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A tetravalent single-chain variable fragment antibody for the detection of staphylococcal enterotoxin A

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Staphylococcal enterotoxin A (SEA) synthesized by *Staphylococcus aureus* is a foodborne and heat-stable toxin, which is a great threat to human health (Pexara et al., 2010). Highly sensitive antibodies are a key factor in the immunological detection of SEA, which is one of the most effective ways to detect SEA because of its accuracy, agility, and efficiency (Nouri et al., 2018). In this study, we constructed a tetravalent anti-SEA antibody gene by linking the tetramerization domain of human p53 to the C-terminus of the anti-SEA single-chain variable fragment (scFv), which was then transformed into *Escherichia coli* BL21 (DE3) for the production of a SEA-specific tetravalent antibody. Successful expression of the tetravalent antibody was determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and western blot. An indirect non-competitive enzyme-linked immunosorbent assay (ELISA) revealed that the tetravalent antibody exhibited SEA-specific binding activity. A sandwich ELISA demonstrated that compared to the scFv monomer, the tetravalent antibody was more sensitive in detecting SEA. Molecular docking analysis revealed that the SEA interacted with the scFv mainly on the opposite side of the residue linked to p53. Thus, this study indicated that genetically engineered tetramerization is a potential way to improve the sensitivity of SEA-specific scFv.

Among staphylococcal enterotoxins (SEs), SEA is the most frequent type recovered from foodborne outbreaks. It causes several symptoms, including but not limited to nausea, vomiting, diarrhea, cramps, and abdominal pain (Pinchuk et al., 2010). The close connection between SEA and human infections was confirmed by genome analysis in our study. Genome sequences were downloaded from the United States National Center for Biotechnology Information (NCBI) for *S. aureus* strains whose complete genome sequences are available, and used to determine whether SEA was produced in these strains. A total of 525 strains were tested and 85 of them (16.2%) were SEA-positive (Fig. 1a). Next, we determined the host information of these SEA-producing strains and found that 62 of them (72.9%) were involved in human infections (Fig. 1b). Detailed host information on SEA-producing strains is shown in Fig. 1c. These results indicated that SEA-producing strains were highly risky for humans. Given that SEA is a kind of heat-stable toxin and still has ability to induce severe symptoms in the digestive tract after ingestion (Ortega et al., 2010), accurate detection is undoubtedly important.

The first and most critical step for the immunological detection of toxins is obtaining stable and highly sensitive antibodies and producing these antibodies more quickly (O'Kennedy, 2019). For this study, we chose the multimerization peptide of human tumor suppressor protein p53 to fuse with the *scFv* gene obtained in our previous research and construct the tetravalent antibody against SEA (Chen et al., 2014). The anti-SEA monoclonal antibody (mAb) used for generating the scFv was also obtained from our previous study (Liang et al., 2011) and preserved in our laboratory. The coding regions of the fusion plasmids are shown in Fig. 2a and the schematic diagram of the tetravalent scFv antibody assembly is represented

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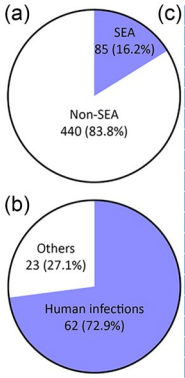
in Fig. 2b. The amplified *scFv* and *p53* gene fragments were digested separately with corresponding restriction enzymes and then were ligated with digested pET-22b plasmid. The successful construction of the pET-22b-scFv/p53 plasmid was confirmed by polymerase chain reaction (PCR) and sequencing (Fig. 2c). The amino acid sequence of anti-SEA scFv revealed that it contains a variable heavy (VH) chain and a variable light (VL) chain, which are connected by a peptide linker (Fig. 2d). Each chain contains three complementarity-determining regions (CDRs) (Fig. 2d), which play a vital role in specific antibody binding (Polonelli et al., 2008).

The constructed expression vector pET-22b-scFv/p53 was then transformed into *E. coli* BL21 (DE3) for protein expression. The soluble target protein was at its highest concentration when isopropyl β-D-1-thiogalactopyranoside (IPTG) concentration was 1 mmol/L and the temperature was 16 °C (data not shown). SDS-PAGE analysis demonstrated that the constructed plasmid expressed an obvious protein band with a relative molecular weight of 30 kDa (Fig. 3a). Western blot yielded two detectable protein bands around 30 and 60 kDa, corresponding to the monovalent products and bivalent form of the antibody, respectively (Fig. 3b). The recombinant protein was purified by metal affinity chromatography using Ni-nitrilotriaceate (Ni-NTA), and the concentration of purified protein was quantified by Bradford assay. The typical yield of nickel resin-purified target protein was about 3.6 mg/L of expression media. The purified protein samples were loaded in the non-reducing buffer and treated at different temperatures (room temperature, 60 and 100 °C) for 10 min. Then they



(c)

Name	ILRI_Eymole1/1	PMB 81-4	PMB196-1	64	85	199	277	16445	5_3949	9_LA_281
Host	Camel	Cattle	Cattle	Human	Human	Human	Human	Human	Human	Human
Name	BPH2003	BPH2019	BPH2056	BPH2869	BPH2947	CMRSA-6	DAR4145	FDAARGOS_2	FDAARGOS_25	FDAARGOS_30
Host	Human	Human	Human	Human	Human	Human	Human	Human	Human	Human
Name	FDAARGOS_35	FDAARGOS_40	FDAARGOS_412	FDAARGOS_42	FDAARGOS_43	FDAARGOS_47	FORC_045	GD487	GD705	H489
Host	Human	Human	Human	Human	Human	Human	Human	Human	Human	Human
Name	JKD6004	JKD6004-DR	JKD6009	Lr6	M2024	MRSA252	MSSA476	NCTC11940	NCTC11965	NCTC13811
Host	Human	Human	Human	Human	Human	Human	Human	Human	Human	Human
Name	Newman_D2C	NYU_Newman	OC8	R50	Sa_Newman_UoM	SAW1	SR153	ST20130944	ST20130945	SVH7513
Host	Human	Human	Human	Human	Human	Human	Human	Human	Human	Human
Name	T0131	UP_1033	UP_1395	UP_1452	UP_1559	UP_1591	UP_1654	UP_248	UP_764	UP_818
Host	Human	Human	Human	Human	Human	Human	Human	Human	Human	Human
Name	USA400-0051	V521	WCH-SK2	WCUH29	Z172	M48	O268	2395 USA500	AR_0219	CFSAN007847
Host	Human	Human	Human	Human	Human	Pig	Sheep	/	/	/
Name	CFSAN007851	ED133	FDAARGOS_766	FORC_001	JKD6008	MRSA107	Mu3	Mu50	MW2	NCTC12233
Host	/	/	/	/	/	/	/	/	/	/
Name	NCTC13277	NCTC13394	ST228	Tokyo12480	TW20					
Host	/	/	/	/	/					

Fig. 1 Host distribution of staphylococcal enterotoxin A (SEA)-producing *Staphylococcus aureus* strains. (a) Percentage of SEA-producing *S. aureus* strains; (b) Host distribution of SEA-producing *S. aureus* strains; (c) Detailed host information on SEA-producing *S. aureus* strains.

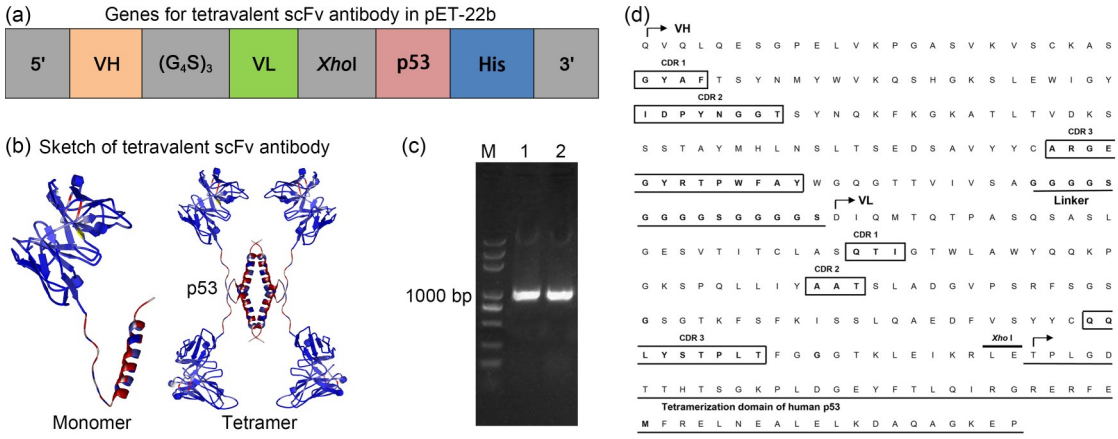


Fig. 2 Genetic components of pET-22b-scFv/p53 plasmid. (a) Constitution of scFv/p53 fragment. (b) Schematic diagram of scFv tetramer assembly. (c) Amplification of scFv/p53 fragment using the recombinant pET-22b-scFv/p53 plasmid. M: marker; Lanes 1 and 2: recombinant plasmid. (d) Amino acid sequence of recombinant pET-22b-scFv/p53 plasmid. scFv: single-chain variable fragment.

were analyzed by SDS-PAGE. The purified protein existed mainly in the form of tetramers and dimers (protein bands at around 120 and 60 kDa which are consistent with the theoretical values) rather than monomers (Fig. 3c). Howeve, when the protein samples were treated with 1% (volume fraction) β-mercaptoethanol, the tetravalent antibodies were reduced from tetramer to dimer form regardless of treatment temperatures (Fig. 3c). These results indicated that the tetramer form could be easily reduced down to dimer form by β-mercaptoethanol but was relatively stable at different temperatures. The tetravalent antibodies were then concentrated and verified by SDS-PAGE (Fig. 3d).

The reactivity of the genetically engineered antibody after purification against SEA was tested by indirect ELISA (iELISA), which revealed that the tetravalent scFv antibody could specifically bind to immobilized SEA in a concentration-dependent manner (Fig. 4a). This result was in accordance with the SEA-binding activity observed in the scFv monomeric form (without binding to p53) (Chen et al., 2014). It should be pointed out that in the previous study we tested the specificity of the scFv monomer and found that the monomer was highly specific to SEA and showed no affinity to other SEs such as SEB and SEC1 (Chen et al., 2014); this provided a foundation for the

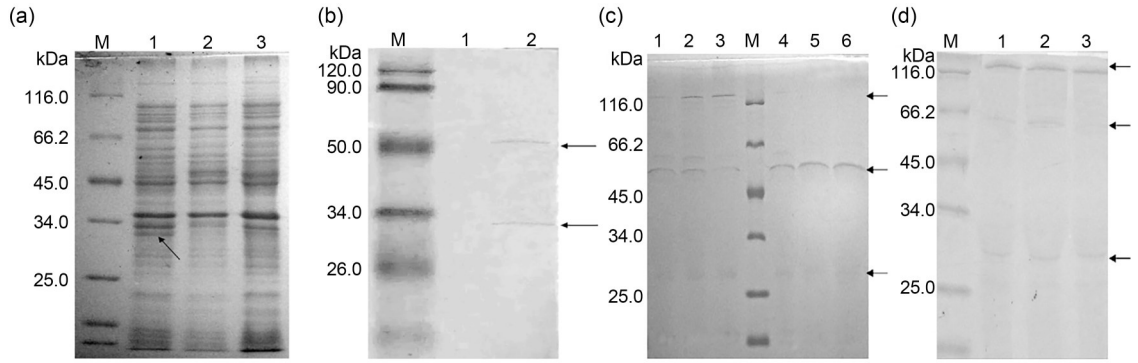


Fig. 3 Expression and purification of the tetravalent single-chain variable fragment (scFv) antibody. (a) Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). M: molecular mass marker; Lane 1: *Escherichia coli* BL21 (DE3) containing plasmid pET-22b-scFv/p53 after induction; Lane 2: *E. coli* BL21 (DE3) containing plasmid pET-22b-scFv/p53 before induction; Lane 3: *E. coli* BL21 (DE3) containing empty plasmid (pET-22b) after induction. (b) Western blot. M: molecular mass marker; Lane 1: *E. coli* BL21 (DE3) containing empty plasmid (pET-22b) after induction; Lane 2: *E. coli* BL21 (DE3) containing plasmid pET-22b-scFv/p53 after induction. (c) Purification of the tetravalent antibody. M: molecular mass marker; Lanes 1–3: purified protein samples placed at different temperatures (room temperature, 60 and 100 °C); Lanes 4–6: purified protein samples treated with 1% (volume ratio) β-mercaptoethanol and placed at different temperatures (room temperature, 60 and 100 °C). (d) Tetravalent antibody after concentration. M: molecular mass marker; Lanes 1–3: concentrated protein samples placed at different temperatures (room temperature, 60 and 100 °C). Arrows indicating protein bands at around 120, 60, and 30 kDa represent tetrameric, dimeric, and monomeric scFv antibodies, respectively.

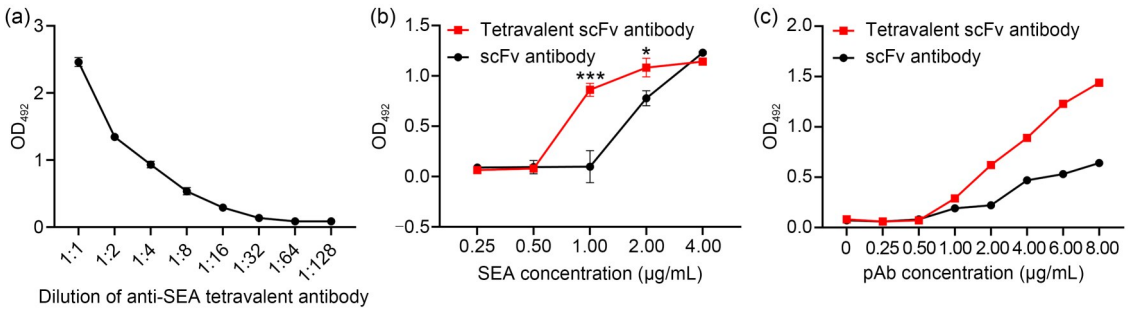


Fig. 4 Characterization and application of the tetravalent single-chain variable fragment (scFv) antibody. (a) Staphylococcal enterotoxin A (SEA)-binding assay; (b) Sandwich enzyme-linked immunosorbent assay (ELISA) detecting different concentrations of SEA; (c) Sandwich ELISA detecting SEA using different concentrations of coating antibody. pAb: polyclonal antibody; OD_{492}: optical density at 492 nm. Data are expressed as mean±standard error of mean (SEM) (n=3) * P<0.05, *** P<0.001 vs. scFv antibody.

SEA-specificity of the scFv tetramer. A sandwich ELISA has been developed for SEA detection, which is more effective than other types of ELISA for the detection of large molecules (Dasgupta, 2019). The sandwich ELISA of our samples indicated that the tetravalent scFv antibody was more sensitive than the scFv monomer at SEA concentrations of 1 and 2 μg/mL (Fig. 4b).

The effect of polyclonal antibody (pAb) concentration on the performance of the tetravalent scFv antibody was then tested. The signal intensity of both scFv tetramers and monomers to SEA was increased following an increase in pAb concentration; and when pAb concentration was higher than 1 μg/mL, the tetravalent scFv antibody consistently generated stronger signals than the scFv monomers (Fig. 4c). These results revealed that the tetramerization of the scFv antibody successfully improved its sensitivity to SEA. However, sensitivity of the tetravalent scFv antibody was still significantly lower than that of its mAb (Liang et al., 2011), which indicated that further attempts, such as point-mutation (Thomas et al., 2002), should be made to improve the sensitivity of the SEA-specific tetravalent scFv.

To understand the effect of tetramerization on scFv sensitivity in a molecular insight, we performed scFv-SEA docking analysis. We used chain A of the scFv antibody 4B08 (Protein Data Bank (PDB) entry 5YD5) as the template to predict anti-SEA scFv structure (Miyanabe et al., 2018). The prediction revealed that 243 residues of anti-SEA scFv (82%) were modeled with 100% confidence by the template (Fig. 5a). Molecular docking analysis is visualized in Fig. 5b. The CDRs play a vital role in the binding of antibodies to specific toxins (Liu et al., 2007; Murase et al., 2014). The specific binding of the anti-SEA scFv monomer to SEA was experimentally confirmed in our previous study (Chen et al., 2014). The current docking analysis revealed that SEA interacted with scFv mainly on the side with CDRs, whereas the residue linked to p53 (C-terminus) was located on the opposite side (Fig. 5b); this indicated that the tetramerization did not negatively affect the scFv-SEA interaction in three dimensions.

In summary, we used the tetramerization domain of human p53 for the multimerization of the anti-SEA scFv antibody. SDS-PAGE and western blot showed that the scFv was successfully expressed and the monomers could self-assemble into tetramers. The iELISA demonstrated that the tetrameric scFv specifically reacted with SEA. The sandwich ELISA for detection of SEA was established using the tetrameric scFv and results showed that the tetramer of scFv was more effective than the monomer in SEA detection. This study revealed that genetically engineered tetramerization is a potential way to improve the sensitivity of the scFv in detecting foodborne heat-stable toxins.

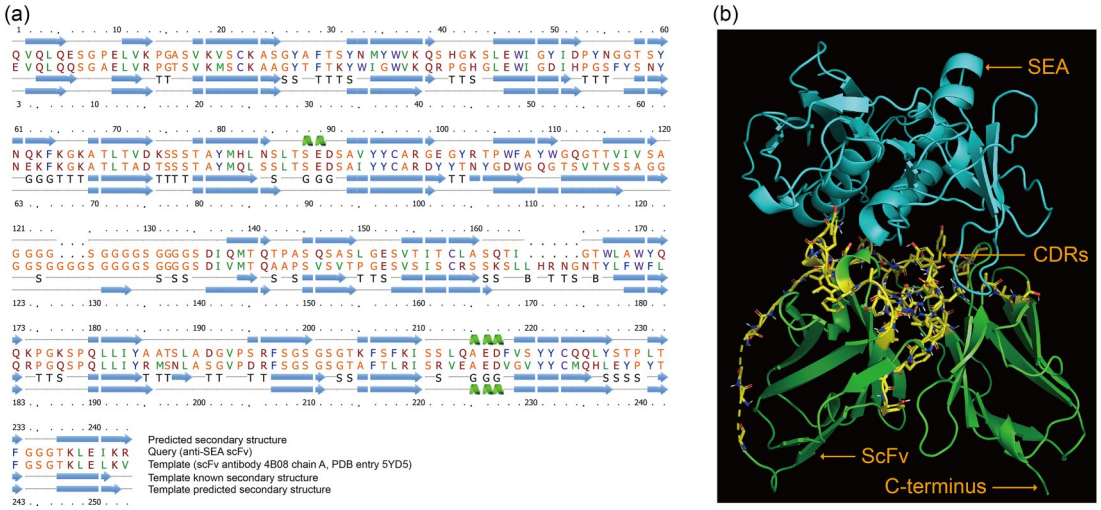


Fig. 5 Interactive mode of single-chain variable fragment (scFv) antibody and staphylococcal enterotoxin A (SEA). (a) Secondary structure prediction of the anti-SEA scFv based on scFv 4b08; (b) Docking analysis of the interaction between anti-SEA scFv and SEA. Blue chain: SEA; Green chain: anti-SEA scFv; Sticks: complementarity-determining regions (CDRs); Yellow: carbon atoms; Blue: nitrogen atoms; Red: oxygen atoms (Note: for interpretation of the references to color in this figure legend, the reader is referred to the web version of this article).

Materials and methods

Detailed methods are provided in the electronic supplementary materials of this paper.

Acknowledgments

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Author contributions

Weifeng CHEN: formal analysis, investigation, data curation, writing original draft, writing review, editing, and funding acquisition. Zhiwei LI: formal analysis, investigation, data curation, writing original draft, writing review, and editing. Xingxing DONG: formal analysis and investigation. Xiaohong WANG: conceptualization, methodology, resources, writing review, editing, supervision, project administration, and funding acquisition. All authors have read and approved the final manuscript and, therefore, have full access to all the data in the study and take responsibility for the integrity and security of the data.

Compliance with ethics guidelines

Weifeng CHEN, Zhiwei LI, Xingxing DONG, and Xiaohong WANG declare that they have no conflict of interest.

This article does not contain any studies with human or animal subjects performed by any of the authors.

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Supplementary information

Materials and methods