Repression Effects of Hydrolysates from Hen-Egg Proteins on Amyloid Fibril Formation

Yukiko Muroi¹,², Izumi Aburaya¹, Takuro Shima¹, Mitsuharu Matsumoto³, Ryo Sasahara⁴, Takahisa Suzuki⁴, Keiichi Watanabe¹,², Koji Wada² and Yasushi Sugimoto¹,²

¹Faculty of Food and Nutrition, Kyushu Nutrition and Welfare University, 5-1-1 Shimoitozu, Kokura-Kita, Kitakyushu 803-8511, Japan
²Department of Food Function Chemistry, The United Graduate School of Agricultural Sciences, Kagoshima University, 1-21-24 Korimoto, Kagoshima 890-0065, Japan
³Joint Faculty of Veterinary Medicine, Kagoshima University, 1-21-24 Korimoto, Kagoshima 890-0065, Japan
⁴R&D Division, Kewpie Corporation, 2-5-7 Sengawa, Chofu, Tokyo 182-0002, Japan

Amyloid fibrils, which are formed from aggregates of aberrant proteins, can cause various forms of amyloidosis (including Alzheimer’s disease). Such disorders often occur in elderly populations and are suspected to be lifestyle related. Thus, it has been speculated that some foodstuffs could be beneficial for preventing amyloidosis. In this study, we determine whether fibril formation by the hen egg white lysozyme (HEWL) could be inhibited by conducting a thioflavin T assay followed by fluorescence and electron microscopy observations. The results demonstrated that four peptide specimens prepared by the hydrolysis of crude proteins from the egg white, egg yolk, chalazae, and eggshell membrane of hen eggs effectively inhibited HEWL fibril formation. Among the four specimens, peptides from chalazae exhibited the highest preventive ability. The superiority of chalaza peptides was also observed when fibril formation was assayed using a full-length human lysozyme and human amyloid β peptide 1-42, which is the key factor for the development of Alzheimer’s disease. Our study of the fibrillization of the human lysozyme also showed that metal ions (Zn²⁺, Ca²⁺, Co²⁺, Mn²⁺ and Al³⁺) promoted fibrillization, and their effects were abolished by the peptide specimens (especially by chalaza peptides). Thus, we conclude that chicken-egg proteins could be a convenient source of therapeutic materials for amyloidosis.

Key words: amyloid fibril, amyloid β1-42, chalaza hydrolysates, HEWL, human lysozyme

J. Poult. Sci., 59: 384–391, 2022

Introduction

Amyloids are disordered proteins that are deposited inside and outside cells as fibrillar aggregates with abnormally insoluble forms that impair the physiological functions of tissues and organs and cause different types of amyloidosis (Knowles et al., 2014; Chiti and Dobson, 2017). These amyloidosis types include Alzheimer’s disease, type 2 diabetes, and spongiform encephalopathy, some of which are major public health problems (Levy, 2007; Chatterjee and Mudher, 2018; Kupfer et al., 2009). The amyloid fibrils involved in these disorders are resistant to degradation and are accompanied by a characteristic β-cross structure (Nelson et al., 2005; Fitzpatrick et al., 2013). Proteins that form amyloid fibrils do not have a common amino acid sequence or tertiary structure (Yoon and Welsh, 2004), which has challenged our understanding of the relationship between amyloid fibrils and diseases.

One way to avoid the development of amyloidosis is to establish a system that inhibits the formation of amyloid fibrils (Ow and Dunstan, 2014). The causes of the onset and progression of amyloidosis may include genetic and physicochemical factors, metabolic conditions, lifestyle factors, and aging (Gomez et al., 2018). Foodstuffs are thus suspected to be beneficial in preventing these diseases. Polyphenols (e.g., catechin and myricetin) with the ability to remove active oxygen have been reported to play a role in the suppression of protein aggregation (Choi et al., 2014; Stefanescu et al., 2020), and food materials that may prevent amyloidosis are
Peptides are expected to be applied in disease therapeutics; numerous benefits of specific peptides have been determined and applied for their ability to lower blood pressure and exert antibacterial and opioid effects. We focused on peptides prepared by the hydrolysis of the crude proteins of chicken eggs as candidate fibrillation inhibitors because there are abundant high-quality nutritional proteins in egg whites, egg yolks, chalazae, and eggshell membranes. In this study, we used an in vivo thioflavin T (ThT) fluorescence assay, fluorescence microscopy, and transmission electron microscopy (TEM) to determine whether these peptides affect fibril formation by the hen egg white lysozyme (HEWL), which is used as a fibrogenic protein/peptide.

The lysozyme is a protein with muramidase and antibacterial activity (Skujiš et al., 1973), and it has been widely studied in terms of its protein structure and function. The lysozyme has also been used to analyze protein fibrillation (Yonezawa et al., 2002; Merlini and Bellotti, 2005). It is known that the lysozyme has a core region for fibril formation and, in humans, lysozyme mutations (some of which cause systemic and intractable amyloidosis) converge in or around an amino acid sequence that appears to be the core region (Pepys et al, 1993). The management and control of this region are presumed to be important for the prevention of fibril formation. Therefore, in addition to HEWL, we used the following fibrillation items: a full-length human lysozyme (h-Lz) and its amyloid β peptide 1-42 (Aβ 1-42); the formation of the latter is a critical step for the advancement of Alzheimer’s disease (Butterfield et al., 2013; Chen et al., 2017).

The results demonstrated that all the protein hydrolysates from the four above-described chicken egg regions/tissues had inhibitory effects on the fibrogenesis of each of the proteins/peptides tested. The hydrolysate specimen from chalazae (hereafter referred to as C-peptides) exhibited the highest ability. Based on these findings, we speculate that hen egg proteins could become possible sources of foodstuffs that prevent amyloid diseases.

Materials and Methods

Materials

HEWL, h-Lz, and Aβ 1-42 were purchased from FUJIFILM Wako Pure Chemical Co. (Osaka, Japan). All other reagents were of biochemical grade.

Preparation of Hydrolysates of Proteins from Egg Whites, Egg Yolks, Chalazae, and Eggshell Membranes

Enzymatic hydrolysates of crude proteins from the egg white, egg yolk, chalazae, and eggshell membrane (EW, EY, C, and ESM peptides, respectively) were prepared by the R&D Division of Kewpie Co. (Tokyo, Japan). The enzymes used to obtain the EW, EY, and C-peptides were Bacillus subtilis protease and papain (both are products of biocatalysts, Cardiff, Wales, UK). ESM peptides were obtained by hydrolysis using Bacillus proteinase (Nagase ChemetX, Osaka, Japan).

The mixture containing the substrate at a concentration of 8% (w/v) and the enzyme with a weight ratio to the substrate of 0.1% was heated at 50°C for 24 h, during which the mixture was maintained at an alkaline pH by sodium carbonate (pH 7.0 for egg yolk). The mixture was then adjusted to pH 7.0 with citric acid and heated at 85°C for 30 min to inactivate the enzyme. Before hydrolysis, the egg whites were diluted with water, the egg yolk was defatted, and the chalazae were thoroughly washed with water by filtration, during which the sialogic acid content was reduced to ~20% of the original level reported by Juneja et al. (1991).

Incubation Conditions for the In Vitro Fibrillation Assay
To investigate lysozyme fibrillation in vitro, HEWL and h-Lz were each dissolved at a concentration of 3 mg/mL in 50 mM glycine-HCl buffer (pH 2.0) and incubated at 35°C for 14 days, as described (Tokunaga et al., 2013). For the fibrillation of Aβ 1-42, each solution was prepared by dissolving in 100% dimethyl sulfoxide, diluted to 2 mg/mL with 50 mM potassium phosphate, pH 7.0 buffer and incubated at 37°C for 48 h.

To observe the effects of the egg-protein hydrolysates on fibrillation, the EW, EY, C-, and ESP peptides were mixed in the above-described incubation system at the concentrations indicated in the figure legends. Quercetin (1 mM) was used as a positive reference for its ability to suppress fibril formation in some experiments.

We also tested whether Zn, Ca, Co, Mn, Al, and Fe ions promoted or inhibited fibril formation using ZnCl2, CaCl2, CoCl2, MnCl2, AlCl3, and FeCl3. Here, only h-Lz was applied as a fibrosis agent, and each metal salt solution was added at a final concentration of 1 mM to the above incubation system, which was incubated at 55°C and pH 2.0 for 10 days.

Measurements of ThT Fluorescence Emission and Observations by Fluorescence Emission Microscopy and TEM

The intensity of ThT fluorescence emission was measured by withdrawing a 200-µL aliquot of the incubated mixture, adding it to 1 mM ThT, and maintaining it at a predetermined temperature in a black 96-microwell plate with a seal to prevent evaporation. The ThT fluorescence intensities were measured using a Corona MTP900Lab multi-microplate reader (Hitachi, Tokyo, Japan). The excitation wavelength was set to 440 nm.

The occurrence and features of the aggregates and/or fibrils were examined using optical observations. Fluorescence emission microscopy was conducted using a 10-µL aliquot of the above-described solution with 1 mM ThT and a Nikon fluorescence microscope. For TEM, a 2 µL aliquot of the incubated mixture (without ThT) was placed on a 200-mesh copper grid coated with formvar/carbon film (Sigma-Aldrich, St. Louis, MO, USA). After negative staining with 2% (w/v) phosphotungstic acid in distilled water for 2 min, the copper grids were dried at 20°C. The grids were viewed using a transmission electron microscope (model 7600, Hitachi, Tokyo, Japan) at 80 kV.

Statistical Analysis

All experiments were repeated five times, unless otherwise indicated. Statistical analyses were performed using IBM SPSS Statistics software. The data were analyzed using...
Tukey’s multiple comparison tests and are presented as the mean±standard error (SE). The statistical significance was set at $P<0.05$ or $P<0.01$ for comparisons.

**Results**

**Effects of Egg-protein Hydrolysates on HEWL Fibrosis and h-Lz Fibrosis**

The formation of fibrils was first tested by the ThT fluorescence assay using the two lysozymes, HEWL and h-Lz as fibrillization proteins (Figs. 1A and 2A, respectively). Both proteins showed a marked increase in the fluorescence intensity from day 7 of incubation, and the value almost peaked on day 14. The effects of all the four egg-protein hydrolysates on the ThT fluorescent emission by HEWL and h-Lz were then examined, and the results are presented in Figures 1A and 2A. The C-peptides exhibited the strongest suppression, the EY peptides were the second most active; however, the suppression by the EY peptides was weak. The ESM peptides showed fairly strong emission inhibition by HEWL but weak emission inhibition by h-Lz.

Thus, the most marked suppressors were the C-peptides, whose inhibitory effects were comparable to those of quercetin, which was used as a positive control. These chalaza-derived peptides showed suppression of ThT fluorescence by h-Lz in a clear-cut HEWL-dependent and concentration-dependent manner, as illustrated in Figures 1B and 2B, respectively. The C-peptide preparations were performed three times independently; each was fractionated by reverse-phase column HPLC, and the chromatographic patterns were compared. All prepared specimens showed suppressed amyloid fibril formation in HEWL (Supplemental Fig. 1A). The reverse-phase HPLC patterns of these three preparations were very similar, each of which is likely to contain an effective peptide (Supplemental Fig. 1B). Therefore, this preparation

**Fig. 1.** Repression effects of egg-protein hydrolysates on HEWL fibrosis. **A**: The ThT assay for the emission of fluorescence by HEWL conducted by incubation at pH 2.0 and 55°C for up to 14 days with the egg-protein hydrolysates (EW peptides etc., each at a concentration of 2.5 mg/ml in the incubation mixture). Other details were as described in the text. Quercetin was used as a positive reference. **B**: The effects of various concentrations (0–5 mg/ml) of C-peptides in the ThT assay for the fluorescence emission by HEWL for 10 days under the above conditions. In panels A and B, each value (a.i. = arbitrary intensity) is the average of five independent experiments; mean±SE ($n=5$), and the control assay was done without peptides. * $P<0.05$ and ** $P<0.01$ versus the control. **C**: Features of the products observed by ThT fluorescence emission microscopy (ThT) and TEM after 14 days of incubation. In panels A and C, the control was used without egg-protein hydrolysates.
method was highly effective.

After the ThT assay, we determined whether protein aggregates/fibrils actually occurred using ThT fluorescence microscopy and TEM (ThT and TEM columns, respectively, of Figs. 1C and 2C; the features on day 10 of incubation are shown). Both HEWL and h-Lz clearly produced aggregates and fibers (top squares in the figures); however, almost no such products were observed when they co-existed with C-peptides (the 4th squares). Neither typical decomposition products nor definite fibers were observed in the specimens with EW peptide (3rd squares); however, some aggregates and/or small amounts of fiber-like substances were observed in the specimens with EY peptides and ESM peptides (2nd and 5th squares, respectively).

Influence of Metal Ions on ThT Fluorescence Emission by h-Lz and Interaction by Egg-protein Hydrolysates

Because there are many reports that metal ions such as Zn$^{2+}$ modulate the formation of amyloid fibrils, we evaluated the influence of six metal ions on the emission of ThT fluorescence by h-Lz, first in the absence of egg protein hydrolysates. Each of the metal ions, except Fe$^{2+}$, accelerated the fluorescence intensity (Fig. 3A). Particularly, Zn$^{2+}$, Mn$^{2+}$, and Ca$^{2+}$ exerted approx. threefold augmentation. In contrast, Fe$^{2+}$ decreased the fluorescence intensity.

We next assayed ThT fluorescence emission by h-Lz in the presence of Zn$^{2+}$, Ca$^{2+}$, Co$^{2+}$, Mn$^{2+}$ or Al$^{3+}$, wherein all four egg protein hydrolysates were separately added to each assay mixture. The results are depicted in Fig. 3B–F and clearly indicate that C-peptides, the chalaza-derived specimen, ex-
tensively prevented the effects of metal ions in the ThT assay, whereas the other three egg-protein hydrolysates did so only moderately. The promotion effects of a metal (Zn$^{2+}$ or Mn$^{2+}$) on the ThT fluorescence emission of h-Lz were suppressed in a C-peptide-concentration-dependent manner (data not shown). Our preliminary TEM observations revealed that C-peptides suppressed the occurrence of fibrous morphology by h-Lz, even under metal-promoted conditions, and the other egg-protein hydrolysates hardly exhibit this phenomenon.

**Effects of Egg-protein Hydrolysates on Aβ1-42 Fibrosis**

We evaluated whether egg-protein hydrolysates exert preventive effects on the fibril formation of Aβ 1-42. In the ThT fluorescence assay, C-peptides exhibited the most extensive inhibition, the EY peptides were moderately inhibitory, and the other two specimens had a slight or no effect (Fig. 4A). Such differing effects among the egg protein hydrolysates were substantially reproducible in morphological observations by fluorescence microscopy and TEM (Fig. 4B).

**Discussion**

Deterioration due to the misfolding of proteins manifests as the accumulation of amyloid fibrils, which leads to serious illnesses including Alzheimer’s disease. As mentioned in the Introduction, eating habits are considered a potential preventive factor for amyloid-related diseases. Here, we assessed the factors in the egg protein hydrolysates and found that the peptide preparations from the egg white, yolk, chalazae, and eggshell membranes (EW peptides, EY peptides, C peptides,
and ESM peptides, respectively) were all active as inhibitors against the fibril formation of the proteins/peptides applied (HEWL, h-L and Aβ1-42), in both the ThT fluorescence assay and microscopic observations. The chalaza-derived C-peptides showed the strongest effect among the four egg-protein-hydrolysate specimens.

The results of this study demonstrate that divalent and trivalent metal ions (Zn$^{2+}$, Ca$^{2+}$, Co$^{2+}$, Mn$^{2+}$ and Al$^{3+}$) promote h-Lz fibrillization. Generally, these results are compatible with previous findings that certain metal ions, including Zn$^{2+}$, stimulate protein fibrosis (Bolognin et al., 2011; Huang et al., 2004). However, metal ions have been reported to retard the fibrillization process (Abelein et al., 2015; Innocenti et al., 2010), as observed in the present study for Fe$^{2+}$. These complex circumstances may indicate that different metal ions modulate protein processing differently, as Bolognin et al. (2011) pointed out. Metals may crosslink defective proteins and modulate their associations. It is interesting that our experiments demonstrated the C-peptides’ extensive suppression of the metal ions’ promotion of fibril formation, probably via the attenuation of the metal ions’ cross-linking effects. The other peptide specimens scarcely exhibited suppression, similar to that exerted by the C-peptides, suggesting the superiority of the chalazae-derived preparation.

The amyloidogenesis of proteins occurs through numerous steps, including degradation, association, aggregation, protofilament formation, and final fibrillization. The stage at which egg protein hydrolysates respond is yet to be elucidated. Details on the effective factors are also unknown; when C-peptides were fractionated by high-performance liquid chromatography (HPLC) reverse-phase column chromatography, anti-fibrogenic signals were observed in multiple peaks (Supplemental Fig. 1). Challazae in chicken eggs are a pair of bands suspended in the yolk at the center of the egg white, and they have abundant proteins resembling those of the egg white. The challazae also possess sialic acid, which is also present in the other egg fractions in smaller w/w proportions (Juneja et al., 1991; Nakano et al., 1994). Because the challazae that we used in the present study were desialized before hydrolysis, as described in the Materials and Methods section, the influence of sialic acid on the present results appears to be small, although the details are yet to be clarified.

Interestingly, it has been reported that protease A-digested crude chicken challaza hydrolysates exert anti-fibrogenic effects in the rat liver (Lin et al., 2021) and antioxidant effects in vitro (Chang et al., 2020). Yang et al. (2014) reported that chicken challazae are rich in antioxidant dipeptides (carnosine/ anserine), free amino acids (leucine, arginine, phenylalanine, valine, and lysine), free radical scavenging, and metal ion-chelating activities. These may be involved, at least partly, in the reaction to block the fibrosis of amyloid β peptide, the occurrence of which is a key step in AD. Thus, it is necessary to separate and identify the peptides, amino acids, and/or other constituents that are effective in suppressing fibrosis.
Egg proteins contain HEWL, and it is of interest that the human lysozyme itself was reported to inhibit the aggregation of amyloid \( \beta \) peptides (Luo et al., 2013). The mechanisms of agglutination/fibrillization inhibition are essential for future research.

In summary, we conclude that chicken-egg protein hydrolysates (particularly those from the chalazae) are a treasure house of therapeutic factors for the prevention of amyloid-related degenerative diseases. Therefore, it is necessary to further test their effects in animal and human intervention experiments.

Data Availability

The study data are available upon reasonable request from the corresponding author (YS).

Author Contributions

Yukiko Muroi, Koji Wada, and Yasushi Sugimoto designed this study. Yukiko Muroi, Izumi Aburaya, Takuro Shima, Mitsuharu Matsumoto, Ryo Sasahara, Takahisa Suzuki, Keiichi Watanabe, and Yasushi Sugimoto performed the experiments and analyzed the data. Yukiko Muroi and Yasushi Sugimoto wrote this manuscript. All the authors have read and approved the final manuscript.

Acknowledgments

This work was supported in part by a grant from the Japan Society for the Promotion of Science (JSPS) KAKENHI (no. 19K06376 (to Yasushi Sugimoto)).

Conflicts of Interest

The authors declare no conflicts of interest.

References

Abelein A, Gräsland A and Danielsson J. Zinc as chaperone-mimicking agent for retardation of amyloid \( \beta \) peptide fibril formation. Proceedings of the National Academy of Sciences of the United States of America, 112: 5407–5412. 2015.

Bolognin S, Messori L, Drago D, Gabbiani C, Cendron L and Zatta P. Aluminum, copper, iron and zinc differentially alter amyloid-\( A_\beta \) (1–42) aggregation and toxicity. International Journal of Biochemistry & Cell Biology, 43: 877–885. 2011.
Butterfield DA, Swomley AM and Sultana R. Amyloid β-peptide (1-42)-induced oxidative stress in Alzheimer disease: Importance in disease pathogenesis and progression. Antioxidants & Redox Signaling, 19: 823–835. 2013.

Chang CJ, Tseng JK, Wang SY, Lin YL, Samuel Wu YH, Chen JW and Chen YC. Ameliorative functions of functional chalaza hydrolysates prepared from protease-A digestion on cognitive dysfunction and brain oxidative damages. Poultry Science, 99: 2819–2832. 2020.

Chatterjee S and Mudher A. Alzheimer’s disease and type 2 diabetes: A critical assessment of the shared pathological traits. Frontiers in Neuroscience, 12: 383. doi: 10.3389/fnins.2018.00383. 2018.

Chen GF, Xu TH, Yan Y, Zhou YR, Jiang Y, Melcher K and Xu HE. Amyloid beta: Structure, biology and structure-based therapeutic development. Acta Pharmacologica Sinica, 38: 1205–1235. 2017.

Chiti F and Dobson CM. Protein misfolding, amyloid formation, and human disease: A summary of progress over the last decade. Annual Review of Biochemistry, 86: 27–68. 2017.

Choi SM, Kim BC, Choi YH, Choi KH, Chang J, Park MS, Kim MK, Cho KH and Kim JK. Effects of flavonoid compounds on β-amyloid-peptide-induced neuronal death in cultured mouse cortical neurons. Chonnam Medical Journal, 50: 45–51. 2014.

Fitzpatrick AWP, Debelouchina GT, Bayro MJ, Clare DK, Caporini MA, Bajaj VS, Jaroniec CP, Wang L, Ladizhansky V, Müller SA, MacPhee CE, Waubdy CA, Mott HR, De Simone A, Knowles TPJ, Saibil HR, Vendruscolo M, Orlova EV, Griffin RG and Dobson CM. Atomic structure and hierarchical assembly of a cross-β amyloid fibril. Proceedings of the National Academy of Sciences of the United States of America, 110: 5468–5473. 2013.

Gomez G, Beason-Held LL, Bilgel M, An Y, Wong DF, Studenski S, Ferrucci L and Resnick SM. Metabolic syndrome and amyloid accumulation in the aging brain. Journal of Alzheimer’s Disease, 65: 629–639. 2018.

Huang X, Atwood CS, Moir RD, Hartshorn MA, Tanzi RE and Bush AI. Trace metal contamination initiates the apparent auto-aggregation, amyloidosis, and oligomerization of Alzheimer’s Aβ peptides. Journal of Biological Inorganic Chemistry, 9: 954–960. 2004.

Innocenti M, Salvietti E, Guidotti M, Casini A, Bellandi S, Foresti ML, Gabbanini C, Pozzi A, Zatta P and Messori L. Trace copper (II) or zinc (II) ions drastically modify the aggregation behavior of amyloid-β 1-42: An AFM study. Journal of Alzheimer’s Disease, 19: 1323–1329. 2010.

Juneja LR, Koketsu M, Nishimoto K, Kim M, Yamamoto T and Itoh T. Large-scale preparation of sialic acid from chalaza and eggyolk membrane. Carbohydrate Research, 214: 179–186. 1991.

Knowles TPJ, Vendruscolo M and Dobson CM. The amyloid state and its association with protein misfolding diseases. Nature Reviews Molecular Cell Biology, 15: 384–396. 2014.

Kupfer L, Hinrichs W and Groschup MH. Prion protein misfolding. Current Molecular Medicine, 9: 826–835. 2009.

Levy DE. Type 2 diabetes and Alzheimer’s disease: From common pathologies to potential new therapeutics. Journal of Diabetes Science and Technology, 1: 590–594. 2007.

Lin YL, Lu CF, Wu YHS, Yang KT, Yang WY, Chen JW, Tseng JK and Chen YC. Protective effects of crude chalaza hydrolysates against liver fibrogenesis via antioxidation, anti-inflammation/anti-fibrogenesis, and apoptosis promotion of damaged hepatocytes. Poultry Science, 100: 101175. 2021.

Luo J, Wärmånlander SKTS, Gräslund A and Abrahams JP. Human lysozyme inhibits the in vitro aggregation of Aβ peptides, which in vivo are associated with Alzheimer’s disease. Chemical Communications, 49: 6507–6509. 2013.

Merlini G and Bellotti V. Lysozyme: A paradigmatic molecule for the investigation of protein structure, function and misfolding. Clinica Chimica Acta, 357: 168–172. 2005.

Nakano K, Nakano T, Ahn DU and Sim JS. Sialic acid contents in chicken eggs and tissues. Canadian Journal of Animal Science, 74: 601–606. 1994.

Nelson R, Sawaya MR, Balbirnie M, Madsen AO, Riekel C, Grothe R and Eisenberg D. Structure of the cross-β spine of amyloid-like fibrils. Nature, 435: 773–778. 2005.

Ow SY and Dunstan DE. A brief overview of amyloids and Alzheimer’s disease. Protein Science, 23: 1315–1331. 2014.

Pepys MB, Hawkins PN, Booth DR, Vigushin DM, Tennent GA, Soutar AK, Totty N, Nguyen Q, Blake CCF, Terry CJ, Feest TG, Zalin AM and Hsuan JJ. Human lysozyme gene mutations cause hereditary systemic amyloidosis. Nature, 362: 553–557. 1993.

Skujins J, Pukite A and McLaren AD. Adsorption and reactions of chitinase and lysozyme on chitin. Molecular and Cellular Biochemistry, 2: 221–228. 1973.

Stefanescu R, Stanciu GD, Luca A, Padurarul I and Tamba BI. Secondary metabolites from plants possessing inhibitory properties against beta-amyloid aggregation as revealed by thioflavin-T assay and correlations with investigations on transgenic mouse models of Alzheimer’s disease. Biomolecules, 10: 870. doi: 10.3390/biom10060870. 2020.

Tokiunaga Y, Sakakibara Y, Kamada Y, Watanabe K and Sugimoto Y. Analysis of core region from egg white lysozyme forming amyloid fibrils. International Journal of Biological Sciences, 9: 219–227. 2013.

Yang KT, Lin C, Liu CW and Chen YC. Effects of chicken-liver hydrolysates on lipid metabolism in a high-fat diet. Food Chemistry, 160: 148–156. 2014.

Yonezawa Y, Tanaka S, Kubota T, Wabayashi K, Yutani K and Fujiwara S. An insight into the pathway of the amyloid fibril formation of hen egg white lysozyme obtained from a small-angle X-ray and neutron scattering study. Journal of Molecular Biology, 323: 237–251. 2002.

Yoon S and Welsh WJ. Detecting hidden sequence propensity for amyloid fibril formation. Protein Science, 13: 2149–2160. 2004.