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Review article

Production and characterization of a biotinylated single-chain variable fragment antibody for detection of parathion-methyl

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ABSTRACT

In this article, we reported the development of a biotinylated single-chain variable fragment (scFv) antibody based indirect competitive enzyme-linked immunosorbent assay (IC-ELISA) for parathion-methyl (PM) detection. Firstly, a phage display library was generated using a pre-immunized BALB/C mouse against a specific hapten of PM. After four rounds of panning, the scFv gene fragments were transferred into a secreted expression vector. Then, the scFv antibodies were secreted expressed and screened by IC-ELISA against PM. The selected scFv antibody was fused with a biotin acceptor domain (BAD) and inserted into pET-28a(+) vector for high-level expression in *Escherichia coli* BL2 (DE3). After optimizing expression conditions, the scFv-BAD antibody was expressed as a soluble protein and biotinylated *in vitro* by the *E. coli* biotin ligase (BirA). Subsequently, the biotinylated scFv-BAD antibody was purified with a high yield of 59.2 ± 3.7 mg/L of culture, and was characterized by SDS-PAGE and western blotting. Finally, based on the biotinylated scFv-BAD, a sensitive IC-ELISA for detection of PM was developed, and the 50% inhibition value (IC_{50}) of PM was determined as 14.5 ng/mL, with a limit of detection (LOD, IC_{10}) of 0.9 ng/mL. Cross-reactivity (CR) studies revealed that the scFv antibody showed desirable specificity for PM.

Contents

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1. Introduction

Parathion-methyl (*O,O*-dimethyl-*O*-4-nitrophenyl phosphorothioate, PM), which is widely used as an insecticide and acaricide to control many insect pests of agricultural products, is a highly toxic insecticide in EPA (United States Environmental Protection Agency) toxicity class I. It could inhibit acetylcholinesterase activity in erythrocytes and brain even at a low concentration [1]. Due to its high toxicity and extensive application, PM has been classified as a restricted pesticide with a strict maximum residue limited (MRLs) standard in many countries [2].

For analysis of PM, some traditional methods, such as gas chromatography (GC) and high-performance liquid chromatography (HPLC), have been successfully developed due to their high sensitivity and reliability [3,4]. However, these classical methods require high cost, skilled analysts, and time-consuming sample preparation steps. Electroanalytical sensors exhibited high sensitivity but showed poor selectivity in determination of practical samples, which limited the application and promotion of electroanalytical methods [5]. Compared with these analysis strategies, immunoassay technology is gaining acceptability as a simple, cost-effective screening method for pesticides analysis in many samples [6,7]. Several immunoassay methods based on polyclonal antibodies (PAbs) and monoclonal antibodies (MAbs) for PM detection have been reported [8–10]. But PAbs sometimes react non-specifically while the preparation of MAbs is time consuming and costly. Recently, the production of recombinant antibodies (RAbs), such as single-chain variable fragment (scFv), has been regarded as an alternative way to obtain low-cost antibodies with desirable affinity and specificity [11–13]. An increasing numbers of scFv antibodies against small molecule contaminants, such as pesticides, fungal toxins, clenbuterol and some other food pollutants, have been produced [14–16].

The low affinity of the scFv antibody always hindered its application [17]. But for the pesticide residue analysis, an alternative approach is improving the affinity of the detection tag of the scFv antibody. Biotin which could be detected by biotin–streptavidin system with extremely strong affinity will be an appropriate tag. For biotinylation of scFv antibody, compared with the random modification of chemical methods, *Escherichia coli* biotin ligase (BirA) which could catalyze the site-specifically attachment of biotin to a 15 amino acid acceptor peptide (known as Avi-tag) is preferable [18].

In this paper, we selected a scFv antibody against PM from a pre-immunized phage display library. The scFv was fused with a biotin acceptor domain (BAD) and high-level expressed in *E. coli* BL21 (DE3) as soluble protein. Then, the fused protein was biotinylated *in vitro* by BirA before purification. The purified biotinylated scFv-BAD antibody was characterized by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS–PAGE) and western blotting. Based on the biotinylated scFv-BAD, a sensitive indirect competitive enzyme-linked immunosorbent assay (IC-ELISA) for detection of PM was developed.

2. Materials and method

2.1. Materials and instruments

Pesticides were purchased from National Standards. (China). *E. coli* BL21 (DE3) and plasmid pET-28a(+) were preserved in our laboratory. Phagemid vector pIT2, *E. coli* TG1 and M13KO7 helper phage were purchased from Amersham Biosciences (Germany). Horseradish Peroxidase-labeled goat anti-mouse immunoglobulin (IgG-HRP), horseradish peroxidase-labeled streptavidin (SA-HRP), complete Freund's adjuvants, incomplete Freund's adjuvants, isopropyl-β-D-thiogalactoside (IPTG), 3,3′-diaminobenzidine (DAB) and tetramethylbenzidine (TMB) were purchased from Sigma Chemical Co. (St. Louis, USA). RNAiso Plus Kit, Prime ScriptII 1st Strand cDNA Synthesis Kit, dNTP, Prime STAR®GXL DNA Polymerase, T4 DNA ligase and restriction enzymes were purchased from Takara Biotechnology Co. Ltd. (Dalian, China). Ni-IDA agarose was purchased from GenScript. (Nanjing, China). All chemical reagents were analytical grade.

Polystyrene 96-well microtiter plates were purchased from Costar. (Corning, MA, USA). ELISA plates were washed with the KHB ST-36W plate washer (Shanghai, China), and well absorbance was determined with the Model 680 plate reader (Bio-Rad, USA).

2.2. Mice immunization

The specific hapten of PM was synthesized and characterized as the previous study [8]. The structures of hapten and PM are shown in Fig. 1. The hapten was covalently attached to bovine serum albumin (BSA) or ovalbumin (OVA) to use as immunogen (hapten-BSA) or coating antigen (hapten-OVA) by active ester method [19]. Five BALB/C female mice (6–8 weeks) were immunized intraperitoneally with 1 mg/mL of immunogen in 100 μL phosphate-buffered saline (PBS, 10 mM, pH 7.4) mixed with 100 μL complete Freund's adjuvant. Immunizations were repeated two times with incomplete Freund's adjuvant at two-week intervals. One week after the last boost, the titer of each mouse was determined by noncompetitive ELISA, the spleen cells of the mouse which showed the highest titer were harvested and used for total RNA preparation by RNAiso Plus Kit according to the manufacturer's instructions.

2.3. Construction and panning of phage-display library

The cDNA was synthesized by PrimeScript II 1st Strand cDNA Synthesis Kit and used as template for PCR amplification of variable heavy (VH) and light (VL) chain genes. The primers and PCR procedures are the same as previously described [20]. The VL and VH genes were then assembled by SOC-PCR to obtain the scFv genes. The scFv gene fragments were purified by gel extraction kit, digested by *Sfi* I, inserted into pIT2-P phagemid and electro-transformed into *E. coli* TG1 to obtain the antibody library. The phagemid pIT2-P (Fig. 2B), which contain a tetracycline resistance gene (Tet) and two *Sfi* I sites between the pelB and full length gIII gene was transformed from pIT2 (Fig. 2A). The antibody library was