Chapter 7
Optimization of the Fermentation Conditions of Pep-1-Fused EGF in *Escherichia coli*

Tong-Cun Zhang, De-Yun Ma, Xue-Gang Luo and Yue Wang

Abstract Human epidermal growth factor (hEGF), a well-known polypeptide agent which has been widely used in the medicine and cosmetics, is a 6.2 kDa single-chain polypeptide consisting of 53 amino acids. Here, to enhance its transmembrane ability, a recombinant EGF fused with Pep-1, a cell-penetrating peptides (CPP) that has been previously shown to be powerful transport vector tool for the intracellular delivery of a variety of cargos through the cell membrane, was expressed in *Escherichia coli*. Furthermore, The expression conditions was optimized, and the results showed that the Pep-1-fused EGF (P-EGF) could be successfully expressed in *E. coli* BL21-TrixB (DE3) using an expression vector, pGEX-6P-3, which contains a GST tag. The recombinant product reached the highest soluble expression when the expression strain was induced by 0.2 mmol/l IPTG and cultivated at the temperature of 20 °C with a rotation speed of 200 rpm for 8 h.

Keywords Epidermal growth factor (EGF) · P-EGF · Optimization · Fermentation conditions

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7.1 Introduction

Epidermal growth factor (EGF) is a 6.2 kDa single-chain small polypeptide containing 53 amino acids secreted from various tissues [1] and it has huge potential application value and broad market prospects both as medicines and cosmetics. However, the transdermal absorption of EGF is very weak because of the intestine mucosal and stratum corneum which functions as barriers to prevent penetration of most foreign large molecules [2, 3]. To solve this problem, the EGF could be fused with Pep-1, a cell-penetrating peptide (CPP) that has been previously shown to be a powerful transport vector tool for the intracellular delivery of a variety of cargos through the cell membrane [4, 5].

In our previously study, we have successfully constructed a recombinant strain to express the protein P-EGF, a fusion of EGF and PEP-1. The gene encoding EGF and DNA fragment encoding Pep-1 were combined together using overlapping PCR technology and then inserted into the vector pGEX-6P-3 with a GST tag to construct the expression vector. In order to obtain the maximum soluble expression of the fusion protein P-EGF, the expression conditions were optimized in this study.

7.2 Materials and Methods

7.2.1 Bacterial Strain and Plasmid

Escherichia coli BL21-TrixB (DE3) was used as the expression host. pGEX-6P-3 with a GST tag was used as the expression vector. The recombination plasmid P-EGF was constructed by our laboratory.

7.2.2 Expression of the Fusion Protein

Escherichia coli BL21-TrixB (DE3) harboring recombinant plasmid was cultured in LB medium containing 50 μg/ml ampicillin and 15 μg/ml kanamycin at 37 °C to OD_{600} of 0.6. The cells were then induced with 0.2 mM isopropy-beta-D-thiogalactopyranoside (IPTG) at 20 °C for 12 h. After harvest, cells were centrifuged at 5,000 × g for 20 min, and then lysed by sonication in phosphate buffer. After centrifugation at 13,000 × g for 30 min, the supernatant was taken out as the soluble protein sample for the subsequent experiment.

7.2.3 Protein Quantification

The total protein concentration was determined by BCA assay. The protein was detected by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). The proportion of fusion protein P-EGF in total protein was analyzed using specialized software Quantity One.

7.3 Results

7.3.1 Optimization of Temperature

The optimum growth temperature for *E. coli* is 37–39 °C, but this temperature was not suitable to express P-EGF for the formation of inclusion bodies. In order to find optimal temperature for the soluble expression of protein, several different temperature gradients were tested, including 37, 30, 25, and 20 °C. P-EGF expressed under different temperature conditions showed in Fig. 7.1 and the result demonstrated that 20 °C was the most suitable temperature tested.

7.3.2 Optimization of Rotation Speed

Different speeds will result in different dissolved oxygen and then will lead to different protein expression. Therefore, four rotation speeds, including 250, 200, 150, and 100 rpm, were chosen to be analyzed. The result showed that highest expression of the soluble protein could be obtained when the rotation speed was 200 rpm (Fig. 7.2).

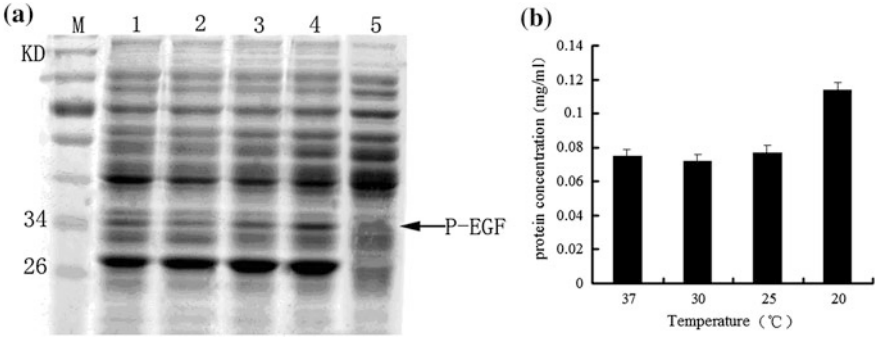

Fig. 7.1 Optimization of temperature for the expression of P-EGF **a** SDS-PAGE analysis of P-EGF expressed under different temperature conditions. *M* protein marker; *1* expression of P-EGF at 37 °C; *2* expression of P-EGF at 30 °C; *3* expression of P-EGF at 25 °C; *4* expression of P-EGF at 20 °C; *5* expression of P-EGF without induction. **b** Quantitative analysis of P-EGF expressed under different temperature conditions

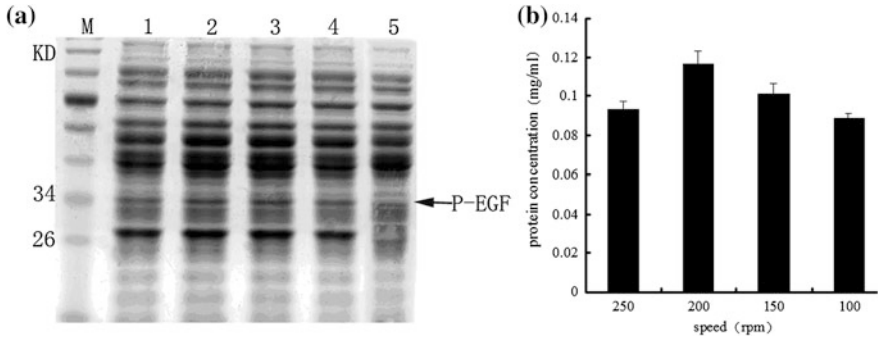

Fig. 7.2 Optimization of rotation speed for the expression of P-EGF **a** SDS-PAGE analysis of P-EGF expressed under different revolutions per minute. *M* protein marker; *1* expression of P-EGF at 250 rpm; *2* expression of P-EGF at 200 rpm; *3* expression of P-EGF at 150 rpm; *4* expression of P-EGF at 100 rpm; *5* expression of P-EGF without induction. **b** Quantitative analysis of P-EGF expressed under different rotation speeds

7.3.3 Optimization of IPTG Concentration

The inducer IPTG could reduce protein expression for its certain toxicity to cells when it was added too much. Therefore, the concentration of IPTG is also very important for the expression of fusion protein P-EGF. In this study, the effect of different concentration of IPTG, including of 0.1, 0.2, 0.4, and 0.6 mmol/l, on the yield of the recombinant protein, were detected. As shown in Fig. 7.3, the optimal IPTG concentration for the expression of the fusion protein was 0.2 mmol/l.

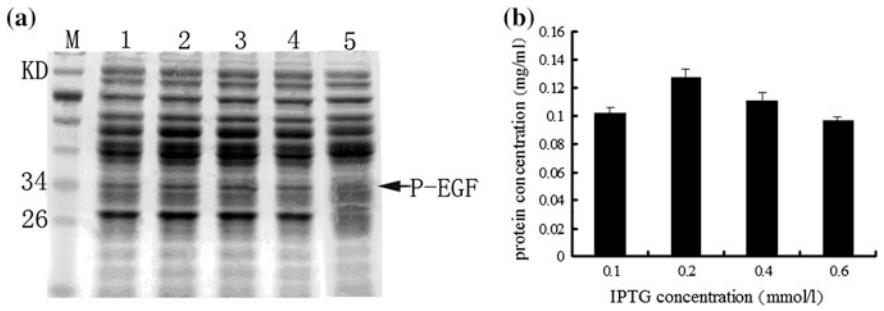

Fig. 7.3 Optimization of IPTG concentration for the expression of P-EGF **a** SDS-PAGE analysis of P-EGF expressed with different IPTG concentrations. *M* protein marker; *1* expression of P-EGF with 0.1 mmol/l IPTG; *2* expression of P-EGF with 0.2 mmol/l IPTG; *3* expression of P-EGF with 0.4 mmol/l IPTG; *4* expression of P-EGF with 0.6 mmol/l IPTG; *5* expression of P-EGF without induction. **b** Quantitative analysis of P-EGF expressed with different IPTG concentrations

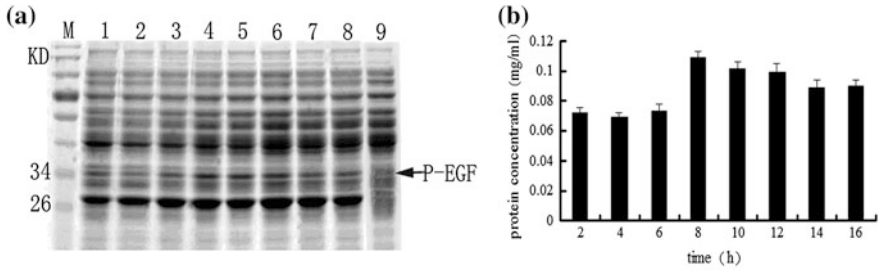

Fig. 7.4 Optimization of induction time for the expression of P-EGF **a** SDS-PAGE analysis of P-EGF expressed with different induction times. *M* protein marker; *1* expression of P-EGF for 2 h; *2* expression of P-EGF for 4 h; *3* expression of P-EGF for 6 h; *4* expression of P-EGF for 8 h; *5* expression of P-EGF for 10 h; *6* expression of P-EGF for 12 h; *7* expression of P-EGF for 14 h; *8* expression of P-EGF for 16 h; *9* expression of P-EGF without induction. **b** Quantitative analysis of P-EGF expressed for different induction times

7.3.4 Optimization of Induction Time

The IPTG was added when then the OD_{600} of the culture reached 0.6. After induction, the sample was collected every 2 h. The expression condition at different time was showed in Fig. 7.4 and the result demonstrated that the optimal induction time was 8 h.

7.4 Conclusion

Administration of macromolecule drugs nonintravenously always exhibited low bioavailability due to the penetration limitation of cell membrane barrier [6]. Increasing evidence has indicated that CPPs could facilitate proteins' transmembrane transport [7]. In our previous work, we have successfully constructed the recombinant plasmid P-EGF. And in this study, we mainly carried out the work to optimize the fermentation conditions for the soluble expression of P-EGF. The expression conditions were optimized, and the results showed that the P-EGF reached the highest soluble expression when the expression strain was induced by 0.2 mmol/l IPTG and cultivated at the temperature of 20 °C with a rotation speed of 200 rpm for 8 h.

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