Abstract

A growing body of current research has revealed that living cells can regulate the complex biomolecular chemistry by the spatiotemporal organization of a wide variety of different cellular components into functionally distinct intracellular liquid-like compartments or membrane-less organelles having different chemical environments. Under certain circumstances, protein misfolding occurs inside the cells, which leads to the accumulation of highly ordered cross-β sheet rich amyloid aggregates that have been implicated in many deadly neurodegenerative diseases such as Alzheimer's, Parkinson's, prion diseases, etc. However, recent studies have identified the beneficial role of amyloids in a multitude of organisms ranging from bacteria to humans' performing an array of physiological functions. Human Pmel17, a melanocyte-specific glycoprotein, forms functional amyloid that plays an essential role in melanosome development by creating a fibrillar amyloid matrix in the organelle, which acts as a template for melanin deposition underneath the skin and in the eyes. The amyloid matrix serves a beneficial role in mitigating the toxicity by sequestering and minimizing the diffusion of highly reactive quinone precursors that are required during melanin biosynthesis. It is known that an intrinsically disordered region (IDR) of Pmel17, the repeat domain (RPT) forms the amyloid core and promotes melanin formation in vitro. Several studies have shown that the deletion of the RPT ablates fibril formation in vivo. However, the molecular mechanism of amyloid formation, as well as the organization of individual protein molecules within the supramolecular assembly, remains elusive. An increasing body of work reveals that under certain physicochemical conditions, IDRs in proteins undergo liquid-liquid phase separation to form dense insoluble phases that have implications in both physiology and disease. These IDRs have an intrinsic preference for conformational disorder and are often characterized by low complexity (LC) domains. While numerous studies have discovered that LC-IDRs in proteins phase separate into mesoscopic liquid droplets, and the phase-separated state predisposes the protein toward the formation of aggregates, the fundamental molecular drivers, and the sequence of events that govern the phase transitions is poorly understood. In this thesis, efforts were directed towards elucidating the molecular mechanism of amyloid formation and phase transitions of the RPT under various physicochemical conditions. The conformational dynamics, heterogeneity, and intermolecular association that drives RPT phase transitions were studied using a multidisciplinary approach involving a combination of biophysical, biochemical, molecular biology, and imaging tools.