Title of Thesis: A study on the fibrillation of human serum albumin in the presence of external factors

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Abstract

The *in vivo* misfolding of proteins and its consequences in amyloid fibril formation is known to be associated with the pathogenesis of severe neurodisorders such as Alzheimer's, Parkinson's, Huntington's and Prion disease. The finding that proteins and polypeptide chains can form amyloid fibrils *in vitro* with characteristics similar to that formed *in vivo* unveils the scope of further research to explore the structure and organization of amyloid fibrils. In this regard human serum albumin (HSA) has found wide usage as a suitable model for protein aggregation studies due to its propensity to easily aggregate *in vitro*. The work included in this thesis has focused on HSA fibrillation under various *in vitro* conditions in the presence of external factors.

Various spectroscopic and microscopic techniques have been employed to monitor the fibrillation propensity of HSA. UV-vis spectroscopy was used to study the congo red binding affinity of HSA aggregates and to monitor the oligomerization process of HSA via a turbidity assay. Thioflavin T (ThT) binding studies and steady state anisotropic measurements were performed by means of fluorescence spectroscopy. Circular dichroism and Fourier transform infrared studies were utilized to probe the secondary structural transitions during the HSA fibrillation process under different *in vitro* conditions. The possible coordination environment around the Cu(II) ion in HSA fibrils was monitored via electron paramagnetic resonance (EPR) measurements. The morphological evolution of HSA under various conditions was examined via fluorescence microscopy and different electron microscopic techniques. The effect of Cu(II) on HSA fibrillation has been investigated *in vitro* where Cu(II) was found to enhance HSA fibrillation with increasing metal ion concentration. Higher Cu(II) concentration shows nonspecific coordination as revealed by EPR measurements. The effect of ethanol and temperature on HSA fibrillation was examined. Ethanol promotes fibrillation of HSA as revealed by ThT binding and corroborates results from other techniques. This effect was found to be more pronounced at higher temperature. The disaggregating ability of surfactants and electric field towards preformed HSA fibrils has been investigated. Cationic surfactants were found to effectively disintegrate preformed HSA fibrils in comparison to anionic surfactant. A static electric field of strength ~8×10^6 V/m was found to be capable of disrupting preformed HSA fibrils. The inhibitory potency of sugars such as fructose, glucose, ribose and sucrose and major green tea polyphenols (GTPs) has also been looked into. Fructose was found to inhibit HSA fibrillation more effectively compared to the other sugars used. Stabilization of the native state of HSA in the presence of sugars governs the inhibition process. ECG was found to inhibit HSA fibrillation more than EGCG and EGC where hydrophobic interactions play a crucial role. The work presented in this thesis is central to the understanding of the fibrillation propensity of a model globular protein (HSA) under varying external factors and additives. Therefore, it offers productive information regarding protein aggregation in general which can help in understanding the underlying factors behind fibrillation of proteins under various conditions.

Keywords: Human serum albumin; fibrillation; Cu(II); ethanol; surfactants; sugars, polyphenols