Abstract

Protein aggregation, particularly amyloid fibrils, poses challenges for biopharmaceutical production and contributes to incurable diseases such as neurodegeneration, transthyretin amyloidosis, and type II diabetes. Amyloid fibrils are highly stable aggregates composed of cross-β structures. They have vast polymorphism and complex formation pathways, involving intermediates like liquid-liquid separation (LLPS) droplets, oligomers, and protofilaments, that complicate structural characterization. A new nucleated conformational conversion (NCC) model proposes that fibrils arise from diverse intermediates along an energy funnel, akin to protein folding.

Both intrinsic and extrinsic factors, such as temperature, pH, protein concentration, and co-solutes, influence aggregation. Amphiphilic protein β-lactoglobulin (BLG) is a widely available whey protein with high concentration of β-structures that serves as an excellent amyloid model. This study systematically varied process conditions and co-solutes to explore BLG aggregation across pH values using spectroscopic, microscopic, biochemical, and diffraction techniques.

At pH 2 in glycine buffer and 80 °C, a novel fibrillization pathway was discovered involving LLPS, α-helix rearrangement, and reduced acid hydrolysis susceptibility. Hexameric oligomers aligned on LLPS surfaces and formed nanotubes and fibrils. Stirring promoted transitions from worm-like to rigid amyloid fibrils.

At pH 7 and high temperatures (80 °C), BLG's Cys121 initiates disulfide scrambling, preventing fibril formation. However, at low temperatures (50 °C), this scrambling was suppressed, leading to large colored microribbons and protein vesicles that formed from LLPS droplets. With added 2 M KSCN, unfolded BLG formed tapes, fibrils, and ring-like aggregates, showing NCC-driven kinetics and cross-α interactions.

This work presents a new framework for amyloid fibrillization, linking LLPS, oligomerization, vesicle formation, and secondary structure transitions. It offers novel avenues for therapeutic development and the design of self-assembled biomaterials. BLG-derived structures also show promise in photonic applications, mimicking amelogenin-based enamel formation and enabling use in drug delivery, sensors, coatings, and optical devices.

Key Words: β-lactoglobulin, amyloid fibrils, polymorphism, co-solutes, phase separation, amphiphilicity, protein vesicles, thiol groups, microscopy, aggregation, photonic crystals