Anti-Ciprofloxacin ScFv in Animal-Derived Food

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Ciprofloxacin (CIP) is a synthetic third-generation fluoroquinolone (FQ) antibiotic that has been developed and is widely used to treat bacterial infections in humans and animals. This antibiotic exerts effects by inhibiting DNA gyrase or topoisomerase II in susceptible bacteria and exhibits high activity against a broad spectrum of Gram-negative and Gram-positive bacteria. However, the unreasonable and extensive use of antibiotics has resulted in the potential for residual antibiotics in food of animal origin, which can damage multiple systems in the body and cause bacterial resistance. Therefore, the European Union, the Joint FAO/WHO Expert Committee on Food Additives (JECFA, Rome, Italy) and China established maximum residue limits of CIP in animal-derived food to prevent the accumulation of antimicrobial residues, e.g., 100 μg/kg in milk and meat.

scFv ciprofloxacin recognition mechanism directional mutagenesis IC-ELISA

1. Overview

An immunized mouse phage display scFv library with a capacity of 3.34×10^9 CFU/mL was constructed and used for screening of recombinant anti-ciprofloxacin single-chain antibody for the detection of ciprofloxacin (CIP) in animal-derived food. After four rounds of bio-panning, 25 positives were isolated and identified successfully. The highest positive scFv-22 was expressed in *E. coli* BL21. Then, its recognition mechanisms were studied using the molecular docking method. The result showed the amino acid residue Val160 was the key residue for the binding of scFv to CIP. Based on the results of virtual mutation, the scFv antibody was evolved by directional mutagenesis of contact amino acid residue Val160 to Ser. After the expression and purification, an indirect competitive enzyme-linked immunosorbent assay (IC-ELISA) based on the parental and mutant scFv was established for CIP, respectively. The IC50 value of the assay established with the ScFv mutant was 1.58 ng/mL, while the parental scFv was 26.23 ng/mL; this result showed highly increased affinity, with up to 16.6-fold improved sensitivity. The mean recovery for CIP ranged from 73.80% to 123.35%, with 10.46% relative standard deviation between the intra-assay and the inter-assay. The RSD values ranged between 1.49% and 9.81%. The results indicate that we obtained a highly sensitive anti-CIP scFv by the phage library construction and directional evolution, and the scFv-based IC-ELISA is suitable for the detection of CIP residue in animal-derived edible tissues.

2. Phage Display Technology

Ciprofloxacin (CIP) is a synthetic third-generation fluoroquinolone (FQ) antibiotic that has been developed and is widely used to treat bacterial infections in humans and animals. This antibiotic exerts effects by inhibiting DNA gyrase or topoisomerase II in susceptible bacteria and exhibits high activity against a broad spectrum of Gram-negative and Gram-positive bacteria [1]. However, the unreasonable and extensive use of antibiotics has resulted in the potential for residual antibiotics in food of animal origin, which can damage multiple systems in the body [2][3] and cause bacterial resistance [4][5]. Therefore, the European Union, the Joint FAO/WHO Expert Committee on Food Additives (JECFA, Rome, Italy) and China established maximum residue limits of CIP in animal-derived food to prevent the accumulation of antimicrobial residues, e.g., 100 μg/kg in milk and meat.

By now, many physicochemical methods have been reported for the detection of residues of FQs in foods of animal origin. These analytical methods are highly sensitive and dependable; however, such methods require specialized instrumentation, trained personals, and are time consuming. They are unsuitable for the rapid evaluation of large numbers of samples. Immunoassays, especially the indirect competitive enzyme-linked immunosorbent assay (IC-ELISA), which is based on the principle that antibodies specifically bind to antigens, are considered the most reliable method for detecting antibodies [6][7]. In previous studies, researchers have developed IC-ELISA based on monoclonal antibodies (MAbs) to determine fluoroquinolone in food of animal origin [8][9][10]. Although ELISA is a mature and widely used method, it has many rigorous programs for preparing traditional antibodies (PAbs and MAbs) from antigen-immunized animals [11]. Hence, a simple, rapid, and effective technology for preparing novel antibodies must be developed.

The development of gene engineering techniques facilitated the production of various gene recombinant antibodies, and single-chain variable fragment (scFv) is the most popular format of recombinant antibody that has been successfully constructed by assembling the variable-heavy (VH) region and light chain (VL) domain of an antibody with a flexible linker [12]. The intrinsic properties of scFv antibodies can be improved by various mutagenesis techniques [13]. The recognition property of an scFv antibody can be evolved in vitro [14]. For the evolution of the scFv antibody, its recognition mechanism should be studied first, and binding sites, contact amino acids, and intermolecular forces should be determined [15]. In recent years, molecular docking has been used in analyzing the interactions between ligands and scFv antibodies, and random mutagenesis and site-directed mutagenesis have been used in obtaining scFv mutants [16][17].

Phage display technology (PDT) is the integration of foreign genes into specific coat protein genes of phage and fusion, with coat protein to promote ligand recognition and binding [18][19]. It is considered to be the most suitable technology for the production of single-chain antibodies. The phage antibody library uses genetic engineering methods to amplify VH and VL genes. After random combination, it is inserted into the phage coat protein gene and fused and expressed on the surface of the phage [20]. Specific single-chain antibodies are obtained through specific panning, which is extensively used for preparing antigen-specific artificial antibodies in biomedicine, environmental pollutants analysis, and food safety detection fields. For example, Xu et al. [21] and Zhao et al. [22] obtained the broad-specificity domain antibodies for Bt Cry toxins and pyrethroid pesticides by rounds of specific phage library biopanning, respectively, which are all based on phage antibody library technology.

3. Conclusions

A highly sensitive anti-CIP single-chain antibody was obtained through phage display and directional evolution, and a rapid and highly sensitive IC-ELISA method for detecting CIP residues in products of animal origin was developed. The method showed good stability, reproducibility, and accuracy for detecting CIP, indicating a wide application prospect for the rapid and sensitive detection of antibiotic residues in animal-derived food.

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