleic acids) based on their biological elements. The presence of this biological element makes biosensor system very specific and highly sensitive. This gives an upper edge over the conventional methods and bioassay in environmental sensing and detection [32][33][34]. Moreover, an ideal biosensor incorporates features of minimal training, power requirements, portability and presents meaningful results using less sample volumes and reagents. Biosensors can achieve low detection limits of MC-LR in dietary supplements as well as various aquatic settings such as drinking water, lakes, and reservoirs due to the selective binding or reaction of the biological recognition element to the target analyte. The technique can also demonstrate good recovery, precision, and accuracy through the evaluation of the spiked water samples and can be readily extended toward the on-site real-time sensitive detection of other targets in the field of environment, food and medical diagnosis [32][33][34][35]. It is worth-knowing that the aptamers can easily be labeled and fabricated into diverse aptasensors to acquire rapid, sensitive, and specific MC-LR detection. Aptamers demonstrate high affinity, and most of the developed aptasensors are simple to perform with miniaturized instruments to attain on-site monitoring of the toxins. Aptamers also show significant advantages in terms of low generation cost, low molecular weight and quick chemical synthesis and modification. Moreover, they can offer rapid and accurate determination of MC-LR and can be referred to detect other hazardous substances in water products [42][43].

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