AN INVESTIGATION OF THE AGGREGATION BEHAVIOR OF SOME PROTEINS AND THEIR MODULATION BY COSOLVENTS

BY

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SUBMITTED

IN FULFILLMENT OF THE REQUIREMENTS OF THE DEGREE OF

DOCTOR OF PHILOSOPHY

TO THE

INDIAN INSTITUTE OF TECHNOLOGY DELHI

JUNE 2010

CERTIFICATE

This is to certify that the thesis entitled, "**An investigation of the aggregation behaviour of some proteins and their modulation by cosolvents**", being submitted by **Mr. Anurag Sharma** to the Indian Institute of Technology Delhi for the award of the degree of Doctor of Philosophy in Chemistry is a record of bonafide research work carried out by him. Mr. Anurag Sharma has worked under my guidance and supervision and has fulfilled the requirements for the submission of this thesis, which to my knowledge has reached the requisite standard.

The results contained in this dissertation have not been submitted in part or full to any other University or Institute for the award of any degree or diploma.

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ACKNOWLEDGEMENTS

It gives me immense pleasure and honour to thank Dr. Shashank Deep, my supervisor and Assistant Professor in the Department of Chemistry, IITD under whose guidance I have realised the dream of doing research. I am grateful to him for providing a thousand ideas for doing experiments and helping to execute them by providing state-of-the-art facilities and thought provoking discussions. I am also indebted to him for providing help and support in critical times of my career.

Dr.Bishwajit Kundu, Department of Biochemical Engineering and Biotechnology, Dr S K Khare and Dr. Siddharth Pandey deserve special thanks as my research committee members.

I would like to thank the all the past and present Heads of the Department, DRC Chairperson and all the faculty and staff members of the Department of Chemistry especially Dr. Pramit Choudhary for the thoughtful discussions.

I would like to thank all my colleagues (past and present) Unnati Ahluwalia for helping me to learn many experimental techniques, Komal Saini for the wonders of curve-fitting and discussions, Shahid Nayeem for his wisdom and support, M Ashhar I. Khan for his jovial nature, Chetan Saini, Pratibha Agarwal, Subhankar, Sandeep, Aritrika, Subroto, Brahma, Javeed, Kamala, Abhro, Maroof, Shruti, Nagaraj whose cooperation and support in one way or the other has helped me to realise this goal.

I would like to express my sincere thanks to Indian Institute of Technology Delhi for providing me financial assistance during the course of research.

Last but not the least I thank my parents for giving me a scientific and truthful thinking that has inspired me to do a good research, my grandparents for nurturing my first neurons, my brother and sister for the love and belief they have in me, my wife and

daughter on whose advent things started turning good and smoother, my friends Manoj, Manish and Shahnawaz who believed that I can do it.

I dedicate this work and thesis to my uncle Late. Sh Adesh Sharma, who was a powerful orator, quiz master of my childhood days and probably and unknowingly an inspiration to take up bio related sciences as my research field. Thank you.

Anurag Sharma
(2006CYZ8182)

ABSTRACT

The ability to control or reverse protein aggregation is vital to the production and formulation of therapeutic proteins and may be the key to prevention of a number of neurodegenerative diseases. The thesis entitled '**An investigation of aggregation behaviour of some proteins and their modulation by cosolvents**' is concerned with the understanding the mechanism of aggregation behaviour of model proteins. Thesis also deals with ways to modulate the protein environment with an eye towards minimizing its aggregation for technological use. The strategy not only includes an exploration of the structural features of the intermediates responsible for the aggregation of proteins but also an effort towards understanding the mechanism of the aggregation through different thermodynamic and kinetic tools available in literature. The main aim of this work is to develop a much needed fundamental molecular-level view of the aggregation.

The thesis has been divided into seven chapters. Chapter 1 provides a brief introduction to types of aggregation, different hypothesis proposed in literature to explain the phenomena of aggregation, structural, kinetics and thermodynamic tools used (a) to differentiate between the different mechanisms and (b) to determine various thermodynamic and kinetic parameters associated with different stages of the aggregation processes. Chapter 1 also describes the combined energy landscape of folding and aggregation, the factors affecting aggregation and a review of strategies used to inhibit the aggregation. Chapter 2 titled "Materials and Methodologies" is about protein and chemical procurement, purification as well as techniques and methodologies used in the investigation. The chapter also describes the procedures for analyzing the data. Chapter 3

(Characterization of different conformations of bovine serum albumin and their propensity to aggregate in the presence of N-cetyl-N,N,N-trimethyl ammonium bromide) lays out details of the investigation on the aggregation of BSA in presence of CTAB and establishes the role of unfolded conformation in the aggregation. Chapter 4 (Effect of the sugar and polyol additives on the aggregation kinetics of BSA in the presence of N-cetyl-N,N,N-trimethyl ammonium bromide) presents the investigation of the kinetics of the BSA-CTAB aggregation. It also describes the effect of addition of sugar and polyol additives on the aggregation of BSA-CTAB system. Based on the results, the plausible mode of actions of these additives is discussed. Chapter 5 (Effects of additives on the aggregation kinetics of the insulin) presents the investigation of aggregation kinetics of insulin at 333 K. The effect of sugar and polyol derivatives on the kinetics of aggregation was also investigated. Apart from sugar and polyol additives, this report includes the inhibitory action of some of the ayurvedic compounds used in the treatment of diabetes. Finally, a plausible mechanism for the inhibition of insulin aggregation was discussed based on the change in kinetic parameters of the protein aggregation, the change in stability data of protein as probed by change in the CD signal and viscosity data on the additive solution. Chapter 6 **(The modulation of the aggregation of Transforming Growth Factor β3 at physiological pH)** describes the inhibitory effect of additives on the aggregation of TGF-β3. In this chapter, we report the inhibitory action of some of the surfactants on the aggregation apart from other usual additives. Ionic and zwitterionic detergents can be used for breaking protein-protein interactions in such cases. Based on the action of these additives, a model of TGF-β3 aggregation has been proposed. **Chapter 7 (Summary and Future Perspectives)** contains salient features of this work.

Taken together, the findings/observation of this thesis will help develop a much needed fundamental and molecular-level view of the aggregation of proteins. In brief, it is concluded that the different conformations of protein may be involved in the aggregation depending on protein of interest. The presence of additives changes the physico-chemical properties of the system alongwith the energy barriers of various nucleation and elongation steps. This results into change in the aggregation propensity of a given protein. The factors influencing the nucleation and elongation do not necessarily work in the same direction.

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