Liquid-liquid phase separation and aggregation of a globular protein SUMO1 under crowded conditions

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Liquid-Liquid Phase Separation (LLPS) is a process where biomolecules separate into distinct liquid phases, leading to various membrane-less organelles in cells. Although LLPS plays a fundamental role in multiple cellular processes, it has also been shown to mediate the formation of pathological aggregates in various neurodegenerative diseases. Intrinsically disordered proteins (IDPs) found in these pathological aggregates are highly flexible and lack a well-defined three-dimensional structure enabling them to undergo conformational changes required for LLPS. Interestingly, many IDPs are often colocalized with structured protein SUMO1 (Small Ubiquitin-like Modifier 1) in inclusion bodies isolated from brains of patients While most of the research on LLPS has focused on IDPs, LLPS of globular proteins is yet to be studied. In this work, we demonstrate the phase separation of a globular protein, SUMO1, under crowded conditions, at physiological pH and room temperature. We also show that SUMO1 undergoes conformational changes inside the phase-separated droplets and form solid aggregates with time. Using various spectroscopic probes (Thioflavin T fluorescence, Raman Spectroscopy and TEM) we show that SUMO1 aggregates are rich in beta-sheet content, but predominantly amorphous. To verify whether the structured part of the protein is indeed forming the LLPS, we have also studied the role of the disordered N-terminal segment of SUMO1 in driving its phase separation. Our study highlights the fact that structured proteins can also undergo LLPS despite their low conformational flexibility, albeit underlying mechanisms are not well understood and may differ from those of IDPs. Moreover, LLPS of SUMO1 protein may have important biological implications for neurodegenerative disease. We hypothesize that presence of SUMO1 tag on IDPs can modulate their phase separation and aggregation propensity under diseased conditions.

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