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Conclusions

The main aim of this thesis was to understand the self-assembly process of selected proteins into higher order structures and to hunt for potential inhibitors against such processes. Important outcomes from this work are summarized below.

- In the first section, the effect of the process of amyloid formation of a particular protein on the aggregation properties of other proteins under *in vitro* conditions was examined. The results indicated a rapid coaggregation process among globular proteins and the kinetics of coaggregation reaction was found to be faster than the kinetics of individual reactions. This work also revealed the occurrence of cross seeding among globular proteins during amyloid formation where amyloid fibers of a protein were found to drive aggregation of other protein monomers. These results on coaggregation and cross-seeding offer new opportunities to understand the mechanism of amyloid formation and to shed light on the critical role of amyloid-linked higher-order entities such as inclusions, plaques, and Lewy bodies.

- In the second part of the first section, studies on the protective effect of type I collagen on amyloid formation of lysozyme was investigated. Strong inhibition of both spontaneous and seed-induced aggregation of lysozyme was observed in the presence of collagen. Both chemical and thermal denaturation experiments showed increased lysozyme stability in the presence of collagen. These findings confirmed that type I collagen is capable of blocking or interfering with amyloid formation of lysozyme, and the results may have significant implications for the design of collagen-based therapeutics against aggregation of disease-linked amyloidogenic proteins.

- Some natural compounds (eugenol and capsaicin) were tested against the onset of protein aggregation process. Eugenol was found to be a strong inhibitor of the amyloid formation of both insulin and serum albumin under *in vitro* conditions. Evidences obtained from both experimental and computational data indicated that stabilization of native protein structures and on-pathway oligomers may be crucial for the inhibition of amyloid formation in the presence of eugenol. Studies on capsaicin-collagen interaction clearly indicated that capsaicin is capable of suppressing the fibril formation process of type I collagen. Results suggest that capsaicin enhances the stability of collagen fibers and it protects the collagen fibers from enzymatic degradation. These results may have significant implications for capsaicin and eugenol based therapeutics against diseases linked to collagen and amyloid activated diseases.

- In the last section of this thesis, inhibitory effect of strategically synthesized nanoparticles against amyloid formation of insulin was tested. Strong inhibition of both spontaneous and seed-induced aggregation of insulin was observed in the presence of gold and silver nanoparticles coated with either tyrosine or tryptophan. Both experimental and computational work suggested that interaction between the surface-functionalized aromatic residues and the key residues of the amyloidogenic region of insulin may be crucial for the inhibition effect. The results also confirmed that surface-functionalization of these aromatic residues was vital for the inhibition mechanism

because isolated amino acids did not show any such inhibition effect. Data clearly indicate that surface functionalization of nanoparticles may offer an attractive approach for both production and formulation of effective anti-amyloid candidates.

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