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The Effect of Deep Eutectic Solvent on the Stability of LCD-TDP-43 Protein Liquid Droplets

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Abstract

The distinctive characteristics of deep eutectic solvents (DESs) offer the potential to stabilize proteins. They can prevent the aggregation of proteins, such as LCD-TDP-43, which serves as a model for prion-like protein [1]. Essentially, DESs impede denaturation caused by external stressors by encapsulating proteins within a protective barrier [2]. DESs fulfill their function by enhancing solvation around proteins, reducing hydrophobic interactions, and maintaining protein structure during phase separation [3.4]. This study aims to develop and refine DES formulations to enhance protein stability during the phase separation pathway [5]. In this study, the pJ-411 vector was used to express the recombinant LCD-TDP-43 protein in *E. coli* BL21 (DE3). Affinity chromatography with Ni-NTA was employed to purify the protein. Dialysis was used to study phase transitions from liquid droplets to amyloid fibrils. After 72 hours of dialysis, the LC domain transitioned from liquid droplets to amyloid fibrils. Liquid-liquid phase transitions occurred first, followed by liquid-solid phase transitions, with a white precipitate indicating amorphous mature droplets and amyloid fibrils. Five DESs were prepared to investigate their effect as chemical chaperones on the aggregation process of LCD-TDP-43 protein. These DESs included betaine: glycerol (1:2), betaine: sorbitol (1:2), betaine: citric acid (1:1), betaine: tartaric acid (1:1), and betaine: xylose (1:2). Protein aggregation in the phase separation pathway was examined, and amyloid fibrils were identified using turbidity measurements and ThT fluorescence. Turbidity measurements revealed a decrease in UV-visible absorption in the presence of several DESs, indicating their potential role in inhibiting amyloid growth in LCD-TDP-43 proteins. This finding suggests that DESs may play a crucial role in improving protein stability during liquid-phase separation. Consequently,

DESs may be considered as promising candidates in the search for new therapeutic agents to treat diseases associated with protein aggregation.

Key words: DESs, LCD-TDP-43, LLPS, Protein aggregation, Neurodegeneration, ALS

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