THESIS ABSTRACT

Optineurin (OPTN) is a multifunctional, ubiquitously expressed cytoplasmic protein. The name 'Optineurin' has been derived from OPTIc NEURopathy INducing protein. The mutants of OPTN are linked with primary open-angle glaucoma (POAG), a pathological condition that causes irreversible blindness and amyotrophic lateral sclerosis (ALS). The presence of OPTN in ocular tissues like the iris, retina, cornea, trabecular meshwork, and lenses is intriguing. This is because mammalian ocular tissues, particularly eye lenses, often experience various stress conditions. Interestingly, the ocular protein OPTN also harbors heat shock elements in its promoter region. Sequence analysis of OPTN exhibits intrinsically disordered regions and nucleic acid binding domains. These properties hinted that OPTN might be endowed with sufficient thermodynamic stability and chaperoning activity. However, these attributes of OPTN have not yet been explored. In the first part of this thesis, we have studied these properties in OPTN and its ALS-related mutant through thermal and chemical denaturation experiments and monitored the processes using CD, fluorimetry, differential scanning calorimetry, and dynamic light scattering. We found that upon heating, OPTN reversibly forms higher order multimers.

OPTN also displayed a chaperone-like function by reducing the thermal aggregation of bovine carbonic anhydrase. It regains its native secondary structure, RNA-binding property, and melting temperature (Tm) after refolding from a thermally as well as chemically denatured state.

Considerable progress has been made over a decade in understanding the physiological and pathological functions of OPTN. It is well reported that OPTN is an aggregation prone protein that is involved in neurodegenerative disease progression. OPTN tends to form aggregates under certain stress conditions such as oxidative stress. Moreover, OPTN is associated with the with the RNA binding proteins and stress granules. However, despite such advancements, there remains a lacuna of knowledge in the fundamental biophysical and aggregation mechanisms of OPTN's involvement in disease progression. To understand the aggregation kinetics of OPTN and its ALS-related mutants E478G and Q398X, we explored their aggregation behaviour in the second part of this thesis. It is known that intrinsically disordered proteins form multivalent interactions which leads to polymerization which eventually undergo phase separation.

Similarly, RNA binding protein, under stress conditions, form multivalent interactions with other proteins and RNA molecules to undergo LLPS in the form of stress granules. On sequence and structure analysis it was found that OPTN is intrinsically disordered glutamine rich protein, consisting of both zinc finger and leucine zipper domains, which are known to bind oligonucleotides. This inclined us to propose that OPTN may act as an RNA binding protein having a significant role in stress granule dynamics and phase separation.

Therefore, we studied its RNA-binding and phase separation properties as well. From our study we found that OPTN is a protein with significant structural reversibility after denaturation. OPTN is a versatile protein that could mould into a conformational multimer. We found that it interacts with RNA in its native state and also it regains this functionality after refolding. Similar phenomenon was not observed with the mutants, which does not show complete reversibility. Reversible thermal transition of OPTN to multimeric form is of special interest as it displays chaperone-like activity, presumably to perform a myriad of cellular functions. The thermodynamic properties of OPTN presented here lays the foundation for further explorations and validation with additional experiments.

Our study gives a reasonable correlation of OPTN's existence in the ocular tissues, pertaining to its chaperoning potential. We have shown that prolonged stress makes the wild type OPTN and its mutants prone to aggregation and we have established aggregation kinetics in-vitro. We found that the wild type OPTN forms worm-like fibers while the mutants form amorphous aggregates. The initial study of liquid-liquid phase separation and formation of gel like particle further throws light on the complexity of this protein and its progressive role in neurological disorders in the cell. Further studies and exploration of LLPS to solid structure or aggregation pathway will be helpful in designing potential therapeutics to inhibit or delay in OPTN- associated disease progression.