1
Introduction

Protein aggregation is a fundamental process in biology in which soluble protein monomers self-assemble into insoluble higher order structures. This phenomenon is usually observed in structural proteins as well as amyloidogenic proteins. The self-association of triple helical collagen molecules to form supramolecular assemblies becomes very important for both structural and functional properties of extracellular matrix (Kielty and Grant 2003). However, the process of aggregation of many amyloidogenic proteins is known to generate toxic amyloid species. Amyloids are very stable higher order entities that exhibit cross-β structures and the process of amyloid fibril formation has been implicated in severe pathologies including a series of neurodegenerative diseases such as Alzheimer's disease, Huntington's disease and Parkinson's disease (Sipe 2005).

Over the past hundred years, life threatening diseases like dementia and Alzheimer's disease are known to us, which mostly appear in the old age. Every year large number of clinical reports related to dementia are recorded and this number is rising exponentially which is a great concern (Alzheimer's 2015). According to World Alzheimer Report, it is reported that by the year 2015 almost 46.8 million patients were suffering from dementia and Alzheimer's diseases and by the year 2050 this count will rise to 131.5 million, which seems an alarming situation. Until now, no effective cure is available for such neurodegenerative diseases, neither to treat nor to suppress the progression of the diseases (Alzheimer's 2015; Prince, Wimo et al. 2015). The mechanistic understanding of the process of amyloid formation of protein is largely unknown. Hence, the knowledge of underlying principles of aggregation mechanism would be very helpful to solve issues related to amyloid linked medical complications.

1.1 PURPOSE OF THE STUDY

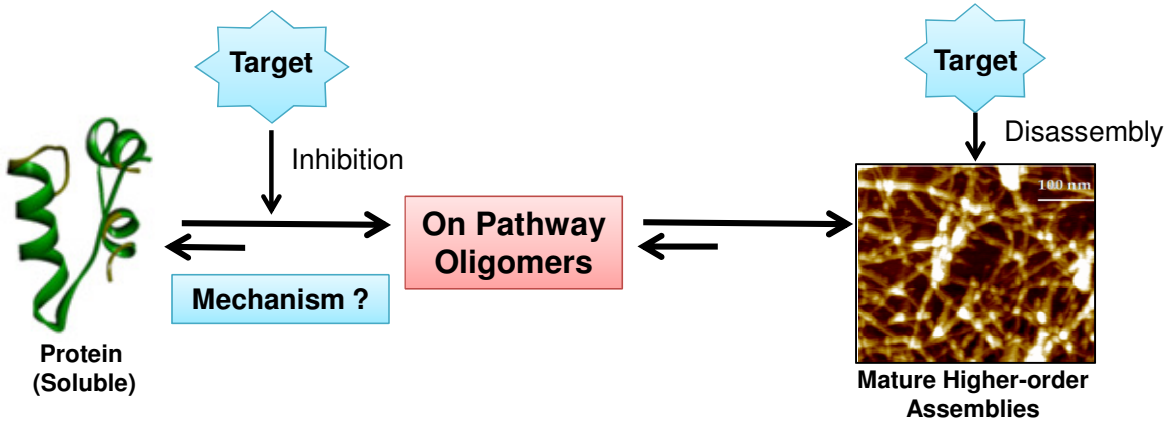

Figure 1.1 : Schematic representation of the protein aggregation process. Main objectives of the current research work are highlighted in blue.

Since the fundamental understanding of protein aggregation process is unclear, here an attempt has been made to understand the mechanism of protein aggregation process. Apart from understanding the mechanism of aggregation it is also important to find out inhibitors of such aggregation processes (Figure 1.1). It is also important to find candidates which can promote dissociation of protein aggregates. Therefore the current research work has focused on elucidating the inhibition effect of selected proteins, natural compounds, amino acids and surface-functionalized nanoparticles on the self association of both collagen and selected amyloidogenic proteins under *in vitro* conditions (Figure 1.1).

1.2 BRIEF RESULTS, SCOPE AND FUTURE PERSPECTIVE OF THE WORK

In order to achieve the desired aim of the present work, relevant experiments were performed using established protocols. The obtained results and outcomes of different studies of this thesis are summarized as below:

Protein-protein interaction during *in vitro* amyloid formation (chapter 3: published as Dubey and Kar, 2014; BBRC and Dubey, et al., 2014; Biochemistry)

In this chapter an attempt has been made to understand the basic principles of protein aggregation process. How does the aggregation process begin? What are the interactions that promote the aggregation process and yield insoluble higher order stable structures? Clear answers to these questions are largely unknown. Since, protein-protein interaction and protein complex formation are vital to life processes, it is important to understand the effect of the process of self-association of a particular type of protein on the biological properties of other proteins. The question of whether amyloid formation of a particular protein would influence the aggregation properties of the other proteins located in its vicinity is particularly significant as many proteins coexist in the cells or in subcellular compartments. In first study, effect of globular proteins was examined on amyloid formation process. Here, *in vitro* protein aggregation was carried out using different combination of globular proteins (lysozyme, serum albumin, insulin and cytochrome *c*). The obtained results reveal the occurrence of aggressive coaggregation among proteins during amyloid formation. Interestingly, the obtained data clearly showed that the kinetics of coaggregation process were significantly faster than the aggregation kinetics of individual proteins. This study also revealed that the amyloid fibrils of a particular protein can drive aggregation of other proteins, confirming the occurrence of cross-seeding. The morphology and the stability of the coaggregated fibrils were found similar to that of individual protein fibrils. While carrying out the sequence similarity search among these proteins, a low level of sequence similarity was observed. All these results indicate that the population of partially folded intermediate species is very critical for the occurrence of the rapid coaggregation and cross-seeding processes. Since amyloid-linked inclusions and plaques are recognized as multi-component entities originated from aggregation of the associated protein, these findings on co-aggregation may add new insights into the underlying principles of amyloid formation process and pathologies related to them.

In the second part of this work, amyloid formation process of lysozyme was studied in the presence of a fibrous protein, collagen. Both collagen and amyloidogenic proteins have an inherent ability to undergo a self-assembly process leading to formation of supramolecular structures. Though our understanding of collagen–amyloid interaction is very poor, a few experimental evidences have indicated the protective nature of collagen against amyloid fibril formation. Hence to gain further insights into collagen–amyloid relationship, the role of type I collagen was examined on amyloid-aggregation of lysozyme. Thioflavin-T assay data indicated strong inhibition of both spontaneous and seed-induced aggregation of lysozyme by collagen. Both chemical and thermal denaturation experiments have showed increased lysozyme stability in the presence of collagen. However, the presence of collagen did not alter lysozyme activity.

These findings confirm that type I collagen is capable of blocking or interfering with the amyloid formation of lysozyme, and the results may have significant implications for the design of collagen based therapeutics against amyloid-linked diseases.

Effect of selected natural compounds on self-assembly of proteins (chapter 4: published as Dubey, et al., 2014; European Biophysics Journal and Dubey, et al., 2017; Scientific Reports 7)

Since the process of aggregation of many proteins is known to trigger the onset of severe diseases, it is important to find inhibitors against such process. In this section, selected natural compounds were used to test their potential to inhibit protein aggregation process. From the obtained results it was found that both eugenol and capsaicin are capable of inhibiting the aggregation of studied proteins.

Eugenol has attracted considerable attention because of its potential for many pharmaceutical applications including anti-inflammatory, anti-tumorigenic and anti-oxidant properties. Here, the effect of eugenol was examined on amyloid formation of selected globular proteins. From the results it was found that both spontaneous and seed-induced aggregation processes of insulin and serum albumin (BSA) are significantly suppressed in the presence of eugenol. Fluorescence quenching data predicted a single binding site for eugenol-protein complexes and the molecular docking studies further revealed eugenol's ability to bind with high affinity to insulin and BSA, particularly through CH-π and H-bonding interactions. CD data also indicated that eugenol helps insulin and BSA to retain their native states during aggregation. These findings reveal the inherent ability of eugenol to stabilize native proteins and to delay the conversion of oligomers into mature fibrils.

In another study of this chapter, the effect of capsaicin on molecular stability, self-assembly and fibril stability of type I collagen was examined. Capsaicin is a versatile plant product which is known to penetrate the skin and have several health benefits such as its anti-inflammatory and analgesic properties. During the investigation it was found that capsaicin suppresses collagen fibril formation, increases stability of collagen fibers in tendons and causes no effect on molecular stability of collagen. Turbidity assay showed that capsaicin does not promote disassembly of collagen fibrils. However, capsaicin moderately protects collagen fibrils from enzymatic degradation. Computational studies revealed the role of aromatic moiety and amide region of capsaicin in collagen-capsaicin interaction. The results may have significant implications in capsaicin based therapeutics that target excess collagen accumulation linked pathologies such as thrombosis, fibrosis and sclerosis.

Targeting amyloid formation of proteins through surface-functionalized nanoparticles (chapter 5: Dubey, et al., 2015; Amino Acids)

In this section, stable gold ($AuNPs^{Tyr}$, $AuNPs^{Trp}$) and silver ($AgNPs^{Tyr}$) nanoparticles (NPs) were synthesized strategically where these nanoparticles are surface functionalized with either tyrosine or tryptophan residues. Further the effect of the synthesized NPs was examined to check their potential against aggregation of insulin. It was observed that both spontaneous and seed-induced aggregation of insulin was significantly suppressed in the presence of $AuNPs^{Tyr}$, $AgNPs^{Tyr}$, and $AuNPs^{Trp}$. These nanoparticles were also found to promote disassembly of insulin amyloid fibrils. Surface functionalization of amino acids appear to be important for the inhibition effect since isolated tryptophan and tyrosine molecules did not prevent insulin aggregation. Bioinformatics analysis predict involvement of tyrosine in H-bonding interactions mediated by its C=O, –NH2, and aromatic moiety. These results offer significant opportunities for developing nanoparticle-based therapeutics against diseases related to amyloid fibril formation of proteins.

...