（学位第３号様式）

学　位　論　文　要　旨

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題　目	Studies on quality control and ER stress of amyloidogenic lysozyme. （アミロイドジェニックリゾチームの品質管理と小胞体ストレスに関する研究）

Accumulations of abnormal proteins that are misfolded and fibrotic have been observed in case of pathological amyloidosis such as Alzherimer's disease, Parkinson's diseases. None of the proteins involved in these diseases have common amino acid and sequences or tertiary structures, but each of them can form amyloid fibrils consisting of a cross-ß-sheet structure and can be deposited in various tissue. Lysozyme is an omnipresent enzyme attacking the peptidoglycan cell wall of certain microorganisms. There are four pathogenic mutants of human lysozyme that cause non-neuropathic systematic amyloidosis. Naturally occurring single mutants, I56T, F57I, W64R and D67H, have been known to form abnormal protein aggregates (amyloid fibrils) and to accumulate in several organs, including the liver, spleen and kidney, resulting amyloidosis. However, the pathogenesis of the disease is not well understood. The mutants may aggregate in the cell, and exhibit cytotoxicity.

In this study, I examined the effects of the above-described four pathogenic lysozyme mutant (I56T, F57I, W64R, D67H) in cultured human embryonic kidney (HEK) 293 cells.

Western blot analyses showed the showed lesser amounts of these mutants proteins in the medium compared to the wild type, but they were abundant in the cell pellets, indicating that the modified lysozyme protein scarcely secreted into the medium but were retained in the cells. Immunocytochemistry revealed that these mutant proteins resided in the restricted regions that were stained by an endoplasmic reticulum (ER) marker. Moreover, the overexpression of the mutant lysozymes were accompanied by marked increases in XBP-1s and GRP78/BiP, which are downstream agents of the IRE1 signaling pathway responding to the unfolded protein response (UPR) upon ER stress. Also, the mutant lysozymes accumulate in the ER with the ER chaperone GRP78/BiP.

Next, I investigated the region of lysozyme that is critical to its association with GRP78/BiP. In addition to above-described mutants, I constructed lysozyme truncation or substitution mutants. These were co-expressed with GRP78/BiP (tagged with FLAG) in HEK293 cells, and I analyzed the interaction by immunoprecipitation. The mutants were confirmed to be strongly associated with GRP78/BiP, whereas N-terminal pruned mutants (1-41del, 1-51del) were found not to be associated with the chaperone. Single amino acid substitutions for the leucine array along the α-helices in the N-terminal region resulted in wild-type lysozyme remaining attached to GRP78/BiP. These mutations also tended to show lowered secretion ability.

I conclude that the N-terminal α-helices region of the lysozyme is pivotal for its strong adhesion to GPR78/BiP. I suspect that lysozyme interacts with the GRP78/BiP at this region as a step in the proper folding. However, in mutants lysozyme, because they are not able to make proper structure, mutants remain interact to GRP78/BiP strongly and induced ER stress.