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Expression of human epidermal growth factor using *Ssp* DnaB mini-intein as fusion partner in *Escherichia coli* BL21(DE3)

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ABSTRACT

The human epidermal growth factor (hEGF) is widely used clinically as a wound healer, as it has a vital role in stimulating cell proliferation, differentiation, and migration. Consequently, the large-scale production of recombinant hEGF in *E. coli* has been developed to meet the high demand for hEGF clinically. However, intracellular proteins in *E. coli*, especially small proteins like hEGF, are degraded by proteases. To overcome this issue, hEGF was fused with CBD-*Ssp* DnaB to construct a fusion protein CBD-*Ssp* DnaB-hEGF. This study was conducted to obtain refolded hEGF from the inclusion bodies of CBD-*Ssp* DnaB-hEGF. The experiment was carried out using *E. coli* BL21(DE3) containing plasmid pD861-CBD-*Ssp* DnaB-hEGF. The CBD-*Ssp* DnaB-hEGF gene was constructed by fusing CBD-*Ssp* DnaB and hEGF gene and then was optimized. The method was started with *E. coli* transformation, CBD-*Ssp* DnaB-hEGF expression, inclusion bodies solubilization, refolding, and simultaneous cleavage to release hEGF. The CBD-*Ssp* DnaB-hEGF was expressed as inclusion bodies, which can then be purified by washing with Triton X-100 and 1 M urea. The inclusion bodies were solubilized in 8 M urea, the solubilized CBD-*Ssp* DnaB-hEGF was reformed by dialysis, and then hEGF was spliced by shifting the pH from 8.5 to 6.0 to yield a concentration of 0.163 mg/ml. Therefore, we concluded that hEGF was obtained from the solubilized CBD-*Ssp* DnaB-hEGF from inclusion bodies produced by *E. coli* BL21(DE3).

INTRODUCTION

Human epidermal growth factor (hEGF) is a protein with 53 amino acid residues with 6.2 kDa of molecular weight. This polypeptide has three intramolecular disulfide bonds and heat resistance properties (Eissazadeh *et al*., 2017; Sriwidodo *et al*., 2019; Tang *et al*., 2016). hEGF plays a role in stimulating cell proliferation, differentiation, and migration in the wound-healing process. This has led to the high demand for hEGF in the clinical field, thus encouraging efforts to increase hEGF production through recombinant DNA technology (Ma *et al*., 2016; Zheng *et al*., 2016).

E. coli was one of the most widely used hosts for recombinant protein expression (Hayat *et al*., 2018). It has a high rate of protein expression and rapid growth, is inexpensive, and produces culture in the high-density cell (Kaur *et al*., 2018; Kim *et al*., 2017; Maksum *et al*., 2019). There were previous studies on the extracellular expression of hEGF in *E. coli* using various signal peptides (Indriyani *et al*., 2019; Maksum *et al*., 2017; Melati *et al*., 2019; Sriwidodo *et al*., 2017). Nevertheless, the extracellular expression system has a lower expression rate than the intracellular system (Su *et al*., 2006). To overcome this problem, the intracellular expression approach was currently used to increase hEGF expression.

Intracellular protein expression in *E. coli* especially small protein such as hEGF mostly did not work well because the small protein was easily degraded by proteases from the host cell. This causes a significant loss in yields and made it difficult in the purification process. Alternatively, the protein was fused with a fusion partner so the target protein could avoid degradation by proteases (Sun *et al*., 2005; Zhang *et al*., 2015). Several fusion tags were commonly used in fusion protein expression systems such as glutathione S-transferase, small ubiquitin-related modifier, thioredoxin, and maltose-binding protein. However, there were

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some difficulties in fusion tag removal such as unspecific cleavage site, the steric hindrance at the cleavage site, and the expensive cost of specific protease (Costa *et al*., 2014; Zheng *et al*., 2016).

The intein is a protein segment that can cleavage itself from protein precursors where it fuses two segments of flanking proteins or amino acids (Li, 2011). The intein-mediated expression system has been developed as a powerful tool for protein expression that utilized its ability to self-cleavage. Target protein synthesized in the fusion of intein form which has been genetically designed to do controllable cleavage from peptide bond at either N- or C-terminal of the intein. After protein cleavage by intein, the target protein was produced with the desired terminal amino acid (Jiang *et al*., 2015). *Ssp* DnaB mini-intein derived from *Synechocystis* sp. was used in the commercial plasmid, such as IMPACT™ system (Ding *et al*., 2003). The release of the target protein can be induced simply by changing pH, giving the advantage of cleaving peptide bonds without using specific protease (Sun *et al*., 2005).

The expression of recombinant protein in *E. coli* frequently leads to the formation of inclusion bodies. Inclusion bodies are protein aggregate that consists of partially folded and misfolded protein that interacts via hydrophobic interaction. Their formation is often undesirable because they generally lack biological activity (Gomes *et al*., 2016; Silaban *et al*., 2019; Singh and Panda, 2005). This approach can give the advantage by refolding the inactive protein in inclusion bodies into its active form (Upadhyay *et al*., 2016). Moreover, inclusion bodies are produced in high amount and easy to separate because they were denser than the other cellular components (Singh and Panda, 2005; Singh *et al*., 2015). To recover active protein from inclusion bodies, inclusion bodies were solubilized in presence of chaotropic agents such as urea and guanidine chloride and afterward solubilized protein was refolded by decreasing the concentration of chaotropic agents (Singh *et al*., 2015; Vallejo and Rinas, 2004).

In this report, we construct fusion protein CBD-*Ssp* DnaB-hEGF by fused hEGF gene with the CBD-*Ssp* DnaB gene in pD861 plasmid. *E. coli* BL21(DE3) was transformed with recombinant plasmid. CBD-*Ssp* DnaB-hEGF was successfully expressed as inclusion bodies. Inclusion bodies were isolated, solubilized, and refolded. *Ssp* DnaB mini-intein splicing activity could be achieved by gradually shifting pH from 8.5 to 6.0. After *Ssp* DnaB mini-intein cleavage, hEGF protein was obtained with a concentration of 0.163 mg/ml. The result demonstrated that hEGF protein could be recovered by inclusion bodies obtained from intracellular expression in *E. coli*.

MATERIALS AND METHODS

Bacterial strain, plasmid, and materials

E. coli BL21(DE3) was taken from our laboratory stock. Plasmid pD861-CBD-*Ssp* DnaB-hEGF was constructed by fused *CBD-Ssp DnaB* gene [National Center for Biotechnology Information (NCBI) access code HG792069.1] and *hEGF* gene (NCBI access code GQ214314.1) into plasmid pD861 and synthesized by ATUM, Newark, CA. Citric acid, sodium chloride, urea, Tris base, β-mercaptoethanol, and calcium chloride were purchased from Merck, Kenilworth, NJ. Yeast extract was purchased from Oxoid, United Kingdom. Agar bacto, ethylenediaminetetraacetic acid (EDTA), tryptone, and glycerol were purchased from 1st base, Singapore. L-rhamnose, tricine, Triton X-100, kanamycin sulfate, and N,N,N',N'-tetramethylethylenediamine (TEMED) were purchased from Sigma-Aldrich, Singapore.

Expression of recombinant CBD-Ssp DnaB-hEGF fusion protein

To express CBD-*Ssp* DnaB-hEGF, $CaCl_2$ competent *E. coli* BL21(DE3) cells were transformed with pD861-CBD-*Ssp* DnaB-hEGF plasmid using the heat shock method. Transformed cells were grown in LB agar plates supplemented with 50 µg/ml kanamycin at 37°C for 16–18 hours. Single colonies were replicated and the selected replica was grown in 5 ml LB medium supplemented with 50 µg/ml kanamycin at 37°C for 16–18 hours. A total of 1 ml of starter culture was transferred into 100 ml LB medium and incubated at 37°C with shaking at 200 rpm until OD_{600} 0.6 was reached and then was induced by adding L-rhamnose until the final concentration of 4 mM and incubated for 5 hours. The culture was centrifuged at 6,000 g for 20 minutes at 4°C. The cell pellet was stored at −20°C for further analysis.

Inclusion bodies preparation

Cell pellet (approximately 2 g wet weight) were resuspended in 1:4 ratio with lysis buffer (20 mM Tris-Cl, 1 mM EDTA, and pH 8.5); then, the suspension was sonicated for 1 minute (1 second on and 1 second off pulse). Sonication was performed on ice for 10 cycles with 1 minute gap for each cycle. The lysed cell suspension was centrifuged at 12,000 g for 20 minutes at 4°C. The pellet obtained was washed twice using washing buffer I (20 mM Tris-Cl, 1 mM EDTA, 1% Triton X-100, 1 M urea, and pH 8.5), once using washing buffer II (20 mM Tris-Cl, 1 mM EDTA, 1% Triton X-100, and pH 8.5), and twice using washing buffer III (20 mM Tris-Cl and pH 8.5). Purified inclusion bodies were resuspended in 20 mM Tris-Cl and pH 8.5 and used for solubilization.

Optimization of urea concentration for solubilization of inclusion bodies

Purified inclusion bodies' suspension was solubilized with 20 mM Tris-Cl pH 8.5 containing different urea concentrations to optimize solubilization conditions for inclusion bodies of CBD-*Ssp* DnaB-hEGF fusion protein. Inclusion bodies' suspension was solubilized with 1:10 solubilizing buffer (20 mM Tris-Cl and pH 8.5) with different concentrations of urea (0–8 M urea). The mixture was incubated for 2 hours at room temperature with shaking at 150 rpm and after that centrifuged at 13,000 rpm for 20 minutes at 4°C. Solubilized inclusion bodies were characterized by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) and analyzed by *ImageJ* software.

Solubilization and refolding of solubilized CBD-Ssp DnaB-hEGF

Purified inclusion bodies were solubilized with 25 ml solubilizing buffer (10 mM Tris-Cl, 5 mM β-mercaptoethanol, 8 M urea, and pH 8.5) (Zhang *et al*., 2015) for 2 hours at room temperature with shaking at 150 rpm and centrifuged at 10,000 g for 30 minutes at 4°C. Solubilized inclusion bodies were refolded using the dialysis method according to Zhang *et al*. (2015) with

some modification. Solubilized inclusion bodies (10 ml) was dialyzed with 1 L refolding buffer I (10 mM Tris-Cl, 0.5 mM EDTA, 50 mM NaCl, 5 mM β-mercaptoethanol, 2 M urea, and pH 8.5) for 24 hours at 4°C, followed by 1 L refolding buffer II (10 mM Tris-Cl, 0.5 mM EDTA, 50 mM NaCl, and pH 8.5). Refolded protein was cleaved by incubating it in buffer with different pH (8.0, 7.5, 7.0, 6.5, 6.0, and 5.6) for 24 hours at 4°C, respectively. The refolded protein and cleaved protein were characterized by SDS-PAGE and analyzed by *ImageJ* software.

RESULTS AND DISCUSSION

Construction CBD-Ssp DnaB-hEGF gene in recombinant plasmid

CBD-Ssp DnaB-hEGF gene was constructed from the sequence of the *hEGF* (GeneBank GQ214314.1) and *CBD-Ssp DnaB* (GeneBank HG792069.1). The first methionine from hEGF was eliminated and fused to asparagine at the end of the C-terminal of CBD-*Ssp* DnaB (Fig. 1). Asparagine residue on C-terminal of *Ssp* DnaB mini-intein was directly fused to alanine residue on N-terminal of hEGF. The expression rate of heterologous protein on *E. coli* was closely related to the codon usage bias phenomenon (Gomes *et al*., 2016). Bias codon could be avoided by optimizing the *CBD-Ssp DnaB-hEGF* gene to have high CAI value and GC content in the range of 30%–70% (Parret *et al*., 2016; Silaban *et al*., 2014; Sriwidodo *et al*., 2017). *CBD-Ssp DnaB-hEGF* gene was optimized by using *E. coli* preference codon to achieve better protein expression. The optimized *CBD-Ssp DnaB-hEGF* gene had a length of 843 bp. CAI value of gene was 1.00 with GC content of 51.89%. *CBD-Ssp DnaB-hEGF* gene was inserted into plasmid pD861 and synthesized by ATUM (Fig. 2). The promoter was a key component in protein expression because it plays a role in regulating the expression of the gene of interest. L-rhamnose promoters provide more tight regulation which will prevent leakage of protein (Kaur *et al*., 2018). Therefore, it is assumed to be able to increase protein acquisition after the addition of an inducer. The use of plasmid with high copy numbers and a strong ribosomal binding site can also increase the rate of protein expression (Hu *et al*., 2012; Marschall *et al*., 2016).

Expression of recombinant CBD-Ssp DnaB-hEGF and inclusion bodies preparation

Recombinant CBD-*Ssp* DnaB-hEGF was successfully expressed as 31.2 kDa protein in *E. coli*. CBD-*Ssp* DnaB-hEGF was majorly expressed in inclusion bodies form compared to soluble form (Fig. 3a). Induction at mid-log phase (OD_{600} 0.6) provides a high expression rate due to *E. coli* rapid growth which leads to a higher protein expression rate (Fazaeli *et al*., 2019; Kaur *et al*., 2018). In a previous study, the L-rhamnose promoter was successfully expressing recombinant protein extracellularly using signal peptide (Indriyani *et al*., 2019; Maksum *et al*., 2019). However, a recent study showed that the L-rhamnose promoter

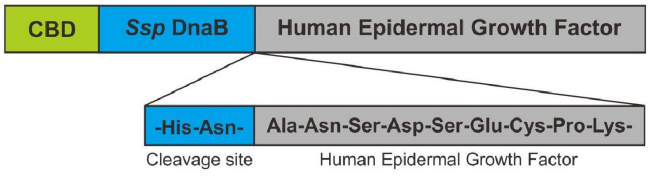

Figure 1. Scheme of CBD-*Ssp* DnaB-hEGF fusion protein assembly (modified from Király *et al*., 2006).

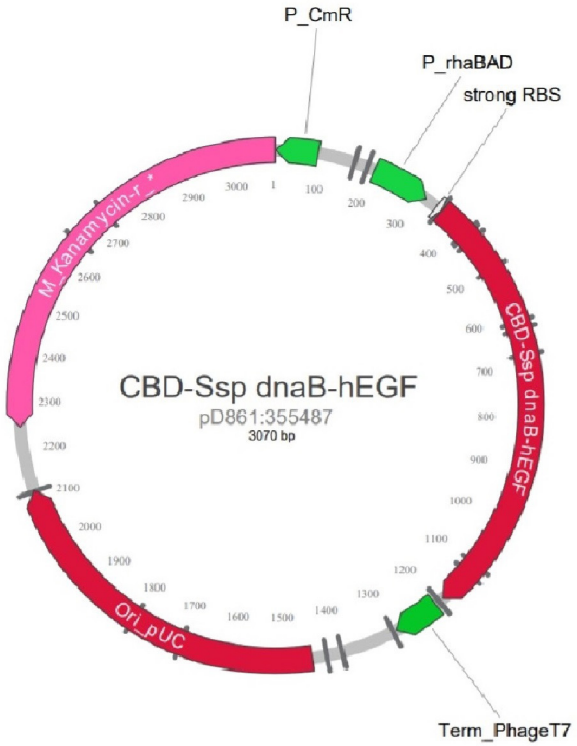

Figure 2. Construction of plasmid pD861-CBD-*Ssp* DnaB-hEGF. The plasmid's characteristics were rhamnose promoter, kanamycin selection marker, Ori pUC, and strong RBS.

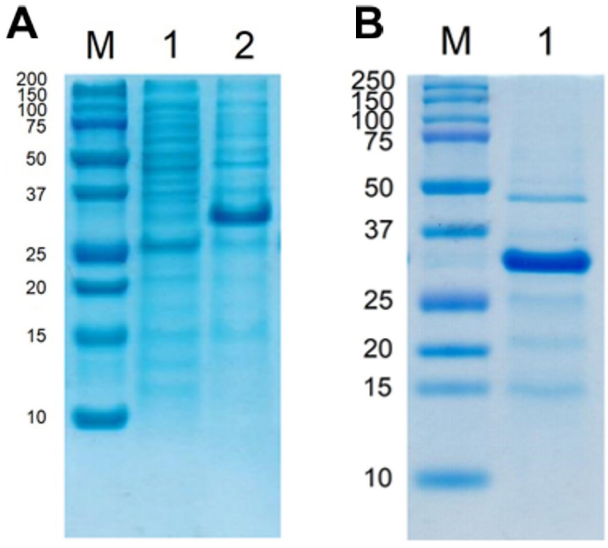

Figure 3. Expression of CBD-*Ssp* DnaB-hEGF and inclusion bodies preparation. (a) Tricine SDS-PAGE analysis of CBD-*Ssp* DnaB-hEGF expression as inclusion bodies. Lane 1: soluble fraction, lane 2: insoluble fraction, and lane M: protein marker. (b) Tricine SDS-PAGE analysis of purified inclusion bodies after several washing steps. Lane 1: purified IB and lane M: protein marker (size of protein in kilodalton is shown on the left).

can also be utilized for intracellular expression of CBD-*Ssp* DnaB-hEGF as well. Inclusion bodies were washed by detergent to remove contaminants such as cell membrane and cellular protein following aggregate with inclusion bodies. Triton-X100 was used to wash cell membranes and also the addition of a low concentration of urea could help to remove cellular protein (Qi *et al*., 2015; Singh *et al*., 2015). Purified inclusion bodies were obtained after several washing steps (Fig. 3b) and it was used for solubilization and refolding.

Optimization of urea concentration for solubilizing inclusion bodies

Different concentration of urea (0–8 M) was used to solubilize CBD-*Ssp* DnaB-hEGF from inclusion bodies. Urea would help to disrupt hydrophobic interaction between proteins in inclusion bodies resulting in solubilized CBD-*Ssp* DnaB-hEGF (Patra *et al*., 2000; Singh and Panda, 2005). CBD-*Ssp* DnaB-hEGF was slightly solubilized from inclusion bodies in the presence of 2 M urea and conversely showed better solubility in the presence of 6–8 M urea. CBD-*Ssp* DnaB-hEGF solubility tended to increase when the concentration of urea was increased (Fig. 4a). The highest amount of solubilized CBD-*Ssp* DnaB-hEGF was achieved by using a solubilization buffer containing 8 M urea (Fig. 4b). This result showed that the solubilization buffer containing 8 M urea could give better solubilization of CBD-*Ssp* DnaB-hEGF from inclusion bodies. The use of higher concentration of urea will give more solubilized protein that would be in denatured state, so refolding was necessary to convert protein into its native conformation by removal of denaturant through dialysis (Yamaguchi *et al*., 2013).

Refolding and simultaneous cleavage of solubilized CBD-Ssp DnaB-hEGF

Solubilized CBD-*Ssp* DnaB-hEGF was refolded by lowering the urea concentration using a stepwise dialysis method. The dialysis method could prevent protein loss because of the uncontrolled cleavage of *Ssp* DnaB mini-intein (Zhang *et al*., 2015). The concentration of urea was lowered from 8 to 2 M then

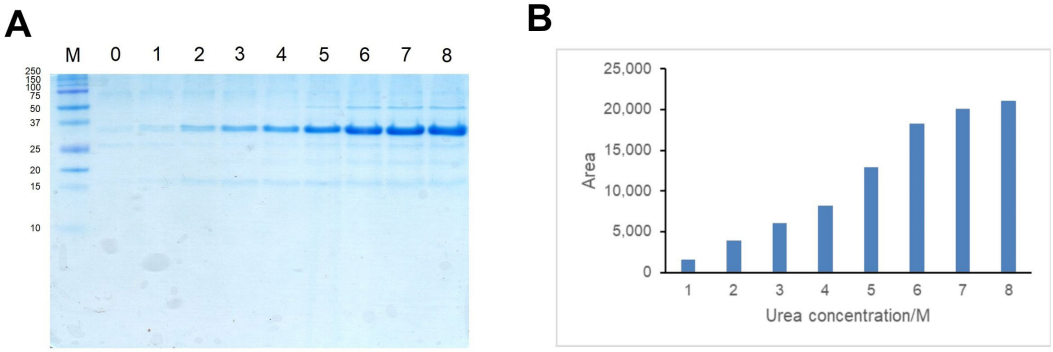

Figure 4. Optimization of solubilization condition of inclusion bodies in different concentrations of urea. (a) Tricine SDS-PAGE analysis of CBD-*Ssp* DnaB-hEGF inclusion bodies solubilized in different concentrations of urea. Lanes 0–8: CBD-*Ssp* DnaB-hEGF inclusion bodies solubilized in urea with a concentration of 0–8 M. Lane M: protein marker (size of protein in kilodalton is shown on the left). (b) Solubilized CBD-*Ssp* DnaB-hEGF quantification by using *ImageJ* software.

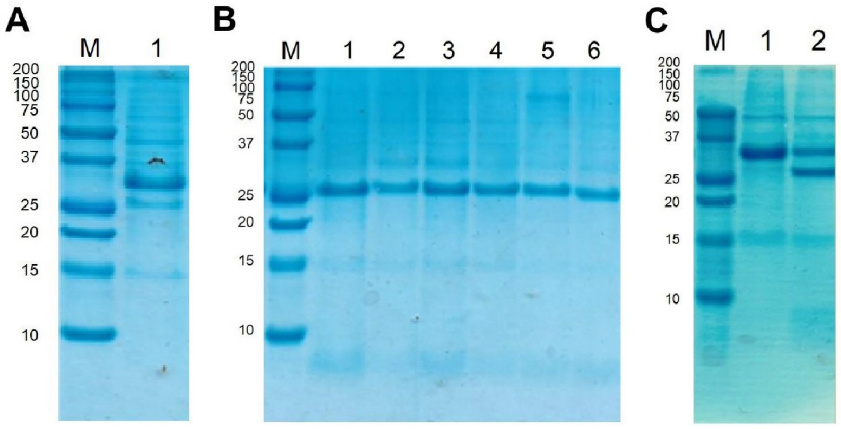

Figure 5. Refolding and cleavage of solubilized CBD-*Ssp* DnaB-hEGF using dialysis method. (a) Refolded CBD-*Ssp* DnaB-hEGF. Lane 1: refolded CBD-*Ssp* DnaB-hEGF. Lane M: protein marker. (b) Optimization of pH condition for induced splicing of refolded CBD-*Ssp* DnaB-hEGF. Lane 1–5: induced splicing by lowering the pH to 8.0; 7.5; 7.0; 6.5; 6.0. Lane 6: induced splicing by unmodified cleavage buffer pH 6.0. Lane M: protein marker. (c) Supernatant of cleavage of refolded CBD-*Ssp* DnaB-hEGF. Lane 1: refolded CBD-*Ssp* DnaB-hEGF. Lane 2: cleavage result of CBD-*Ssp* DnaB-hEGF. Lane M: protein marker (size of protein in kilodalton is shown on the left).

until approximately reaching 0 M. No aggregate was observed after dialysis; this showed that the refolding process was successful. Solubilized CBD-*Ssp* DnaB-hEGF started to refold as the urea concentration was decreasing (Singh *et al*., 2015; Yamaguchi and Miyazaki, 2014). On the other side, a proper disulfide bond was formed from reduced disulfide bond through disulfide shuffling while stepwise dialysis was performed (Yamaguchi *et al*., 2013). Even stepwise dialysis was taking more time and "slow," this method could give good results. There was no aggregation observed after dialysis was performed.

CBD-*Ssp* DnaB-hEGF shows *Ssp* DnaB splicing activity which is characterized by a band at 25 kDa; this band belonged to CBD-*Ssp* DnaB (Fig. 5a). The pH condition for splicing was investigated (pH 8.0-6.0) to ensure the pH optimum for splicing. Protein splicing occurred even at pH 8.0 and so did at the lower pH until pH 6.0 (Fig. 5b). This showed that gradually lowering pH until 6.0 could induce *Ssp* DnaB splicing activity. hEGF was released from CBD-*Ssp* DnaB-hEGF, which showed by a band at 6.2 kDa (Fig. 5c). Splicing activity of *Ssp* DnaB intein involved charge relay between amino acid residues and was ended by asparagine cyclization at C-terminal of intein (Ding *et al*., 2003; Topilina and Mills, 2014). Uncontrolled splicing activity was caused by *Ssp* DnaB intein which was sensitive to pH changes (Chen *et al*., 2002) and the cleavage was observed even at pH 8.0. By gradually decreasing pH from 8.0 to 6.0, hEGF was obtained. hEGF was obtained with a concentration of 0.163 mg/ml which was quantified by the densitometry method.

CONCLUSION

In summary, CBD-*Ssp* DnaB-hEGF was successfully expressed as inclusion bodies in *E. coli* BL21(DE3). Refolded hEGF was recovered from inclusion bodies after isolation, solubilization, and refolding steps with a concentration of 0.163 mg/ml. The solubilization and refolding method presented in this study could be used for the recovery of refolded hEGF from CBD-*Ssp* DnaB-hEGF inclusion bodies. The result shows a great opportunity to produce large amounts of hEGF from bacterial inclusion bodies. This finding leads to the optimization and development method in the future for maximizing the recovery of refolded hEGF. So, it could be applicable for a larger scale, especially at the industrial scale.

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AUTHOR CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be an author as per the international committee of medical journal editors (ICMJE) requirements/guidelines.

CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

ETHICAL APPROVALS

Not applicable.

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REFERENCES

Chen XH, Xu MQ, Ding Y, Ferrandon S, Rao ZH. Purification and initial crystallization studies of a DnaB intein from *Synechocystis* sp PCC 6803. Acta Crystallogr Sect D-Biol Crystallogr, 2002; 58:1201–3.

Costa S, Almeida A, Castro A, Domingues L. Fusion tags for protein solubility, purification, and immunogenicity in *Escherichia coli*: the novel Fh8 system. Front Microbiol, 2014; 5:1–20.

Ding Y, Xu MQ, Ghosh I, Chen X, Ferrandon S, Lesage G, Rao Z. Crystal structure of a mini-intein reveals a conserved catalytic module involved in side chain cyclization of asparagine during protein splicing. J Biol Chem, 2003; 278(40):39133–42.

Eissazadeh S, Moeini H, Dezfouli MG, Heidary S, Nelofer R, Abdullah MP. Production of recombinant human epidermal growth factor in *Pichia pastoris*. Braz J Microbiol, 2017; 48(2):286–93.

Fazaeli A, Golestani A, Lakzaei M, Varaei SSR, Aminian M. Expression optimization, purification, and functional characterization of cholesterol oxidase from *Chromobacterium* sp. DS1. PLoS One, 2019; 14(2):1–15.

Gomes A, Byregowda S, Veeregowda B, Vinayagamurthy B. An overview of heterologous expression host systems for the production of recombinant proteins. Adv Anim Vet Sci, 2016; 4:346–56.

Hayat SMG, Farahani N, Golichenari B, Sahebkar A. Recombinant protein expression in *Escherichia coli* (*E. coli*): what we need to know. Curr Pharm Des, 2018; 24(6):718–25.

Hu Y, Feng L, Li Y, Zhang Y, Lu P, Rayner S, Chen S. Ribosomal binding site switching: an effective strategy for high-throughput cloning constructions. PLoS One, 2012; 7(11):e50142.

Indriyani A, Anggraeni NI, Sriwidodo, Maksum IP. Optimization extracellular secretion of recombinant human epidermal growth factor (hEGF) in *Escherichia coli* BL21(DE3) pD881-OmpA-hEGF by using Response Surface Method (RSM). Int J Res Pharm Sci, 2019; 10(3):1824–31.

Jiang A, Jin W, Zhao F, Tang Y, Sun Z, Liu J-N. Split *Ssp* DnaB mini-intein-mediated production of recombinant human glucagon-like peptide-1/7-36. Biotechnol Appl Biochem, 2015; 62(3):309–15.

Kaur J, Kumar A, Kaur, J. Strategies for optimization of heterologous protein expression in *E. coli*: roadblocks and reinforcements. Int J Biol Macromol, 2018; 106:803–22.

Kim S, Jeong H, Kim EY, Kim JF, Lee SY, Yoon, SH. Genomic and transcriptomic landscape of *Escherichia coli* BL21(DE3). Nucleic Acids Res, 2017; 45(9):5285–93.

Kiraly O, Guan L, Sepessy E, Tóth M, Kukor Z, Sahin-Tóth M. Expression of human cationic trypsinogen with an autenthic N terminus using intein-mediated splicing in aminopeptidase P (*pepP*) deficient *Escherichia coli*. Protein Expr Purif, 2006; 48:104–11.

Li Y. Self-cleaving fusion tags for recombinant protein production. Biotechnol Lett, 2011; 33(5):869–81.

Ma Y, Yu J, Lin J, Wu S, Li S, Wang J. High efficient expression, purification, and functional characterization of native human epidermal growth factor in *Escherichia coli*. Biomed Res Int, 2016; 2016.

Maksum I, Lestari A, Fauzia R, Rachman S, Soedjanaatmadja U. *Escherichia coli* BL21(DE3) expression system using TorA signal peptide for Recombinant Human Albumin (rHA) secretion. Int J Res Pharm Sci, 2019; 10:3319–24.

Maksum IP, Utama E, Sriwidodo Subroto T. Extracellular secretion of recombinant human epidermal growth factor by using trimethylamine N-Oxide reductase a (TorA) signal peptide in *Escherichia coli* BL21(DE3). J Pharm Sci Res, 2017; 9(6):1007–16.

Marschall L, Sagmeister P, Herwig C. Tunable recombinant protein expression in *E. coli*: enabler for continuous processing? Appl Microbiol Biotechnol, 2016; 100(13):5719–28.

Melati R, Indriyani A, Gaffar S, Sriwidodo, Maksum IP. Comparison of extracellular secretion of recombinant human epidermal growth factor using TorA and PelB signal peptides in *Escherichia coli* BL21 (DE3). Asian J Pharm Clin Res, 2019; 12(11):81–4.

Parret AH, Besir H, Meijers R. Critical reflections on synthetic gene design for recombinant protein expression. Curr Opin Struct Biol, 2016; 38:155–62.

Patra AK, Mukhopadhyay R, Mukhija R, Krishnan A, Garg LC, Panda AK. Optimization of inclusion body solubilization and renaturation of recombinant human growth hormone from *Escherichia coli*. Protein Expr Purif, 2000; 18(2):182–92.

Qi X, Sun Y, Xiong S. A single freeze-thawing cycle for highly efficient solubilization of inclusion body proteins and its refolding into bioactive form. Microb Cell Fact, 2015; 14(1):1–12.

Silaban S, Gaffar S, Simorangkir M, Maksum IP, Subroto T. Effect of IPTG concentration on recombinant human prethrombin-2 expression in *Escherichia coli* BL21(DE3) ArcticExpress. IOP Conf Ser Earth Environ Sci, 2018; 217(1):1–6.

Silaban S, Maksum I, Gaffar S, Hasan K, Enus S, Subroto T, Soemitro S. Codon optimization and chaperone assisted solubilization of recombinant human prethrombin-2 expressed in *Escherichia coli*. Microbiol Indones, 2014; 8(4):170–5.

Singh A, Upadhyay V, Upadhyay AK, Singh SM, Panda AK. Protein recovery from inclusion bodies of *Escherichia coli* using mild solubilization process. Microb Cell Fact, 2015; 14(1):1–10.

Singh SM, Panda AK. Solubilization and refolding of bacterial inclusion body proteins. J Biosci Bioeng, 2005; 99(4):303–10.

Sriwidodo S, Maksum I, Riswanto N, Rostinawati T, Subroto T. Extracellular secretion recombinant of human epidermal growth factor (hEGF) using pectate lyase B (PelB) signal peptide in *Escherichia coli* BL21(DE3). Int J Res Pharm Sci, 2017; 8:33–40.

Sriwidodo S, Subroto T, Maksum IP, Wathoni N, Rostinawati T, Ulya H, Putri IU. Optimization of secreted recombinant human epidermal growth factor production using pectate lyase B from *Escherichia Coli* BL21(DE3) by central composite design and its production in high cell density culture. J Pharm Bioallied Sci, 2019; 11(Suppl 4):S562–6.

Su Z, Huang Y, Zhou Q, Wu Z, Wu X, Zheng Q, Ding C, Li X. High-level expression and purification of human epidermal growth factor with SUMO fusion in *Escherichia coli*. Protein Pept Lett, 2006; 13(8):785–92.

Sun Z, Chen J, Yao H, Liu L, Wang J, Zhang J, Liu JN. Use of *Ssp* dnaB derived mini-intein as a fusion partner for production of recombinant human brain natriuretic peptide in *Escherichia coli*. Protein Expr Purif, 2005; 43(1):26–32.

Tang X, Liu H, Yang S, Li Z, Zhong J, Fang, R. Epidermal growth factor and intestinal barrier function. Mediators Inflamm, 2016;2016:27–30.

Topilina NI, Mills KV. Recent advances in *in vivo* applications of intein-mediated protein splicing. Mob DNA, 2014; 5(1):1–14.

Upadhyay V, Singh A, Panda AK. Purification of recombinant ovalbumin from inclusion bodies of *Escherichia coli*. Protein Expr Purif, 2016; 117:52–8.

Vallejo LF, Rinas U. Strategies for the recovery of active proteins through refolding of bacterial inclusion body proteins. Microb Cell Fact, 2004; 3:1–12.

Yamaguchi H, Miyazaki M. Refolding techniques for recovering biologically active recombinant proteins from inclusion bodies. Biomolecules, 2014; 4(1):235–51.

Yamaguchi S, Yamamoto E, Mannen T, Nagamune T. Protein refolding using chemical refolding additives. Biotechnol J, 2013; 8(1): 17–31.

Zhang Y, Zhang K, Wan Y, Zi J, Wang Y, Wang J, Wang L, Xue X. A pH-induced, intein-mediated expression and purification of recombinant human epidermal growth factor in *Escherichia coli*. Biotechnol Prog, 2015; 31(3):758–64.

Zheng X, Wu X, Fu X, Dai D, Wang F. Expression and purification of human epidermal growth factor (hEGF) fused with GB1. Biotechnol Biotechnol Equip, 2016; 30(4):813–8.

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