LIQUID-LIQUID PHASE SEPARATION OF S100A4 PROTEIN

Arijus Jaloveckas[1], Darius Šulskis[1]

[1] Vilnius University, Life Sciences Center, Institute of Biotechnology, Lithuania
arijus.jaloveckas@gmc.stud.vu.lt

The main indications of Alzheimer's disease are the accumulation of aggregates called amyloids in the brain, which cause inflammation and neuronal damage [1]. One example are calcium binding and neuroinflammation associated S100A proteins, whose aggregates are found in Corpora Amylacea, spherical bodies of unknown function and origin, that form during the ageing process and several neurodegenerative disorders [2]. One of the initial causes that accelerates amyloid fibril formation is the liquid-liquid phase separation (LLPS) process, where proteins form membraneless droplets based on their affinity for each other and a crowded environment [3]. Presently, the relationship between LLPS and amyloid aggregation of S100 protein is unknown, therefore, the goal of this study is to determine whether a specific S100A4 member undergoes LLPS in a crowded environment and if it causes protein aggregation.

A construct S100A4 fused with an N-terminal eEGP construct was formed using restriction-free cloning. After successfully selecting positive colonies, plasmids containing eGFP-S100A4 insert were purified and sequenced. For protein expression, BL21 Star™ (DE3) E. coli cells were transformed with the pET28b plasmid, containing eGFP-S100A4. Cells were grown until 0.6 optical density and protein production was induced with 0.1 mM IPTG. Harvested cells were lysed using sonication, and the soluble medium was separated via centrifugation. Then, the protein was applied to the Ni-NTA column, and the eluted fractions were concentrated. eGFP-S100A4 was further purified by size exclusion and ion exchange chromatography. Amyloid aggregation was monitored using amyloid-specific ThT dye. LLPS assay was conducted using different concentrations of eGFP-S100A4 and S100A4 mixture (1:99 ratio) and different polyethylene glycol (PEG) concentrations, which were used to imitate cellular environment. LLPS assay was observed using Olympus IX83 fluorescence microscope.

Initially, we observed that S100A4 does not aggregate in a non-crowded environment, except at low pH conditions. The addition of PEG induced the formation of S100A4 droplets at all 4 different protein concentrations (25, 50, 75, 100 µM), although the most prominent droplets were found in 10% and 20% PEG solutions. After successfully perceiving LLPS of S100A4, further research will include characterization of S100A4 state (aggregates, oligomers) in the droplets and their effect on the neuronal cells.

[1] D. J. Selkoe and J. Hardy, 'The amyloid hypothesis of Alzheimer's disease at 25 years', EMBO Mol. Med., vol. 8, no. 6, pp. 595–608, Jun. 2016, doi: 10.15252/emmm.201606210.

[2] E. Augé, I. Bechmann, N. Llor, J. Vilaplana, M. Krueger, and C. Pelegrí, 'Corpora amylacea in human hippocampal brain tissue are intracellular bodies that exhibit a homogeneous distribution of neo-epitopes', Sci. Rep., vol. 9, no. 1, p. 2063, Feb. 2019, doi: 10.1038/s41598-018-38010-7.

[3] S. Mukherjee, M. Poudyal, K. Dave, P. Kadu, and S. K. Maji, 'Protein misfolding and amyloid nucleation through liquid–liquid phase separation', Chem. Soc. Rev., vol. 53, no. 10, pp. 4976–5013, 2024, doi: 10.1039/D3CS01065A.