Aggregation kinetics of amylin and its optical and electrochemical properties

Thesis Abstract

Amylin is a peptide hormone co-secreted with insulin from the pancreatic β-cell. The formation of intermediate oligomers is a seminal event in the eventual self-assembled fibril structures of amylin, believed to have a central role in Type-II diabetes mellitus. Therefore, it is imperative to develop a deeper understanding of structural changes during its aggregation pathways for developing therapeutic strategies.

In this thesis, we observe the kinetics of amylin aggregation experimentally from monomers to non-identical multi-stranded fibrils that follow a sigmoidal growth, and the shape changed from globular to rod-like entities. The repulsive interaction in the globular form switches to attractive to initiate the aggregation process. The microscopy data shows that the distance between protofibril is <5 Å, confirming its stacking through hydrogen bonds. Next, we observe the vital signature for conversion of α-helix into β-sheet secondary structures during aggregation through various spectroscopy techniques by measuring the spectrum from the di-tyrosine linkage at the initial stages; emission bands corresponding to tyrosinate, oligomer interaction, and structure-specific intrinsic fluorescence. The elongation and widening of fibrils due to stacking of oligomers and monomers were implied in red/blue- shifts. Further, we report a concentration-dependent competitive role of inhibitors/ligands on aggregation rates and fluorescence quenching during aggregation and quantifying the conversion percentage of β-sheets into less aggregation-prone secondary structures.

The matured fibrils of amylin were mechanically and chemically perturbed to access their stability. A sufficient population of specific size intermediate amylin oligomers under diffusion-limited conditions could self-assemble into fractal-like structures. The interactions between the anisotropically distributed hydrophobic and polar/ionic residues on the solvent-accessible surface of oligomers were crucial for self-assembly and aggregation. The electrochemical and optical properties of amylin-denaturant complexes were quantified by electron transfer rates, the irreversibility of oxidation-reduction, and photoluminescence spectra. The results captured the structural heterogeneity and different disruption mechanisms of the denaturants.

The thesis details the structural aspects of amylin aggregates, their self-assembly, and fibril stability which may help fabricate bioinspired nanomaterials with enhanced electrochemical and optoelectronic properties. In addition, the information of stable intermediates and their modulations in the presence of different ligands is critical for designing drugs for protein misfolding diseases.

Keywords:

Amylin, Type-II diabetes mellitus, Aggregation kinetics, Interaction parameter, Ligands, Intrinsic fluorescence, Fluorescence quenching, Fractal self-assembly, Fibril stability