Abstract

Title: Structural and mechanistic insights of amyloid protein and designed inhibitor interaction in presence or absence of membrane interface

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Amyloidogenic disorders are currently rising as a global health issue, prompting the requirement of extensive studies dedicated to the development of effective targeted therapeutics. Protein amyloidogenesis, involving proteins or peptides such as amyloid β, α-synuclein, and insulin, is crucial in the progression of several human diseases, including neurodegenerative disorders like Alzheimer's (AD) and Parkinson's (PD), and metabolic disorders like Type-2 Diabetes, respectively. The inherent tendency of these amyloidogenic proteins to interact with biological membranes introduce additional complexities to the system. Several literature reports have enhanced our understanding of the system, through the application of *in vitro*, *in vivo*, and *in silico* methods. However, achieving success with these methods has been challenging, partly because of the intricate nature of the aggregation process as well as due to the poor understanding of the mechanism and the targets of the inhibitors.

In this context, the goals of this study are outlined through five chapters. (1) The first chapter provides an overview of amyloid formation in association with membrane, alongside mentioning various therapeutic agents designed for treating or preventing the studied amyloid diseases. (2) In second chapter, inhibition of insulin aggregation was performed using two small molecules, namely Coomassie Brilliant Blue G-250 (CBBG) as well as PAD-S. It's worth mentioning that insulin has been shown to form amyloid deposits at the site of administration. So, inhibition of insulin amyloid not only preserves insulin's therapeutic efficacy but also reduces complications associated with insulin administration in patients. The present investigation established that CBBG and PAD-S, serve as a chemical chaperone that effectively prevents the fibrillation of insulin and disrupted matured fibrils into nontoxic fragments. (3) In the next chapter, PWWP motif, which is known to inhibit the amyloidogenesis, was used in designing a small peptide library and their effect was elucidated in inhibition of insulin. The *de novo* designed cyclic peptide KR7CC (KCPWWPCRR-NH_2), was non-toxic, stable in serum, and effectively inhibited insulin fibrillation in both membrane and non-membrane environments. Several biophysical spectroscopic studies successfully revealed the specific mechanism of inhibition at the atomistic level. (4) In Chapter 4, small peptide and peptidomimetics were designed and synthesized by altering the "RWSLMRPF" recognition motif to specifically target amyloid β peptides (Aβ) in AD. Peptide1 (P1) and Peptide2 (P2) were synthesized by substituting S and RWS residues, respectively, with β-breaker element (2-aminobenzoicacid) in the RF8 peptide sequence, resulting in novel, serum-stable, and non-toxic peptides with anti-AD activity. Further, P1, a potent inhibitor effectively prevented in late-stage AD by inhibiting Aβ42 fibril formation and converting mature fibrils into nontoxic aggregates, followed by an off-pathway aggregation kinetics. (5) Chapter 5 focuses on repurposing Lasunadya Ghrita (LG), an ancient Indian medicine traditionally used to treat gut dysregulation and mental disorder, for AD. Different extracts of LG were analyzed for their potential to inhibit Aβ aggregation, revealing that the water extract exhibited greater efficacy in preventing Aβ peptide aggregation and disintegrating mature fibrils. Non-toxic to both neuronal cells and mouse models, LG (water extract) effectively rescues Aβ toxicity in neuronal SH-SY5Y cells by decreasing ROS generation, membrane leakage, cellular apoptosis, and calcium dyshomeostasis. This research provides a pathway for the development of future medications to combat amyloidosis, offering promising prospects for treating amyloid disorders.

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