The study of Phycoerythrin as natural pink protein

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ABSTRACT

Phycoerythrin (PE) is the pink pigment-protein complex for light-harvesting of the red algae and widely used as indicator. PE has an emerging application as natural, nontoxic pigments in industrial product since synthetic dyes have been argued against maleficence in human health. However, the yield in traditional system is very low. Although it has been tried to obtain PE by genetic engineering, but it has not been optimized yet. In this study, we reported the possibility of PE mass production from genetically engineered cells. After optimizing the culture condition, we purified PE by Ni-IMAC. The purified PE had maximum absorbance at 556 nm. The yield of PE was 10.06 mg/g of cells and its purity index was 10.49. In the effectiveness evaluation *in vitro*, there were non-cytotoxicity in various concentration of PE in HaCaT cells. Besides, PE had gradually increased as its concentrations for the protection of oxidative damage.

Consequently, PE had a great effect in oxidative stress without any toxicity and could be effectively produced in our strategy with low cost compared to conventional method. Thus, our present study shows the PE could be applied into cosmetic or food industrial fields as natural pink protein with a great commercial value.

KEYWORDS: PE, pink protein, natural dye, cosmetic, bioreactor

INTRODUCTION

Phycobiliprotein is a kind of light-harvesting proteins that are found in some eukaryotic alga, including cyanobacteria. It can strongly absorb in the visible lights by carring various phycobilins, linear tetrapyrroles, linked to their particular cysteinyl residues. Phycoerythrin (PE) is one of the phycobiliproteins and water-soluble and have intensively pink. Many studies have shown that PE had various physiological and pharmacological activities[1]. Thus, PE is widely used as a natural pigment in food and cosmetics and as a fluorescent probe. It is, therefore, interesting to develop mass production of PE for use in the industrial fields.

Currently, PE has been mainly produced by the extraction from alga, but this process is complicated and accomplished a low yield[2]. It is necessary to improve the production yield of PE by the engineered cells. PE is a tightly associated αβ heterodimer, in which each subunit carries phycoerythrobilin (PEB). The process of the PE biosynthesis which phycobilin is attached to phycobiliprotein subunits have been inferred from diverse studies[3]. In that report, five genes, Hox1 encoding hemeoxygenase, PebS encoding PEB synthase, CpcA encoding PE-α subunit, CpcE encoding phycocyanin-α-subunit phycocyanobilin lyase E, and CpcF encoding phycocyanin-α-subunit phycocyanobilin lyase F, are involve and consequently produced in PE biosynthesis.

In this study, we examined the biosynthetic pathway of PE in cyanobacteria leading from heme to the formation of PEB and its attachment at cysteine residues of CpcA and tried to reconstitute our strategy in *E. coli*. After expression of PE, it was purified by Ni-IMAC and evaluated its cytotoxicity, anti-inflammation, whitening effect and protective effect on the oxidative damage.

EXPERIMENTAL

Expression and Purification of PE. The three expression plasmids (pETDuet-HT-CpcA, pACYC-Duet1-pebS-hox1, pCDF-Duet-CpcE-CpcF) were transformed into *E. coli* BL21(DE3) cells and cultured in LB broth containing antibiotics at 37°C overnight. Then transferred to ZYP-5052 medium for auto-induction[4] at a ratio of 1:100. The culture was grown in a bioreactor with the temperature maintained at 37°C for 10 hr and decreased to 18°C for 24 hr and then cells were harvested. Cell pellets were resuspended in buffer A (50 mM Tris-HCl, pH 8.0, 500 mM NaCl) and disrupted by sonication. Clear cell lysate was collected and it was loaded onto a Ni-NTA column (Takara) pre-equilibrated with buffer B (Buffer A, 20 mM Imidazole), washed with 15 column volume (CV) of buffer B and then protein samples were completely eluted with 5 CV of buffer C (Buffer A, 300 mM Imidazole). The eluted sample were desalted with PD-10 desalting column (GE healthcare) and then lyophilized.

MTT assay. The cytotoxic effect of the PE was evaluated by MTT assay. Briefly, HaCaT cells were incubated with DMEM containing 1.2 mM MTT at 37 °C for 4 hr. The media were then removed and washed with PBS three times. The produced formazan salts were dissolved with DMSO and the absorbance was measured at 540 nm.

Anti-oxidant assay. HaCaT cells were treated with 1 mM H_2O_2 wither PE or N-acetylcystein (NAC) as a positive control for 24 hr. The media were then removed and performed MTT assay as described above or methylene blue staining to evaluate the H_2O_2 scavenging power of the PE.

RESULTS AND DISCUSSION

In order to produce PE, we used biosynthetic pathway leading from heme to the formation of the cysteinyl residue of PEB with CpcA and were presented in Fig. 1. As shown in Fig 1., *E. coli* cells were co-transformed with Hox1/PebS to produce PEB and CpcE/CpcF to attach PEB at cysteine residue of CpcA. After induction of the PE by auto-induction at 18°C overnight, these cultures gradually turned to pink, implying that PE had been successfully produced.

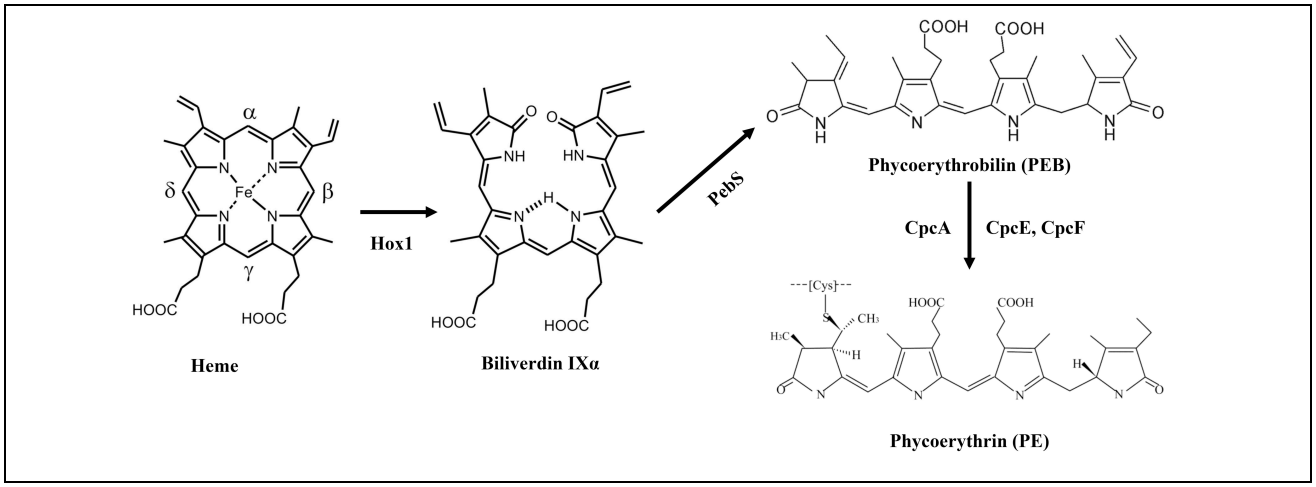

Figure 1: Biosynthetic pathway of PE production.

To examine the growth of cells expressing PE in bioreactor, and we measured optical density 600 (OD 600) and estimated the growth curves (Fig. 2A). As shown in Fig. 2, it was shown in the typical bacteria growth pattern, including the lag phase, exponential phase, stationary phase. After 34 hr, we obtained *E. coli* cells total 7.28 g (wet weight) /L and examined its efficient production in bioreactor by SDS-PAGE (Fig. 2). PE (19kDa) was induced after 6.5 hr and increased in time dependent manner. To purify PE protein, we performed the Ni-IMAC chromatography and acquired highly pure PE in elution fraction (Fig. 2C).

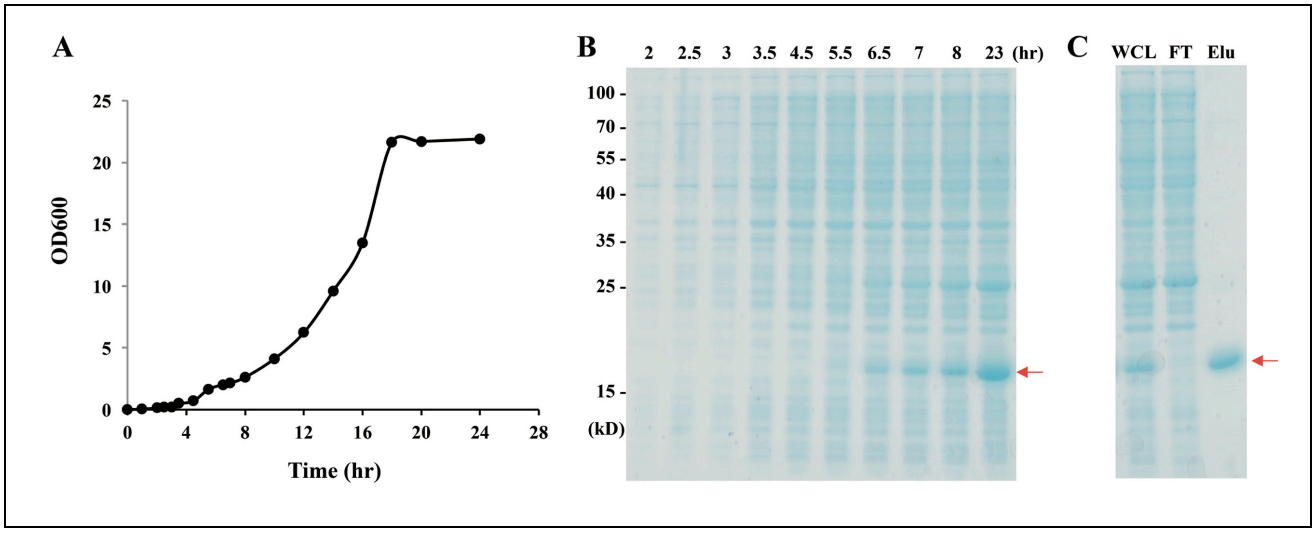

Figure 2: (A) E. coli cell growth curve. (B) Time course expression profile of PE. (C) Purification of PE

After affinity purification of the PE, we measured absorption spectra of PE. It had an absorption from approximately 500 nm to 600 nm and maximum at 556 nm which similar to those of naturally produced PE (Fig. 3). Next, we calculated the yield and the purity index. We purely obtained 10.06 mg/g (wet weight) of lyophilized PE which had 10.58 (A556/A280) of the purity index, this amount was 25 times higher than previous report by Aaron J. et al[5]. These results indicate our strategy is very efficiently produced in PE.

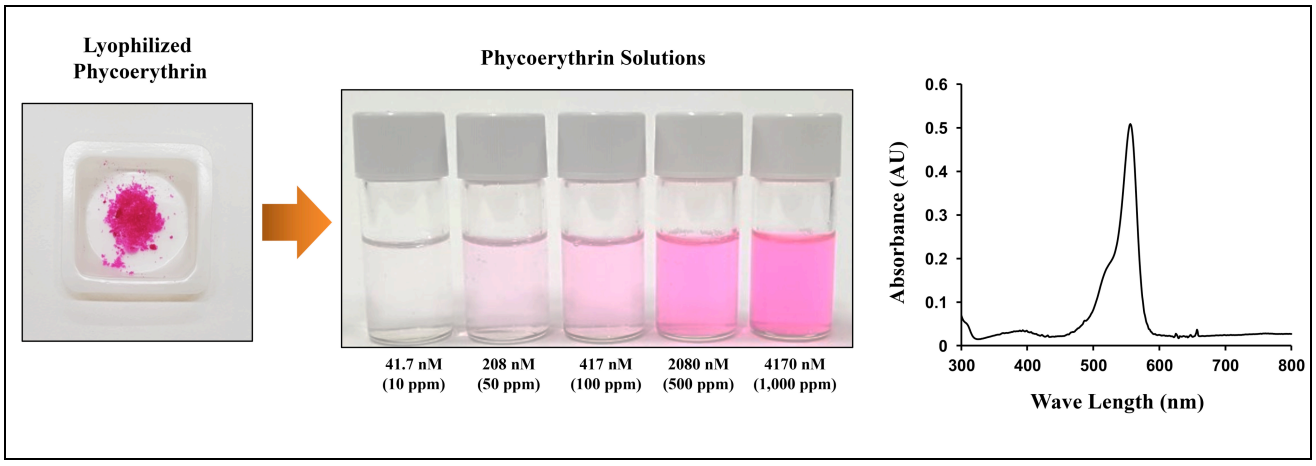

Figure 3: The color of PE solution with different concentrations and its absorbance spectra.

To determine any effect on cellular toxicity for the purified PE, we examined the cytotoxicity of PE in human keratinocytes, HaCaT cells through MTT assay (Fig. 4A). The viability was improved at high concentration (208 and 417 nM), but there was not affected at lower concentrations (4.17, 20.8 and 41.7 nM). This suggest that PE could be used as wound-healing materials without any toxicity at high concentration.

In the effectiveness of oxidative stress, we examined the protection of the cellular oxidative damage of purified PE and the results was shown in Fig. 4B, C. After H_2O_2 treatment, MTT and methylene blue staining assays were performed to analyze cell viability. When HaCaT cells were treated with H_2O_2, it had an oxidative damage including cell death. However, the pretreatment with NAC, a strong antioxidant, was significantly protected cells from H_2O_2-induced oxidative damage (positive control, Fig. 4B, C). In the experiment of purified PE, there had significant improvement at the high concentration (208 and 417 nM) compared to NAC, but had no effect at the low concentration (4.17, 20.8 and 41.7 nM). This imply that the purified PE play an important role in preventing the oxidative damage originated from H_2O_2.

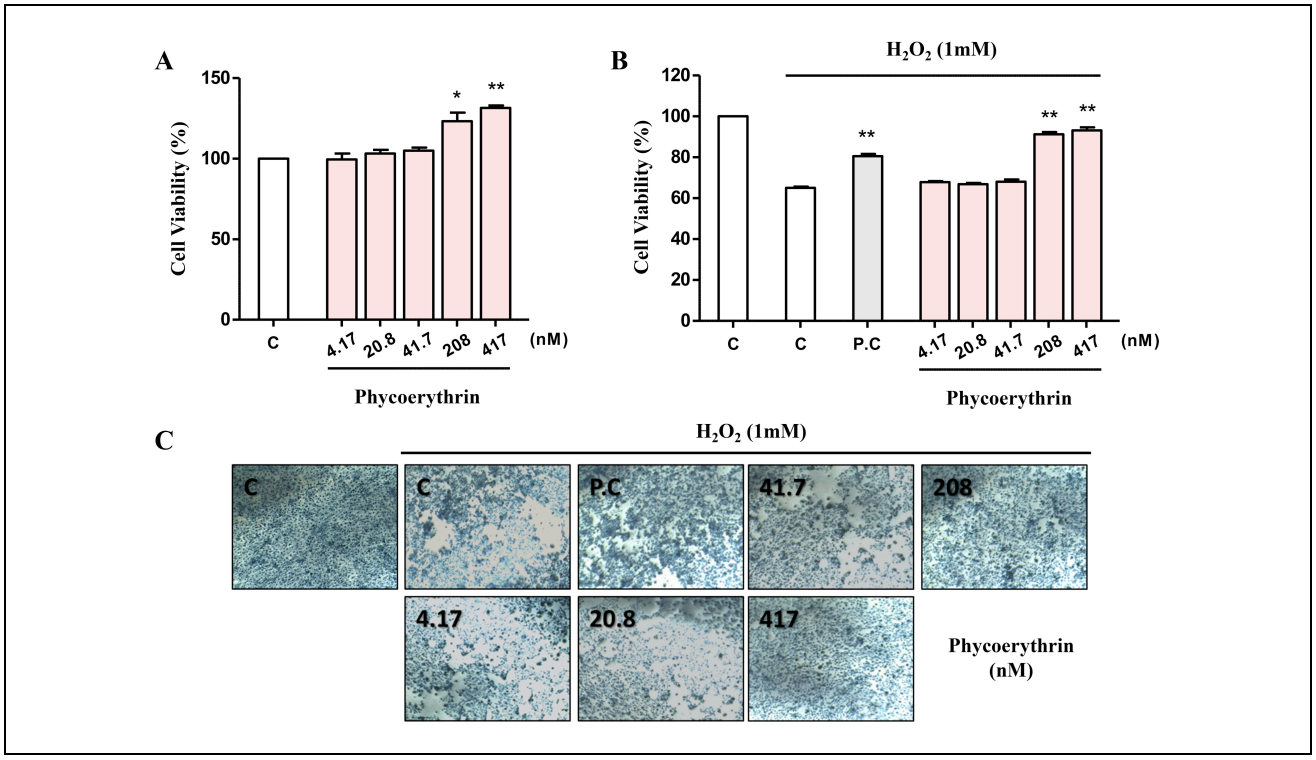

Figure 4: (A) Cell cytotoxicity assay (B, C) Oxidative damage protection assay

CONCLUSION

In conclusion, we propose the PE production through the recombinant *E. coli* bearing genes involved in its biosynthesis. It could be effectively produced in our strategy with low cost. Synthesized and purified PE had non-cytotoxicity and improved the cell viability at high concentration (208 and 417 nM). Besides, it was exhibited in a strong ability to scavenge free radicals produced by H_2O_2. These results suggest that purified PE in our strategy could be used in wound-healing and anti-oxidant materials. Consequently, our present study shows the PE could be applied into cosmetic or food industrial fields as natural pink protein with a great commercial value

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