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Optimization of Quercetin Concentration for Studying Liquid-Liquid Phase Separation

Nahid Zamani-Moghaddam*, Amirhossein Latifi, Elnaz Hosseini, Payam Arghavani,
Ahmad Amiri, Ali Akbar Moosavi-Movahedi, Ali Akbar Saboury

Institute of Biochemistry and Biophysics (IBB), University of Tehran, Tehran, Iran. Email:
nahid.zamani.m@ut.ac.ir

Abstract

Liquid-liquid phase separation (LLPS) of proteins is crucial for understanding the mechanisms of protein aggregation. LLPS acts as an initial stage in protein aggregation, enabling proteins to transition from a uniform solution to distinct phases or concentrated droplets. This study examines the effects of varying concentrations of quercetin on the structural properties of bovine serum albumin (BSA) and aims to identify optimal conditions for studying LLPS. To determine suitable quercetin concentrations for analyzing BSA's structural integrity and interactions, we utilized fluorescence spectroscopy and circular dichroism (CD) spectroscopy. Changes in the protein's secondary structure were evaluated using CD in the far-UV range, while alternations in its tertiary structure were examined through the intrinsic fluorescence of tryptophan and ANS (8-anilino-1-naphthalenesulfonic acid). Our findings reveal that quercetin concentrations of 4.5 μM and 45 μM have minimal affect on the tertiary structure of the BSA monomer, establishing these concentrations optimal for LLPS studies. Additionally, the role of these concentrations can be explored in the context of protein droplet formation during phase separation and the resulting aggregates.

Key words: Liquid-liquid phase separation, Liquid droplet, Quercetin, BSA