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Short communication

A nanosensor based on quantum-dot haptens for rapid, on-site immunoassay of cyanotoxin in environmental water

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ABSTRACT

A nanoprobe based on quantum-dot (QD) haptens was synthesized by conjugating carboxyl quantum dots with aminoethyl-microcystin (MC)–leucine–arginine (LR). A two-alkyl group was introduced to supply a spacer between the QD nanoprobe and anti-MC–LR antibody to reduce the steric hindrances of immunoreaction. The sensor system based on a portable optofluidic platform exhibited a liner range of 0.10–4.0 μg/L for MC–LR with a detection limit of 0.03 μg/L. The proposed sensor has potential application in the rapid, on-site detection of MC–LR in real water samples.

1. Introduction

The frequency of occurrence of cyanobacterial blooms has become a global concern because of the resulting liberation of cyanobacterial toxins and the deterioration of water quality (Ye et al., 2009). Microcystins (MCs), a group of cyclic heptapeptide hepatotoxins consisting of a seven-amino-acid peptide ring, have been proven to be highly toxic to vertebrates (Campo and Ouahid, 2010). Many reported cases of animal poisoning, human diseases, and even death are attributed to MC exposure through drinking and surface waters (Amé et al., 2010). MC–leucine–arginine (MC–LR; MW=995.2) is one of the most frequently detected and toxic cyanotoxins (Amé et al., 2010; Campo and Ouahid, 2010; Dennis and Bao, 2008; Herranz et al., 2012). As a result, the World Health Organization (WHO) has set a guideline value of 1.0 μg/L for MC–LR in drinking water to minimize the risk to the public (World Health Organization, 2003). A number of detection methods, including high-performance liquid chromatography, invertebrate bioassays, and protein phosphatase inhibition assays, are available. However, these techniques generally require long analysis times and may be strongly affected by matrix effects (Herranz et al., 2012). Recently, immunosensors based on mono-/polyclonal antibodies have been developed as screening techniques because of their specificity, sensitivity, ease of use and rapidity. However, the sensitivity of an immunoassay strongly depends on the affinity of specific antibodies and the sensitivity of the detection method.

Fluorescence detection techniques have the advantages of versatility, high sensitivity, and the simplicity of signal detection. In particular, fluorescence resonance energy transfer (FRET)-based nanoscale biosensors are widely used detection systems that rely on the fluorescence response (Jaiswal et al., 2003). In this technique, one of the optimum donors is quantum dots (QDs) because of their unique optical properties, including high quantum yield, photostability, narrow emission spectrum, and broad absorption. Fluorescent dyes are generally used as acceptors. QD-FRET based biosensors have been widely used in immunoassays, clinical/diagnostic assays, and biomolecular binding assays. Morevoer, QDs-antibody (Ab) conjugates are the most developed and most widely used detection bioprobes for QD integration in bioanalyses (Dennis and Bao, 2008). For example, a QD/Ab probe was used for the immunological recognition of MC–LR as well as for electrochemical transduction (Yu et al., 2010). QD responses were amplified and converted to electrochemical signals by measuring the release of cadmium ions from the QDs. However, this method is complex and requires a long analysis time (> 5 h). More importantly, when QDs are conjugated with antibodies by covalent or noncovalent methods, antibody activity loss occurs. Moreover, the number of antibodies per QD and their orientation and position relative to the QDs are difficult to control (Medintz et al., 2004). In addition, these bioprobes

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have to be freshly prepared because antibodies require cryopreservation but QDs cannot be frozen. Therefore, challenges remain for the fabrication of new types of QD-based nanosensors for MC–LR detection with high sensitivity and high stability.

In the present study, a hapten-coupled QD nanoprobe protocol was developed for the rapid and sensitive detection of MC–LR in real water samples. QD-hapten nanoprobes were prepared by conjugating carboxyl QDs with aminoethyl-MC–LR (H_2N-etMC–LR), which is responsible for the immunological recognition of the anti-MC–LR antibody and for optical transduction. The nanosensor's sensitivity, specificity, stability, and resistance to background interferents were determined.

2. Experiments

2.1. Materials and chemicals

Bovine serum albumin (BSA), ovalbumin (OVA), and 1-ethyl-3 (-3dimethylamino-propyl)carbodiimide hydrochloride (EDC) were purchased from Sigma-Aldrich (Steinheim, Germany). MC–LR, MC–YR, MC–RR, MC–LW, and MC–LF were obtained from Alexis (Läufelfingen, Switzerland). All other reagents, unless otherwise specified, were supplied by the Beijing Chemical Agents; they were also analytical grade and used without further purification. Distilled deionized water was used throughout the investigation. About 1 mg/mL MC–LR stock solutions were prepared in 0.01 mol/L phosphate-buffered saline (PBS) and stored at 4 °C. Standard concentrations of the analyte were prepared from the stock solution by serial dilutions in 0.01 mol/L PBS.

Monoclonal anti-MC–LR-MAb (MC-LR-MAb, reference no. 8C10) was produced by our research group and labeled by Cy5.5 as previously described (Long et al., 2008).The estimated number of Cy5.5 molecules attached to each antibody determined was 2.4 according to producer's guide.

2.2. Preparation of QD nanoprobe

First, aminoethyl-MC–LR was synthesized by the reaction of 2-mercaptoethylamine with the seventh amino acid residue (dehydroAla) of MC–LR (Fig. 1A). This residue introduces a primary amino group to MC–LR and allows its easy conjugation to the QDs. The reason for choosing the seventh amino acid residue is its location, which is the farthest from both of the variable amino acid residues and from 3-amino-9-methoxy-10-phenyl-2,6,8-trimethyl-deca-4(E) 6(E)-dienoic acid (Adda). The latter plays an important role in toxicity and is recognized by most of the currently available mono-/polyclonal antibodies. A two-alkyl group was added to supply a suitable and biologically compatible spacer between the QD nanoprobe and the anti-MC–LR antibody to reduce steric hindrances for immunoreaction (Moorhead et al., 1994). QD-hapten nanoprobes were prepared by conjugating carboxyl QDs with H_2N-etMC–LR (Fig. 1B), which is responsible for the immunological recognition of the anti-MC–LR antibody and for optical transduction.

2.3. Sensing mechanism

Fig. 1C shows the competitive immunoassay mechanism for the QD-FRET-based MC–LR detection. During one detection cycle, different concentrations of MC–LR solutions, the fluorescence-labeled antibodies, and a fixed concentration of the QD nanoprobe were mixed and incubated for 5 min. During incubation, the free MC–LR in solution and the antigens immobilized onto the QD nanoprobe surface simultaneously and competitively bound with the fluorescence-labeled antibodies. Once the equilibrium state of this competitive antigen-antibody reaction was reached, the mixture was introduced into the optofluidic channel for detection. The fluorescence intensity was inversely proportional to the MC–LR concentration in the samples.

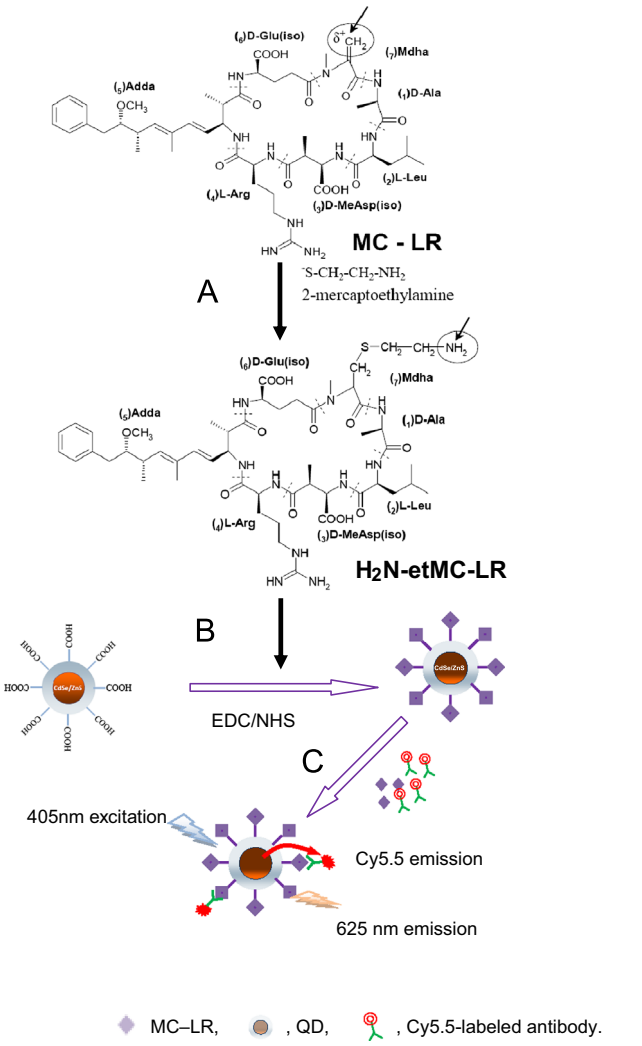

Fig. 1. (A) Synthesis of aminoethyl-MC–LR using the reaction of 2-mercaptoethylamine with the seventh amino acid residue of MC–LR; (B) Preparation of the QD nanoprobe by conjugating amine of BSA to carboxyl-coated Qdot 605 through the EDC/sulfo-NHS chemistry; and (C) Sensing mechanism of MC–LR detection based on indirect competitive immunoassay.

2.4. Instruments: Portable optofluidic platform

A portable optofluidic platform was developed to achieve rapid, on-site MC–LR detection (Fig. S3). In this system, the 405 nm, 20 mW pulse diode laser with a pigtail was used as the excited light source, whereas a single multifiber optic coupler was used for the transmission of the excitation light and the collection and transmission of fluorescence. The emission signal of the QD nanoprobe was collected by the multimode optical fiber, filtered by a bandpass filter, and then detected by photodiodes through a digital lock-in amplifier that was interfaced to a minicomputer.

3. Results and discussions

3.1. Characterization of QD-hapten nanoprobe

The QD-hapten nanoprobe was prepared by conjugating carboxyl QDs with H_2N-etMC–LR conjugate, which is regarded as