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Introduction

Self-assembly process of proteins and peptides into defined higher order structures is a fundamental process in biology which influences both structural and functional properties of many tissues in human body system. In case of structural proteins such as collagen, the self-assembly of triple helical collagen molecules into higher order entities becomes important for both structural and functional properties of the extracellular matrix. Though collagen self-assembly is vital to the body system, excess collagen fibril formation is known to cause several pathologies (Steplewski and Fertala 2012). On the other hand, formation of amyloid fibrils due to protein aggregation is known to trigger the onset of several neurodegenerative diseases. More than about 35 different proteins are known to form amyloid aggregates causing devastating diseases such as Aβ-linked Alzheimer's disease, α-synuclein-linked Parkinson's disease, polyglutamine-linked Huntington's disease and Islet Amyloid PolyPeptide (IAPP) associated type II Diabetes(Aguzzi and O'Connor 2010)(Chiti and Dobson 2006)(Greenwald and Riek 2010). One of the straight forward strategies to target amyloid linked diseases is to prevent the process of amyloid fibril formation (Figure 1.1). Hence, a clear mechanistic understanding of the process of protein aggregation, particularly focusing on the issue of how the aggregation process is initiated, is very important not only to identify the key factors for protein aggregation, but also to successfully design specific inhibitors to prevent such aggregation process.

In vitro aggregation studies of amyloidogenic proteins and peptides have been very helpful in providing valuable information for the development of potential inhibitors against amyloid fibril formation. Candidates ranging from single amino acids and natural products to selected peptides have been reported to inhibit the aggregation process of proteins into amyloid fibrils (Greenwald and Riek 2010). Few investigations have also looked at the effect of various nanoparticles on amyloid fibril formation of proteins (Álvarez et al. 2013)(Dubey et al. 2015)(Siposova et al. 2012)(Palmal, Jana, et al. 2014). Over the past decade, extensive research has been devoted to surface functionalization of nanoparticles with selected compounds to target several biological processes, including amyloid formation of proteins. Some studies have also proved that the nanoparticles coated with hydrophobic molecules can inhibit amyloid aggregation (Dubey et al. 2015) (Palmal, Jana, et al. 2014). Hence, making of nanoparticles which are strategically surface functionalized with potential inhibitors has become one of the most useful tools to target amyloid-linked medical complications.

This research work has primarily focused on unraveling the effect of surface functionalized nanoparticles on the process of amyloid formation under *in vitro* conditions. An attempt has been made to successfully synthesize metallic nanoparticles surface-functionalized with selected natural compounds, and their effects on the aggregation process of selected amyloidogenic globular proteins have been investigated. Using different biophysical tools, a detailed characterization and anti-amyloid activity of the synthesized nanoparticles have been examined. This work has also designed gold nanoparticles with aromatic amino acids to target collagen fibril formation under *in vitro* conditions.

1.1 PURPOSE OF THE STUDY

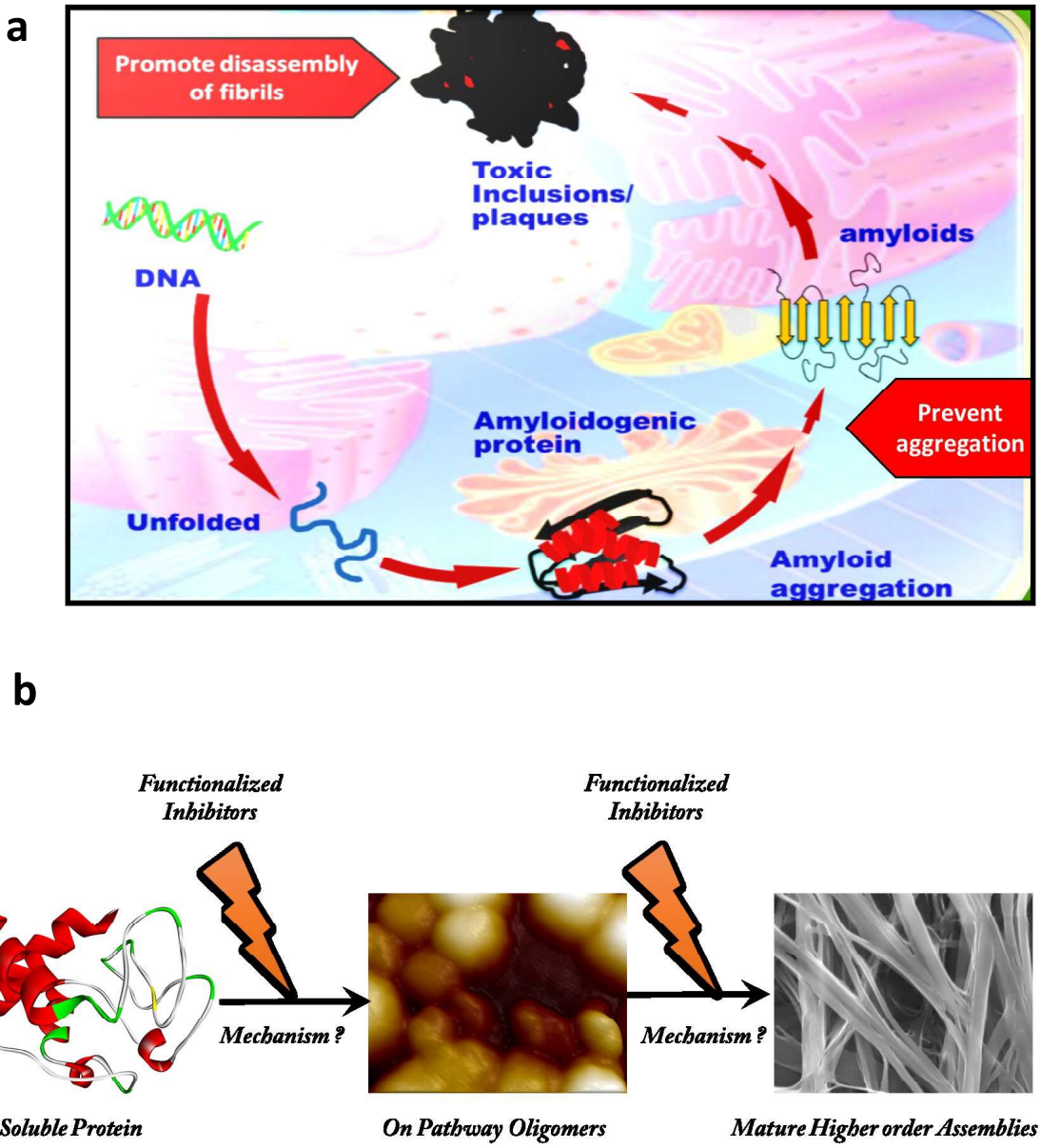

Figure 1.1: (a) Schematic representation of the general overview protein aggregation pathway beginning from its synthesis to formation of amyloid inclusions or plaques in the cell. The red shaded area shows one of the effective targets to interfere with this process of protein aggregation. **(b)** Schematic representation of the protein aggregation process under *in vitro* conditions. Main objectives of the current research work are highlighted ( ).

This work begins with the investigation of aggregation of amyloidogenic phenylalanine molecules as well as proteins to understand the critical role of aromatic amino acids on the occurrence of cross-seeding and co-aggregation during amyloid fibril formation(Dubey et al. 2014)(Anand et al. 2016). The fundamental information gained from this study, particularly understanding the critical role of exposed aromatic and hydrophobic residues in triggering the onset of amyloid aggregation of proteins has been used for designing nanoparticle based inhibitors. Stable gold and silver nanoparticles which are surface-functionalized with selective biomolecules including aromatic amino acids and phytochemicals have been synthesized and characterized. Capsaicin coated silver nanoparticles and piperine coated gold nanoparticles have been designed to target amyloid aggregation of serum albumin and insulin respectively. In addition to anti-amyloid nanoparticles, this work has also explored designing of gold nanoparticles coated with aromatic residues to target collagen fibril formation. Experiments have been performed to understand whether the surface functionalization of inhibitor molecule is vital for its inhibitory effect against aggregation of both amyloidogenic and collagen proteins. Interpretation of both experimental and computational results of this work has been carried out to understand the inhibition mechanism.

1.2 BRIEF RESULTS, SCOPE AND FUTURE PERSPECTIVE OF THE WORK

To achieve the desired aims of the present work, a combination of both biophysical tools and computational methods have been employed to carry out the relevant experiments. The goal was to target protein aggregation process through stable and uniform surface-functionalized nanoparticles under *in vitro* conditions. The obtained results and outcomes of different studies of this thesis are summarized as below:

Self-assembly of phenylalanine molecules under physiological conditions and its biological significance. (chapter 3: published as Anand, et al., 2017; Scientific Reports)

This work was performed to understand the amyloid aggregation of phenylalanine and to see what effects phenylalanine fibrils would have on the aggregation propensities of different globular proteins. Excess accumulation of phenylalanine is known to cause Phenylketonuria (PKU), a well-known genetic abnormality, which triggers several neurological, physical and developmental severities. However, the fundamental mechanism behind the origin of such diverse health problems, particularly the issue of how they are related to the build-up of phenylalanine molecules in the body, is largely unknown. This work reveals that phenylalanine-fibrils can effectively initiate the process of amyloid formation in proteins under physiological buffer condition in which most of these proteins retain their functional native, converting native protein structures to β-sheet assembly. The resultant fibrils were found to cause severe hemolysis, yielding a plethora of deformed erythrocytes that is highly relevant to phenylketonuria. Single amino acids were also trapped into an amyloid aggregation pathway through phenylalanine fibrils. Unique arrangement of zwitterionic phenylalanine molecules in their amyloid-like higher order entities is predicted to promote both hydrophobic and electrostatic interaction, sufficient enough to trap proteins and to preferentially interact with the membrane components of RBCs. Since the prevalence of hemolysis and psychoneurological severities are mostly observed in PKU patients, this work predicts that the inherent property of phenylalanine fibrils to trigger hemolysis and to induce protein aggregation may have direct relevance to the disease mechanism of PKU.

Effect of capsaicin coated silver nanoparticles on amyloid fibril formation of serum albumin (chapter 4: published as Anand, et al., 2016; Biochemistry)

In this chapter, an attempt has been made to target amyloid fibril formation of serum albumin protein by capsaicin- coated silver nanoparticles. Using established protocol, capsaicin-coated silver nanoparticles ($AgNPs^{Cap}$) have been synthesized and their anti-amyloid activity, considering serum albumin (BSA) as a model protein, has been tested. It was found that

amyloid formation of BSA was strongly suppressed in the presence of $AgNPs^{Cap}$ nanoparticles. However, isolated capsaicin and uncapped control nanoparticles did not show such inhibition effect. Bioinformatics analysis reveals CH-π and H-bonding interactions between capsaicin and BSA in the formation of protein-ligand complex. Native gel data have also confirmed the stabilization of native protein structures in the presence of these nanoparticles. These results suggest the significance of surface functionalization of nanoparticles with capsaicin which probably enables capsaicin to effectively interact with the key residues of the amyloidogenic core of BSA.

Effect of Piperine coated gold nano particles on amyloid fibril formation of insulin (chapter 5: published as Anand, et al., 2017 ACS-Biomaterials Science and Engineering)

In this chapter uniform, polycrystalline and thermostable piperine-coated gold nanoparticles have been synthesized to target insulin fibril assembly. Since the process of insulin fibril assembly is linked to a multitude of medical problems, finding effective and biocompatible inhibitors against such aggregation process could be beneficial. Targeting aggregation prone residues of insulin may work as an effective strategy to prevent the onset of insulin fibril assembly. In this work, thermostable gold nanoparticles ($AuNPs^{piperine}$) surface-functionalized with piperine have been synthesized to target amyloid-prone residues of insulin. It was observed that the process of both spontaneous and seed-induced amyloid formation of insulin was strongly inhibited in the presence of $AuNPs^{piperine}$. Surface functionalization of piperine was found to be critical for its inhibition effect because no such effect was observed for isolated piperine as well as for uncoated control gold nanoparticles. Fluorescence quenching data revealed binding of $AuNPs^{piperine}$ with insulin's native structure which was further validated by docking studies that predicted viable H-bond and CH-π interactions between piperine and key aggregation-prone residues of insulin's B-chain. Results obtained from hemolysis assay studies confirmed that these nanoparticles were hemocompatible. Data obtained from both experimental and computational studies suggest that the retention of native structure of insulin and the interaction of piperine with the aggregation-prone residues of insulin are the key factors for the inhibition mechanism. The findings of this work may help in the development of nanoparticle-based formulations to prevent medical problems linked to insulin aggregation.

Strategically designed antifibrotic gold nanoparticles to prevent collagen fibril formation (chapter 6: published as Anand, et al., 2017; Langmuir)

This work was performed to design nanoparticle based inhibitors against collagen fibril formation under *in vitro* conditions. Collagen is an important structural protein in human body system that provides crucial mechanical frame work for many tissues. However excess collagen fibril formation has been reported to cause many medical complications such as thrombosis, restenosis and tissue stiffness in hypertensive heart diseases. Since excess accumulation of collagen is linked to many diseases it becomes important to find potential inhibitors of collagen fibril formation and such a strategy to target collagen fibril formation may perhaps be useful in treating collagen linked diseases. The aromatic interaction is known to promote the self-association of collagen molecules to higher order structures. Hence, in this chapter, thermostable gold nanoparticles surface-functionalized with selected aromatic/hydrophobic residues ($AuNPs^{PHE}$ and $AuNPs^{TRP}$) have been synthesized and the inhibition effect of these nanoparticles on fibril formation of type I collagen has been studied using a combination of biophysical and computational methods. In addition to aromatic amino acids gold nanoparticles coated with hydroxyproline ($AuNPs^{HYP}$) and proline ($AuNPs^{PRO}$) were also synthesized. Strong inhibition effect of these functionalized nanoparticles on fibril formation of type-I collagen (both from Rat Tail Tendon and calf skin) under physiological conditions was observed. The molecular interaction between model collagen triple helical peptides and aromatic residues has

been studied using computational docking tools, which suggests a possible interaction between collagen molecules with the aromatic residues. However, surface functionalization was critical to the inhibition effect because no such inhibition effect on collagen fibril formation was observed in the presence of uncoated control nanoparticles and isolated amino acids. Further, glycine (a non-aromatic residue) coated gold nanoparticles ($AuNPs^{GLY}$) did not show any such inhibition effect. The results of this work may have direct relevance to the development of nanoparticle based inhibitors to target collagen activated pathologies.

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