学位（博士）論文要旨

(Doctoral thesis abstract)

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論 文 題 目 (Title)	Control of BPTI's aggregation states using Solubility Controlling Peptide tags and their effect on its *in vivo* immunogenicity

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)

Aggregation is known to improve a protein's immunogenicity, which is a desired trait for protein based vaccine candidates. However, in the field of protein therapeutics sub-visible aggregates are suspected to cause adverse or undesired immune response, and a recent FDA guideline has recommended the monitoring of micrometer-sized aggregates (2-10 μm) though recognizing that the underlying mechanism behind aggregation and immunogenicity remains unclear. To this end, I first report a correlation between the immunogenicity and the size of nanometer-scale aggregates of a small 6 kDa model protein, Bovine Pancreatic Trypsin Inhibitor (BPTI) variant, BPTI-19A, by attaching four hydrophobic solubility controlling peptide (SCP) tags to its C-terminus. Dynamic light scattering (DLS) and static light scattering (SLS) measurements indicated that hydrophobic SCP-tags did merely affect the particle size of BPTI except the C5I tag which formed sub-visible aggregates with a hydrodynamic radius (R_h) of ~4 nm. Moreover, the polydispersity and oligomeric status of the subvisible aggregates of BPTI-C5I were also confirmed by analytical ultra-centrifugation (AUC) experiments. Tyrosine fluorescence and circular dichroism (CD) indicated that all tagged BPTI variants had the same tertiary and secondary structure contents as the untagged BPTI, except BPTI-C5I (and BPTI-C5L to some extent), which had a partially folded

structure. Immunization experiment with a near “real-time” monitoring of hydrodynamic radius showed that BPTI-C5I significantly increased the IgG titer over 55 fold, as assessed by the ELISA. Overall, the study emphasizes that subvisible aggregates, as small as a few nanometers, which are presently ignored, are worth monitoring for deciphering the origin of undesired immunogenicity of therapeutic proteins. Then, I report the effects of ten other SCP-tags for improving BPTI’s immunogenicity that are generally hydrophilic and thus do not induce protein oligomerization. CD, fluorescence, DLS, SLS measurements, and AUC experiments indicated that the addition of the SCP-tags did not change the secondary and tertiary structure of the protein and its monomeric state. Moreover, ThT assay show no signal for fibrillary aggregates. Finally, ELISA indicated that the 5-proline (C5P) tag quite unexpectedly increased the immunogenicity (IgG level) of BPTI-19A by up to 240 fold while the 5-arginine tag (C5R) improved the antibody titer by up to 73 fold. Overall, these results suggested that not only oligomerizing tags but also non-oligomerizing short peptide tags could efficiently increase the immune response of a non-immunogenic protein, and thus provide a further tool to facilitate the antibody production for biopharmaceutical usage or improve the immunogenicity of poorly immunogenic proteins.