

High-Level Expression and Purification of Human Epidermal Growth Factor with SUMO Fusion in *Escherichia coli*

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Abstract: Human epidermal growth factor (hEGF) can stimulate the division of various cell types and has potential clinical applications. However, the high expression of active hEGF in *Escherichia coli* has not been successful, as the protein contains three intra-molecular disulfide bonds that are difficult to form correctly in the bacterial intracellular environment. To solve this problem, we fused the hEGF gene with a small ubiquitin-related modifier gene (SUMO) by synthesizing an artificial SUMO-hEGF fusion gene that was highly expressed in Origami (DE3) strain. The optimal expression level of the soluble fusion protein, SUMO-hEGF, was up to 38.9% of the total cellular protein. The fusion protein was purified by Ni-NTA affinity chromatography and cleaved by a SUMO-specific protease to obtain the native hEGF, which was further purified by Ni-NTA affinity chromatography. The result of the reverse-phase HPLC showed that the purity of the recombinant cleaved hEGF was greater than 98%. The primary structure of the purified hEGF was confirmed by N-terminal amino acid sequencing and MALDI-TOF mass spectroscopy analysis. Using the method of methylthiazole tetrazolium, the mitogenic activity on Balb/c 3T3 cells of the purified hEGF was comparable to that of commercial hEGF.

Keywords: Human epidermal growth factor, SUMO fusion, protein expression, *Escherichia coli*.

INTRODUCTION

Human epidermal growth factor (hEGF) is a powerful and broad-spectrum mitogen and can stimulate the division of a number of cell types including epithelial, fibroblast and endothelial cells. It is also capable of preventing gastric acid secretion in human [1]. Therefore, hEGF has been widely used not only for wound healing, corneal transplantation, and gastric ulcer treatment, but also in the cosmetic industry [2]. Synthetic hEGF gene has been successfully expressed in various heterologous hosts. Expression of hEGF in *Escherichia coli* is considered to be the simplest and most inexpensive means to obtain the recombinant protein for research or commercial purposes. However, as hEGF is a small protein with three disulfide bonds, it is almost impossible to fold well in the intracellular environment of *E. coli*. Several protein-fusion systems, such as His₆-tagged, glutathione-S-transferase (GST) and thioredoxin (Trx), have been applied to express and purify hEGF, but these methods have shortcomings in efficient soluble expression, cleavage and purification [3, 4]. To solve these problems, biologically active hEGF was efficiently secreted to the extracellular environment of both *E. coli* by using either the signal sequence of ompA gene or the signal sequence of alkaline phosphatase gene [5, 6, 7] and several yeasts including *Saccharomyces cerevisiae* [8], *Hansenula polymorpha* [9] and *Yarrowia lipolytica* [10]. However, the expression level of the resulting secreted EGF was much lower than that of the

intracellular expressed EGF. Moreover, EGF expressed in yeast was C-terminally cleaved from the pre-pro leader peptide resulting in a significant reduction of the yield of intact EGF and producing truncated forms of EGF1-51 and EGF1-52 [11]. Recently, EGF has also been produced by chemical synthesis but this method is not widely used due to the high cost involved [12].

Small ubiquitin-related modifier (SUMO) family proteins function as post-translation modifiers by covalently and reversibly attaching to other proteins [13]. SUMO modifies many proteins which are involved in a wide range of cellular processes such as transcriptional regulation, nuclear transportation, chromosome organization, DNA repair, and signal transduction [14]. SUMO and its associated enzymes are present in all eukaryotes and are highly conserved from yeast to human, but are absent from prokaryotes [14]. Although SUMO has only 18% sequence identity with ubiquitin, the data of structure analysis reveals that they share a common three-dimensional structure [14, 15]. Unlike ubiquitination, SUMOylation does not target proteins for degradation [16]. Recently, SUMO, fused at the N-terminus with heterologous proteins, was found to improve protein folding, to enhance expression level, and to protect the protein from degradation via its chaperoning properties. SUMO-fusion protein can be cleaved by the SUMO protease according to SUMO protein 3D structure and the target protein was obtained with native N-terminus [13,17]. Several proteins including matrix metalloproteinase (MMP13), green fluorescent protein (GFP), SARS-CoV 3CL, SARS-CoV Nc and SARS-CoV Spike C, were successfully expressed and purified using this fusion strategy [17]. In the present study, we report the high-level

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expression and rapid purification of hEGF with SUMO-fusion strategy.

MATERIALS AND METHODS

Reagents

Restriction enzymes Nde I and BamH I were purchased from NEB Company (USA); Pyrobest DNA Polymerase was from Dalian Takara Company (China); PCR purification kit, gel extraction kit, and plasmid miniprep kit were obtained from Shanghai Biocolors Company (China); hEGF standard was purchased from Guangzhou Weijia company (China); Ni-NTA Agarose was from Invitrogen (USA); The expression vector pET3c and *Escherichia coli* strain Origami (DE3) were kept by Biopharmaceutical Research and Development Center of Jinan University; The SUMO protease, and hEGF antibody were purchased from Invitrogen Company (USA). Primers were synthesized by Shanghai Sangon Company (China).

Artificial Synthesis of Fusion Gene Composed of SUMO and hEGF

The fusion gene synthesis strategy is illustrated in Fig. 1. The core fragment of SUMO (SI) was first obtained by polymerase chain reaction (PCR) performed as follows: The reaction mixture containing SUMO-F4 and SUMO-R4, dNTP and 10x PCR pfu buffer was incubated at 94 °C for 4min. Pyrobest DNA polymerase was added when the temperature was slow down to 55°C. The annealing reaction was conducted at 55°C for 5min and the elongation was taken at 72°C for 60s to get the core fragment SI. Using SI as the template, SUMO-F3 and SUMO-R4 as the forward and reverse primer, the fragment SII was obtained by the second round using standard PCR techniques. The third round PCR

was run to amplify the fragment SIII from SII by using SUMO-F2 and SUMO-R2 as the forward and reverse primer. The fragment SIV with full length SUMO cDNA and 6x His-tag was obtained by the last round PCR with SIII as the template, and with SUMO-F1 and SUMO-R1 as the forward and reverse primer.

The full-length hEGF cDNA was obtained via the same procedure as shown above and the PCR products were named EI, EII and EIII respectively. Using fragments of SIV and EIII as the templates, the target fusion gene was amplified by a standard PCR with SUMO-F1 and EGF-R1 as the forward and reverse primer. The fusion gene was digested with Nde I and BamH I, and then ligated into previously digested pET-3c vector to create the corresponding expression vector pET-SUMO-hEGF. Accuracy of the inserted cDNA was confirmed by automated DNA sequencing.

Induction and Expression of SUMO-hEGF

Protein expression was performed as following: a single transformed colony was grown in 4ml LB media containing 100 µg/ml ampicillin and 1% glucose at 37°C with shaking at 250 rpm. The cells were grown overnight and then 100 µl culture was transferred into 50ml fresh LB medium without 1% glucose to permit exponential growth. When the OD600 value reached 0.6, the culture was divided into four tubes and Isopropyl-β-D-thiogalactopyranoside (IPTG) was added to a final concentration of 0.4mmol/L. Four divided tubes were continued to grow for 4h at 37°C, 30°C and 20°C, respectively or for 24 h at 20°C to determine optimal induction conditions. To determine the effects of temperature shifts on the expression of SUMO-EGF fusion protein, a single colony was grown at 30°C using the same methods described above. When the OD600 value reached 0.6, the temperature of incubation was shifted to 42°C and the culture was con-

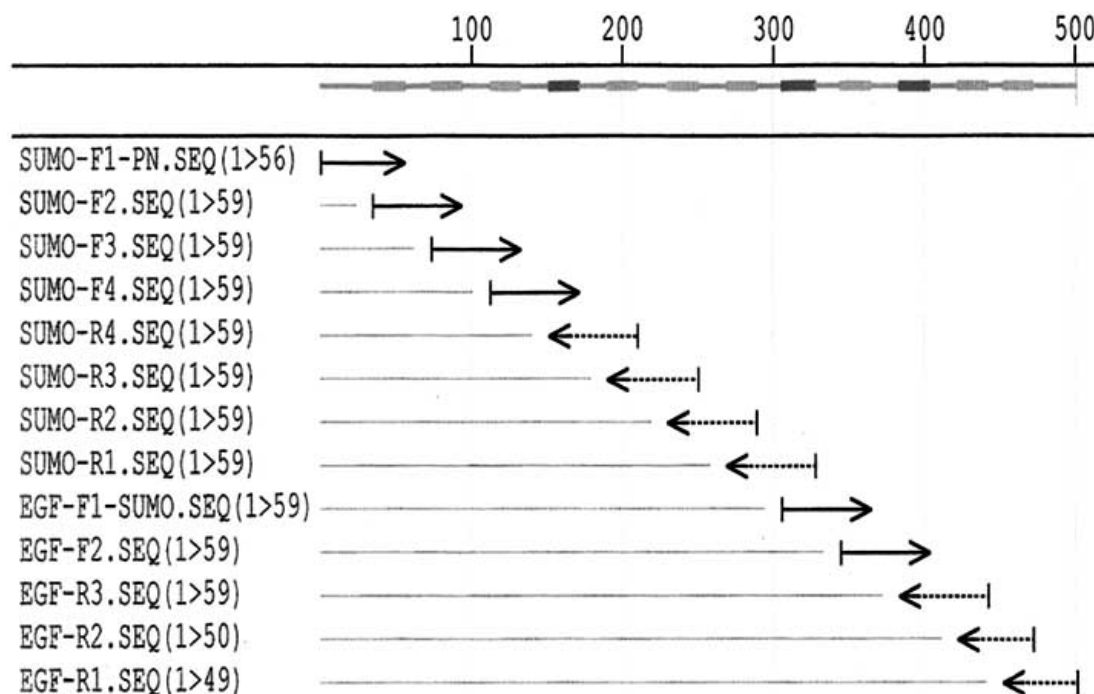


Figure 1. PCR Strategy for artificial synthesis.

tinued to grow with a final IPTG concentration of 0.4mmol/L. For protein purification, cultures were scaled up to 1.0L medium.

Detection of Soluble Situation of SUMO-hEGF

Cells of 10 ml induced at different conditions were collected by centrifugation at 6000 rpm for 5 min at 4°C. The cell pellets were freeze-thawed once and resuspended in 20mmol/L PBS buffer of 7ml containing 10,000 IU lysozyme for 1 g of the cells. The suspensions were incubated at 30°C for 30min and then centrifuged at 18,000 rpm for 20 min at 4°C. The pellets were discarded and the supernatant was mixed with SDS-PAGE sample buffer and heated at 95°C for 5 min. The soluble proteins were analysed by 12 % SDS-PAGE and the expression level of SUMO-hEGF was detected by densitometer scanning.

Purification of SUMO-hEGF

The cell lysate was applied to a column containing 10 ml Ni-NTA resin (Invitrogen) equilibrated with 20 mmol/L PBS buffer. The resin was washed with 100ml wash buffer I (20 mmol/L PBS containing 10mmol/L imidazole and 150mmol/L NaCl, pH 8.0) until OD280 reached base line. The contaminated proteins were eluted with 100-150ml wash buffer II (20mmol/L PBS containing 20mmol/L imidazole and 150mmol/L NaCl, pH 8.0). Finally, the 6xHis-tagged SUMO-fusion proteins were eluted with elution buffer (20 mmol/L PBS containing 300mmol/L imidazole and additional 150mmol/L NaCl, pH 8.0). Fractions of purification steps were pooled at the elution peak. The purity of SUMO-hEGF was assessed using SDS-PAGE and the concentration was evaluated by Bradford method.

Cleavage of SUMO-hEGF and Purification of hEGF

The purified fusion protein was diluted to the concentration of 1mg/ml, 10U SUMO protease was added to the protein and the mixture was incubated in high salt buffer (500mmol/L Tris-HCl, pH 8.0, 2% Igepal, 1.5mol/L NaCl, 10mmol/L DTT) at 4°C or 30°C for 30 min to 1h. The cleaved sample was applied to the Ni-NTA resin to obtain the recombinant hEGF, which was further concentrated by using ultrafilter with 3kDa cutoff membrane at 4°C. The protein (SUMO-hEGF, SUMO and SUMO protease) bound to the resin was eluted as described above. The immunogenic activity of hEGF was checked by Western blotting. The purified protein was desalted and then loaded onto a C18 column. The elution was carried out using a linear gradient of 30–70% acetonitrile at a flow rate of 0.8mL/min in the presence of 0.1% trifluoroacetic acid (TFA). The fractions containing hEGF were pooled and subjected to N-terminal amino acid sequencing, amino acid composition analysis and MALDI-TOF mass spectroscopy analysis. The concentration of hEGF was evaluated by Bradford method.

Bioassay of Mitogenic Activity of Recombinant hEGF

Balb/c 3T3 cells were grown in Medium 1640 supplemented with 10% fetal bovine serum, 100 U/ml ampicillin and 100 U/ml streptomycin. When the culture reached at the

mid-logarithm time, cells were transferred to a 96-well plate (7000/well) and incubated in Medium 1640 containing the above supplements for 24h. The medium was replaced with Medium 1640 without fetal bovine serum and the cells were cultured for 24h. The cells were washed once with PBS, treated with recombinant hEGF, SUMO-hEGF and commercial hEGF with different concentrations (form 0.006-25 ng/ml), and incubated for 24-48h. The number of viable cells was determined by adding 20µl methylthiazoleterazolium (MTT) (5mg/ml) to each well and incubated for 4h. After discarding the medium, 150µl dimethyl sulfoxide (DMSO) was added to each well. The plate was kept at room temperature for 30 min. The absorbance was measured by absorption at 570 nm immediately.

RESULTS

Artificial Synthesis of Fusion Gene and Construction of SUMO-hEGF Expression Strain

To synthesize the DNA fragment coded fusion protein composed of SUMO and hEGF, thirteen special primers were designed (Table 1). The strategy of synthesis is described in the Material and Methods, and the molecular weight of PCR products of each step was shown in Fig. 2a and 2b. Final PCR product digested with two restriction enzymes (Nde I and BamH I) was cloned in the expression vector pET-3c and the sequence of fusion gene was confirmed by automated DNA sequencing. Recombinant plasmid pET-SUMO-hEGF containing accurate fusion gene was transform into *E. coli* Origami (DE3).

Expression of SUMO-hEGF

When the culture reached at the mid-logarithm time, *E. coli* cells harboring pET-SUMO-hEGF were treated with 0.4mmol/L IPTG. The expression of a ~27 kDa protein corresponding to the predicted size was induced in the presence of IPTG. The soluble expression levels of SUMO-hEGF were 2.3% at 37°C, 19.3% at 30°C, 13.6% at 20°C for 4 h and 38.9% at 20°C for 24 h respectively (Fig. 3 and 4). The optimal soluble expression condition was conducted at 20°C for 24 h. According to Fig. 5, there was only about 5.5% in the supernatant with acute heat shock (30-42°C) during induction.

Purification of SUMO-hEGF

The cells lysate was applied to an affinity column. Proteins without 6x His tags were removed from the Ni-NTA resin using PBS containing 10mmol/L and 20mmol/L imidazole, and the SUMO-hEGF was eluted using PBS containing 300mM imidazole. The result of SDS-PAGE electrophoresis showed that the purity of SUMO-hEGF was 87.2% (Fig. 6).

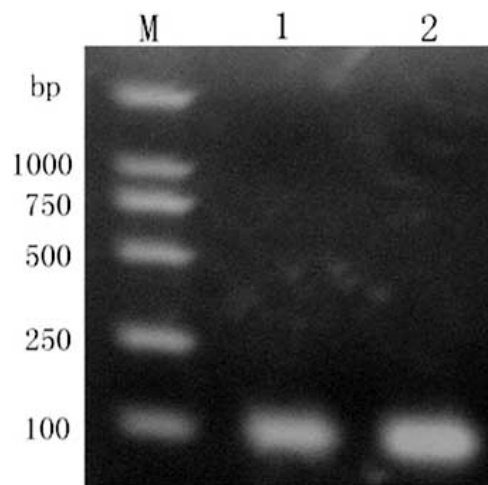
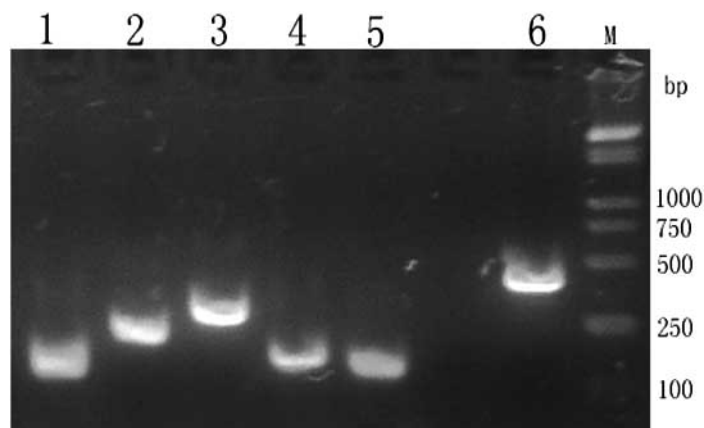
Cleavage of SUMO-hEGF and Purification of hEGF

About 5g cell pellets were collected from 1 liter of culture with 0.4mM IPTG inducement. The pellets were treated according to material and methods. About 54.3mg of fusion protein was obtained from 1 L culture. The fusion protein was diluted and cleaved by SUMO protease. The result of SDS-PAGE showed that about 65% of SUMO-hEGF protein

Table 1. PCR Primers for Amplifying the SUMO-EGF Fusion Protein Genes

	Primers
SUMO-F4	AAGGTGTCCGATGGATCTTCAGAGATCTTCTTCAAGATCAAAAAGACCACTCCTTTAAG
SUMO-R4	TCCTTACCCTGTCTTTTAGCGAACGCTTCCATCAGCCTTCTTAAAGGAGTGGTCTTTTT
SUMO-F3	GTCAAGCCAGAAGTCAAGCCTGAGACTCACATCAATTTAAAGGTGTCCGATGGATCTTC
SUMO-F2	ATGTCGGACTCAGAAGTCAATCAAGAAGCTAAGCCAGAGGTCAAGCCAGAAGTCAAGCC
SUMO-R3	ATTCTAATACCGTCGTACAAGAATCTTAAGGAGTCCATTTCCCTTACCCTGTCTTTTAGC
SUMO-R2	TCCTCCATGTCCAAATCTTCAGGGGTCTGATCAGCTTGAATTCTAATACCGTCGTACAA
SUMO-R1	CCACCAATCTGTTCTCTGTGAGCCTCAATGATATCGTTATCCTCCATGTCCAAATCTTC
SUMO-F1-PN	AACTGCAG CATATG CATCATCATCATCACGGCATGTCCGACTCAGAAGTCAAT
EGF-F2	GTCCTCTGTCCCACGATGGTTACTGTTACACGATGGTGTGTGTATGTACATTGAAGCT
EGF-R3	GATGTAACCGACGACACAGTTACAAGCGTACTTGTCCAAAGCTTCAATGTACATACACA
EGF-F1	CTCACAGAGAACAGATTGGTGGTAATTCAGACTCTGAATGCCTCTGTCCCACGATGGT
EGF-R2	CTTCAGGTCTCTGTACTGACATCTCTCACCGATGTAACCGACGACACAGT
EGF-R1	GAG GGATCC TCATCATCTCAGTTCACCACCACTTCAGGTCTCTGTACTGAC

Restriction enzyme recognition sites used for cloning are *Nde*I in SUMO-F₁-PN and *Bam*HI in EGF-R₁ and indicated in boxed letters. The sequence of bold letters showed in SUMO-F₁-PN is 6xHis tag.

**Figure 2a.** The first-step PCR amplified products. M: PCR marker; 1: core fragment of SUMO (SI); 2: core fragment of EGF (EI).**Figure 2b.** The PCR products amplified from second step to final step. 1. PCR product of fragment SII; 2. PCR product of fragment SIII; 3. PCR product of fragment SIV; 4. PCR product of fragment EIII; 5. PCR product of fragment EII; 6. PCR product of SUMO-hEGF fusion gene using SIV and EIII as templates; M: PCR marker.

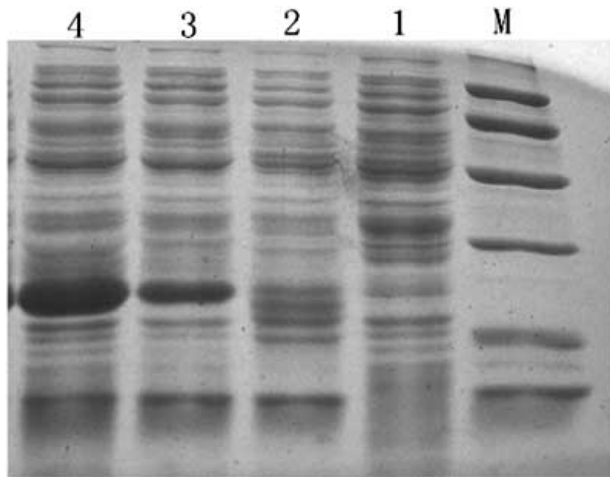


Figure 3. SDS-PAGE analysis of the supernatant of Origami (DE3)/ pET-SUMO-hEGF induced at different temperatures. M: low molecular protein marker (from up to down: 97.4, 66.2, 42.7, 31.0, 20.5, 14.4 kDa); 1. uninduced Origami (DE3)/ pET-SUMO-hEGF; 2. Supernatant of Origami (DE3)/ pET-SUMO-hEGF induced at 37°C for 4 h; 3. Supernatant of Origami (DE3)/ pET-SUMO-hEGF induced at 30°C for 4 h; 4. Supernatant of Origami (DE3)/ pET-SUMO-hEGF induced at 20°C for 24 h.

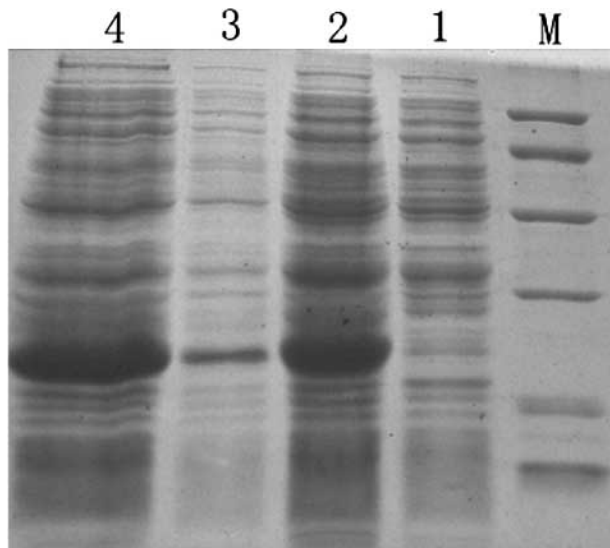


Figure 4. SDS-PAGE analysis of the supernatant of Origami (DE3)/ pET-SUMO-hEGF induced at different temperature and time. M: low molecular protein marker (from up to down: 97.4, 66.2, 42.7, 31.0, 20.5, 14.4 kDa); 1. uninduced Origami (DE3)/ pET-SUMO-hEGF; 2. Induced Origami (DE3)/ pET-SUMO-hEGF induced at 30°C for 4h; 3. Supernatant of Origami (DE3)/ pET-SUMO-hEGF induced at 20°C for 4 h; 4. Supernatant of Origami (DE3)/ pET-SUMO-hEGF induced at 20°C for 24 h.

was cleaved within 1h at 4°C (Fig. 7) and 90% was digested within 1h at 30°C (data not shown). The cleaved SUMO-fusion sample was then reloaded to the Ni-NTA resin to obtain the recombinant hEGF. The purity of hEGF detected by SDS-PAGE was higher than 95%. The western blotting result indicated that the hEGF antibody could react with puri-

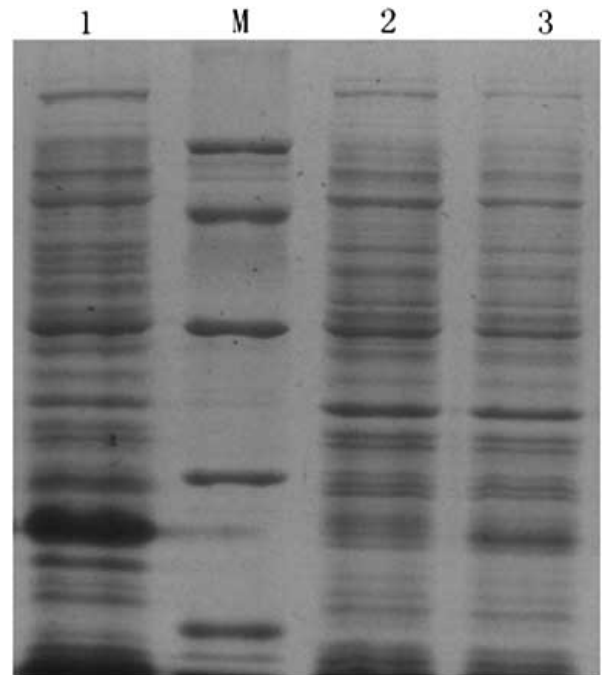


Figure 5. SDS-PAGE analysis of effects of heat shock on the expression. M: low molecular protein marker (from up to down: 97.4, 66.2, 42.7, 31.0, 20.5, 14.4 kDa); 1: Supernatant of Origami (DE3)/ pET-SUMO-hEGF induced at 30°C for 4 h; 2: Supernatant of uninduced Origami (DE3)/ pET-SUMO-hEGF; 3: Supernatant of Origami (DE3)/ pET-SUMO-hEGF which cultured at 30°C and then shifted 42°C to heat shock during induction with 0.4mmol/L IPTG for 4 h.

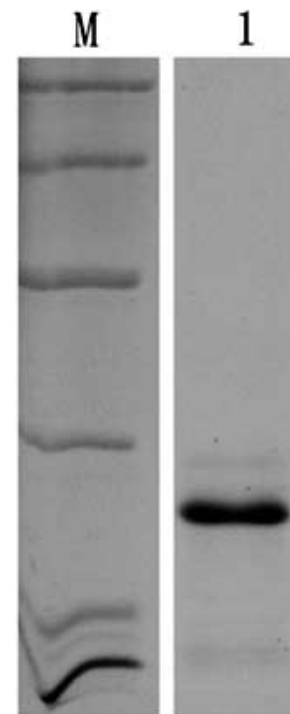


Figure 6. SDS-PAGE analysis of purified SUMO-hEGF. M: low molecular protein marker (from up to down: 97.4, 66.2, 42.7, 31.0, 20.5, 14.4 kDa); 1. Purified SUMO-hEGF eluted with PBS containing 300mmol/L imidazole and additional 150mmol/L NaCl.

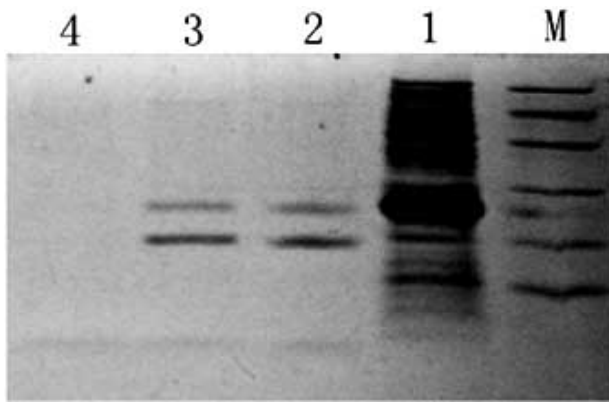


Figure 7. SDS-PAGE analysis of SUMO-hEGF digested by SUMO protease and purified hEGF. M: low molecular protein marker (from up to down: 97.4, 66.2, 42.7, 31.0, 20.5, 14.4 kDa); 1. Supernatant of Origami (DE3)/pET-SUMO-hEGF induced at 20°C for 24h; 2. Digestion of purified fusion SUMO-hEGF using SUMO protease at 4°C for 0.5h; 3. Digestion of purified fusion SUMO-hEGF using SUMO protease at 4°C for 1 h; 4. The purified hEGF obtained after reloading the digested sample through the Ni-NTA column.



Figure 8. The western blotting analysis of SUMO-hEGF and purified hEGF.
1. SUMO-hEGF; 2. hEGF.

fied hEGF but not with fusion protein (Fig. 8). The reason may be exist in that the SUMO blocked the antigenic site of the hEGF. After concentration and desalting, the recombinant hEGF was further purified by reverse-phase HPLC. The HPLC chromatograms of the recombinant and the commercial hEGF indicated that the time of emergent peak of recombinant hEGF was as same as the commercial hEGF and the purity of the recombinant hEGF was higher than 98% (Fig. 9). The final yield of purified recombinant hEGF from one liter of culture was about 16.67mg.

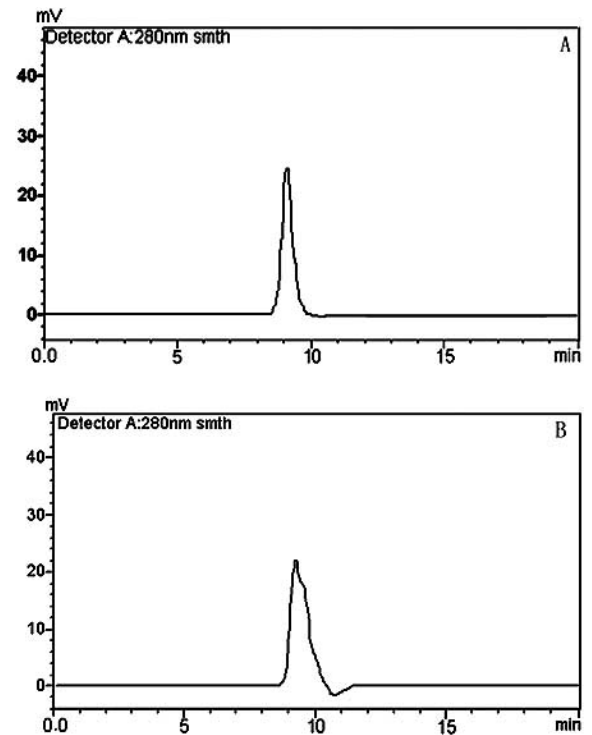


Figure 9. The purity of recombinant hEGF analyzed by RP-HPLC
A: commercial hEGF; B: recombinant hEGF.

The Authenticity of the recombinant hEGF

In order to confirm its authenticity, recombinant hEGF was subjected to N-terminal amino acid sequencing, amino acid composition and MALDI-TOF mass spectroscopy analysis. According to the sequencing result, the first six amino acid of N-terminal of recombinant hEGF was N-S-D-S-E-C, which was as same as the native hEGF sequence. On the other hand, the result of amino acid composition analysis indicated that the amino acid composition of the recombinant hEGF was also in accordance with the anticipated composition (data not shown). Moreover, the molecular weight of cleaved recombinant hEGF analyzed by MALDI-TOF mass spectroscopy was 6216.94 Dalton, which was similar to the theoretical molecular weight of hEGF (6221.2 Dalton) (Fig. 10).

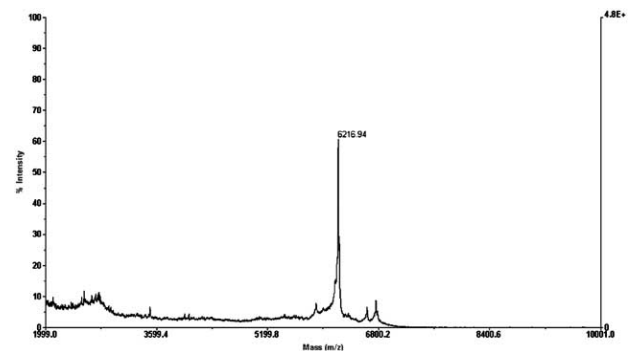


Figure 10. MALDI-TOF mass spectroscopy analysis of recombinant hEGF.

Mitogenic Activity of hEGF and Fusion Protein

The result of mitogenic assay showed that the mitogenic activity of hEGF cleaved from SUMO-fusion protein was comparable to that of the commercial hEGF (Fig. 11). The value of ED50 of purified and standard hEGF was 1.77×10^4 $\mu\text{mol/L}$ and 1.61×10^4 $\mu\text{mol/L}$ respectively. Whereas, the mitogenic activity of the fusion protein, SUMO-hEGF, was much lower (ED50= 4.36×10^{-4} $\mu\text{mol/L}$) than that of the commercial hEGF.

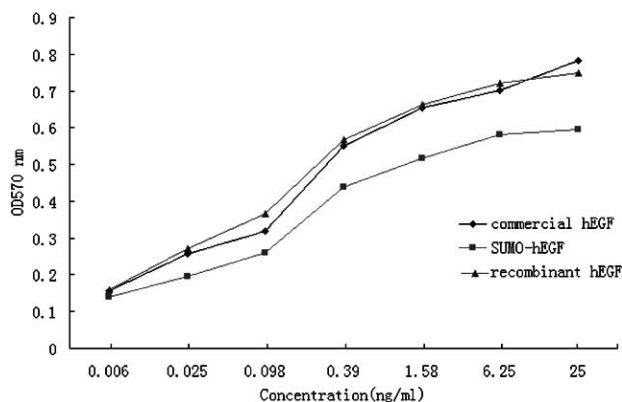


Figure 11. The stimulation effect of commercial hEGF, recombinant hEGF and SUMO-hEGF on Balb/c 3T3 cells.

DISCUSSION

EGF is a member of a large family of closely related proteins that can modulate the function of a wide range of cell types. It is well known that EGF is difficult to be highly solubly expressed in intracellular in *E. coli*, even with a regular fusion protein partner [3]. Recently, SUMO, based on its unique characters, has been used as a novel fusion partner for the synthesis of recombinant proteins. Several valuable and difficult-to-express proteins have been expressed successfully in *E. coli* expression system by using this technology [16]. The exact mechanism whereby SUMO elicits its beneficial effects has not been described clearly. There are two hypotheses to explain these results. First, SUMO acts as a chaperone, or, second, SUMO acts as a nucleation site for the folding of the target protein [17]. In this paper, we successfully highly expressed hEGF with SUMO fusion in *E. coli*. Moreover, since the SUMO fusion proteins and the SUMO protease bear an N-terminal 6x His tag, the cleaved SUMO-fusion samples could be re-applied to the Ni-NTA column to rapidly obtain the purified native hEGF. The purity and authenticity of recombinant hEGF were assessed by four independent methods: RP-HPLC, amino acid composition, protein N-terminal sequencing and MALDI-TOF mass spectroscopy analysis. The result of HPLC manifested that the purity of hEGF was higher than 98%. The results of the other three methods revealed that the recombinant hEGF was identical to the native one, indicating that this strategy of producing EGF was feasible. The results of the expression and mitogenic activity indicated that the SUMO could significantly enhance the expression and efficiently improve the accurate folding of hEGF. However, the mitogenic activity of the fusion protein, SUMO-hEGF, was

much lower than that of the hEGF probably because SUMO prevents hEGF from binding to the EGF receptor. The final yield of recombinant hEGF is 16.67 mg/L, which is much higher than other published methods. For example, Oka *et al.* [5] obtained a yield of 2.4 mg/L of secreted EGF from *E. coli*. Soler *et al.* [3] obtained 3-10 mg/L of fusion expressed EGF from *E. coli*. Heo *et al.* [9] had a yield of 0.57mg/L from methylotrophic yeast (*Hansenula polymorpha*).

Ubiquitin is also used as fusion tag to enhance the expression and improve the recombinant proteins folding [18]. The fusion proteins can be induced to highly express in regular temperatures [18, 19]. Pilon *et al.* have reported that a heat shock during fermentation could substantially increase the production of fusion protein. The ubiquitin fusion protein comprised 16% of the wet weight of the cell paste and remained soluble [19]. They thought that ubiquitin played a role in bacterial host survival [19]. Compared with ubiquitin, SUMO fusion is more suitable for large-scale expression induced at 20°C overnight, since a shorter time (e.g., 4 h) or higher temperature (37°C) resulted in lower yields of soluble proteins. Furthermore, heat shock during fermentation led to decrease the yield of soluble fusion protein significantly and produced mass of inclusion bodies (data not shown). For this phenomenon, we speculate that SUMO gene used in the experiment origins from *Saccharomyces cerevisiae*, which seems to be related to the SUMO-1 genes of vertebrate in phylogenetic relationship using methods of maximum parsimony (MP) and minimum evolution (ME), and may not have same function as ubiquitin when temperature elevations [15]. However, SUMO-2/3 may be potential candidates. It is well known that yeast and invertebrates contain a single SUMO gene, whereas vertebrates contain three paralogues: SUMO-1, SUMO-2 and SUMO-3 [14]. Saitoh and Hinchey reported that there were some functional differences between SUMO-2/3 and SUMO-1 [20]. On one hand, the conjugation of SUMO-2/3 with substrates is strongly induced in response to various cellular stresses, such as acute heat elevation, but SUMO-1 conjugation is not. On the other hand, SUMO-2/3 can form polymeric chains to conjugate substrates of interest like ubiquitin does [21].

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