Biosensors and Bioelectronics 255 (2024) 116269

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

journal homepage: www.elsevier.com/locate/bios

Nanozyme-assisted molecularly imprinted polymer-based indirect competitive ELISA for the detection of marine biotoxin

Chae Hwan Cho [a], Ji Hong Kim [a], Navnath S. Padalkar [a], Y. Veera Manohara Reddy [b], Tae Jung Park [c], Jinyoung Park [d], Jong Pil Park [a,*]

[a] Department of Food Science and Technology, GreenTech-Based Food Safety Research Group (BK21 Four), Chung-Ang University, Anseong, 17546, Republic of Korea
[b] Department of Chemistry, Sri Venkateswara College, University of Delhi, New Delhi, India
[c] Department of Chemistry, Institute of Interdisciplinary Convergence Research, Research Institute of Chem-Bio Diagnostic Technology, Chung-Ang University, 84 Heukseok-ro, Dongjak-gu, Seoul, 06974, Republic of Korea
[d] Department of Polymer Science & Engineering, Kyungpook National University, 80 Daehak-ro, Daegu, 41566, Republic of Korea

ARTICLE INFO

Keywords:
Molecularly imprinted polymer (MIP)
Affinity peptide
Marine toxin
ELISA
Nanozyme

ABSTRACT

Saxitoxin (STX), which is produced by certain dinoflagellate species, is a type of paralytic shellfish poisoning toxin that poses a serious threat to human health and the environment. Therefore, developing a technology for the convenient and cost-effective detection of STX is imperative. In this study, we developed an affinity peptide-imprinted polymer-based indirect competitive ELISA (ic-ELISA) without using enzyme–toxin conjugates. AuNP/Co_3O_4@Mg/Al cLDH was synthesized by calcining AuNP/ZIF-67@Mg/Al LDH, which was obtained by combining AuNPs, ZIF-67, and flower-like Mg/Al LDH. This synthesized nanozyme exhibited high catalytic activity (K_m = 0.24 mM for TMB and 132.5 mM for H_2O_2). The affinity peptide-imprinted polymer (MIP) was imprinted with an STX-specific template peptide (STX MIP) on a multi-well microplate and then reacted with an STX-specific signal peptide (STX SP). The interaction between the STX SP and MIP was detected using a streptavidin-coated nanozyme (SA-AuNP/Co_3O_4@Mg/Al cLDH). The developed MIP-based ic-ELISA exhibited excellent selectivity and sensitivity, with a limit of detection of 3.17 ng/mL (equivalent: 0.317 μg/g). Furthermore, the system was validated using a commercial ELISA kit and mussel tissue samples, and it demonstrated a high STX recovery with a low coefficient of variation. These results imply that the developed ic-ELISA can be used to detect STX in real samples.

1. Introduction

Saxitoxin (STX) is a potent neurotoxin that has a low molecular weight (299 g/mol) and belongs to the paralytic shellfish poisoning (PSP) toxin group. When humans consume STX-contaminated shellfish, such as mussels, clams, and oysters, the STX acts as a sodium channel blocker. This blockage disrupts the normal flow of sodium ions through the membranes of nerve cells, leading to paralysis and, in severe cases, respiratory failure (Campbell et al., 2011; Epstein-Barash et al., 2009; van der Merwe, 2014). Thus, the regulatory limit for STX (PSP group) in seafood is 80 μg equivalent per 100 g (Food and Drug Administration, 2011). Accordingly, detecting STX in shellfish is crucial for public health and environmental monitoring.

Traditional STX detection methods include mouse bioassay, liquid chromatography–mass spectrometry, and high-performance liquid chromatography (HPLC)–mass spectrometry (Campbell et al., 2007; Humpage et al., 2010). However, these methods have limitations, including the need for specialized training, time-consuming processes, and expensive equipment. The enzyme-linked immunosorbent assay (ELISA) has been widely employed for STX detection because of its cost-effectiveness and ease of use (McCall et al., 2019; Teepoo et al., 2019). Commercial ELISA kits for STX detection, such as Euro-Proxima®STX ELISA and ABRAXIS®Saxitoxins (PSP), offer high selectivity and sensitivity but rely on antibodies and STX–enzyme (or carrier protein) conjugates, making them complex, expensive, and unstable (Cho et al., 2019). Consequently, it is necessary to develop cost-effective and easy-to-use ELISA systems for STX detection.

Short affinity peptides and molecularly imprinted polymers (MIPs) have been widely studied as receptors for the recognition of various targets, including proteins (Agrawal et al., 2016; Cho et al., 2021, 2022;

* Corresponding author.
E-mail address: jppark@cau.ac.kr (J.P. Park).

https://doi.org/10.1016/j.bios.2024.116269
Received 8 February 2024; Received in revised form 21 March 2024; Accepted 1 April 2024
Available online 2 April 2024