

A homogeneous assay for rapid detection of cyanobacterial peptide hepatotoxins: microcystins and nodularins

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ABSTRACT

BACKGROUND-AIM

Toxic cyanobacterial blooms creates local and global problems by contaminating surface water resources with their potent toxins having adverse health effect for both humans and animals. Two structurally related families of cyclic peptides, microcystins (MC) and nodularins (Nod), are the most commonly reported and troublesome cyanobacterial hepatotoxin. For the assessment of water quality and safety, simple and rapid screening methods are required for analysis of water samples to detect the possible presence of MC/Nod. We report a mix-and-measure type simple and rapid non-competitive homogenous screening assay for MC/Nod based on time resolved fluorescence resonance energy transfer (TR-FRET).

METHODS

To demonstrate the homogenous assay a generic anti-immunocomplex (anti-IC) scFv (single-chain variable fragment) isolated from our in house synthetic antibody library was crucial together with a generic anti-adda specific antibody recognizing the common adda (3-Amino-9-methoxy-2,6,8-trimethyl-10-phenyldeca-4(E),6(E)-dienoic acid) part of the microcystins and nodularins. The anti-IC scFv labeled with alexa 680 and the anti-adda

antibody labeled with europium enabled the FRET assay to occur in the presence of MC or Nod. In the presence of toxin in sample, FRET occurs only at the close proximity of the two fluorophores when anti-IC scFv binds specifically to the anti-adda-antibody:MC/Nod immunocomplex and sensitized emission of fluorescence signal was detected at 730 nm in time resolved mode.

RESULTS

Using only 20 μ l of water sample, the rapid (15 min or less) wash-free assay was capable of detecting all the tested nine major hepatotoxins (microcystin-LR, -dmLR, -RR, -dmRR, -YR, -LY, -LF -LW, and nodularin-R) with sensitivities well below the World Health Organization guideline limit of 1 μ g/L.

CONCLUSION

The mix and measure type assay without requiring any washing step has a great potential as a quick screening tool for MC/Nod detection from a large number of water samples.

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