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# Efficient silkworm expression of single-chain variable fragment antibody against ginsenoside Re using *Bombyx mori* nucleopolyhedrovirus bacmid DNA system and its application in enzyme-linked immunosorbent assay for quality control of total ginsenosides

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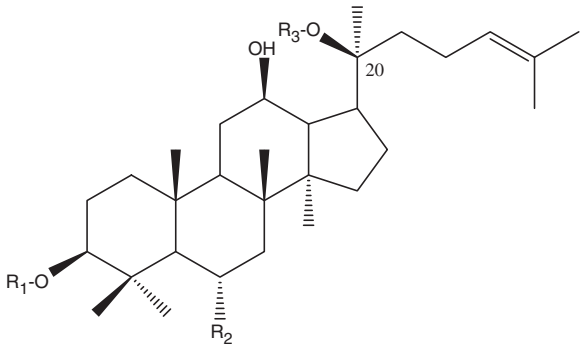
**A single-chain variable fragment (scFv) antibody against ginsenoside Re (G-Re) have been successfully expressed in the silkworm larvae using *Bombyx mori* nucleopolyhedrovirus (BmNPV) bacmid DNA system. The baculovirus donor vector for expression of scFv against G-Re (GRe-scFv) was constructed to contain honeybee melittin signal sequence to accelerate secretion of the recombinant GRe-scFv into the haemolymph of silkworm larvae. Functional recombinant GRe-scFv was purified by cation exchange chromatography followed by immobilized metal ion affinity chromatography. The yield of purified GRe-scFv was 6.5 mg per 13 silkworm larvae, which is equivalent to 650 mg/l of the haemolymph, exhibiting extremely higher yield than that expressed in Escherichia coli (1.7 mg/l of culture medium). It was revealed from characterization that GRe-scFv retained similar characteristic of the parental monoclonal antibody (MAb) against G-Re (MAb-4G10), making it possible to develop indirect competitive enzyme-linked immunosorbent assay (icELISA) for quality control of total ginsenosides in various ginsengs. The detectable range for calibration of G-Re by developed icELISA shows 0.05–10 μg/ml. These results clearly suggested that the silkworm expression system is quite useful for the expression of functional scFv that frequently required time- and cost-consuming re-folding when it expressed in *E. coli*.**

*Keywords*: *Bombyx mori* nucleopolyhedrovirus/enzyme-linked immunosorbent assay/ginsenoside Re/silkworm/single-chain variable fragment antibody.

*Abbreviations*: BmNPV, *Bombyx mori* nucleopolyhedrovirus; CR, cross-reactivities; ELISA, enzyme-linked immunosorbent assay; G-Re, ginsenoside Re; GRe-scFv, single-chain variable fragment antibody against G-Re; HMSS, honeybee melittin signal sequence; HSA, human serum albumin; HRP, horseradish peroxidase; PBS, phosphate buffered saline; scFv, single-chain variable fragment.

*Panax ginseng* and its related species such as *P. japonicas*, *P. quinquefolium* and *P. notoginseng* are as well known as important traditional medicines which has been widely used in Asia, especially China, Japan and Korea, for thousands of years. Recently, it has also been widely used all over the world as an ingredient of dietary health supplements and an additive in foods and beverages owing to its pharmacologic activities such as tonic, immunomodulatory, antimutagenic and anti-ageing activities *(1, 2)*. Clinical studies have also demonstrated that ginseng may improve psychological function, immune function and conditions associated with diabetes *(3, 4)*. Ginsenosides, which contain protopanaxatriol or protopanaxadiol (which possess a dammarane skeleton) in their molecules, are well known as major bioactive compounds containing in ginseng (Fig. 1). The variety of total and individual ginsenoside concentrations has increased the demand of monitoring ginseng samples for quality control. Therefore, a method for standardizing ginseng samples is required.

In our previous studies, we prepared monoclonal antibodies (MAbs) against G-Re (MAb-4G10) for development of enzyme-linked immunosorbent assays (ELISA) *(5–7)* and chromatographic immunostaining methods *(5, 8)* for the determination of various ginsenosides. The ELISA using MAb-4G10 was shown to be an effective tool for determining total ginsenosides in ginseng and the assay showed potential as a fast and reliable method for assessing the total ginsenosides concentration of plant samples. However, it is time-consuming and labour intensive to obtain MAb. Furthermore, certain techniques are required to deal with the hybridoma cells to avoid the cultures being contaminated with microorganisms. These disadvantages of MAb can be overcome by a genetically engineered antibody. A single-chain variable (scFv) antibodies, which have variable regions of heavy (VH) and light chains (VL) with a short flexible peptide $(Gly_4Ser)_3$, strategy has become one of the most popular methods in antibody engineering because antibody production with bacteria is much simpler, cheaper and




| Ginsenoside | $R_1$ | $R_2$ | $R_3$ |
|---|---|---|---|
| *Protopanaxatriol* | | | |
| **Re** | H | $Rha^1$-$^2$Glc-O- | Glc- |
| **$Rg_1$** | H | Glc-O- | Glc- |
| *Protopanaxadiol* | | | |
| **$Rb_1$** | $Glc^1$-$^2$Glc- | H | $Gluc^1$-$^6$Glc- |
| **Rc** | $Glc^1$-$^2$Glc- | H | $Ara(f)^1$-$^6$Glc- |
| **Rd** | $Glc^1$-$^2$Glc- | H | Glc- |

**Fig. 1 Structure of major ginsenosides in ginseng.**

faster than with hybridomas once the construction of recombinant antibodies is established. Thus, we have been focused on construction of scFv against bioactive plants secondary metabolites such as plumbagin, paeoniflorin, berberine, solasodine and G-Re *(9–13)* for quality control of their host plants. However, these scFvs expressed in *Escherichia coli* was obtained as the bacterial cytoplasmic inclusion body that required time- and cost-consuming re-folding. In addition, there was yield problem in the case of GRe-scFv.

In this present study, *Bombyx mori* nucleopolyhedrovirus (BmNPV) bacmid DNA system using silkworm larvae had been used for expression of GRe-scFv to overcome disadvantage of these refolding process and yield problem. Nowadays silkworm larvae are used for expression of eukaryotic proteins having complicated structure because their protein expression level is 10- to 100-fold higher than that using insect cell. Moreover, the expressed protein in this system retains their functional structure. From these points of view, *B. mori* silkworm larvae infected with BmNPV has been widely used instead of insect cell. Unfortunately, however, the traditional preparation of recombinant baculoviruses that express exogenous genes needs at least 40 days, because multiple rounds of purification and amplification of viruses are required. Furthermore, large-scale cultivation and virus handling techniques are required as well. Recently, the speedy bacmid (a baculovirus shuttle vector) system had been developed for BmNPV *(14)*. The bacmid can be replicated in *E. coli* BmDH10Bac strain as a large plasmid and generate the recombinant virus DNA by the site-specific transposition in *E. coli*, and retains infectious with silkworm larvae. Since the replication of bacmid DNA and transposition of the target genes of the transfer vector onto the bacmid DNA can be occurred in *E. coli*, the time-consuming preparation of recombinant virus is not required.

Efficient expression of GRe-scFv using novel BmNPV bacmid DNA system and development of icELISA for quality control of ginseng were demonstrated in this article.

## Materials and Methods

### *Chemicals and immunochemicals*

G-Re, ginsenoside-Rg1 (G-Rg1), ginsenoside-Rb1 (G-Rb1), ginsenoside-Rc (G-Rc) and ginsenoside-Rd (G-Rd) were purchased from Wako Pure Chemical (Osaka, Japan). Human serum albumin (HSA) and anti-T7-tag MAb produced in mice were purchased from Sigma-Aldrich (Steinheim, Germany). T7-tag horseradish peroxidase (HRP) labelled conjugate and DMRIE-C reagent were obtained from Invitrogen (CA, USA). Peroxidase labelled anti-mouse IgG goat antibody and HRP-labelled anti-mouse IgG goat antibody were purchased from Organon Teknika Cappel Products (West Chester, PA, USA) and Santa Cruz Biotechnology (CA, USA), respectively. DNA polymerase and DNA restriction enzymes were purchased from Takara (Kyoto, Japan). All other chemicals were standard commercial products of an analytical grade.

### *Plant materials and sample preparation*

*Panax ginseng* (white ginseng), processed *P. ginseng* (red ginseng), *P. japonicus* (Japanese ginseng), *P. quinquefolium* (American ginseng) and *P. notoginseng* (Tienchi ginseng) were purchased from Uchida (Tokyo, Japan). Dried powdered samples (50 mg) were extracted with 0.5 ml MeOH in an ultrasonic bath for 15 min. The extracts were then centrifuged at 6,000*g* for 1 min, and the supernatants were transferred to microtubes. This extraction step was repeated three times. The extracted solutions were then evaporated to dryness in a water-bath at 60°C, and the residues were re-constituted in 1 ml of MeOH and diluted appropriately for ELISA.

### *Construction of a baculovirus donor vector*

The honeybee melittin signal sequence (HMSS) peptide used to promote the secretion of GRe-scFv into the haemolymph of silkworm was amplified by the PCR from pMelBac A vector (Invitrogen) and ligated downstream of the polyhedrin promoter of the pFastBac 1 vector (Invitrogen) to generate the pFastBacMel (pFBM) vector. The GRe-scFv gene which contain flexible standard 15-amino acid linker $(Gly_4Ser)_3$ between VH and VL domains with a format of VH-linker-VL was amplified by fusing it with the N-terminal His6-tag and T7-tag of pET28a vector encoding the GRe-scFv gene by PCR and ligated into the pFBM vector to generate the pFBM/GRe-scFv vector.

The primers used for the construction of the pFBM/GRe-scFv were as follows: forward primer for HMSS: 5′-CGCGGATCC**ATGAAATTCTTAGTCAAC**-3′; reverse primer for HMSS: 5′-AGCGAATTC**CGCATAGATGTAAGAAA**-3′; forward primer for GRe-scFv: 5′-CGCGTCGACACATGAGCAGC**CATCATCATCAT**-3′; and reverse primer for GRe-scFv: 5′-TTTGCGGCCGCCTA**ACGTTTTATTTCCAA**-3′. In these primers, the nucleotides coding restriction sites (*Bam*H I, *Eco*R I, *Sal* I and *Not* I) are underlined. The nucleotides contributing to the coding sequence of the HMSS, His6-tag and GRe-scFv are shown in bold.

### *Transposition of pFBM/GRe-scFv in E. coli BmDH10Bac cells*

Transposition was carried out by transforming the donor plasmid pFBM/GRe-scFv into *E. coli* BmDH10Bac cells *(14)* (Fig. 2). It contains a parent bacmid that recombines with the donor plasmid, pFBM/GRe-scFv, to create an expression bacmid DNA. The resultant transformed *E. coli* BmDH10Bac cells were grown on Luria–Bertani (LB) agar plates containing kanamycin (50 μg/ml), gentamycin (7 μg/ml), tetracycline (10 μg/ml), isopropyl-β-D-thiogalactopyranoside (IPTG) (40 μg/ml) and 5-bromo-4-chloro-3-indolyl-β-D-Galactopyranoside (300 μg/ml). White antibiotics-resistant colonies were selected, and then the BmNPV bacmid, designed as BmNPV bacmid/GRe-scFv was isolated and its identities were confirmed by PCR using universal primer as follow: M13 primer: 5′-GTTTTCCCAGTCACGAC-3′; M13 primer RV: 5′-CAGGAAACAGCTATGAC-3′.

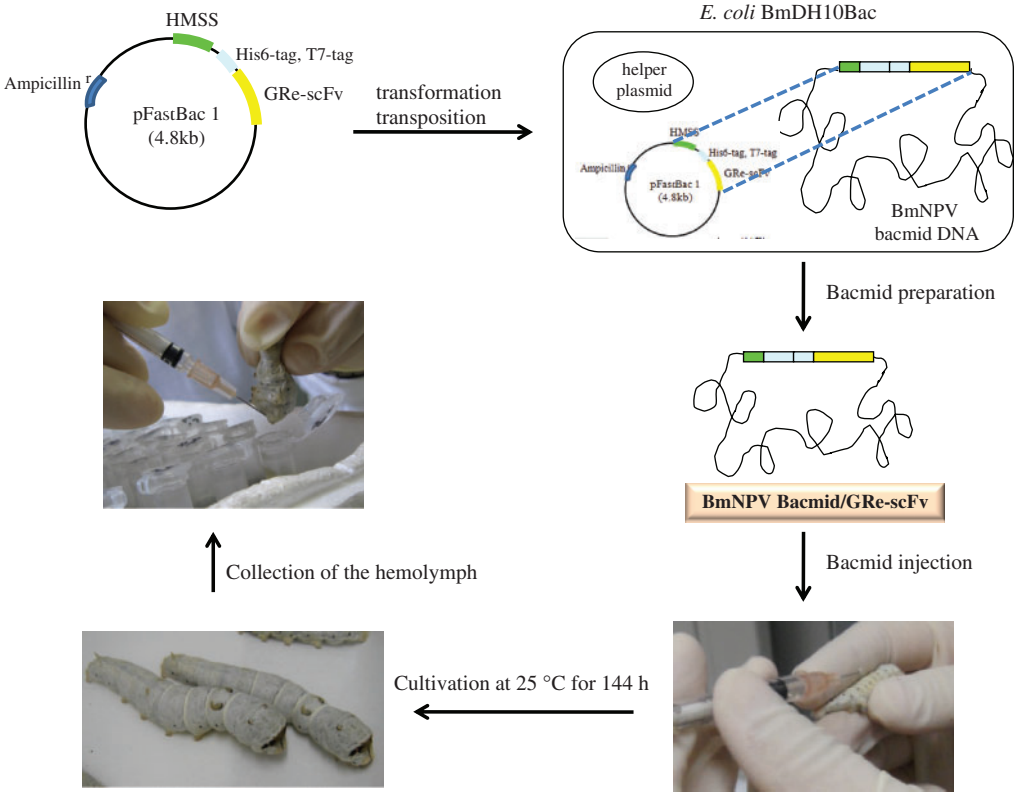



**Fig. 2 Construction of the recombinant BmNPV bacmid DNA, BmNPV Bacmid/GRe-scFv and expression strategy using silkworm larvae.**

### *Expression of the GRe-scFv gene in silkworm larvae*

The first day of fifth instar silkworm larvae was used for the infection of BmNPV bacmid/GRe-scFv. BmNPV bacmid/GRe-scFv (1 μg) was suspended with 3 μl of DMRIE-C reagent (Invitrogen) and kept at room temperature for 45 min, and resultant mixture was then directly injected into the dorsal side of the larvae. After the larvae were cultured for 144 h at 25°C, the haemolymph was collected in the microtube containing 5% sodium thiosulphate (50 μl) (Fig. 3). The collected haemolymph (10 ml) from 13 silkworm larvae was diluted with 39.5 ml of starting buffer (10% glycerol in 50 mM Tris–HCl, pH 6.8) and 500 μl of protease inhibitor cocktail (Nacalai, Japan) for further purification.

### *Purification of the GRe-scFv expressed in the haemolymph of silkworm larvae*

Functional GRe-scFv expressed in the haemolymph of silkworm larvae was purified by cation exchange chromatography using TOYOPEARL CM-650M (Tosoh Crop.) followed by immobilized metal ion affinity chromatography (IMAC) using His-bind resin (Novagen).

First, 10 ml of CM-650M cation exchanger were packed into a column (1.1 × 23 cm) and equilibrated with starting buffer (10% glycerol in 50 mM Tris–HCl, pH 6.8). Then, the samples (50 ml) treating with starting buffer and protease inhibitor were directly applied to a cation exchanger with filtrating through a 0.45 μm polyvinylidene difluoride (PVDF) membrane (Milipore). After washing the column with starting buffer to remove unadsorbed proteins, the bound proteins were then eluted with a continuous gradient of NaCl from 0 to 500 mM in starting buffer. Indirect ELISA was carried out to follow GRe-scFv in the fractionated test tube.

Subsequently, 6 ml of His-bind resin were packed into the column (1.1 × 23 cm) and charged with 50 mM $NiSO_4$ in binding buffer (10 mM imidazole, 500 mM NaCl, 0.1% Nonidet P-40 and 10% glycerol in 50 mM Tris–HCl, pH 8.0). The positive fractions detected in indirect ELISA (350 ml) were collected and adjusted so that they had almost the same constitution as the binding buffer using 50 ml of 8-fold concentrated binding buffer (80 mM imidazole and 4 M NaCl in 50 mM Tris–HCl, pH 8.0). These samples were then applied on resin and it were washed with binding buffer

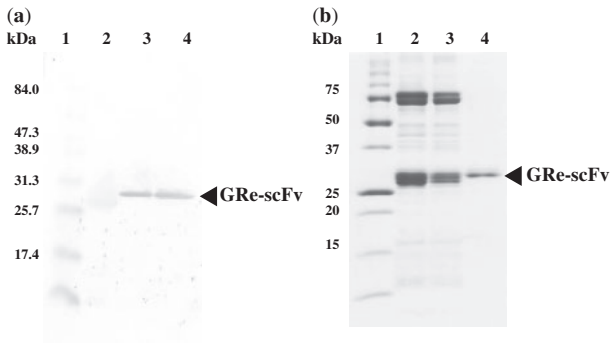



**Fig. 3 Western blotting analysis and SDS–PAGE analysis of GRe-scFv.** (A) Western blotting analysis using the haemolymph before/after infection with BmNPV Bacmid/GRe-scFv and purified GRe-scFv. The collected haemolympth was two-times diluted with distilled water and used as a sample for lanes 2 and 3. Anti-T7 tag MAb (mouse) and HRP-labelled anti-mouse IgG goat antibody were used to visualize immunoreactive bands. Lane 1, molecular protein marker; lane 2, the haemolymph before infection; lane 3, the haemolymph after infection; lane 4, purified GRe-scFv (1.0 μg). (B) SDS–PAGE analysis under reducing condition. The collected haemolymph was 50-times diluted with distilled water and used as a sample for lanes 2 and 3. Lane 1, molecular protein marker; lane 2, the haemolymph before infection; lane 3, the haemolymph after infection; lane 4, purified GRe-scFv (1.0 μg).

followed by washing buffer (40 mM imidazole, 500 mM NaCl, 0.1% Nonidet P-40 and 10% glycerol in 50 mM Tris–HCl, pH 8.0) to remove non-specifically bound proteins. The bound protein were then eluted with elution buffer (100 mM imidazole, 500 mM NaCl, 0.1% Nonidet P-40 and 10% glycerol in 50 mM Tris–HCl, pH 8.0) and analysed by indirect ELISA. The yield of purified GRe-scFv was determined according to the method of Bradford *(15)*.

#### *SDS–PAGE and western blotting analysis*

SDS–PAGE and western blotting analysis were performed according to the methods of Laemmli (*16*) and Towbin *et al. (17)*, respectively. Protein samples were separated by 12.5% SDS–PAGE under reducing conditions and then transferred electrophoretically onto a PVDF membrane (Millipore) at 100 V, 90 mA for 3 h in an ice water bath. The immunoreactive band was visualized by using anti-T7-tag MAb produced in mice (Sigma) as a primary antibody and HRP-labelled anti-mouse IgG goat antibody (Santa Cruz) as a secondary antibody followed by 4-chrolo-1-naphthol (1 mg/ml) in phosphate buffered saline (PBS) containing 0.003% (v/v) $H_2O_2$.

#### *Indirect competitive ELISA using purified GRe-scFv*

An indirect competitive ELISA (icELISA) was carried out to analyse inhibitory activity against G-Re. A 96-well immunoplate (Nunc, Maxisorb, Roskilde, Denmark) was coated with GRe-HSA conjugates (2 μg/ml), which were revealed to have five molecules of G-Re per one molecule of HSA in our previous study *(6)*. The plate was incubated for 1 h to coat GRe-HSA and then treated with 300 μl of PBS containing 10% (w/v) skimmed milk (PBS-sm) for 1 h to block the plate. After washing the blocked-plate, 50 μl of various concentrations of G-Re in 5% MeOH were incubated with 50 μl of GRe-scFv solution for 1 h. After competitive reaction of GRe-scFv between free antigen, G-Re and coated antigen, GRe-HSA, the GRe-scFv bound to GRe-HSA was combined with 100 μl of a 5,000-fold diluted solution of HRP-labelled anti-T7-tag conjugates (Invitrogen) for 1 h. After washing the plate three times with PBS-T, 100 μl of substrate solution [0.3 mg of 2, 2′-azinobis 3-ethylbenzothiazoline-6-sulphonic acid ammonium salt (ABTS) in 100 mM citrate buffer containing 0.003% (v/v) $H_2O_2$] were added to each well and incubated for 20 min. Absorbance at 405 nm was measured using a microplate reader (Immuno Mini NJ-2300, Nalge Nunc International). All incubation steps of this icELISA were carried out at 37°C. And the PBS containing 0.05% (v/v) Tween 20 (PBS-T) was used for washing the plate three times between each step.

The dissociation constant ($K_D$) was used to evaluate the binding affinities of the recombinant GRe-scFv and MAb-4G10 and the cross-reactivities (CR) of both antibodies against various compounds were calculated to investigate specificity according to the method of Friguet *et al. (18)* and Weiler and Zenk *(19)*, respectively.

#### *IcELISA using parental MAb-4G10*

IcELISA using MAb-4G10 was carried out to compare total ginsenosides determined by icELISA using GRe-scFv. In the icELISA using MAb-4G10, 1,000-fold diluted solution of peroxidase labelled anti-mouse IgG (Organon Teknika Cappel Products) which recognize Fc fragment of mouse IgG was used as secondary antibody. And other procedures were same as used in icELISA using GRe-scFv.

## Results and discussion

#### *Construction of baculovirus donor vector and recombinant baculovirus*

The baculovirus donor vector, pFBM/GRe-scFv, was successfully constructed for the expression of GRe-scFv in the silkworm larvae by cloning the HMSS (63 bp) from the pMelBac A vector (Invitrogen) and the GRe-scFv (702 bp) gene from the pET-28a vector. A recombinant bacmid containing the GRe-scFv gene, BmNPV bacmid/GRe-scFv, was obtained through transposition in *E. coli* BmDH10Bac cells developed by Motohashi *et al. (14)* (Fig. 2).

#### *Expression and purification of recombinant GRe-scFv*

Isolated BmNPV bacmid/GRe-scFv was directly injected into the dorsal side of the larvae with DIMRIE-C reagent (Invitrogen) (Fig. 2). Western blotting analysis showed that the haemolymph of silkworm larvae infected with BmNPV bacmid/GRe-scFv exhibited an immunoreactive band of chimera protein containing His6-tag and T7-tag with a molecular mass of 28.8 kDa including the molecular mass of GRe-scFv (24.7 kDa) (Fig. 3A). The N-terminal amino acid sequence of GRe-scFv was analysed on an Applied Biosystems 494 protein sequencer. The results of western blotting (Fig. 3A) and the N-terminus amino acid sequence of GRe-scFv showed EFKGL, suggesting that the HMSS incorporated into the gene construct had successfully targeted GRe-scFv with the N-terminal His6-tag and T7-tag.

Purification of GRe-scFv from the haemolymph was performed by cation exchange chromatography using TOYOPEARL CM-650M (Tosho Corp.) followed by IMAC using His-bind resin (Novagen). In this purification process, the purity of purified GRe-scFv was estimated at >90% based on Coomassie brilliant blue staining (Fig. 3B). The yields of purified GRe-scFv were 6.5 mg per 13 silkworm larvae (500 μg/silkworm larvae), which is equivalent to about 41 of *E. coli* culture medium *(13)*.

#### *Characterization of recombinant GRe-scFv*

An icELISA was carried out to investigate the specificity of the recombinant GRe-scFv against structure related compounds and their binding affinity against G-Re.

In this assay, GRe-HSA conjugates (2.0 μg/ml) were used as a solid-phase antigen. After competition, free GRe-scFv (15 μg/ml) was bound to a polystyrene micro-immunoplate precoated with GRe-HSA. After washing the plate, the amount of GRe-scFv antibodies bound to the GRe-HSA conjugates was measured using the HRP-labelled anti-T7-tag conjugates and a substrate added to develop colour. The detectable range of G-Re concentrations in this assay was 0.05–10 μg/ml. It became evident from this experiment that the icELISA using GRe-scFv expressed in the silkworm larvae displayed the same sensitivity as that using parental MAb, MAb-4G10 secreted from hybridoma cells (4G10) (Fig. 4).

The ELISA method described by Friguet *et al.* (*18*) was used to estimate the dissociation constant ($K_D$) of the MAb-4G10 and GRe-scFv in solution. This method is equally available for antibodies against small (hapten) and large molecular weight antigens but does not label either the antibodies or antigens involved. Briefly, various concentrations of G-Re were incubated with either MAb-4G10 or GRe-scFv at 37°C for 1 h until they reached equilibrium. The amounts of free antibodies in the incubation mixture were then determined by indirect ELISA. The $K_D$ of MAb-4G10 and GRe-scFv in solution were $1.69 \times 10^{-8}$ and $4.21 \times 10^{-7}$ M, respectively, as determined by typical Scatchard plots. When the $K_D$ of MAb-4G10 was compared with that of the GRe-scFv, MAb-4G10 ($1.69 \times 10^{-8}$ M) exhibited ~25-fold higher binding affinity than GRe-scFv ($4.21 \times 10^{-7}$ M).

To evaluate the specificity of GRe-scFv, the CR of GRe-scFv antibody with other compounds were determined using the developed icELISA and the calculation described by Weiler and Zenk *(19)*. Table I shows

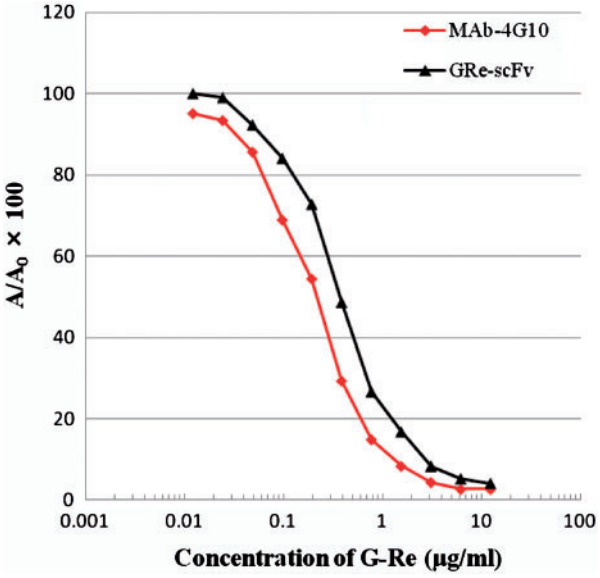



**Fig. 4 Standard icELISA curve for the determination of total ginsenosides using MAb-4G10 and purified GRe-scFv.** The red squares and red curve show the standard curve produced using MAb-4G10 (300 ng/ml). The black triangles and black curve show the standard curve produced when GRe-scFv (15 μg/ml) was used. $A/A_0$, $A_0$ is the absorbance with no G-Re, and $A$ is the absorbance with G-Re present.

**Table I. CR of GRe-scFv and MAb-4G10 against various compounds.**

| | CR (%) | |
|---|---|---|
| **Compound** | **GRe-scFv** | **MAb-4G10** |
| G-Re | 100 | 100 |
| G-Rd | 71.77 | 76.23 |
| G-Rg1 | 73.46 | 70.94 |
| G-Rc | <0.001 | 0.046 |
| G-Rb1 | <0.001 | 0.045 |
| Saikosaponin A | <0.001 | <0.009 |
| Digitonin | <0.001 | <0.009 |
| Deoxycholic acid | <0.001 | <0.009 |
| Glycyrrhizin | <0.001 | <0.009 |
| Glycyrrhetinic acid | <0.001 | <0.009 |
| Saponin | <0.001 | <0.009 |
| Sennoside A | <0.001 | <0.009 |
| Sennoside B | <0.001 | <0.009 |
| Swertiamarin | <0.001 | <0.009 |

The CR of both GRe-scFv and MAb-4G10 against various compounds were calculated using the method of Weiler and Zenk, as follow:

$$\mathrm{CR}(\%) = \frac{\text{Concentration of G - Re yielding, } A/A_0 = 50\ \%}{\text{Concentration of test compound yielding, } A/A_0 = 50\ \%} \times 100$$

where $A$ is the absorbance in the presence of the test compound and $A_0$ is the absorbance in the absence of the test compound.

that the CR of GRe-scFv against various compounds including structurally related compounds. The CR of GRe-scFv against G-Rd and G-Rg1 were 71.8 and 73.5%, respectively; whereas no CR was exhibited against other compounds (<0.001%). The CR of GRe-scFv is almost identical to that of MAb-4G10, the parental antibody, suggesting that GRe-scFv could be used as an alternative approach for analysing the total ginsenoside concentration in ginseng samples.

**Table II. Intra- and inter-assay CV for precision of ginsenoside analysis using ELISA.**

| | CV (%) | |
|---|---|---|
| **G-Re (μg/ml)** | **Intra-assay ($n = 5$)** | **Inter-assay ($n = 3$)** |
| 12.50 | 6.3 | 0.1 |
| 6.25 | 4.5 | 0.6 |
| 3.13 | 4.6 | 2.8 |
| 1.56 | 6.2 | 4.3 |
| 0.78 | 2.9 | 3.3 |
| 0.39 | 4.2 | 6.2 |
| 0.20 | 2.7 | 2.8 |
| 0.10 | 3.9 | 3.9 |
| 0.05 | 3.2 | 0.2 |

**Table III. Determination of total ginsenoside concentrations in ginseng samples using MAb-4G10 and GRe-scFv.**

| | Concentration (mg/g dry weight) | |
|---|---|---|
| **Samples ($n = 5$)** | **MAb-4G10** | **GRe-scFv** |
| Red ginseng | 4.05 ± 0.31 | 2.95 ± 0.39 |
| White ginseng | 4.24 ± 0.40 | 3.34 ± 0.35 |
| Japanese ginseng | 2.06 ± 0.18 | 1.33 ± 0.12 |
| American ginseng | 9.67 ± 0.54 | 10.38 ± 2.10 |
| Tienchi ginseng | 40.46 ± 1.41 | 38.40 ± 4.57 |

Five samples were used to calculate concentration of total ginsenoside in various ginsengs.

### *Intra- and inter-assay precision of developed icELISA*

To validate the developed icELISA using GRe-scFv, intra- and inter assay precision was evaluated by testing nine different G-Re concentration samples in five assays performed together on the same day and on three consecutive days, respectively. Intra- and inter-assay coefficients of variation (CV) for precision were determined based on the ratios of standard deviations (SD) and means from five assays. From the results shown in Table II, the maximum intra assay CV was 6.3%, while the maximum inter assay CV was 6.2%. All CV values were <10%, indicating that the developed icELISA system using GRe-scFv has good accuracy.

### *Correlation between total ginsenoside concentration in ginseng measured by icELISA using GRe-scFv and MAb-4G10*

To investigate the accuracy of this assay, the total ginsenoside concentrations of various ginseng samples determined by icELISA using GRe-scFv were compared with those determined by ELISA using MAb-4G10, which showed a high correlation with HPLC analysis in our previous report *(6)*. Table III shows the results of quantitative ELISA for total ginsenosides using GRe-scFv and MAb-4G10. Our findings show that the highest concentration of ginsenosides was obtained from Tienchi ginseng, which agreed well with that determined using MAb-4G10. The total ginsenoside concentrations

calculated using the two methods showed a good correlation, with a coefficient of determination ($r^2$) of 0.997. These data indicate that GRe-scFv could be used as an alternative tool for ELISA used to determine total ginsenoside concentrations of ginseng samples.

## Conclusion

Recombinant GRe-scFv was successfully expressed in the haemolymph of silkworm larvae using BmNPV bacmid DNA system and applied it for development of icELISA to determine total ginsenoside for quality control of various ginsengs. The GRe-scFv expressed in this system overcome disadvantage of time-, cost-consuming re-folding and yield problem when it is expressed in bacterial system. In recent year, antibody drug including scFv and Fab fragment have a tendency to increase year by year. Silkworm, as a 'biofactory' for producing recombinant antibody would be alternative to the conventional bacterial system due to its advantages of low feeding cost, high production, easy purification and high safety for biohazard.

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**Conflict of interest**
None declared.

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