

- Kuznetsova I.M., Sulatskaya A.I., Uversky V.N., Turoverov K.K.* 2012c. A new trend in the experimental methodology for the analysis of the thioflavin T binding to amyloid fibrils. *Mol. Neurobiol.* V. 45. P. 488.
- Nelson R., Eisenberg D.* 2006. Recent atomic models of amyloid fibril structure. *Curr. Opin. Struct. Biol.* V. 16. P. 260.
- Nelson R., Sawaya M.R., Balbirnie M., Madsen A.O., Riekel C., Grothe R., Eisenberg D.* 2005. Structure of the cross-beta spine of amyloid-like fibrils. *Nature.* V. 435. P. 773.
- Otzen D., Riek R.* 2019. Functional Amyloids. *Cold Spring Harb. Perspect. Biol.* V. 11. a033860. <https://doi.org/10.1101/cshperspect.a033860>
- Pham C.L., Kwan A.H., Sunde M.* 2014. Functional amyloid: Widespread in nature, diverse in purpose. *Essays Biochem.* V. 56. P. 207.
- Provencher S.W., Glockner J.* 1981. Estimation of globular protein secondary structure from circular dichroism. *Biochemistry.* V. 20. P. 33.
- Prusiner S.B., McKinley M.P., Bowman K.A., Bolton D.C., Bendheim P.E., Groth D.F., Glenner G.G.* 1983. Scrapie prions aggregate to form amyloid-like birefringent rods. *Cell.* V. 35. P. 349.
- Rovnyagina N.R., Sluchanko N.N., Tikhonova T.N., Fadeev V.V., Litskevich A.Y., Maskevich A.A., Shirshin E.A.* 2018. Binding of thioflavin T by albumins: An underestimated role of protein oligomeric heterogeneity. *Int. J. Biol. Macromol.* V. 108. P. 284.
- Sen P., Fatima S., Ahmad B., Khan R.H.* 2009. Interactions of thioflavin T with serum albumins: spectroscopic analyses. *Spectrochim. Acta A Mol. Biomol. Spectrosc.* V. 74. P. 94.
- Sneideris T., Darguzis D., Botyriute A., Grigaliunas M., Winter R., Smirnovas V.* 2015. pH-Driven Polymorphism of insulin amyloid-like fibrils. *PLoS One.* V. 10. e0136602. <https://doi.org/10.1371/journal.pone.0136602>
- Sreerama N., Woody R.W.* 2000. Estimation of protein secondary structure from circular dichroism spectra: Comparison of CONTIN, SELCON, and CDSSTR methods with an expanded reference set. *Anal. Biochem.* V. 287. P. 252.
- Stefani M.* 2010. Structural polymorphism of amyloid oligomers and fibrils underlies different fibrillization pathways: Immunogenicity and cytotoxicity. *Curr. Prot. Pept. Sci.* V. 11. P. 343.
- Sulatskaya A.I., Maskevich A.A., Kuznetsova I.M., Uversky V.N., Turoverov K.K.* 2010. Fluorescence quantum yield of thioflavin T in rigid isotropic solution and incorporated into the amyloid fibrils. *PLoS One.* V. 5. e15385. <https://doi.org/10.1371/journal.pone.0015385>
- Sulatskaya A.I., Rodina N.P., Kuznetsova I.M., Turoverov K.K.* 2017. Different conditions of fibrillogenesis cause polymorphism of lysozyme amyloid fibrils. *J. Mol. Struct.* V. 1140. P. 52.
- Sulatskaya A.I., Rodina N.P., Polyakov D.S., Kuznetsova I.M., Turoverov K.K.* 2015. Investigation of amyloid fibrils on the basis of full-length and truncated forms of beta-2-microglobulin with the use of equilibrium microdialysis. *Proc. Europ. Conf. Biol. Med. Sci.* P. 11.
- Sulatsky M.I., Sulatskaya A.I., Povarova O.I., Antifeeva I.A., Kuznetsova I.M., Turoverov K.K.* 2020. Effect of the fluorescent probes ThT and ANS on the mature amyloid fibrils. *Prion.* V. 14. P. 67.
- Sunde M., Serpell L.C., Bartlam M., Fraser P.E., Pepys M.B., Blake C.C.* 1997. Common core structure of amyloid fibrils by synchrotron X-ray diffraction. *J. Mol. Biol.* V. 273. P. 729.
- Tycko R.* 2015. Amyloid polymorphism: Structural basis and neurobiological relevance. *Neuron.* V. 86. P. 632.
- Valentine J.S., Doucette P.A., Zittin Potter S.* 2005. Copper-zinc superoxide dismutase and amyotrophic lateral sclerosis. *Ann. Rev. Biochem.* V. 74. P. 563.
- Vassar R., Bennett B.D., Babu-Khan S., Kahn S., Mendiaz E.A., Denis P., Teplow D.B., Ross S., Amarante P., Loeloff R., Luo Y., Fisher S., Fuller J., Edenson S., Lile J. et al.* 1999. Beta-secretase cleavage of Alzheimer's amyloid precursor protein by the transmembrane aspartic protease BACE. *Science.* V. 286. P. 735.
- Vernaglia B.A., Huang J., Clark E.D.* 2004. Guanidine hydrochloride can induce amyloid fibril formation from hen egg-white lysozyme. *Biomacromol.* V. 5. P. 1362.
- Vladimirov Y.A., Litvin F.F.* 1964. Photobiology and spectroscopic methods. *Handbook Gen. Biophys.* V. 8. P. 88.
- Warby S.C., Montpetit A., Hayden A.R., Carroll J.B., Butland S.L., Visscher H., Collins J.A., Semaka A., Hudson T.J., Hayden M.R.* 2009. CAG expansion in the Huntington disease gene is associated with a specific and targetable predisposing haplogroup. *Am. J. Hum. Genet.* V. 84. P. 351.
- Westermark P., Benson M.D., Buxbaum J.N., Cohen A.S., Frangione B., Ikeda S., Masters C.L., Merlini G., Saraiva M.J., Sipe J.D.* 2005. Amyloid: Toward terminology clarification. Report from the Nomenclature Committee of the International Society of Amyloidosis. *Amyloid.* V. 12. P. 1.

Structural Polymorphism of Lysozyme Amyloid Fibrils

N. M. Melnikova^a, M. I. Sulatsky^a, I. M. Kuznetsova^a, K. K. Turoverov^{a,*}, and A. I. Sulatskaya^{a,**}

^aInstitute of Cytology Russian Academy of Sciences, St. Petersburg, 194064 Russia

*e-mail: kkt@incras.ru

**e-mail: ansul@mail.ru

According to the modern concepts, polymorphism of amyloid fibrils can be the cause of the differences in its cytotoxicity, as well as the variability of amyloidosis. The aim of this work was to study the structure and properties of the lysozyme amyloid fibrils obtained under various conditions (at different concentrations of the denaturing agent guanidine hydrochloride) using a wide range of physicochemical methods, including specially elaborated ones. As a

result, the difference was shown: 1) the propensity of amyloid fibers to interact with each other and the size of their clusters; 2) secondary structure and microenvironment of tryptophan residues of amyloid-forming proteins; 3) the characteristics of the fibrils interaction with the amyloid-specific probe thioflavin T (ThT), as well as 4) the resistance of amyloids to the action of the ionic detergent sodium dodecyl sulfate and boiling. Our results indicate the polymorphism of the studied protein aggregates. The results of the work allowed us to conclude that the obtained amyloid fibrils are an attractive object for further research aimed at identifying the relationship between the structure of amyloids and their cytotoxicity.

Keywords: amyloid fibrils, lysozyme, structural polymorphism, thioflavin T, guanidine hydrochloride, equilibrium microdialysis