学位（博士）論文要旨 (Doctoral thesis abstract)

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論 文 題 目 (Title)	Development of short peptide tags for solubilizing biologically relevant proteins without altering their activities

(Abstract(400 words))

Protein solubility remains a challenge in biophysical and biochemical studies of many recombinant proteins. Low solubility limits the wider usage of recombinant proteins in various biotechnology and pharmaceutical industries, especially in the production and storage of therapeutic proteins. Here, we report the effect of a nine arginine (C9R) solubility enhancing (SEP) tag on two important recombinant proteins

.First, we designed and characterized TEV-C9R, a novel Tobacco etch virus protease (TEV). The attachment of C9R tag, improved its expression, refolding, yield, and solubility after purification without affecting its activity. The yield of HPLC purified TEV-C9R expressed in *E.*coli was ~ 6.5 times higher than the yield of the untagged TEV. TEV-C9R was active over a pH range of 5-8, which was wider than that of the commonly used Thrombin, and it remained active upon incubation at 60 °C much longer than the untagged TEV, which aggregated at this temperature. Static and dynamic light scattering (SLS and DLS) demonstrated the higher solubility of purified TEV-C9R. Furthermore, the thermal unfolding of TEV-C9R, as assessed by Circular Dichroism (CD) at pH 4.7, was almost perfectly reversible in contrast to that of untagged TEV, which aggregated at high temperature.

As a second example, we applied the same strategy, i.e. attaching a C9R SEP tag to C terminal, for increasing the solubility of an anti-epidermal growth factor receptor (Anti-EGFR) single chain variable fragment (ScFv). To this end, we first estimated the solubility increase by running 500-ns Brownian dynamics (BD) simulations and computing the fraction of monomeric proteins. We then experimentally evaluated the predictions by producing recombinant Anti-EGFR ScFv with and without a SEP tag in E. coli, and measured their biochemical and biophysical properties. At a lower temperature of 20 ° C, ~85% of Anti-EGFR ScFv-C9R expressed in the soluble fraction whereas all of the Anti-EGFR ScFv remained in the insoluble fraction. In addition, the yield of Anti-EGFR ScFv-C9R purified by Ni-NTA was 17.15 mg, which was ~3 times higher than that of the untagged Anti-EGFR ScFv. SLS and DLS demonstrated the higher solubility of the purified Anti-EGFR ScFv-C9R and CD analysis indicated a high thermal stability for Anti-EGFR ScFv-C9R, whereas the untagged protein aggregated at 37 ° C (pH 6). Finally, the binding activity of Anti-EGFR ScFv-C9R to EGFR was confirmed by surface plasmon resonance (SPR). Altogether, these results illustrate the improved biophysical and biochemical characteristics of TEV-C9R and Anti-EGFR ScFv-C9R and emphasize the potentials of SEP-tags for enhancing the solubility of recombinant proteins.