Talanta 179 (2018) 646–651

Contents lists available at ScienceDirect

Talanta

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

An ultrasensitive electrochemical immunosensor for Cry1Ab based on phage displayed peptides

Xin Lu[a,b,c], Dong-jian Jiang[a,b,c], Jia-xiang Yan[a,b,c], Zhen-e Ma[a,b,c], Xiu-er Luo[c], Tai-long Wei[c], Yang Xu[a,b], Qing-hua He[a,b,*]

[a] State Key Laboratory of Food Science and Technology, Nanchang University, No. 235 Nanjing East Road, Nanchang 330047, China
[b] Sino-German Joint Research Institute, Nanchang University, No. 235 Nanjing East Road, Nanchang 330047, China
[c] School of Food Science and Technology, Nanchang University, No. 999 Xuefu Avenue, Nanchang 330047, China

ARTICLE INFO

Keywords:
Cry1Ab protein
Phage displayed peptide
Electrochemical immunosensor
Bacillus thuringiensis

ABSTRACT

The rapid and widespread adoption of *Bacillus thuringiensis* (Bt) proteins in genetically modified (GM) crops has raised concerns about the impact of GM crops on environment and food safety. A sensitive and specific method for detecting Bt proteins in GM crops is of great significance for environment and food risk assessment. In this study, using Cry1Ab as a model Bt protein, an ultrasensitive electrochemical immunosensor for Cry1Ab protein has been developed based on phage displayed peptide. Phage displayed peptides against Cry1Ab protein were obtained from a phage displayed peptide library without animal immunization process through biopanning-elution strategy. After modification of electrode with gold nanoparticles, selected phage displayed peptide was applied to electrochemical immunosensor for Cry1Ab. Under the optimized conditions, the peptide-based immunosensor showed a dynamic range of 0.01–100 ng/mL and a limit detection of 7 pg/mL. Specific measurement of this established method was conducted by testing cross-reactivity of Cry1Ac (88% amino acid sequence homology to Cry1Ab protein), and the result showed that peptide-based immunosensor has negligible cross-reactivity with analogue. In addition, the accuracy and reproducibility of this established immunosensor was evaluated by testing the recovery of spiked samples and assay coefficients of variation, respectively. The results showed that the average recovery of corn and wheat sample was 90–120% and 86.7–120%, respectively; the intra-assay coefficient of variation was 7.4% (n = 6), and the inter-assay coefficient of variation was 6.9% (n = 6) at 1 ng/mL Cry1Ab solution. Furthermore, the novel concept of peptide-based immunosensor may provide a potential application in general method for the ultrasensitive detection of various Bt proteins.

1. Introduction

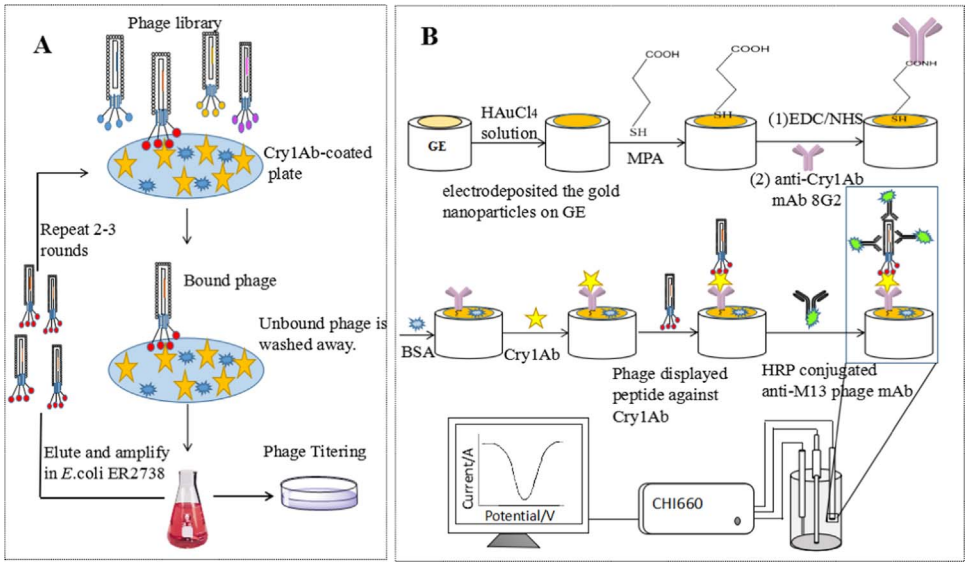
Bacillus thuringiensis (Bt) is a spore-forming bacterium with one of its most important features being the formation of intracellular inclusions comprised of protein protoxins (δ-endotoxins). These are very effective and highly specific biological insecticides [1–3]. To date, researchers use modern biotechnology to introduce insect-resistant gene into the plant genome to achieve the purpose of improving plant insect resistance. Cry1Ab genes have been widely introduced into genetically modified (GM) crops, such as corn, potato, cotton and rice, whose planting areas have increased every year globally [4–6]. However, the increasing areas of GM crops also bring safety concerns surrounding the potential risks of GM crops to human health and nature. In many countries, the labeling of grains, feed and foodstuffs is mandatory if the GMO content exceeds a certain threshold level [7,8].

Therefore, the qualitative and quantitative determination of transgenic components in products is an urgent problem to be solved. Currently, the detection of transgenic products is mainly focused on nucleic acid and protein analyses. The most common method based on genetic level is the polymerase chain reaction assay (PCR) [8–10]. For example, Kumar et al. [11] established a real-time immuno-PCR (IPCR) assay method for Cry1Ab with minimum detection limit of 100 pg/mL; Kqueen et al. [12] proposed a multiplex-PCR for Cry1Ab and EPSPS genes in soy and maize samples and 70.0% samples were found containing Cry1Ab or EPSPS genes. On the other hand, another common method for Bt protein detection is based on immunoassays such as ELISA [13,14], Western-blot [15], test strip method [16,17]. In addition, new methodologies have been developed, including the use of microarray [18], and Surface Plasmon Resonance [19] , loop-mediated isothermal amplification (LAMP) [20,21].

The core of immunological analysis is the specific identification and binding of antigen and antibody, so the preparation of antibodies

* Corresponding author at: State Key Laboratory of Food Science and Technology, Nanchang University, No. 235 Nanjing East Road, Nanchang 330047, China.
E-mail address: heqinghua@ncu.edu.cn (Q.-h. He).

https://doi.org/10.1016/j.talanta.2017.11.032
Received 3 September 2017; Received in revised form 30 October 2017; Accepted 16 November 2017
Available online 22 November 2017
0039-9140/



Scheme 1. The schematic representation of biopanning of phage displayed peptides against Cry1Ab protein (A); the schematic representation of electrochemical immunosensor formation for Cry1Ab detection based on phage displayed peptide (B).

specifically binding to the antigen becomes the prerequisite and the core of the development of immunoassays. To date, antibody research has been developing rapidly in recent years, ranging from traditional polyclonal and monoclonal antibodies to genetically engineered antibodies represented by single chain antibodies, phage display antibodies, and single domain heavy chain antibodies [22–26]. For example, Zhang et al. [27] developed scFvs against Cry1B toxin and the linear range of detection for standards in an indirect competitive enzyme-linked immunosorbent assay (ic-ELISA) was approximately 0.19–1.1 mg/mL and 50% inhibition of control (IC_{50}) was 0.84 mg/mL for Cry1B; Zhu et al. [28] obtained anti-Cry1Ab nanobody, and the sensitivity of nanobody-based electrochemical immunoassay method with a limit of detection value 0.07 ng/mL. Furthermore, with the development of synthetic materials technologies, numerous non-immunoglobulin-based affinity materials which remain the antigen-binding elements have also been reported in recent years. Compared to conventional antibodies, these synthetic materials (e.g. aptamer, molecular imprinted polymers, peptide) can be obtained without animal immunization process, and have the benefits include greater stability, rapid preparation, low cost of production, improved tolerance to solvents or extremes of pH or ionic strength [29–32].

Phage displayed peptides have many advantages, such as good biological activity, high affinity, low cost, infinite amplification, which show that phage displayed peptides as promising immunoassay recognition molecules will have a very wide range of application prospects. A vast repertory of candidate peptides can be expressed in phage displayed peptide libraries, where randomly generated amino acid sequences are genetically fused to coat proteins of the filamentous phage M13 of the fd family [33]. Moreover, the phage displayed peptide contained multiple capsid protein copies (about 2700 copies of pVIII). When constructing a signal probe with this structural advantage and used for immunoassays, the markedly amplification effect for the specific signal of the target molecule will be generated [34,35].

In this study, using Cry1Ab as a study model, anti-Cry1Ab phage displayed peptides were obtained from a phage displayed peptide library by biopanning-elution strategy. The anti-Cry1Ab phage displayed peptide was used as recognition element and applied to electrochemical immunoassay for detection of Cry1Ab protein. The approach to develop highly sensitive phage displayed peptide based electrochemical immunoassay for Cry1Ab has not been reported to the best of our knowledge. Furthermore, the novel concept might provide potential applications to a general method for the electrochemical immunoassay of various Bt proteins.

2. Materials and methods

2.1. Chemicals and reagents

The Ph.D.-12 Phage Display Peptide Library Kit was purchased from New England Biolabs (Beverly, MA, USA). Cry1Ab, Cry1Ac protein were obtained from Youlong Co., Ltd. (Shanghai, China). The HRP-conjugated anti-M13 phage antibody was from GE Healthcare Inc. (Piscataway, NJ, USA). Bovine serum albumin (BSA), ovalbumin (OVA), 5-bromo-4-chloro-3-indolyl-β-D-galactoside (X-gal), isopropylthio-β-D-galactoside (IPTG), 1-ethyl-3-(3-dimethylamino-propyl) carbodiimide (EDC), N-hydroxysuccinimide (NHS), 3-mercaptopropionic acid (3-MPA), and gold(III) chloride hydrate were obtained from Sigma Aldrich Co. (St. Louis, MO, USA). 2-(N-morpholino) ethanesulfonicacid (MES), Pyrocatechol (H_2Q) and Hydrogen peroxide (H_2O_2) were purchased from Aladdin (Shanghai, China). The anti-Cry1Ab monoclonal antibodies (mAb) were prepared in our laboratory. All organic solvents and inorganic chemicals were of analytical grade and were prepared using ultra-pure water.

2.2. Apparatus

Electrochemical measurements, including cyclic voltammetry (CV), electrochemical impedance spectroscopy (EIS) and square wave voltammetry (SWV), were performed on a computer-controlled CHI660E electrochemical workstation (Shanghai CH Instrument Co., China). A three-electrode system (Shanghai CH Instrument Co., China) was used, including fabricated gold electrode (CHI101, 2 mm in diameter), platinum wire electrode (CHI115), and saturated calomel electrode (CHI150), which was used as working electrode, counter electrode and reference electrode, respectively. All measurements were conducted at room temperature.

2.3. Biopanning of phage displayed peptide against Cry1Ab

Biopanning of phage displayed peptide against Cry1Ab was performed according to the instruction manual of peptide library kit with some modification (Scheme 1A). Briefly, in the first round of biopanning, 100 μL of Cry1Ab protein (30 μg/mL) in 0.01 M phosphate-buffered saline (PBS, pH = 7.2) was added to microtiter plate wells and incubated overnight at 4 °C with gentle agitation in a humidified container. To eliminate the nonspecific binding sites, Cry1Ab coated wells were blocked with 300 μL of PBS containing 3% BSA for 1 h at 37 °C. After discarding the blocking solution and washing six times with PBST