

Trypsin Induced Degradation of Amyloids

Subjects: Biochemistry & Molecular Biology

Contributor: Anna Sulatskaya

Proteolytic enzymes are known to be involved in the formation and degradation of various monomeric proteins, but the effect of proteases on the ordered protein aggregates, amyloid fibrils, which are considered to be extremely stable, remains poorly understood. In this work we study resistance to proteolytic degradation of lysozyme amyloid fibrils with two different types of morphology and beta-2-microglobulin amyloids.

Keywords: amyloid fibrils ; proteolytic degradation ; trypsin ; stability ; fragmentation ; cytotoxicity ; florescent probes ; thioflavin T (ThT) ; 1-anilinonaphthalene-8-sulphonate (ANS)

1. Introduction

The development of a number of serious diseases, so-called conformational diseases, which include Alzheimer's, Parkinson's, prion diseases, etc.^{[1][2][3][4][5][6][7]} is associated with the accumulation of amyloid fibrils in various organs and tissues of the human body. The appearance of an amyloidogenic protein in the body may be due to the proteolytic cleavage of its precursor, which itself does not tend to aggregate to form amyloid fibrils^{[8][9][10][11]}. For example, β -secretase generates several short peptides from the amyloid precursor protein (APP). The formation of amyloid fibrils from one of these peptides (42 amino acid residues long) is associated with Alzheimer's disease^[8]. Thus, the processing of precursor proteins with the participation of proteolytic enzymes leads to the formation of native proteins and peptides^[12], including those with amyloidogenic properties.

In addition, proteases can modify native proteins and induce, accelerate, slow down, or even inhibit their aggregation^{[13][14][15]}. In particular, it was shown that the rate of whey protein concentrate (WPC) aggregation increases significantly upon treatment with trypsin and noticeably decreases upon treatment with protease A, pepsin, and protease M^[13]. This indicates the possible involvement of proteolytic enzymes in the fibrillogenesis of various proteins. Recent studies also indicate that proteases, in particular trypsin, NET-associated elastase, proteinase K, and HTRA1, can lead to degradation of mature amyloid fibrils, including those accumulating in amyloid plaques in vivo^{[16][17][18][19][20][21]}. This is probably due to the main function of proteolytic enzymes, which consists of the degradation of misfolded proteins^{[22][23][24][25][26][27]}. Assessment of fibril resistance to proteolysis is currently used as an effective approach to the analysis of external factors influence on the structure and stability of amyloids^[18]. However, despite the widespread use for the study of amyloids, the mechanisms of fibril degradation under the influence of proteases and changes in the structure and cytotoxicity of amyloids, induced by the treatment with these enzymes, remain poorly understood. The study of these issues was the aim of this work.

2. Results and discussion

In this work, we investigated the process of degradation of amyloid fibrils formed from two amyloidogenic proteins with significantly different primary, secondary, and tertiary native structure after the action of the trypsin serine protease, which is widely used to estimate the resistance of fibrils to proteolysis. Trypsin is one of the main digestive enzymes and effectively catalyzes the hydrolytic cleavage of proteins and peptides at the carboxyl side of L-lysine or L-arginine residues (Figure 1). Lysozyme and beta-2-microglobulin were chosen as amyloidogenic proteins in this study. Accumulation of amyloid fibrils of these proteins leads to the development of hereditary lysozyme systemic amyloidosis (ALys amyloidosis^[28]) and dialysis-related amyloidosis (DRA^[29]), respectively. Fibrillogenesis of lysozyme was induced using two alternative methods, which made it possible to obtain protein aggregates with a polymorphic structure^[30].

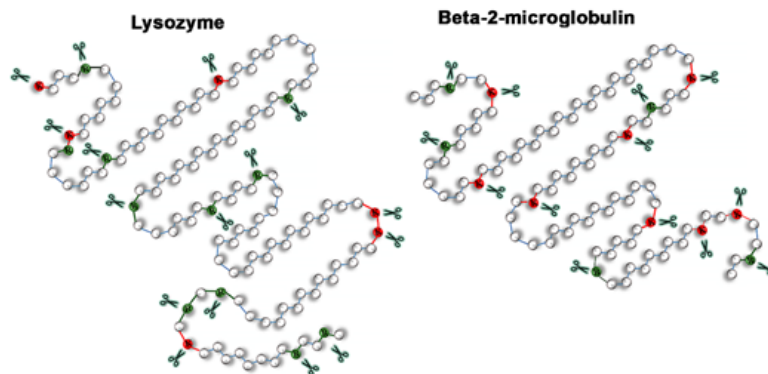


Figure 1. Trypsin cleavage sites in the lysozyme and beta-2-microglobulin amino acid sequences. Lysine (K) and Arginine (R) amino acid residues in the primary sequence of the proteins are highlighted in red and green color, respectively.

We revealed changes in the structure and stability of amyloid fibrils after the action of trypsin using a number of spectroscopic approaches, including fluorescence of the amyloid-specific dye thioflavin T (ThT) and the hydrophobic probe 1-anilidonaphthalene-8-sulfonic acid (ANS), as well as transmission electron microscopy (TEM) for amyloid fibrils imaging (at the [Figure 2](#) presented for lysozyme amyloids). It was demonstrated that trypsin can lead to degradation of amyloid fibrils, which results in fragmentation of their fiber and a decrease in the ordering of amyloid-forming proteins with the formation of non-fibrillar aggregates of various sizes. In this case, trypsin facilitates the degradation of fibrils across the entire length of their fiber rather than starting from the fibril ends, suggesting that proteolysis is not directional. These ideas are in good agreement with the results of the work Chander et al.^[19], where through the electron microscopic analysis of immunogold labeling of trypsin binding to A β 40 and A β 42, the protease binding to fibrils along the entire length of their fiber and not only to the ends of the fibrils was shown. It should be noted that the mechanism of proteolytic degradation of amyloid fibrils observed by us is similar to that previously proposed by Poepsel et al.^[17] in the study of fragmentation of the tau protein fibrils under the action of serine protease HTRA1 (belongs to the high temperature requirement A (HtrA) family of serine proteases), implying the universality of protease action. According to the observed degradation mechanism, trypsin can affect both the structure of protein molecules forming β -sheets and intermolecular hydrogen bonds involved in the formation of amyloid fibers.

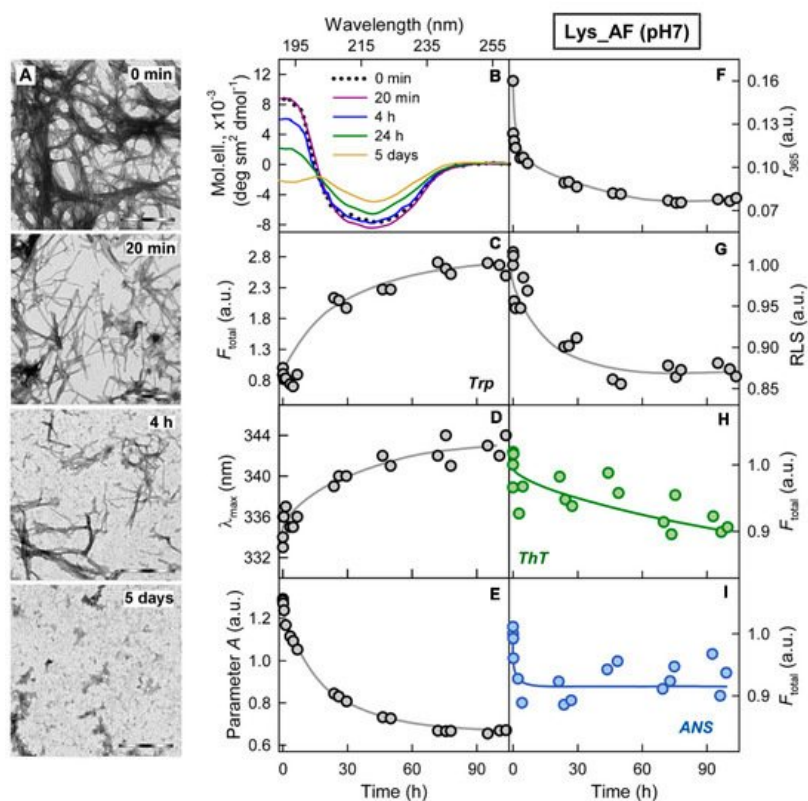


Figure 2. Trypsin-induced degradation of amyloid fibrils formed from lysozyme at pH 7. **(A)** The electron micrographs of amyloid fibrils in the absence and at different time points after the addition of trypsin to the samples. Scale bars are equal to 1 μ m. **(B)** Changes in far UV CD spectra of amyloid samples. **(C)** Changes in total fluorescence intensity of amyloid fibrils at the excitation wavelength of 295 nm. **(D)** Changes in wavelength of the fluorescence spectrum maximum of

amyloid fibrils (λ_{\max}). (E) Changes in parameter A of amyloid fibrils, $\lambda_{\text{ex}} = 295$ nm. (F) Changes in fluorescence anisotropy of amyloid fibrils, $\lambda_{\text{ex}} = 295$ nm, and $\lambda_{\text{em}} = 365$ nm. (G) Changes in Rayleigh Light Scattering of amyloid fibrils. (H) Changes in the total fluorescence intensity of ThT and (I) ANS in the presence of amyloid fibrils. The values at the panels (C,H,I) are normalized to the values for fibrils in the absence of the protease. The experiments were performed under physiological conditions (in 20 mM sodium phosphate buffer solution, pH 7.4) and weight ratio of fibrils to trypsin was kept at 125:1. The standard error of the mean is determined for a confidence interval of 0.95 and does not exceed 10%.

Our data for two amyloidogenic proteins (and three polymorphic structures (Figure 3 A-F)) show the reliance of the effectiveness and rate of trypsin action on amyloid fibrils on the structure of the amyloid-forming protein (primary, secondary, and tertiary), which determines the number and localization of proteolytic cleavage sites, as well as on the morphology and degree of clustering of amyloid fibrils, which determine the availability of trypsin binding sites. This is in line with the literature data^[17], indicating that the disintegration activity of HTRA1 significantly increases the rate of proteolytic degradation of densely packed areas of fibrils, which leads to more efficient fragmentation of amyloid fibers.

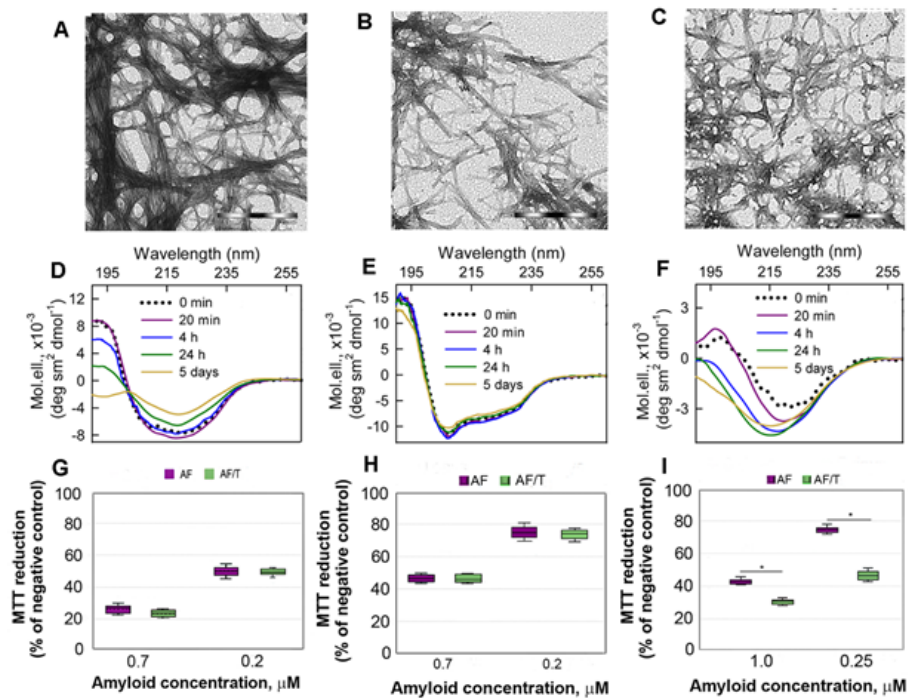


Figure 3. Cytotoxicity of amyloids formed from different proteins. (A-C) The electron micrographs, (D-F) CD spectra and (G-I) dose-dependent inhibition effect on HeLa cells (determined by MTT assay) of amyloid fibrils formed from lysozyme at pH7 (A, D, G) and pH2 (B, E, H) and from beta-2-microglobulin (C, F, I). Scale bars at the electron micrographs are equal to 1 μm . At the Panels (G-I) data are expressed as medians and interquartile ranges, whiskers denote $1.5 \times \text{IQR}$. ANOVA, Tukey post hoc test, * $p < 0.01$.

Binding of proteases to amyloids and proteolytic degradation of fibrils, at first glance, seems to be a positive effect in terms of amyloidosis treatment; however, this is actually not entirely true. The interaction of trypsin with abnormal aggregates amyloid fibrils, which was shown in our work, as well as in the work^[19], can lead to the inability of the enzyme interaction with other substrates. For example, trypsin is required for $\text{A}\beta$ -peptide catabolism, and its interaction with $\text{A}\beta$ -peptide amyloids can reduce clearance of soluble $\text{A}\beta$ -peptide, and an increased concentration of $\text{A}\beta$ -peptide enhances its aggregation. A similar conclusion was reached in the study of the trypsin binding to fibrillar $\text{A}\beta$ -peptide^[19]. This assumption is also in a good agreement with the results according to which mice deficient of proteolytic enzymes exhibited elevated levels of $\text{A}\beta$ while overexpression of neprilysin in APP-transgenic mice could reduce the deposition of fibrillar $\text{A}\beta$ ^{[31][32]}.

In addition, we showed that proteolytic degradation of amyloids did not reduce cytotoxicity of the protein aggregates for HeLa cells, as might be expected (at the Figure 3 G-I). We attribute this to the formation of highly toxic non-fibrillar aggregates along with the formation of non-toxic protein monomers and their fragments induced by trypsin. A high cytotoxicity of large disordered aggregates forming after exposure of the lysozyme and beta-2-microglobulin fibrils to the protein with chaperone activity alpha-B-crystallin was already demonstrated by us earlier^[33].

In conclusion, we show that the proteolytic enzyme of the pancreas, trypsin, can induce degradation of amyloid fibrils, and the mechanism of this process is qualitatively the same for amyloids with different structure and morphology (Figure 4). At the same time, the efficiency and rate of fibril degradation are dependent on the structure of the amyloid-forming protein as well as on the morphology and clustering of amyloid fibrils, which determines the number, localization, and availability of protease cleavage sites. Proteolytic degradation of amyloids not only does not reduce, as might be expected, but, in some cases, even increases cytotoxicity of the amyloid fibrils, presumably due to the formation of more toxic non-fibrillar aggregates.

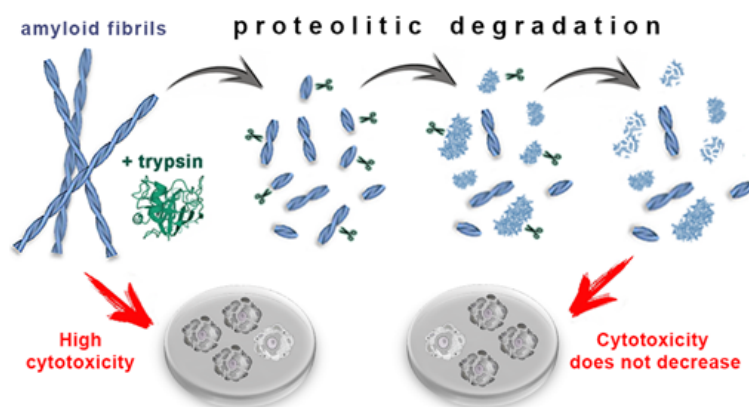


Figure 4. Mechanism of trypsin induced degradation of amyloids formed from different proteins: 1) first, amyloid clots are declustering, and amyloid fibers are fragmented; 2) then, the structure of shortened fibril fragments “decompacts” and becomes less ordered, 3) after which these aggregates degrade to monomers, which, in turn, degrade into short fragments. The cytotoxicity of amyloids treated with trypsin not only does not decrease, but can even increase (this has been demonstrated for beta-2-microglobulin fibrils).

Funding. This work was supported by the Russian Science Foundation (NO. 18-74-10100).

References

1. Giampaolo Merlini; Vittorio Bellotti; Molecular Mechanisms of Amyloidosis. *New England Journal of Medicine* **2003**, 349, 583-596, [10.1056/nejmra023144](https://doi.org/10.1056/nejmra023144).
2. Alessandra Recchia; Patrizia Debetto; Alessandro Negro; Diego Guidolin; Stephen D. Skaper; Pietro Giusti; α -Synuclein and Parkinson's disease. *The FASEB Journal* **2004**, 18, 617-626, [10.1096/fj.03-0338rev](https://doi.org/10.1096/fj.03-0338rev).
3. Jun-Xia Lu; Wei Qiang; Wai-Ming Yau; Charles D. Schwieters; Stephen C. Meredith; Robert Tycko; Molecular Structure of β -Amyloid Fibrils in Alzheimer's Disease Brain Tissue. *Cell* **2013**, 154, 1257-1268, [10.1016/j.cell.2013.08.035](https://doi.org/10.1016/j.cell.2013.08.035).
4. Naiki, H.; Okoshi, T.; Ozawa, D.; Yamaguchi, I.; Hasegawa, K.; Molecular pathogenesis of human amyloidosis: Lessons from beta2 -microglobulin-related amyloidosis. *Pathol. Int.* **2016**, 66, 193, [10.1111/pin.12394](https://doi.org/10.1111/pin.12394).
5. Jean D. Sipe; Merrill D. Benson; Joel N. Buxbaum; Shu-Ichi Ikeda; Giampaolo Merlini; Maria J. M. Saraiva; Per Westermark; Amyloid fibril proteins and amyloidosis: chemical identification and clinical classification International Society of Amyloidosis 2016 Nomenclature Guidelines. *Amyloid* **2016**, 23, 209-213, [10.1080/13506129.2016.1257986](https://doi.org/10.1080/13506129.2016.1257986).
6. G. Esposito; R. Michelutti; G. Verdone; P. Viglino; H. Hern Ández; C. V. Robinson; A. Amoresano; F. Dal Piaz; M. Monti; P. Pucci; et al. Removal of the N-terminal hexapeptide from human β 2-microglobulin facilitates protein aggregation and fibril formation. *Protein Science* **2000**, 9, 831-845, [10.1110/ps.9.5.831](https://doi.org/10.1110/ps.9.5.831).
7. P. T. Sattianayagam; S. D. J. Gibbs; D. Rowczenio; J. H. Pinney; A. D. Wechalekar; J. A. Gilbertson; P. N. Hawkins; H. J. Lachmann; J. D. Gillmore; Hereditary lysozyme amyloidosis - phenotypic heterogeneity and the role of solid organ transplantation. *Journal of Internal Medicine* **2011**, 272, 36-44, [10.1111/j.1365-2796.2011.02470.x](https://doi.org/10.1111/j.1365-2796.2011.02470.x).
8. Valérie Wilquet; Bart De Strooper; Amyloid-beta precursor protein processing in neurodegeneration. *Current Opinion in Neurobiology* **2004**, 14, 582-588, [10.1016/j.conb.2004.08.001](https://doi.org/10.1016/j.conb.2004.08.001).
9. Martijn F. B. G. Gebbink; Emile E. Voest; Arie Reijerkerk; Do antiangiogenic protein fragments have amyloid properties?. *Blood* **2004**, 104, 1601-1605, [10.1182/blood-2004-02-0433](https://doi.org/10.1182/blood-2004-02-0433).
10. Han Zhang; Qilin Ma; Yun-Wu Zhang; Huaxi Xu; Proteolytic processing of Alzheimer's β -amyloid precursor protein. *Journal of Neurochemistry* **2011**, 120, 9-21, [10.1111/j.1471-4159.2011.07519.x](https://doi.org/10.1111/j.1471-4159.2011.07519.x).

11. Sebastian Høgl; Peer-Hendrik Kuhn; Alessio Colombo; Stefan F. Lichtenthaler; Determination of the Proteolytic Cleavage Sites of the Amyloid Precursor-Like Protein 2 by the Proteases ADAM10, BACE1 and γ -Secretase. *PLOS ONE* **2011**, 6, e21337, [10.1371/journal.pone.0021337](https://doi.org/10.1371/journal.pone.0021337).
12. 12. Mirgorodskaya, O.A.; Kazanina, G.A.; Mirgorodskaya, E.P.; Shevchenko, A.A.; Mal'tsev, K.V.; Miroshnikov, A.I.; Roipstorff, P.; [Proteolysis of human proinsulin catalysed by native, modified, and immobilized trypsin]. *Bioorganicheskaya Khimiya* **1997**, 23, 91-97, .
13. Yu-Zhe Gao; Hong-Hua Xu; Ting-Ting Ju; Xin-Huai Zhao; The effect of limited proteolysis by different proteases on the formation of whey protein fibrils. *Journal of Dairy Science* **2013**, 96, 7383-7392, [10.3168/jds.2013-6843](https://doi.org/10.3168/jds.2013-6843).
14. P. Patrizia Mangione; Riccardo Porcari; Julian D. Gillmore; Piero Pucci; Maria Monti; Mattia Porcari; Sofia Giorgetti; Loredana Marchese; Sara Raimondi; Louise C. Serpell; et al. Proteolytic cleavage of Ser52Pro variant transthyretin triggers its amyloid fibrillogenesis. *Proceedings of the National Academy of Sciences* **2014**, 111, 1539-1544, [10.1073/pnas.1317488111](https://doi.org/10.1073/pnas.1317488111).
15. Julien Marcoux; P Patrizia Mangione; Riccardo Porcari; Matteo T Degiacomi; Guglielmo Verona; Graham W Taylor; Sofia Giorgetti; Sara Raimondi; Sarah Cianferani; Justin L P Benesch; et al. A novel mechano-enzymatic cleavage mechanism underlies transthyretin amyloidogenesis. *EMBO Molecular Medicine* **2015**, 7, 1337-1349, [10.15252/emmm.201505357](https://doi.org/10.15252/emmm.201505357).
16. Thuzar M Shin; J Mario Isas; Chia-Ling Hsieh; Rakez Kaye; Charles G Glabe; Ralf Langen; Jeannie Chen; Formation of soluble amyloid oligomers and amyloid fibrils by the multifunctional protein vitronectin. *Molecular Neurodegeneration* **2008**, 3, 16-16, [10.1186/1750-1326-3-16](https://doi.org/10.1186/1750-1326-3-16).
17. Simon Poepsel; Andreas Sprengel; Barbara Sacca; Farnusch Kaschani; Markus Kaiser; Christos Gatsogiannis; Stefan Raunser; Tim Clausen; Michael Ehrmann; Determinants of amyloid fibril degradation by the PDZ protease HTRA1. *Nature Chemical Biology* **2015**, 11, 862-869, [10.1038/nchembio.1931](https://doi.org/10.1038/nchembio.1931).
18. Linda Söderberg; Camilla Dahlqvist; Hiroyoshi Kakuyama; Johan Thyberg; Akira Ito; Bengt Winblad; Jan Näslund; Lars O. Tjernberg; Collagenous Alzheimer amyloid plaque component assembles amyloid fibrils into protease resistant aggregates. *The FEBS Journal* **2005**, 272, 2231-2236, [10.1111/j.1742-4658.2005.04647.x](https://doi.org/10.1111/j.1742-4658.2005.04647.x).
19. Harish Chander; Abha Chauhan; Jerzy Wegiel; Mazhar Malik; Ashfaq Sheikh; Ved Chauhan; Binding of trypsin to fibrillar amyloid beta-protein. *Brain Research* **2006**, 1082, 173-181, [10.1016/j.brainres.2006.01.079](https://doi.org/10.1016/j.brainres.2006.01.079).
20. Estefania P. C. Azevedo; Anderson B. Guimarães-Costa; Guilherme S. Torezani; Carolina A. Braga; Fernando Palhano; Jeffery W. Kelly; Elvira M. Saraiva; Debora Foguel; Amyloid Fibrils Trigger the Release of Neutrophil Extracellular Traps (NETs), Causing Fibril Fragmentation by NET-associated Elastase*. *Journal of Biological Chemistry* **2012**, 287, 37206-37218, [10.1074/jbc.M112.369942](https://doi.org/10.1074/jbc.M112.369942).
21. Eugene E. Emeson; Yutaka Kikkawa; Boris Gueft; NEW FEATURES OF AMYLOID FOUND AFTER DIGESTION WITH TRYPSIN. *Journal of Cell Biology* **1966**, 28, 570-577, [10.1083/jcb.28.3.570](https://doi.org/10.1083/jcb.28.3.570).
22. Ayenachew Bezawork-Geleta; Erica J. Brodie; David A. Dougan; Kaye N. Truscott; LON is the master protease that protects against protein aggregation in human mitochondria through direct degradation of misfolded proteins. *Scientific Reports* **2015**, 5, 17397-17397, [10.1038/srep17397](https://doi.org/10.1038/srep17397).
23. Wagner, I.; Arlt, H.; van Dyck, L.; Langer, T.; Neupert, W.; Molecular chaperones cooperate with PIM1 protease in the degradation of misfolded proteins in mitochondria. *EMBO J* **1994**, 13, 5135-5145, .
24. Satoshi Ninagawa; Kazutoshi Mori; Trypsin Sensitivity Assay to Study the Folding Status of Proteins. *BIO-PROTOCOL* **2016**, 6, e1953, [10.21769/bioprotoc.1953](https://doi.org/10.21769/bioprotoc.1953).
25. John Kay; A Possible Role for Neutral Proteolysis in the Degradation of Intracellular Proteins. *Novartis Foundation Symposia* **2008**, 75, 219-225, [10.1002/9780470720585.ch14](https://doi.org/10.1002/9780470720585.ch14).
26. Jose M. Sanchez-Ruiz; Protein kinetic stability. *Biophysical Chemistry* **2010**, 148, 1-15, [10.1016/j.bpc.2010.02.004](https://doi.org/10.1016/j.bpc.2010.02.004).
27. Jessica Merulla; Elisa Fasana; Tatiana Soldà; Maurizio Molinari; Specificity and Regulation of the Endoplasmic Reticulum-Associated Degradation Machinery. *Traffic* **2013**, 14, 767-777, [10.1111/tra.12068](https://doi.org/10.1111/tra.12068).
28. Christopher Pleyer; Jan Flesche; Fahad Saeed; Lysozyme amyloidosis – a case report and review of the literature. *Clinical Nephrology. Case Studies* **2015**, 3, 42-45, [10.5414/cnecs108538](https://doi.org/10.5414/cnecs108538).
29. Roberto Scarpioni; Marco Ricardi; Vittorio Albertazzi; Sara De Amicis; Francesco Rastelli; Lara Zerbini; Dialysis-related amyloidosis: challenges and solutions. *International Journal of Nephrology and Renovascular Disease* **2016**, ume 9, 319-328, [10.2147/ijnrd.s84784](https://doi.org/10.2147/ijnrd.s84784).
30. Anna I. Sulatskaya; Natalia P. Rodina; Olga I. Povarova; Irina M. Kuznetsova; Konstantin K. Turoverov; Different conditions of fibrillogenesis cause polymorphism of lysozyme amyloid fibrils. *Journal of Molecular Structure* **2017**, 1140, 52-58, [10.1016/j.molstruc.2016.10.037](https://doi.org/10.1016/j.molstruc.2016.10.037).

31. Malcolm A Leissring; Wesley Farris; Alice Y Chang; Dominic M Walsh; Xining Wu; Xiaoyan Sun; Matthew P Frosch; Dennis J Selkoe; Enhanced Proteolysis of β -Amyloid in APP Transgenic Mice Prevents Plaque Formation, Secondary Pathology, and Premature Death. *Neuron* **2003**, 40, 1087-1093, [10.1016/s0896-6273\(03\)00787-6](https://doi.org/10.1016/s0896-6273(03)00787-6).
32. Robert A. Marr; Edward Rockenstein; Atish Mukherjee; Mark S. Kindy; Louis B. Hersch; Fred H. Gage; Inder M. Verma; Eliezer Masliah; Nepilysin Gene Transfer Reduces Human Amyloid Pathology in Transgenic Mice. *The Journal of Neuroscience* **2003**, 23, 1992-1996, [10.1523/jneurosci.23-06-01992.2003](https://doi.org/10.1523/jneurosci.23-06-01992.2003).
33. Olga V. Stepanenko; M. I. Sulatsky; E. V. Mikhailova; Olesya V. Stepanenko; O. I. Povarova; I. M. Kuznetsova; K. K. Turoverov; A. I. Sulatskaya; Alpha-B-Crystallin Effect on Mature Amyloid Fibrils: Different Degradation Mechanisms and Changes in Cytotoxicity. *International Journal of Molecular Sciences* **2020**, 21, 7659, [10.3390/ijms21207659](https://doi.org/10.3390/ijms21207659).
34. Olga V. Stepanenko; M. I. Sulatsky; E. V. Mikhailova; Olesya V. Stepanenko; O. I. Povarova; I. M. Kuznetsova; K. K. Turoverov; A. I. Sulatskaya; Alpha-B-Crystallin Effect on Mature Amyloid Fibrils: Different Degradation Mechanisms and Changes in Cytotoxicity. *International Journal of Molecular Sciences* **2020**, 21, 7659, [10.3390/ijms21207659](https://doi.org/10.3390/ijms21207659).

Retrieved from <https://encyclopedia.pub/entry/history/show/25670>