学位（博士）論文要旨(Doctoral thesis abstract)

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論 文 題 目 (Title)	**Short Peptide Tag-Assisted Purification and Immunogenicity Modulation of Virus-Derived Recombinant Proteins as a Novel Platform for Vaccine Development**

論文要旨(Abstract)

Protein subunit vaccines have several advantages over other vaccine types. However, the conventional protein purification techniques have time and cost concerns, while protein subunit vaccines have relatively poor immunogenicity. To address these challenges, a novel protein purification technique was developed, and protein aggregation was applied to enhance the immunogenicity of a small viral antigen protein.

First study presents a simpler, faster, and handier method for purifying His-tagged proteins using free Ni^{2+}-induced selective precipitation. The technique has been applied to purify four His_6-tagged recombinant proteins overexpressed in *E. coli*. Ni^{2+} ions at a final concentration of as low as 1 mM precipitates the His-tagged proteins with near-complete specificity. The final yields were similar to or even higher than purification using conventional Ni-NTA chromatography. The purified proteins exhibited natively folded characteristics and binding activity demonstrating the method's potential in both small and large-scale settings.

Second study addresses effectively overcoming the low immunogenicity of *E. coli*- produced envelope protein domain III of the Japanese encephalitis virus (JEV-ED3) by employing a five-isoleucine tag at C-terminus (C5I). C5I-tagged JEV-ED3 formed soluble oligomers, whereas the untagged JEV-ED3 remained monomeric. The JEVED3-C5I significantly enhanced anti-JEV ED3 IgG titers and increased the population of memory T cells. Most notably, the C5I-tagged JEV-ED3 elicited live JEV-neutralizing antibodies. These findings suggest that use of C5I-tag offers a promising, adjuvant-free approach for developing *E. coli*-produced, protein domain-based vaccines.

Third study addresses how metal-mediated His-tagged protein oligomers overcome the poor immunogenicity of a small protein. The JEV-ED3 with a six-histidine tag at N-terminus and five divalent metal ions (Co, Cu, Mn, Ni, and Zn) were utilized. All metals, except Mn, resulted in native subvisible aggregates of His-tagged JEV-ED3. Surprisingly, only cobalt-mediated oligomers increased the anti-JEV-ED3 IgG titers and T-memory cell population. Importantly, cobalt-mediated oligomers induced live JEV-neutralizing anti-JEV IgG antibody generation. These results indicate that use of free cobalt may act as an adjuvant-free method to greatly enhance the immunogenicity of recombinant proteins.

According to the investigations, the Ni^{2+}-precipitation approach may reduce production time and cost, while the C5I-tag or trace amount of cobalt can improve the efficacy of *E coli*-expressed protein subunit vaccine candidates.