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学 位 の 種 類	博 士 (工学)
学 位 記 番 号	博 甲 第　8964　号
学位授与年月日	平成 31年 3月 25日
学位授与の要件	学位規則第4条第1項該当
審 査 研 究 科	数理物質科学研究科
学 位 論 文 題 目	

Protein aggregation in heterogeneous system
（混合系におけるタンパク質凝集）

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論　文　の　要　旨

Protein aggregation is an important issue for various fields, such as pharmaceutics, bioengineering, and food engineering. Abundant and diverse researches to control of proteins have been performed for decades, though fundamental understanding of the mechanisms has remained elusive. The study of aggregation in heterogeneous protein systems has made little progress compared to that in purified protein systems. Therefore, it is indispensable for understanding of heterogeneous protein systems beyond the single system in conventional protein science. As shown in Chapter 1, this thesis aims to shed light on the aggregation of proteins in a heterogeneous system using hen egg-white proteins.

Chapter 2 described the thermal aggregation of egg-white proteins at various concentrations in the presence of inorganic salts. The thermal aggregation is changed depending on the protein concentration. A chaotrope dramatically promote the thermal aggregation at high protein concentration.

Chapter 3 described the thermal aggregation of co-existed proteins (so-called coaggregation) with ovotransferrin (OVT)–lysozyme (LYZ) and ovalbumin (OVA)–LYZ binary systems. The coaggregation process of OVT and LYZ were determined in terms of protein composition, structure, intermolecular forces, and morphology. The coaggregates of OVT and LYZ formed colloidal particles with a large network, while OVT or LYZ alone did not observe the particles. Similar data were obtained for OVA–LYZ binary systems; heat-labile OVA and relatively-stable LYZ. These data showed that the hierarchical coaggregation of protein mixture started with the thermal unfolding of unstable protein, followed by the coaggregation occurred with

native or partially unfolded stable protein by disulfide crosslinking reactions.

Chapter 4 described the interaction mode and morphology on native and thermally unfolded proteins constituting coacervates and coaggregates using OVA and LYZ. The thermally-unfolded OVA was prepared by pre-heat treatment, and then incubated at ambient temperature with native LYZ. The unfolded molecules were prone to form complexes than native state by hydrophobic interactions, rather than electrostatic attraction. Interestingly, native proteins formed coacervates by liquid–liquid phase separation in the absence of salts. These results indicate that OVA and LYZ formed coacervates by electrostatic attraction and a solid-like aggregates by hydrophobic interaction.

審　　査　　の　　要　　旨

〔批評〕

This thesis investigated coaggregation process and mechanism with heterogeneous proteins. The coaggregation with two kinds of proteins is different from an aggregation with a single kind of proteins as follows. First, coaggregation occurs hierarchical process with the electrostatic attraction between heteromolecules, after that the hydrophobic interaction stabilizes the coaggregates. At high temperature, coaggregates are stabilized by disulfide-bond exchange reaction to form crosslink networks. At low ionic concentration, heteromolecules form coacervates by liquid-liquid phase separation. These mechanisms of coaggregation provide important information about food science and biopharmaceutics to understand the aggregation mechanism in a heterogeneous protein system.

〔最終試験結果〕

The final examination committee conducted a meeting as a final examination on February 18, 2019. The applicant provided an overview of dissertation, addressed questions and comments raised during Q&A session. All of the committee members reached a final decision that the applicant has passed the final examination.

〔結論〕

Based on the results of the above-mentioned dissertation defense and final examination, the final examination committee approved that the applicant is qualified to be awarded Doctor of Philosophy in engineering.