Synthesis of Periplasmic AppA Protein using *ExiProgen*™ Automated Protein Synthesis Equipment

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Introduction

Cell-free protein synthesis (CFPS) enables to synthesize target protein in a short time. And the productivity has been increased significantly with the development of different types of cell lysate and optimization of reaction conditions (1). Especially, *E. coli* based CFPS makes it more suitable for synthesizing protein in terms of cost and productivity. Hence, it allows to synthesize proteins with disulfide bonds such as antibody and therapeutic proteins by creating an oxidized redox environment with glutathione buffer and supplementing chaperones such as disulfide bond C (DsbC) protein family and protein disulfide-isomerase (PDI) (2). *ExiProgen*™, automatic device that produces proteins from nucleic acid (DNA or RNA), combines *in vitro* transcription-translation system and automatic system. It automatically synthesizes proteins like a vending machine if target genes are added.
In this study, we synthesized periplasmic AppA protein having four disulfide bonds with *ExiProgen*™ and measured enzyme activity for confirming synthesized protein's function. We optimized protein synthesis condition for containing disulfide bonds in *E. coli* cell free system using specific buffer containing glutathione and disulfide bond isomerase. And stepwise exchange cell-free protein expression method was adopted.

Methods and results

For expression of AppA using *ExiProgne*™, we constructed pBT7-appA including T7 promoter and terminator, His_6-tag. Expression and purification of AppA protein were carried out using *ExiProgen*™(A-5041, Bioneer, South Korea) and *ExiProgen*™ EC-Disulfide Protein Synthesis Kit (K-7330, Bioneer). The total 8 µg of pBT7-AppA were added in reaction mixture for the protein synthesis. It took about 34 hours to synthesize disulfide protein with expression, purification and dialysis.
Protein analysis was performed with SDS-PAGE analysis, Bradford assay and image analysis with ImageJ. Purity of protein solution was determined by image analysis of SDS-PAGE gel. Then, it introduced into the value of protein quantification and weight of AppA excluding impurities was calculated. The weight of purified AppA protein without impurities was 6.3 µg per reaction (Fig 1).
The acid phosphatase activity assay was performed in 130 µL of 0.25 M glycine-HCl (pH 2.5) using 25 mM *p*-nitrophenyl phosphate as a substrate at 37 ℃ for 10 min. The reaction stopped by addition of 1M sodium hydroxide and the absorbance was measured at 405 nm. The concentration of released *p*-nitrophenol by enzyme reaction was determined using p-nitrophenol standard curve. One Unit (U) of enzyme activity was defined as the amount of the enzyme that released 1 µmol of *p*-nitrophenol per minute at 37℃. The activity of synthesized AppA by *ExiProgen*™ was determined 92.5 U/mg (Fig 2).
In conclusion, we successfully expressed and purified periplasmic AppA using automatic machine and confirmed specific activity of synthesized enzyme which contains four disulfide bonds. Rapid synthesis of disulfide proteins with *ExiProgen*™ would be expected to save the time for researching and pharmaceutical protein in the future.

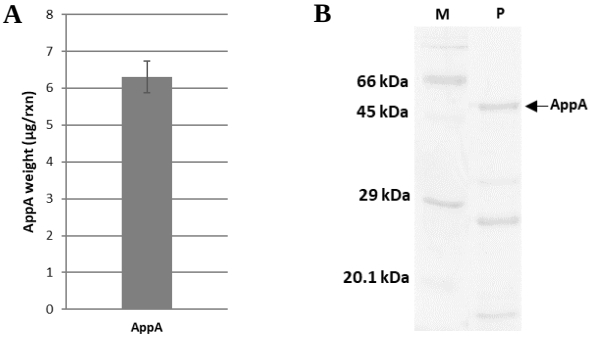

Figure 1. Protein analysis of purified AppA.
(A) Quantification of AppA protein without impurities by bradford assay. (B) SDS-PAGE analysis of purified AppA protein. Protein loading weight was 0.7 µg without impurities. AppA size: 47kDa, Lane M*: AccuLadder*™ Protein Size Marker (Broad), Lane P: Purified AppA

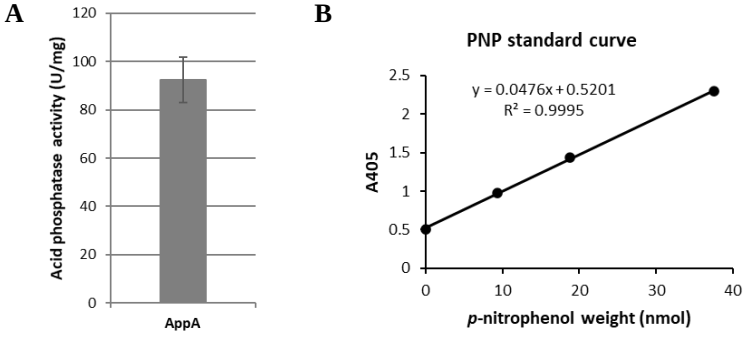

Figure 2. Enzyme activity analysis of purified AppA
(A) Acid phosphatase activity of purified AppA (B) *p*-nitrophenol (PNP) standard curve obtained using colorimetric reading

References

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2. I. S. Oh, D. M. Kim, T. W. Kim, C. G. Park, C. Y. Choi. 2006. Providing an oxidizing environment for the cell-free expression of disulfide-containing proteins by exhausting the reducing activity of *Escherichia coli* S30 extract. Biotechnol. Prog. 22:1225-1228