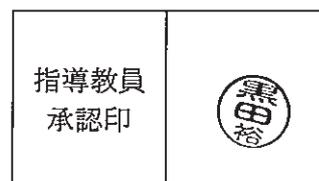


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## 学位（博士）論文要旨

(Doctoral thesis abstract)

論文提出者 (Ph. D. candidate)	工学府生命工学専攻 Department of Biotechnology and Life Science (major) 平成 年度入学 (Admission year) 25 年 04 月 01 日 学籍番号 氏名 印 (Student ID No.) 13871107 (Name) Md. Golam Kabir (Seal) 
主指導教員氏名 (Name of supervisor)	Yutaka Kuroda
論文題目 (Title)	Biophysical characterization of soluble protein aggregates and oligomers controlled using short amino acid peptide tags
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)  Understanding protein aggregation and/or oligomerization is very important both from academic and biotechnological application viewpoints. Here, we report the effects of short peptide tags attached to a simplified bovine pancreatic trypsin inhibitor (BPTI) variant on its sub-visible aggregate formation. First, we report a biophysical and biochemical analysis of a model BPTI (BPTI-19A, a BPTI variant containing 19 alanines out of 58 residues), whose oligomerization were controlled by attaching solubility controlling peptide tags (SCP tags) to its C terminus. Dynamic light scattering (DLS) and Static Light Scattering (SLS) at 25 °C indicated that the hydrophobic SCP tags composed of 5 Ile (C5I) or 5 Leu (C5L) resulted larger sub-visible aggregates compared to other tagged and untagged BPTI variants. These	

larger sub-visible aggregates originated from reversible association native BPTI units through hydrophobic interactions of the tag residues. Circular dichroism indicated that all SCP tagged BPTIs had the same secondary structure contents as the reference BPTI-19A at 25°C and 37°C, except the C5I and C5L which were partially denatured due to the hydrophobic interactions between the tags residues. Moreover, thermal stability of C5I and C5L variants decreased with increasing protein concentration and tag's hydrophobicity and larger aggregates were notably more stable than the untagged BPTI against pepsin. Then, we monitored the time-dependent salt-induced amorphous protein aggregation by fluorescence self-quenching. The light scattering of four peptide-tagged BPTIs labelled with NHS-Fluorescein indicated that the FAM-C2G was the most aggregation prone, followed by FAM-C5A and FAM-C5S and FAM-C5D did not show visible aggregation over 24h. Furthermore, the particle sizes of FAM-C2G, FAM-C5A and FAM-C5S increased significantly over solution incubation time while that of FAM-C5D remained constant. The fluorescence intensity of all FAM-BPTIs decreased immediately, albeit to a different extent, upon addition of salt and became constant after 10 min for 24 h. These observations suggested that FAM-C2G, FAM-C5A and FAM-C5S showed strong molecular interactions at condensation stage resulting large aggregates and the very soluble FAM-C5D would remain in the molecular condensation state without further propagating into insoluble protein aggregates and self-quenching method can be used for monitoring and analyzing even the early stages of protein aggregation. These observations suggested that the short poly-amino-acid peptide tags could be used as a generic tool for both manipulating the sub-visible aggregate formation and their detailed biophysical characterization.

(英訳) ※和文要旨の場合(400 words)