ABSTRACT

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Thesis Title: "To investigate the anti-amyloidogenic activity of different oxygen containing biologically active compounds against the fibrillation of bovine β-lactoglobulin"

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Protein aggregation can be merely a nuisance factor in many *in vitro* studies of proteins or it can cause major economic and technical problems in the biotechnology and pharmaceutical industries. Neurodegenerative diseases such as Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD) and Prion diseases have common molecular and cellular mechanisms including protein aggregation and inclusion body formation. The aggregates generally consist of fibers containing misfolded protein with cross β-sheet conformation, termed as amyloid. Protein stability depends on electrostatic interactions, steric interactions, hydrogen bonding and hydrophobic interactions. The instability of protein structure leads to the formation of pre-molten globule states, molten globules (MGs), partially folded intermediates and aggregates of native proteins. Several proteins like β-lactoglobulin (β-lg), α-lactalbumin, immunoglobulin, bovine serum albumin (BSA), lysozyme and insulin undergo pH-dependant conformational changes during its thermal aggregation forming amyloid fibrillar structure. In my research work I have selected a model carrier protein, bovine β-lactoglobulin (β-lg), having pH dependant opening, encapsulating property and a unique acidic pH resistivity and for its bio-availability. It has a core structural pattern formed by an α-helix and eight strands of antiparallel β-sheets. Moreover, there exists one free, highly reactive -SH group at Cys-121, buried in the hydrophobic core of the protein. It also has two tryptophan residues Trp_{19} and Trp_{61} responsible for its fluorescence properties. In order investigate the influence of small molecules on the thermal aggregation of beta-lactoglobulin (β-lg), I have employed several synthesized oxygen-nitrogen containing heterocycles like coumarin compounds, chalcones and the compounds related to neurotransmission like dopamine, phenylethyl amine and gamma aminobutyric acid **Chapter 1** consists of a brief overview of the source, isolation, purification, structural aspects, chemical modification and biological roles of β-lg. It has major contribution in the field of structural biology as β-lg, serves as a novel model protein system due to its well defined crystal structure. This chapter also deals with the formation of amyloid fibril, cause of several

neurodegenerative diseases and the various factors affecting this amyloid fibril formation due to aggregation.

Chapter 2 contains the review of literature on coumarins compounds. While **Chapter 3** involves the synthesis of five different coumarin derivatives SM0, SM1, SM2, SM3 and SM4 having substituents differing in their positions to the coumarin nucleus. I also have investigated the effect of coumarin derivatives on the structural and aggregation properties of β-lg through a multi spectroscopic and microscopic (AFM) approaches. Results shows these coumarin compounds are effective in the prevention of thermal aggregation of β-lg and the order of their inhibition is SM2> SM4> SM3> SM1> SM0.

Chapter 4 demonstrates the synthesis of the four hydroxychalcones having different substituents. It has been found that chalcones and their derivatives demonstrate a wide range of biological activities. **In chapter 5**, these compounds were tested for the monitoring of the conformational change of β-lg leading to its amyloid fibrillation process by Th-T binding assay, FT-IR, TEM and molecular docking studies. It has been observed that these compounds promotes the aggregation of β-lg in following the order HC1 > HC2 > HC3 > HC4.

Chapter 6 contains the brief accounts of three compounds dopamine, phenylethyl amine and gamma aminobutyric acid (GABA) which are related to the neurotransmmision in the CNS. We employed these compounds to monitor the inhibition and depolymerization of amyloid fibrils formed by β-lg. Our investigation involves in vitro studies ofaggregation mechanism using multi spectroscopic, microscopic, and computational approaches. Th T assay and TEM study clearly shows that DOPA inhibits completely the fibrillation of β-lg by preventing the structural change of native β-lg into the β-sheet rich fibrillar structure in a concentration dependent manner. Other two compounds PEA and GABA inhibit less effectively than DOPA following the order DOPA > PEA > GABA. Results of ANS fluorescence studies confirmed that DOPA is opposing the thermal exposure of hydrophobic patches and thus stabilizes the heat-treated β-lg structure.

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(Signature of the Supervisor date with official seal)

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