（学位第３号様式）

学 位 論 文 要 旨

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題　目	(Studies on the mechanisms of amyloid fibril formation of lysozyme and a halophilic protein) （リゾチーム及び好塩性タンパク質を用いたアミロイド線維形成機構の解明）

Amyloid fibril (AF) are ordered protein aggregates, and are formed by self-association of proteins that undergo protein denaturation or misfolding. AF possess a common cross β-structural motif, and are related to various degenerative diseases, including Alzheimer's. Human lysozyme (hLz) with hereditary mutations (I56T, F57I, D67H, W64R) is known to cause systemic amyloidosis. Its homologue, hen egg white lysozyme (HEWL), has been well studied as a model protein of AF formation. Recently, several native and functional AF was reported in bacteria and other organisms, where the amyloid fold can be recognized as an important part of cellular physiology, but not as aberrant disease-associated pathological structures.

My research aimed at elucidation of the molecular mechanisms of fibril formation and protein aggregations. I have studied AF formation of two type of K peptides derived from HEWL (54^{th}G-62^{th}W) and of hLz (55^{th}G-63^{th}Y), and halophilic histidine-rich protein (HP). HEWL (10 mg/ml of 50 mM Glycine-HCl buffer, pH2.0) was incubated at 58 ^{o}C for 2 weeks. AF formation was examined by thioflavin fluorescence, transmission electron and atomic force microscopic observations. AF formation was enhanced with the addition of seeds (preformed AF with sonication). After ultracentrifugation of the HEWL samples at 150,000 x g for 5 hrs, transparent gel-like AF precipitates (17% of total protein amount) were obtained, showing a typical beta-sheet rich profile by circular dichroism (CD) measurement. K peptide derived from HEWL also formed AF at pH 4 and 37 ^{o}C: it has been found in the studies of interactions between HEWL and ovoalbumin. AF formation of synthetic K peptide (HEWL) with several mutations revealed the important role of 62^{th}W in the inter-molecular structural stacking, leading to formation of AF. Thus, K peptide is crusial as a core region on AF formation of HEWL. The human K peptide was also confirmed to form AF with electron microscopic and CD observations.

I further studied AF formation of a highly soluble and aggregation-resistant halophilic protein, HP. It readily formed AF under conditions of low pH and high temperature, which appeared to be tightly associated with rapid acid hydrolysis of HP and resultant generation of short peptides. HP also formed AF under neutral pH upon addition of moderate concentrations of 2,2,2-trifluoro-ethanol without any protein degradations. The aggregation-resistant characteristics of HP resulted in the formation of homogeneous AF without formation of amorphous protein aggregations, suggesting that HP could be a novel model protein for AF formation.