Strategies for improving the expression of antibody derivatives in *Escherichia coli*

Antibody engineering has undergone intensive development and became a more effective technique for use in the biotechnology, pharmaceutical and therapeutic industries. ScFv and Fab are the most widely used antibody fragments, produced by recombinant processes in prokaryotic and eukaryotic systems such as bacteria, yeast, insect and mammalian cells. The antibodies are commonly generated by hybridoma technology. However, cultivation of eukaryotic cells is difficult, expensive and unsuitable for industrial scale protein production. *Escherichia coli* (*E. coli*) is frequently used as an expression system to generate recombinant proteins, offering several advantages including easy growth and fast generation. Cells can be readily manipulated in the laboratory at a low expense and high cell density cultures are simple to achieve. Overexpression of recombinant proteins in *E.coli* often results in inclusion bodies, inactive proteins which require activation by refolding. However, refolding methods have many disadvantages such as complexity and low reproducibility. Formation of inclusion bodies and the frequent need for refolding represent drawbacks of the *E. coli* expression system.

Our group developed a new Fab derivative named ‘Zipbody’ in which the leucine zipper, LZA and LZB peptides, were fused to the C-terminus of the heavy chain (Hc) and light chain (Lc), respectively. The zipbody format significantly increased the production of active Fab in an *E. coli* cell-free protein synthesis system and *in vivo E. coli* expression system. In addition, we reported the insertion of a short peptide tag with the sequence Ser-Lys-Ile-Lys (SKIK) at the N-terminus of the protein as an effective method for increasing production of the difficult-to-express proteins in *S. cerevisiae* and an *E. coli* cell-free protein synthesis system.

In this study, we aimed to improve the protein expression in *E.coli* by first focusing on insoluble protein and examining several refolding processes to recover the bioactive protein. Second, we explored ways to increase soluble protein expression by constructing a new format of antibody derivative.

In Chapter 2, refolding methods were examined to obtain high yields of Fab derivative from *E. coli* inclusion bodies. The mouse Zipbody (m6FabLZ) against *E. coli* O157 was expressed in *E. coli* BL21 (DE3) cytoplasm at 37°C and isolated by three different methods. Next, we compared five protocols for solubilization, and the solubilized m6FabLZ protein was refolded using three different processes by step-wise dialysis. Finally, inclusion bodies of m6FabLZ were successfully solubilized and refolded. Yield of purified m6FabLZ was 0.25 g from 1 L culture and refolded protein showed high affinity a and specificity toward *E. coli* O157 in ELISA. Refolding procedures used here are easy, inexpensive and time-saving and can be utilized for recovery of bioactive Fab in high yield from inclusion bodies in *E. coli*.

In Chapter 3, a soluble expression of a zipbody fused with an enzyme, resulting in a bifunctional protein of antibody and enzyme named ‘Zipbodyzyme’ was examined using an

E.coli strain with oxidative cytoplasm, SHuffle T7. Five m6Fab derivatives with and without SKIK, leucine zipper or alkaline phosphatase (AP) were constructed for improved affinity and expression yield in an *E. coli* expression system. All constructs were expressed in *E. coli* SHuffle T7 Express strain and affinities were compared using ELISA. The m6FAb derivatives were purified by Ni-NTA affinity chromatography and size-exclusion chromatography. Finally, the soluble antibody derivative, SKIK-m6FabLZ-AP fusion protein showed high affinity and specificity toward *E. coli* O157 in ELISA and had a high expression level in *E. coli*, suggesting that AP fusion to Fab derivatives can increase not only apparent affinity of the antibody but also the solubility of the Fab fragments. Similar results were obtained for a different antibody sequence, suggesting that the new format may be applicable for a wide variety of antibodies.

As far as I know, Fab derivatives are very difficult to express in *E.coli* because its hetero-dimeric structure, complex disulfide bridges, sequence variety of antibodies, etc. Strategies tried in this research will contribute the science and technology of functional protein production in bacterial cells.