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Development of a selective fluorescence nanosensor based on molecularly imprinted-quantum dot optosensing materials for saxitoxin detection in shellfish samples

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ABSTRACT

A new type of molecularly imprinted silica layers appended to quantum dots (MIP-QDs) for saxitoxin (STX) was fabricated through the surface grafting technique. The MIP-QDs were characterized by scanning electron microscopy (SEM), transmission electron microscopy (TEM), fourier transform infrared spectroscopy (FT-IR), and imprinting and selective fluorescence quenching properties in different solutions.

Results demonstrated that the synthesized MIP-QDs exhibited excellent selective fluorescence quenching to STX because of the complementary imprinted cavities on the surface of MIP-QDs. Furthermore, a fluorescence nanosensor based on MIP-QDs was fabricated for the selective detection of STX under optimal experimental conditions. A good linear relationship in the range of 20.0-100.0 μg/L with a correlation coefficient of 0.9988 was obtained. Excellent recoveries ranging from 89.4% to 102.4% with the RSD below 6.3% were obtained for the shellfish samples at three spiked levels of STX. The detection limit of STX in shellfish samples was 0.3 μg/kg. The results indicated that the developed fluorescence nanosensor was highly selective and sensitive enough to detect STX in shellfish samples.

1. Introduction

Paralytic shellfish toxins (PSTs), mainly including saxitoxin (STX) and its analogs, are marine toxins commonly produced by genera *Alexandrium, Gymnodinium,* and *Pyrodinium* [1,2]. Among PSTs, STX is the most common toxic PST with LD_{50} value of 10.0 μg/kg in mice [3,4], which can block the neuronal transmission between nerves and muscle cell membranes by binding to the voltage-gated Na^+ channel, resulting in subsequent disturbance of neuromuscular transmission and voluntary muscle paralysis [4–6]. Particularly, STX has a strong tolerance to high temperatures and acidic environments and can not be destroyed by common treatments [7–9].

Due to the accumulation in filter-feeding bivalves and fish, the STX shows detrimental effects on human health and shellfish industry. Therefore, many countries, such as the United States, Canada, and most countries in Europe, established the STX maximum level of 80.0 μg/100 g in fresh shellfish [10], and mouse bioassay method established by Sommer and Mayer in 1937 is accepted internationally as the standard method for the quantitative measurement of PSP in shellfish according to the Association of Analytical Communities (AOAC) [11]. However, this method shows low sensitivity and accuracy and is accompanied with cumbersome operation and cultivation of animals [12,13]. Now, high-performance liquid chromatography (HPLC) coupled with fluorescence or mass spectrometry (MS) is also adapted by AOAC because they are highly sensitive and accurate [14]. However, these instrumental analysis technologies require complex preprocesses, expensive cost and professional operators. Therefore, the rapid, low-cost, and low-sample consumption sensor method on the basis of fluorescence, electrochemistry, surface plasmon resonance, and enzyme-linked immunosorbent assays for STX detection are being developed and exhibited excellent sensitivity and accuracy [15–18]. Neverthe-

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less, the major drawback of these methods is the antibody usage as recognition element, which commonly requires sophisticated production processes, experimental animals, and special storage conditions for stability [19]. Molecular imprinting is an alternative approach in designing artificial antibodies that possesses specific molecular recognition properties similar or superior to biological antibodies. These artificial antibodies had successfully applied to biomimetic sensors as recognition elements [20,21].

Meanwhile, quantum dots (QDs), a semiconductor nanoparticle, have size-dependent optical and electronic properties, narrow emission spectra, broad absorption spectra, and exhibit high resistance to photobleaching and thus can potentially improve the signal response of sensor-based MIPs [22]. In particular, the MIPs functionalize the surfaces of QDs to realize high selective fluorescence sensing. At present, molecularly imprinted silica layers appended to quantum dots (MIP-QDs) are fabricated and successfully used as recognition and response probes of sensors in detecting analytes, such as metronidazole, pesticides, toxins, and endocrine disruptors [23–26]. These sensors show special selective recognition and sensitive fluorescence signal response to template molecules. However, to the best of our knowledge, an fluorescence nanosensor based on MIP-QDs for STX detection in shellfish samples is not yet reported. The present study first fabricated MIP-QDs through surface imprinting technique and successfully constructed an fluorescence nanosensor to selectively detect STX.

2. Experimental

2.1. Materials and chemicals

STX, okadaic acid (OA), gonyautoxin (GTX), anatoxin-a (ATX), and neosaxitoxin (NEO) were purchased from the National Research Council (Halifax, NS, Canada). Tetraethyl orthosilicate (TEOS), 3-aminopropyl triethoxysilane (APTES) and triton X-100 were obtained from Sigma-Aldrich (Steinheim, Germany). QDs (CdS/CdSe/ZnS) with excitation and emission wavelengths of 270 and 618 nm, respectively, were purchased from Bedajubang Science and Technology Co., Ltd. (Beijing, China). Ammonia (25.0%-28.0%, v/v), acetone, and cyclohexane were obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). High-purity water was obtained from laboratory ultrapure water device (Ulupure, Sichuan, China). All other reagents used in experiments were of analytical grade.

2.2. Synthesis of MIP-QDs

Modified reverse micro-emulsion was performed to prepare MIP-QDs [27]. Briefly, 1.8 mL of TritonX-100 was added to 7.5 mL of cyclohexane, and the resulting mixture was magnetically stirred for 15 min. Subsequently, 400.0 μL of QDs (500.0 μg), 50.0 μL of TEOS, and 100.0 μL of ammonia (25.0%-28.0%, v/v) were sequentially added. After stirring for 2 h, 15.6 μL of STX (1.0 mg/mL, dissolved in methanol) and 22.8 μL of APTES were added to methanol (0.2 mL) and reacted with the above mixture for 12 h at room temperature. Acetone (10.0 mL) was then used to break the micro-emulsion, and the mixture was centrifuged at 8.0×10^3 g for 10 min. The pellets were washed with 6.0 mL of water to remove the unreacted TEOS and APTES. Finally, the template was extracted with ethanol/acetonitrile (8:2, v/v) until no template was detected by LC–MS. Nonimprinted silica layers appended to quantum dots (NIP-QDs) were simultaneously synthesized without the template.

2.3. Instruments and measurements

Fluorescence detection was performed by F-4600 spectrophotometer (Hitachi, Japan) equipped with a quartz cell (1 cm × 1 cm). Fourier transform infrared spectroscopic (FT-IR) measurements were conducted on a Nicolet 6700 Fourier infrared spectrometer (Thermo, USA). Scanning electron microscopy (SEM) and transmission electron microscopy (TEM) images were obtained using Model S-4800 electron scanning microscope (Hitachi, Japan) and Model H-7650 transmission electron microscope (Hitachi, Japan), respectively. The surface area and pore size distribution of MIP-QDs and NIP-QDs were analyzed using a micromeritics ASAP 2020 analyzer instrument (Norcross, GA, USA)

2.4. Fluorescence measurement

For the detection of STX, the conditions of fluorescence measurement were as follows: the slit widths of the excitation and emission were 5.0 and 10.0 nm, the excitation wavelength was set to 270 nm, and the range of the emission wavelength was set to 560–700 nm with a photomultiplier tube voltage of 700 eV. MIP-QDs were dispersed into an appropriate solution with the final concentration of 15.0 mg/mL. An appropriate volume of STX standard solution was added to the cell. The fluorescence intensity at the maximum emission wavelength of 618 nm was measured. The experiments were conducted in triplicate.

2.5. Selectivity analysis of MIP-QDs

Under optimal conditions, the selectivity of the MIP-QDs was investigated by comparing the fluorescence quenching of STX and its analogs including ATX-a, GTX, NEO, and OA. Fluorescence quenching was calculated according to the Stern-Volmer equation: $F_0/F = 1 + Ksv[Q]$ [27], where F_0 and F are the fluorescent intensities of MIP-QDs in the absence and presence of various STX concentrations, respectively, *Ksv* is the Stern-Volmer quenching constant of STX, and [Q] is the STX concentration. The imprinting factor (IF) was defined as the ratio between the *Ksv* values of MIP-QDs and NIP-QDs and used to evaluate the selectivity of the MIP-QDs [27].

2.6. Sample preparation

Shellfish samples were obtained from a local market and stored frozen until analysis. Exactly 2.0 g homogenized shellfish samples were weighed in 15.0 mL polypropylene tubes. Afterwards, 4.0 mL of acetonitrile/water/formic acid (80:20:0.1, v/v/v) were added, ultrasonicated for 10 min, and then centrifuged at 4.5×10^3 g for 10 min. The supernatant was collected and frozen at −20 °C for 1 h. After quick filtration, the filtrate was dried at N_2 atmosphere, and the residues were resuspended in 1.0 mL of water with 0.1% formic acid. Subsequently, 50.0 mg of C_{18} and 50.0 mg of acidic alumina were added, immediately vortexed for 1.0 min and centrifuged at 4.5×10^3 g for 10 min. Finally, the supernatant was filtered through a 0.22 μm nylon filter for analysis.

3. Results and discussion

3.1. Preparation of MIP-QDs based on QDs

The strategy for the synthesis of MIP-QDs based on CdS/CdSe/ZnS QDs is illustrated in Fig. 1. The MIP-QDs were synthesized through reverse microemulsion. The imprinted silica layers were successfully fabricated onto the QDs surface via the hydrolysis and condensation reaction of TEOS and APTES, which provide the $-NH_2$ surface binding sites on the QD surface and effectively improve the fluorescence stability of the QDs by inhibiting photo-oxidation. The fluorescence was quenched after adding the template but was recovered when the template was extracted. In this study, the number of initial QDs was optimized by determining the fluorescence intensity due to the nimiety or