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Research Article

COMPARISON OF EXTRACELLULAR SECRETION OF RECOMBINANT HUMAN EPIDERMAL GROWTH FACTOR USING TORA AND PELB SIGNAL PEPTIDES IN *ESCHERICHIA COLI* BL21 (DE3)

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ABSTRACT

Objective: The objective of this study was to evaluate two signal peptides (TorA and PelB), representing the most common secretion pathways in *Escherichia coli*, for their ability to secrete recombinant human epidermal growth factor (rhEGF) protein in the extracellular expression.

Methods: *E. coli* BL21 (DE3) as the host cell to be transformed using recombinant plasmid pD881-TorA the consensus already containing hEGF gene and the signal peptide TorA or PelB, then expressed by L-rhamnose induction. rhEGF purified by heat treatment and ion-exchange chromatography. The hEGF protein was characterized using sodium dodecyl sulfate-polyacrylamide gel electrophoresis and ELISA.

Results: The result showed that PelB was secreting more hEGF protein compared to TorA with protein expression results of 48.2 µg/L and purification results of 0.360 µg/L, with a purity level of 83%.

Conclusion: The results of this study explain in extracellular expression of hEGF protein in *E. coli*, PelB helps hEGF protein secretion to culture media better than TorA.

Keywords: Human epidermal growth factor, *Escherichia coli,* Extracellular expression, TorA, PelB.

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INTRODUCTION

Escherichia coli is classified as a rod-shaped, Gram-negative bacterium in the family *Enterobacteriaceae* [1]. *E. coli* as a host cell has several disadvantages, including: (1) The accumulation of protein secretion is non-functional, (2) high chances for the formation of inclusion bodies as a result of incorrect folding (misfolding), and (3) the high rate of protein degradation due to their intracellular protease enzymes [2]. To be able to secrete recombinant protein into the membrane human epidermal growth factor (hEGF) periplasm or to the culture medium to be used the signal peptide which is fused with the target protein to be passing lane, sec twin-arginine translocation (TAT) or sec-pathway [3,4].

The general extracellular secretion route, termed Sec-pathway, catalyzes the transmembrane translocation of proteins in their unfolded conformations, whereupon they fold into their native structure at the transitions of the membrane. The twin-arginine translocation pathway, termed Tat pathway, catalyzes the translocation of secretory proteins in their folded state. Although the targeting signals that direct secretory proteins are to these pathways show a high degree of similarity, the translocations and translocations involved are vastly different [3,5].

EGF is a single chain, 53 amino acid residues polypeptide with molecular weight of 6.2 kDa [6]. Specifically, EGF interacts with its receptor across the entire epidermis and particularly in the basal layer, promoting epithelial growth through activation of several pathways [7]. Through binding to the EGF receptor in the cell membrane, EGF can induce its autophosphorylation and initiate signal transduction, thus regulating a wide range of biological functions. EGF is widely used in basic research and clinical medicine, which has created a great demand for it [8].

hEGF recombinant production was carried out in *E. coli*. The previous studies have succeeded in expressing rhEGF extracellular secretion pathways [9]. *E. coli* is also relatively easy for the recombinant plasmid which is inserted into it so that the gene hEGF can be easily inserted into the plasmid vectors of *E. coli* [10]. hEGF had potential as effective wound healing and had function as problems of premature aging, that is, why hEGF used not only for wound healing, corneal transplantation, and gastric ulcer treatment but also in the cosmetic industry [9]. Therefore, the method for producing large and high efficiency of hEGF needed[A]. This study was to evaluate two signal peptides (TorA and PelB), representing the most common secretion pathways in *E. coli*, for their ability to secrete hEGF protein in the extracellular expression.

MATERIALS AND METHODS

Reagent and chemicals

Material used in this study were Bacto agar Bacto (Oxoid), agarose (Sigma-Aldrich), ammonium persulfate (Bio Basic INC), Bromophenol blue (Merk), Coomassie Brilliant blue (SERVA), EDTA (1st Base), DNA 1 kb marker (Thermo Fisher Scientific), *E. coli* BL21 (DE3), Gel Red (Biotium), hEGF synthetic gene inserted in pD881-PelB plasmid, and pD881-TorA (synthesized by DNA 2.0, California, USA), glycerol (1st Base), Kanamycin Sulfate (Sigma-Aldrich), L-rhamnose (Sigma-Aldrich), protein marker (Biorad), sodium chloride (Merk), sodium dodecyl sulfate (SDS) (Merk), tetracycline, and TEMED (Sigma-Aldrich).

Methods

E. coli BL21 (DE3) (pD881-TorA-hEGF) growth and post-induction curve

Transformant was grown in LB medium 5 mL containing kanamycin about 16–18 jam at 37°C with shaking at 200 rpm. 1000 µL of culture was transferred into LB medium 100 mL in shake flask containing kanamycin for 12 h at 37°C with shaking at 200 rpm. OD_{600} was measured every hour. Starter culture 1000 µL was transferred into LB medium 100 mL containing kanamycin and was grown to OD_{600} reach 0.6. Inducer L-rhamnose 4 mM final concentration was added. The culture was incubated for 20 h at 37°C with shaking at 200 rpm. OD_{600}nm was measured every hour.

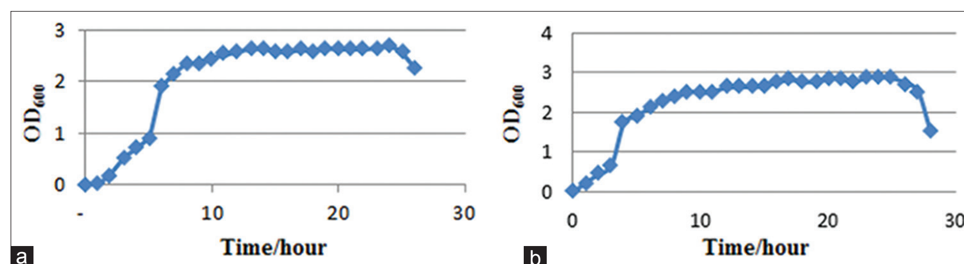

Fig. 1: Growth curve *Escherichia coli* BL21 (DE3) incubation at 37°C using LB shaking speed 200 rpm. (a) *E. coli* BL21 (DE3) (pD881-TorA-human epidermal growth factor (hEGF]). (b) *E. coli* BL21 (DE3) (pD881-PelB-hEGF)

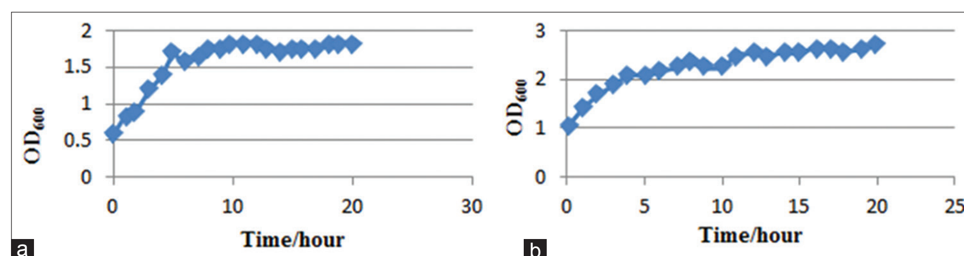

Fig. 2: Post-induction growth curve *Escherichia coli* BL21 (DE3) incubation at 37°C using LB media, shaking speed 200 rpm. (a) *E. coli* BL21 (DE3) (pD881-TorA-human epidermal growth factor [hEGF]). (b) *E. coli* BL21 (DE3) (pD881-PelB-hEGF)

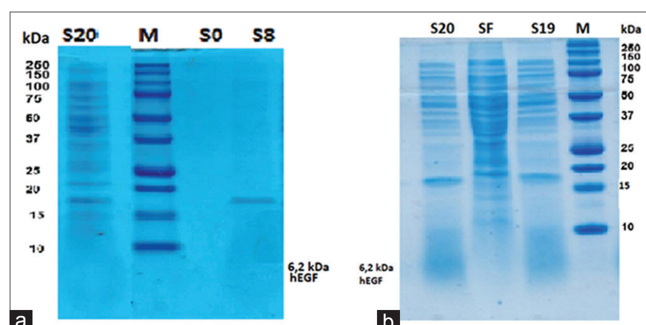

Fig. 3: Characterization of human epidermal growth factor (hEGF) protein expression results in *Escherichia coli* BL21 (DE3) using Tricine-sodium dodecyl sulfate-polyacrylamide gel electrophoresis. M: Protein marker. Sn: Supernatant at n h post-induction (S20: Supernatant at the 20th h post-induction), SF: Soluble fraction. (a) Result of hEGF expression using: *E. coli* BL21 (DE3) (pD881-TorA-hEGF) and (b) using *E. coli* BL21 (DE3) (pD881-PelB-hEGF)

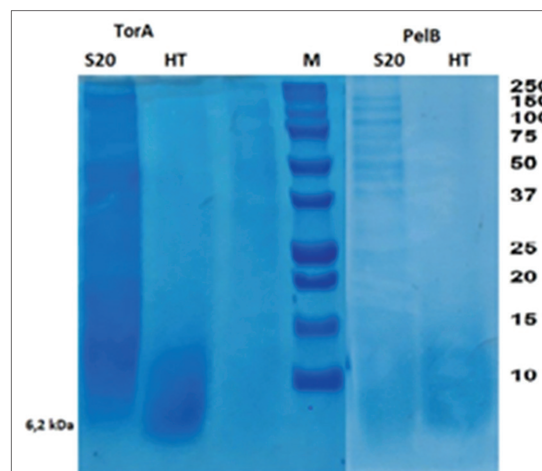

Fig. 4: Human epidermal growth factor protein characterization after heat treatment using Tricine-sodium dodecyl sulfate-polyacrylamide gel electrophoresis. M: Protein marker; S20: Supernatant at the 20th h post-induction. HT: Supernatant fraction resulting from heat treatment

Expression of hEGF by *E. coli* BL21 (DE3) (pD881-TorA-hEGF) and *E. coli* BL21 (DE3) (pD881-PelB-hEGF)

E. coli BL21 (DE3) (pD881-TorA-hEGF) and (pD881-PelB-hEGF) that was characterized, grown in 5 mL of liquid Luria Bertani (LB) media which contained kanamycin (25 µL/mL) for about 16–18 h, in temperature 37°C and 200 rpm shaking speed. Each cell culture 1000 µL was added into different Erlenmeyer flask containing 100 mL liquid LB and kanamycin (25 µL/mL). Then, all cultures in the Erlenmeyer flask incubated at 37°C and shaking speed 200 rpm until OD_{600} reached at 0.6. Then, 1 mL of each culture taken as t_0 (before induction of L-rhamnose) and collected into a 1.5 mL microtube, then cell pellet and it's supernatant separated using centrifugation with velocity 3000 g, 4°C for 20 min. Then, the cultures in Erlenmeyer flasks added by L-rhamnose total concentration of 4 mM in culture, then incubated at 37°C, shaking speed 200 rpm for 20 h. For about 2 mL, each culture was taken as t_i (after induction of L-rhamnose) 1 mL, separated the cell pellet and its supernatant by centrifugation with a velocity of 3000 g, 4°C for 20 min. Pellets are used for cell lysis and extraction processes periplasmic membrane and media can be analyzed using SDS-polyacrylamide gel electrophoresis (PAGE).

Purification of hEGF protein using heat treatment and anion exchange chromatography

Cultures are harvested 20 h after induction, separated the cell pellet and its supernatant by centrifugation with a velocity of 3000 g, 4°C for 20 min. Supernatant (soluble fraction) heated at 80°C for 20 min, then centrifuged at a speed of 20,000 g, for 30 min.

Supernatant was taken and hEGF contained in it was then purified by anion exchange chromatography with DEAE matrix Sephadex A-25, eluen I (citrate-phosphate buffer pH 5.6), and eluen II (citrate-phosphate buffer pH 5.6 with sodium chloride 2 M), which is integrated in the AKTA START. The heat-treated 5 mL sample was filtered using 0.22 mm pore diameter filter units and loaded onto the DEAE sepharose fast flow