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Production of a soluble single-chain variable fragment antibody against okadaic acid and exploration of its specific binding

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ABSTRACT

Okadaic acid is a lipophilic marine algal toxin commonly responsible for diarrhetic shellfish poisoning (DSP). Outbreaks of DSP have been increasing and are of worldwide public health concern; therefore, there is a growing demand for more rapid, reliable, and economical analytical methods for the detection of this toxin. In this study, anti-okadaic acid single-chain variable fragment (scFv) genes were prepared by cloning heavy and light chain genes from hybridoma cells, followed by fusion of the chains via a linker peptide. An scFv–pLIP6/GN recombinant plasmid was constructed and transformed into *Escherichia coli* for expression, and the target scFv was identified with IC–CLEIA (chemiluminescent enzyme immunoassay). The IC_{15} was 0.012 ± 0.02 μg/L, and the IC_{50} was 0.25 ± 0.03 μg/L. The three-dimensional structure of the scFv was simulated with computer modeling, and okadaic acid was docked to the scFv model to obtain a putative structure of the binding complex. Two predicted critical amino acids, Ser32 and Thr187, were then mutated to verify this theoretical model. Both mutants exhibited significant loss of binding activity. These results help us to understand this specific scFv–antigen binding mechanism and provide guidance for affinity maturation of the antibody in vitro. The high-affinity scFv developed here also has potential for okadaic acid toxin detection.

Okadaic acid (OA) is a lipophilic marine algal toxin that was first extracted from the sponges *Halichondria okadai* and *Halichondria melanodocia*. Later it was found to be the same toxin as that isolated from the dinoflagellate *Prorocentrum* and *Dinophysis* spp. OA is one of the major toxins responsible for diarrhetic shellfish poisoning (DSP) and can accumulate in shellfish that feed on toxigenic algae [1,2]. During the past few decades, the frequency, intensity, and geographic distribution of harmful algal blooms have increased along with the number of toxins found in the marine food chain [3], The outbreaks of DSP caused by shellfish contaminated with OA are of worldwide public health concern and pose an economic hazard to global shellfish industries. Therefore, it is very important to develop rapid analytical methods for the detection of this toxin.

Current detection techniques for OA can be classified into three categories: biological methods, physicochemical methods, and biochemical methods. Biological methods have been almost completely replaced by alternative methods owing to insufficient accuracy. Physicochemical methods such as spectrophotometry [4], high-performance liquid chromatography (HPLC) [5,6], and capillary electrophoresis [7] are commonly used to detect OA and its related derivatives. However, these analytical procedures are time-consuming and complex and require trained personnel. Moreover, the instrumentation required for these methods is not suitable for on-site measurement. Therefore, there is a growing demand for more rapid, reliable, and economical methods for the qualification and quantification of OA residues.

Enzyme-linked immunosorbent assay (ELISA) is becoming increasingly popular as a screening methodology to meet the demand for testing. It is capable of surveying larger numbers of samples than can be accomplished with conventional analyses. In many cases, it also exhibits high sensitivity and lower detection limits.

Abbreviations used: OA, okadaic acid; DSP, diarrhetic shellfish poisoning; ELISA, enzyme-linked immunosorbent assay; scFv, single-chain variable fragment; CDR, complementarity-determining region; PDB, Protein Data Bank; PCR, polymerase chain reaction; AP, alkaline phosphatase; IPTG, isopropyl β-D-1-thiogalactopyranoside; SDS–PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis; OVA, ovalbumin; PBS, phosphate-buffered saline; PBST, PBS containing 0.1% Tween 20; IC_{50}, concentration required for 50% inhibition of binding; CLEIA, chemiluminescent enzyme immunoassay; DS, Discovery Studio; MD, molecular dynamics; LOD, limit of detection; RMSD, root mean square deviation.

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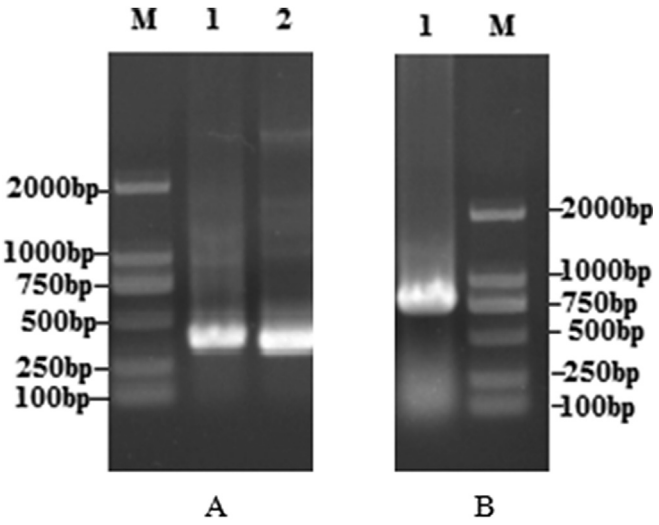

Fig.1. Agarose gel electrophoresis of VH, VL, and scFv genes of OA. (A) PCR amplification of VH and VL. Lane M: marker 2000; lane 1: VH genes; lane 2: VL genes. (B) PCR amplification of scFv genes. Lane M: marker 2000; lane 1: scFv genes.

Most of the previously reported immunoassays for OA are based on conventional polyclonal or monoclonal antibodies. However, both types of antibodies require labeling with enzymes by means of chemical methods, which introduces several drawbacks such as the low stability and safety of the reagents used for labeling. Single-chain variable fragment (scFv) antibodies belong to the class of third-generation antibodies. During recent years, with advances in molecular biology techniques, it has become possible to produce scFvs in bacterial culture using recombinant DNA technology [8,9]. Compared with monoclonal and polyclonal antibodies, the cost of scFv production is very low. In addition, scFvs can be produced in batches and fused with a marker molecule for effective immunological detection. Therefore, the objective of this study was to construct a high-affinity scFv against OA with the potential for toxin detection.

In the past, scFvs have often suffered from low binding affinities compared with macromolecular antigens, although the study of the binding mechanism of scFv with a particular antigen can assist in improving its binding affinity. However, successful protein

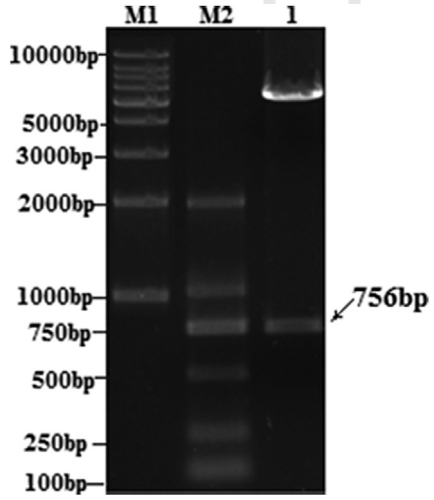

Fig.2. Double enzyme validation for scFv–pLIP6/GN plasmid. Lane M1: marker 10,000; lane M2: marker 2000; lane 1: products of double enzyme.

MAQVKLQESGGGLVKSGGSLKPSCVASGFTFSTYAMSMVRQTPEKRLEWVAS
H-CDR1
ITLGGRYTQSPDSVKGRFTISRDDGKNTLYLQMSSLRSEDTAIYYCARDNRVT
H-CDR2 H-CDR3
MRYFDAWGAGTTVTVSSGGGGSGGGGSGGGGSDIELTQSPAIMSASPGEKVT
Linker
MTCSASSSVNYLHWHQQKSSTSPKLWIYDTSHLASGVPGRFSGSGSGNSYSLT
L-CDR1 L-CDR2
ISSMEAEDVATYYCFQGSGYPYTFGDQAGNQT
L-CDR3

Fig.3. Sequence of anti-OA scFv and partition.

crystallization of anti-OA antibody has not yet been achieved. Thus, there is limited knowledge of the mechanism of the molecular recognition of this specific antigen, and hapten design and immunoassay development have largely been based on trial and error [10], which has restricted progress. During recent years, with the help of computational biology, it has become possible to study receptor–ligand interactions using in silico molecular docking. Antibodies possess several highly conserved framework regions and complementarity-determining regions (CDRs), but the folds adopted by most of the CDR loops are restricted to a few main chain conformations, called canonical structures [11], and a great number of antibody crystal structures have been made available in the Protein Data Bank (PDB). Therefore, these known crystal structures can be used as templates to model a target antibody that has not yet been crystallized, and previous research has proven that homology modeling of a protein structure can yield data with reasonably high confidence levels [12]. Using docking programs, the binding complex and binding interaction can also be modeled where homologous structures are available. Several successful antibody affinity maturation approaches have been reported based on artificial intelligent design, and this is a promising, fast, and efficient technique for enhancing the affinities of antibodies in vitro [13].

Materials and methods

Materials

OA was purchased from Hebei Aquatic Science and Technology Development (Hebei, China). GoScript reverse transcription system was purchased from Promega (USA). PGEM-T-Easy plasmid was purchased from Promega. *Escherichia coli* strain JM109 was purchased from Solarbio Science & Technology (Beijing, China). The oligonucleotide primers [14] were synthesized by Invitrogen and used for polymerase chain reaction (PCR) cloning of the variable

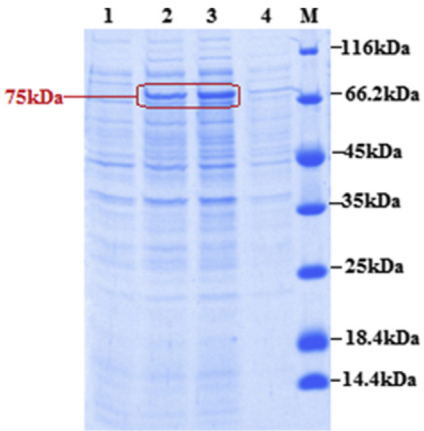

Fig.4. SDS–PAGE analysis of the expressed products. Lane M: marker 116 kDa; lane 1: the precipitation; lane 2: the supernatant; lane 3: after the induction; lane 4: before the induction.

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