(様式 5)

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学位（博士）論文要旨

(Doctoral thesis abstract)

論文提出者 (Ph.D. candidate)	工学府博士後期課程　生命工学専攻 (major) 平成 31 年度入学(Admission year) 2019 年 04 月 01 日 学籍番号　19831003　(student ID No.)　氏名　Subbaian Brindha (Name)
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論 文 題 目 (Title)	Efficient production of multi-disulfide bond proteins in Escherichia coli using solubility enhancing peptide tag

論文要旨（2000 字程度）
(Abstract(400 words))
※欧文・和文どちらでもよい。但し、和文の場合は英訳を付すこと。
(in English or in Japanese)

I showed attaching a short, 12 residue solubility enhancing peptide tag (SEP-Tag) containing 9 arginines (C9R tag) at the C-terminus of EGFR-ECDIII, we can reduce the inclusion body formation with six times higher yield than produced without a C9R tag. The protein was isolated as a soluble form with a single peak by RP-HPLC, with a significantly high yield of 20 mg/L while expressed at 16°C. Multiple biophysical methods like circular dichroism, intrinsic fluorescence, static and dynamic light scattering confirmed the folded state of the protein. The binding activity of EGFR-ECDIII-C9R to antiEGFR-VHH7D12, a single-domain antibody binding specifically to the extracellular domain III of EGFR, is also confirmed. The biological function of prokaryotic expressed EGFR-ECDIII was further confirmed by its ability to generate specific antibodies with high titers in mice model, suggesting the proper presentation of the antigens from recombinant proteins to the animal immune system, which raises specific antibodies with the high affinity toward native proteins in eukaryotic full-length EGFR ECD structure. Furthermore, the effect of EGFR-ECDIII-C9R anti-sera on the growth of A432, an epidermoid carcinoma cancer cell line, was assessed by MTT assay. As a result, EGFR-ECDIII-C9R anti-sera inhibited the growth of A431 cells. Also, in this dissertation, I tried to apply the same strategy for yet another protein having a greater number of disulfides bonds.ie., SARS-CoV-2-RBD containing 4 disulfide bonds, and its expression in *E. coli* is limited by the formation of aberrant disulfide bonds resulting in inclusion bodies. Here we show that a solubility-enhancing peptide (SEP) tag containing

nine arginine residues (RBD-C9R) attached at the C-terminus can overcome this problem. The SEP tag increased the expression in the soluble fraction and the final yield by five times (2mg/L). The native-like properties of the *E. coli* expressed RBD-C9R were demonstrated with biophysical characterization using RP-HPLC, circular dichroism, thermal denaturation, fluorescence, and light scattering. The quartz crystal microbalance (QCM) analysis confirmed the binding activity of RBD-C9R with ACE2, the host cell's receptor. In addition, RBD-C9R elicited a Th-2 immune response with a high IgG titer in Jcl: ICR mice. The RBD-C9R anti-sera interacted with both itself and the mammalian-cell expressed spike protein (S1) as demonstrated by ELISA, indicating that the *E. coli* expressed RBD-C9R harbors native-like epitopes.

Overall, these results emphasize the potential of our SEP tag for the production of natively folded multi disulfide proteins. The SEP tag increases the solubility of a protein in both the folded, as well as the misfolded, unfolded states through repulsive electrostatic interactions. We thus hypothesize that the ability of the SEP tag to maintain the unfolded and misfolded proteins in the soluble fraction provides time for the protein to refold into the native state and disulfide bond isomerase present in the cytoplasm of T7 shuffle cell helps the reshuffling of non-native disulfide bonds in the oxidized cytoplasm. We believe that the strategy presented in this study certainly could also be adapted to produce other multi-SS bonded protein in *E. coli* expression systems to accelerate the drug discovery and protein mechanism studies.

（英訳）※和文要旨の場合(400 words)