CONCENTRATION-DEPENDENT POLYMORPHISM OF INSULIN AMYLOID FIBRILS

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Protein aggregation into insoluble amyloid aggregates is associated with several neurodegenerative diseases, such as Alzheimer's, Parkinson's and Prion diseases [1]. Polymorphism of amyloidogenic proteins is one of the factors that complicate the disease mechanisms which may be the cause of failure in research for anti-amyloid drugs. In this study we show that protein concentration is another factor which leads to formation of structurally distinct insulin fibrils [2]. Experimental data suggests that distinct insulin fibrils self-replicate via elongation, while seed-induced nucleation will lead to environment-defined conformation of fibrils.

We have characterized insulin amyloid fibrils formed at protein concentration range from 0.2 mM to 1.0 mM. Insulin aggregation kinetics were measured using amyloid-specific fluorescent dye – Thioflavin T (ThT). After insulin aggregation, data revealed two different fibrils populations – low concentration fibrils (LCF) and high concentration fibrils (HCF) which had a major difference in ThT fluorescence intensity and fibril concentration ratio values. Further assessment of secondary structures using Fourier-transform infrared spectroscopy confirmed two different fibril conformations formed at 0.2 mM and 1.0 mM insulin concentration. Fibrils morphology was imaged by AFM and fibril height distribution was estimated from the images.

[1] F. Chiti and C. M. Dobson, "Protein Misfolding, Amyloid Formation, and Human Disease: A Summary of Progress Over the Last Decade," *Annu. Rev. Biochem.*, vol. 86, no. 1, pp. 27–68, 2017.

[2] A. Sakalauskas, M. Ziaunys, and V. Smirnovas, "Concentration-dependent polymorphism of insulin amyloid fibrils," *PeerJ*, vol. 7, p. e8208, Dec. 2019.