報告番号	※　　第　　号

主　論　文　の　要　旨

論文題目　Synthesis of difficult-to-express proteins by *Escherichia coli* expression systems (大腸菌発現システムを用いた難発現タンパク質の合成)

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論　文　内　容　の　要　旨

Disulfide bonds are a covalent bond formed upon oxidation of two thiols in two cysteine groups. The presence of disulfide bonds in protein often lead to misfolded, make the protein prone to be aggregated when expressed in *Escherichia coli* host cells. Manganese peroxidase (MnP) is a heme peroxidase oxidizing Mn^{2+} to Mn^{3+}, which is diffusible and strong non-specific oxidant acting on inaccessible and bulky phenolic substrates. It has five disulfide bonds, with three of them are non-consecutive, make it prone to expressed as inclusion bodies in *E. coli* expression system. Another peroxidase, horseradish peroxidase (HRP), are extensively used in biochemistry. It has two consecutive and two non-consecutive disulfide bonds, lead to the inclusion bodies expression in *E. coli*. Fragment antigen binding (Fab) is one of the widely used antibody that has disulfide bonds between its heavy chain and light chain, causing the difficulties of correct binding when expressed in *E. coli*. The purpose of this study is to increase the solubility and productivity of protein with disulfide bonds expressed in *E. coli*.

In chapter 2, soluble MnP expression in *E. coli* was studied. Previous study found the presence of DsbC in *E. coli*-based cell-free protein synthesis increased the solubility and

activity of MnP significantly, compare to PDI or the absence of both. Hence, *E. coli* SHuffle T7 Express was chosen as host cells due to its production of cytoplasmic DsbC. Several chaperone combinations were tested by co-expression with MnP, in which DnaK-DnaJ-GrpE gave the highest soluble ratio towards its insoluble fraction. DnaK-DnaJ-GrpE remain in complex with MnP after purification, required *in vitro* ATP maturation to release the MnP. While ATP and ATP regenerating system help the chaperone to complete the refolding process, the presence of hemin help MnP maintain its activity resulting in 2 folds higher activity compare to the commercial MnP.

In chapter 3, CyDisCo expression used in this study consist of sulfhydryl oxidase Erv1p and disulfide isomerase DsbC was tested for MnP, HRP, and Fab-AP in *E. coli* expression system. While the sulfhydryl oxidase facilitates the formation of disulfide bonds, the presence of disulfide isomerase promotes the correct disulfide formation by isomerize the mis-formed disulfide bonds. CyDisCo and vector containing target protein were transformed into *E. coli* BL21(DE3). MnP and Fab-AP can be detected insolubly by SDS-PAGE analysis. Similarly, various HRP constructs were also expressed as inclusion bodies, with large amount of target protein was detected in SKIK-HRP and HRP. Further study is necessary to obtain reliable activity, but soluble T7-HRP gave 3 folds higher activity after activation compare to the not activated protein.

In chapter 4, various leucine zipper pairs in zipbodyzyme format were constructed to examine the productivity in *E. coli* expression system. Zipbodyzyme is genetically fused antibody with leucine zipper and enzyme, in the presence of SKIK tag insertion in the N-terminus. SKIK-tag is not only increasing the yield of zipbodyzyme, but also increase its activity as previously reported. Through the cell-free protein synthesis (CFPS), both antibody and enzymatic activity of the constructs have been confirmed by one-step and

two-step ELISA. The constructs were transformed into *E. coli* BL21(DE3) and mostly expressed as inclusion bodies. No activity was observed in the soluble fraction, along with the necessity of continuous buffer exchange to properly refolded the inclusion bodies. Within 4 pairs of leucine zipper (Leucine Zipper A/Leucine Zipper B; Jun/Fos; Basic Jun/Acid Fos; Basic Jun/Basic Fos), zipbodyzyme containing Basic Jun/Acid Fos gave the highest activity.

The techniques mentioned in this dissertation could serve as important clue for establishing bacterial production of protein with disulfide bonds.