Title: Studies on Hen egg white lysozyme aggregation in presence of additives: Binding of preformed fibrils with nucleic acids

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ABSTRACT

Misfolding of proteins into toxic aggregates known as amyloid fibrils, are associated with several neurological diseases such as Alzheimer's, Parkinson's, Huntington's, and Prion diseases. Studies have shown distinct similarities between the fibrillar systems studied *in vivo* and *in vitro* for a number of proteins. This has led to research on amyloidogenesis based on *in vitro* analysis of model systems. This thesis has investigated the different aspects of fibrillation considering hen egg white lysozyme (HEWL) as the model template.

pH based fibrillation of HEWL (pI~ 11) was carried out at pH 7 (below pI), pH 11 (near pI) and pH 12.75 (above pI). The effect of Cu(II) ion has also been examined under these conditions. The formation of HEWL fibrillar aggregates were characterized using different probes via fluorescence spectroscopy. Conformational alterations of HEWL were monitored using circular dichroism (CD) spectroscopy. Formation of HEWL oligomers was characterized via SDS-PAGE. The results show that fibrillation of HEWL at pH 7 in the presence of Cu(II) results in complete reduction of Cu(II) to Cu(I). Quantification of Cu(I) was accomplished via UV-vis spectroscopy using bathocuproine disulfonate (BCS). Electron paramagnetic resonance (EPR) and Raman spectroscopy were used to determine the oxidation state and the co-ordination environment of copper during fibrillation of HEWL. At pH 11, experimental observations suggest the presence of both Cu(II) and Cu(I). With increasing pH, the reducing environment created in the course of HEWL fibrillation at pH 7 gradually disappears at pH 12.75. This results in an interesting trend where Cu(II) exhibits a differential role in HEWL fibrillation that ranges from insignificant→prominent inhibition of fibrillation with increase in pH. Fluorescence and transmission electron microscopic (TEM) images were obtained to monitor the growth and morphology of HEWL aggregates/fibrils.

The inhibition of HEWL fibrillation was studied in the presence of green tea polyphenols (GTPs) and polyethylene glycols at alkaline pH (12.75) where (-)-epicatechin gallate (ECG) and PEG 20000 (20PEG) were found to be most effective. UV-vis, fluorescence, CD spectroscopic and fluorescence and high resolution transmission electron microscopic (HRTEM) techniques were employed to monitor the inhibition process.

Glycation of HEWL results in the formation of cross-linked oligomers most effectively in the presence of ribose. The aggregates were found to be non amyloidal in nature. For characterization of glycated HEWL aggregates fluorescence, CD, SDS-PAGE, MALDI-TOF and microscopic techniques were used. Preformed HEWL fibrils (pH 7) exhibit poorer binding affinity with nucleic acids (DNA/RNA) as compared to the *native* analogue. The binding affinity was investigated using an agarose gel based assay, fluorescence and circular dichroism spectroscopic techniques. Binding of HEWL fibrils with RNA was found to lower the availability of RNA for degradation by RNase A. From a protein chemistry standpoint, the fibrillation studies on HEWL provide a general platform which is beneficial in understanding protein aggregation phenomena under the influence of external additives.

Key words: Hen egg white lysozyme; Aggregation; Cu(II); Green tea polyphenols; Polyethylene glycols; Glycation; Nucleic acids