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# Optimisation of production of a domoic acid-binding scFv antibody fragment in *Escherichia coli* using molecular chaperones and functional immobilisation on a mesoporous silicate support

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**Abstract**

Domoic acid is a potent neurotoxin that can lead to amnesic shellfish poisoning in humans through ingestion of contaminated shellfish. We have produced and purified an anti-domoic acid single-chain Fragment variable (scFv) antibody fragment from the *Escherichia coli* periplasm. Yields of functional protein were increased by up to 100-fold upon co-production of *E. coli* DnaKJE molecular chaperones but co-overproduction of GroESL led to a reduction in solubility of the scFv. Co-production of the peptidyl-prolyl isomerase trigger factor resulted in accumulation of unprocessed scFv in the *E. coli* cytoplasm. This was due to an apparent bottleneck in translocation of the cytoplasmic membrane by the recombinant polypeptide. Co-expression of the *E. coli* disulfide bond isomerase *dsbC* increased scFv yields by delaying lysis of the host bacterial cells though this effect was not synergistic with molecular chaperone co-production. Meanwhile, use of a cold-shock promoter for protein production led to accumulation of greater amounts of scFv polypeptide which was predominantly in insoluble form and could not be rescued by chaperones. Purification of the scFv was achieved using an optimised metal affinity chromatography procedure and the purified protein bound domoic acid when immobilised on a mesoporous silicate support. The work outlines the potential benefit of applying a molecular chaperone/folding catalyst screening approach to improve antibody fragment production for applications such as sensor development.


*Keywords:* *Escherichia coli*; Recombinant antibody; scFv; Chaperone; Domoic acid; Mesoporous silicate; Immunosensor

Domoic acid is a naturally occurring neurotoxin produced by species of the diatom genus *Pseudo-nitzschia* and concentrated in shellfish that ingest the algal species. It leads to amnesic shellfish poisoning (ASP)[1] in humans, which is characterised by vomiting and diarrhoea in mild cases but can lead to neurological symptoms and possibly death in more severe attacks. As there is no antidote for ASP, on-site testing of shellfish samples is extremely important in limiting human exposure. Current tests for domoic acid are based on analytical methods such as HPLC [1] and liquid chromatography with tandem mass spectrometry [2] or mouse toxicity assays [3], none of which is suitable for rapid, on-site testing or high throughput screening of samples.

Antibody molecules can be produced in *Escherichia coli* in the form of small, recombinant fragments that retain the binding properties of their parent monoclonal antibodies [4]. This is a more economic and faster process than monoclonal antibody production in mammalian expression systems. In addition, the smaller size of the fragments may also

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[1] *Abbreviations used:* ASP, amnesic shellfish poisoning; TEE, translation enhancer element; TF, trigger factor; scFv, single-chain Fragment variable.

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lead to increased sensitivity of biosensors through more dense packing of binding sites on the sensor surface [5]. Recombinant protein production in *E. coli* is frequently characterised, however, by aggregation of heterologous polypeptides and accelerated host cell lysis [6]. While numerous approaches have been utilised to improve the production of individual proteins in *E. coli*, no universal solution has been identified to date. Thus, painstaking genetic manipulation [7], investigation of physiological conditions [8] and/or molecular chaperone co-production [9] is typically necessary to increase the yield of a poorly produced heterologous protein in *E. coli*.

We previously described the cloning, production in *E. coli* and characterisation of a murine anti-domoic acid scFv antibody fragment [10]. Development of an immunosensor was limited by low functional yields of the antibody fragment in *E. coli* and an inability to purify it to homogeneity. In this work, we describe optimisation of expression and purification of the anti-domoic acid scFv, followed by adsorption and analysis of its activity on a novel mesoporous silicate support with potential in development of an immunosensor for *in situ* domoic acid screening.

## Materials and methods

### *Chemicals, strains, and plasmids*

All chemicals and antibodies were obtained from Sigma (Ireland) unless otherwise stated. Restriction enzymes were from Roche Applied Science (UK) and PCRs were carried out using EHF *Taq* polymerase from the same supplier. Oligonucleotides were obtained from MWG-Biotech (Germany), where sequencing was also carried out. *E. coli* TOP 10 (F′ *mcrA* Δ(*mrr-hsdRMS-mcrBC*) $\phi$80*lacZ*ΔM15 Δ*lacX74 deo*$^r$ *recA1 araD139* Δ(*ara-leu*)7697 *galU galK rpsL* (Str$^r$) *end*A1 *nup*G; Invitrogen Corp., USA) was used for all cloning and protein production work. The genotypes of plasmids used in the study are shown in Table 1. The pIG6 periplasmic expression vector [11] and pCold III cold shock expression vector [12] (Takara Corp., Japan) were used for production of recombinant proteins in the *E. coli* periplasm. Chaperone co-production plasmids pG-KJE8, pGro7, pKJE7, pG-Tf2, and pTf16 from Takara Corp. [13], which are compatible with pIG6, were used to co-produce the relevant *E. coli* chaperones during scFv production (Table 1).

### *Genetic manipulations*

DNA manipulations were carried out according to Sambrook and Russell [14]. The $V_H$-$(Gly_4Ser)_3$-$V_L$ 2H12 anti-domoic acid scFv was cloned in an *ompA* leader–FLAG tag–scFv–hexahistidine tag format in pIG6, as described previously [10], and as a *Sac*I–*Hin*dIII fragment into the pCold III expression vector, leading to its transcription with an additional, vector-encoded 5′ translation enhancer element (TEE) in the latter. The TEE was also removed from the pCold III-2H12 scFv construct by overlap PCR and the new *ompA* leader–FLAG–scFv–$His_6$ construct was cloned as before into pCold III. The *dsbC* gene, encoding the *E. coli* disulfide bond isomerase C enzyme, was cloned into the pIG6-scFv vector downstream of, and under joint control of $P_{lac}$ with, the scFv gene [10].

### *Protein production and detection*

*Escherichia coli* TOP 10 cells containing pIG6-2H12 scFv or pCold III-2H12 scFv and, where appropriate, the compatible chaperone co-expressing plasmids (Table 1), were grown and induced to produce scFv as described elsewhere [10]. Protein production was carried out at 20 and 25 °C for pIG6-containing cultures and at 15 and 25 °C for clones containing pCold III. Cultures containing chaperone plasmids were induced with the relevant chaperone inducer 1 h prior to the addition of 0.3–1 mM IPTG, following which scFv production was typically allowed to continue for up to 6 h before harvesting. In pCold III-containing cultures, scFv production was continued for up to 24 h prior to harvesting and analysis. Culture ODs were measured at hourly intervals for up to 10 h after induction and harvesting of cells was followed by fractionation into soluble and insoluble protein fractions, as described elsewhere [6,15]. SDS–PAGE and Western blotting of protein fractions were carried out according to standard procedures [16] and Western blots were developed using M1 anti-FLAG or anti-polyhistidine peroxidase-conjugated antibodies (Sigma).

Table 1
Genotypes of plasmids used in the work

| Plasmid | Genotype | Source and reference |
|---|---|---|
| pIG6(-scFv) | $P_{lac}$::scFv, *ori* (*colE1*), amp$^R$ | A. Plückthun [11] |
| pIG6(-scFv::*dsbC*) | $P_{lac}$::scFv::*dsbC*, *ori* (*colE1*), amp$^R$ | This lab [10] |
| pCold III | $P_{cspA}$::TEE-scFv, *ori* (*colE1*), amp$^R$ | TakaRa Corp. [12] |
| pCold III/ΔTEE | $P_{cspA}$::scFv, *ori* (*colE1*), amp$^R$ | This study |
| pG-KJE8 | $P_{araB}$::*dnaK/J*::*grpE*, $P_{zt1}$::*groEL/ES*, *ori* (pACYC184), Cm$^R$ | Takara Corp. [13] |
| pGro7 | $P_{araB}$::*groEL/ES*, *ori* (pACYC184), Cm$^R$ | Takara Corp. [13] |
| pKJE7 | $P_{araB}$::*dnaK/J*::*grpE*, *ori* (pACYC184), Cm$^R$ | Takara Corp. [13] |
| pG-Tf2 | $P_{zt1}$::*groEL/ES*::*tig*, *ori* (pACYC184), Cm$^R$, tet$^R$ | Takara Corp. [13] |
| pTf16 | $P_{araB}$::*tig*, *ori* (pACYC184), Cm$^R$ | Takara Corp. [13] |