学 位 論 文 の 内 容 の 要 約
Summary of doctoral dissertation content

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学位論文題目 Thesis title	PSD95-PDZ3 の高温可逆オリゴマー (RO)及びアミロイド形成の変異解析 Mutational analysis of PSD95-PDZ3's formation of high-temperature reversible oligomers and amyloidogenesis

【論文の内容の要約】[Summary of the contents of the doctoral dissertation]

Understanding the mechanism and component of protein aggregation pathways are important to develop the biotherapeutic drug, investigate the cause of the aggregation diseases, and observe protein misfolding. Thermodynamics of protein unfolding and protein stability analysis methods are typically used to investigate each component of protein aggregates. In this research, I aim to study the thermal unfolding of a small globular protein by developing the analysis method to be simple and effective to understand the mechanism of protein aggregation as well as the biophysical characterization of distinct conformation of protein in the unfolding state. I divided my dissertation into 4 chapters.

In chapter 1, I reviewed general information of protein unfolding and aggregation, the study of thermal denaturation, reversible oligomerization and model protein. Protein unfolding is caused by external stress and unfolded to various conformation and particle size. The thermal denaturation and protein stability play an important role in controlling protein unfolding state and/or partially unfolding. In this intermediate state, protein forms in different conformation including, small oligomer, molten globule, etc. and it can be developed to the sub visible aggregate (amorphous, amyloid) and irreversible aggregate. Spectroscopy and fluorescence methods were used for determining the two-state thermal denaturation and three-state thermal denaturation proteins. The unusual unfolding phenomenon that occurred in the three-state thermal denaturation was observed in several proteins, but the studying of the intermediate state is still unclear. Reversible oligomerization was recently observed and

explained the intermediate state of thermal denaturation of protein. The typical thermal denaturation of protein exposed a single endothermic peak in the DSC thermogram and fitted to the two-state thermal denaturation model. However, some protein was observed as the second endothermic peak in the DSC thermogram and fitted with the three-state thermal denaturation model. At the high temperatures, molar fraction of intermediate state oligomerization was observed and it's reversible. I used the PSD95-PDZ3 protein as a model protein because of the precise x-ray crystallography structure and related to the neuron function. The lack of reversible oligomerization and the mechanism of protein unfolding motivated me to develop mutational analysis methods. The understanding of protein stability controlling and protein aggregation mechanisms will be able to develop more protein aggregation resistant and inhibit the aggregation diseases.

In chapter 2, I introduced a mutational analysis method for understanding the inhibition of reversible oligomerization (RO) at high temperatures occurring in the thermal unfolding of PSD95-PDZ3 domain protein. The mini-alanine method was used to design the mutants. After calculating by bioinformatics tool, we substituted the hydrophobic residues adjacent to the interface and apart from the interface when protein form tetrameric native structure. Two variants (PDZ3-F340A and L342A) showed the reducing of hydrophobicity on the interface area can inhibit the RO at high temperatures. The biophysical characteristics and the Transmission electron microscope (TEM) imaging of all mutants were observed and explained the correlation between the RO at high temperatures and amyloidogenicity which reducing the RO can also result in inhibition of amyloid fibril formation. Therefore, this design strategy suggests that the hydrophobic residues located adjacent on the interface play an important role in the RO formation related to amyloidogenicity.

In chapter 3, I applied reverse engineering to the mutational analysis method for producing the new product related to the inhibition and exhibition of RO at high temperatures. The design strategy was like the previous research, but we used the PDZ3-F340A which inhibited the RO as a template protein. We decided on the candidate residues using bioinformatics tools. We substituted the hydrophilic residue with Leucine which is a moderate hydrophobic amino acid. We hypothesize that increasing hydrophobic propensity will increase the formation of RO at high temperatures. The biophysical characteristics of new mutants showed that one out of three variants successfully induced the RO at high temperatures from the template which inhibited RO formation. The RO formation at high temperatures also correlates with the amyloidogenicity in the term of a precursor of amyloid fibrillation. Thus, this design strategy suggests that increasing the hydrophobic propensity on the interface area of

protein predicted from the tetrameric structure of protein ais effectively reproduce the RO at high temperatures concurrent with the amyloidogenicity.

In chapter 4, I conclude that a single point mutation with two different mutational analysis is effectively used for inhibiting and reproducing RO at high temperatures. Because of the relation between RO and amyloidogenicity, the controlling of the RO is also effective to control the amyloidogenicity. The application of the model protein in the biological function still needs to be carried out. This mutational analysis will be validated by conducting the experiments in other control residues or other small globular protein. Especially, the protein that is related to pathogenic and aggregation diseases. This protein stability control can be utilized to the development of protein aggregation resistance and therapeutic drugs.