In vitro directed evolution of TIMP-2 protein for soluble expression from E. Coli

Dong Soon Choi[1], Chun Kyu Lee[2], Sung Su Kim[1], Churl K. Min[2], Hyun Joo[1]†
[1]Dept.of Molecular Science & Technology, Ajou Univ.
[2]Dept.of Biological science, Ajou Univ.
TEL: +82-31-219-2625, FAX: +82-31-219-1615
† Corresponding Author : hjoo@ajou.ac.kr

Abstract

TIMP-2 , the second member of the family of tissue inhibitors of matrix metalloproteinases (TMMPs) is 23KDa protein which inhibit matrix metalloproteinase 2 (MMP-2)[1)]. High-level intracellular expression of many mammalian protein in E.coli, results in the formation of large insoluble aggregates, known as inclusion body[2)]. The TIMP-2 is invariable insoluble when expressed in E.coli[1)], because this folding requires the formation of 6 disulfide bonds being incompatible with the reducing environment of the E. coli. but the soluble recombinant protein can often be recovered from E coli by in vitro recombination and mutagenesis[3)]. In this reason, we tried the staggered extension process(StEP) that is one of the in vitro PCR-based recombination methods for mutagenesis[4)]. C-terminally located CAT gene expression from the mutant genes made us indentify the soluble from the insoluble through linker sequences[5)]. As the results, chloroamphenicol resistant varients were selected and some of the mutant showed dramatic soluble expressin of TIMP-2 compared to the wild type. Therefore, StEP has successfully improved folding and solubility of TIMP-2, and the CAT depedent screening may be a simple and effective method in selecting soluble forms from the insoluble. This study was supported by HTPEB (HTPEB 01 - PJ11 - PG9 - 01NT00-0036).

References

1. Negro A, Onisto M, Masiero L, Garbisa S. Synthesis and refolding of human TIMP-2 from E. coli, with specific activity for MMP-2, FEBS Lett. 1995 Feb 20;360(1):52-6.
2. Williamson R. A., Refolding of TIMP-2 from Escherichia coli inclusion bodies. Methods Mol Biol. 2001;151:257-65
3. Lesley SA, Graziano J, Cho CY, Knuth MW, Klock H. E., Gene expression response to misfolded protein as a screen for soluble recombinant protein Protein Eng. 2002 Feb;15(2):153-60.
4. Aguinaldo A. M., Arnold F. H., Staggered extension process (StEP) in vitro recombination. Methods Mol Biol. 2003;231:105-10.
5. Maxwell K. L., Mittermaier A. K., Forman-Kay J. D., Davidson A. R., A simple in vivo assay for increased protein solubility, Protein Sci. 1999 Sep;8(9):1908-11.