STUDIES ON MODULATION OF GELSOLIN AMYLOIDOGENESIS *IN VITRO*

PRABHA ARYA

DEPARTMENT OF BIOCHEMICAL ENGINEERING & BIOTECHNOLOGY

INDIAN INSTITUTE OF TECHNOLOGY DELHI

JANUARY 2014

STUDIES ON MODULATION OF GELSOLIN AMYLOIDOGENESIS *IN VITRO*

By

PRABHA ARYA

Department of Biochemical Engineering and Biotechnology

Submitted in fulfillment of the requirement of the degree of

DOCTOR OF PHILOSOPHY

to the

INDIAN INSTITUTE OF TECHNOLOGY DELHI

JANUARY 2014

Certificate

This is to certify that the thesis titled **"Studies on modulation of gelsolin amyloidogenesis *in vitro*"** being submitted by Ms. Prabha Arya to the Department of Biochemical Engineering and Biotechnology, Indian Institute of Technology Delhi, New Delhi for the award of the degree of '**Doctor of Philosophy**' is a record of the bonafide research work carried out by her, prepared under our supervision in conformity with the rules and regulations of 'Indian Institute of Technology Delhi'. The research report and the results presented in the thesis have not been submitted to any other University or Institute for the award of any other degree or diploma.

Prof. Prashant Mishra

Department of Biochemical Engineering

and Biotechnology, IIT Delhi

New Delhi-110016

Date

Prof. Bishwajit Kundu

Kusuma School of Biological Sciences

IIT Delhi, New Delhi-110016

Date

Acknowledgements

I express my thanks to ***Indian Institute of Technology Delhi*** *for providing this opportunity to carry out the research at my dream institute. I am thankful towards* ***Council of scientific and Industrial research (CSIR) and University Grants Commission (UGC)*** *for providing me the funds during this tenure.*

Many sincere thanks towards my supervisors ***Prof. Prashant Mishra*** *and* ***Dr. Bishwajit Kundu*** *for their kind support, encouragement and timely check of the progress. Prof. Mishra has been a moral support and witness of various ups and down of my life during Ph.D. I will never forget his confidence in me towards completing the task successfully.*

This journey would have not completed without the kind support of ***Dr. Bishwajit Kundu****. I had regular meetings with him regarding my Ph.D. work and always found him very energetic and full of enthusiasm. He was a good critic also who made me to think in relevant direction. He inspired by discussions and interesting conversations during our meetings.*

I am very thankful to Chairperson of my SRC, first ***Prof V. S. Bisaria*** *and then* ***Prof. (Mrs.) Saroj Mishra*** *for monitoring my research work during the SRC presentation.*

I appreciate the wonderful contribution of my SRC's external expert ***Prof. Rajiv Bhat****, School of Biotechnology, JNU. I am very impressed with his profound knowledge of the field of structural Biology.*

I have many thanks towards my SRC's internal expert ***Prof. Tapan K. Chaudhary*** *who is having wide and deep knowledge of the field of proteomics. I*

assisted him in his lab classes where I found chance to interact with him and found him very enthusiastic.

I express my heartily gratitude towards microbiologists ***Mr. V. K. Ghose*** *and then* ***Mr. Sumeet Kapoor*** *who provided cultures required time to time and also suggested various microbial culture techniques. I want to thank* ***Mrs. Neera Verma*** *for her always ready to help presence in her cubicle room in library for providing reference books which helped in our course work and also data books to make a record of experiments conducted.*

I would like to thank to PA to head, ***Mrs. Suneeta Dang, Mrs. Meena Mathur, Mrs. Pushp Lata*** *and* ***Ms. Keerti*** *for being there to help me in doing all clerical work related to ordering the chemicals and equipments and other administration work. I am thankful to storekeepers namely* ***Mr. G. Prashant, Mr. Kala, Mr. Inderjeet ji*** *for processing the orders and bills of the consumable and non-consumable items bought for the research work. I am very thankful to* ***Mr. Bhagwaan Singh*** *for being there in M.Tech lab and providing me all supporting instrument and chemical I needed for my research without any hesitation. I am thankful to* ***Mr. Mehar Chand, Mr. Ram Ghulam, Mr. Salek Chand*** *and* ***Mr. Devender*** *for cleaning the lab and providing washed glassware on time. I am thankful to* ***Mr. Sitaram*** *for his kindness and providing help in non official hours when I was stuck in some bad experimental situation.*

I would like to thank my seniors ***Dr. Raju Shankarayan, Dr. (Mrs.) Bhawana Aggrawal, Dr. Bhawana Madan, Dr. (Mrs.) Richa Barnwal, Dr. (Mrs.) Meenu Chhabra*** *and specially to* ***Dr. Saurabh Bansal*** *for sharing their very important experience and knowledge based suggestions. Also I have a heartily regards for my friends from DBEB namely* ***Dr. (Mrs.) Sucharita Sen, Mrs. R. Gayathri, Dr.***

Swati Ojha, Mrs. Shweta Kamthan, Ms. Jyoti Batra and Mr. Puneet Juneja for their selfless support while troubleshooting of the work.

I am in debt of my junior Mr. Ankit Srivastava, who has been a big support in carrying out this thesis work. Also I am very thankful to Mrs. Rachana Tomar, whose straight forward attitude made me to force for self assessment and do the right things. Also I have no words to say thanks to Mr. Dushyant Garg, whose deep knowledge and tight grip on fundamentals helped me during discussion for planning correct experiment. I admire his ready to help attitude. I am also very thankful to my other juniors namely, Ms. Rishibha Sachdev, Mr. Pankaj Sharma and Mr. Jasdeep Saggranwala. I want to thank to my juniors in DBEB namely Ms. Surbhi Goel, Ms. Neeti Kalyanai, Ms. Swati Jaiswal, Ms. Pooja singh, Ms. Tenzin Kenzom for their support in various small but important work related to research. I do not have words to say thanks to my spiritual friend Ms. Prakrati Bhargava for becoming constant support during odds of my life as she has been a source of courage to just overcome the dilemma of the situations. I am thankful to my batch mate of M.Sc., Dr. Manoj Kumar, for providing valuable suggestions at times.

I am highly in debt of my husband Mr. Ram Sunil Kumar L. with whom I started second phase of my life at the mid of my Ph.D. and tried to sail on two boats out of which he took care of home when I was busy in pursuing experiments and staying in hostel for days. He had been like a pillar for me whose support I could take during odds of my Ph.D. Also I have no words to say thank to my daughter Ms. Anveshika Ram as like her father, she also supported me most by not troubling me in pursuing my experiments. I have guilt for her that I could not give that time to her for which she had the right. I am thankful

*to my brother **Mr. Rakesh Arya** who being elder to me has been playing role of leader for me whose suggestions I followed and reached at this stage. I am in debt of my parents who helped me to start this journey and my in laws whose constant support helped me in carrying out this thesis work successfully.*

*Last I would like to thank **God** for this almost impossible thing to become possible.*

Prabha Arya

ABSTRACT

Several pathological conditions in humans have been attributed to amyloid deposition. Amyloidosis is cause of number of neurodegenerative and systemic diseases like Alzheimer's disease, Creutzfeldt-Jakob disease and Parkinson's disease etc. Besides several other uncured or partially cured diseases such as AIDS, Hepatitis and cancers; amyloid diseases also remain a major nemesis to mankind. Gelsolin is an actin modulating protein which nucleates, caps and severs actin protein is abundantly present in all cells. Gelsolin amyloidosis resulting from a point mutation (D187N/Y) causes familial amyloidosis disease prevalent in Finland, Japan and southern American continent. Under *in vivo* conditions, mutant gelsolin gets cleaved through a cascade of proteases and releases an 8kDa fragment that forms amyloid deposits in the extracellular space. However, the intact gelsolin protein has not been reported to form amyloid under any condition.

Although several amyloid intervention strategies have been reported for structurally diverse proteins, the molecular mechanisms involved are not fully understood. Growing body of experimental data have reported the presence of amino acid stretches called the 'amyloidic' stretches within protein molecules. These stretches typically get exposed upon unfolding, followed by assembly with identical stretches from other protein molecules. Hence, in the present study induction of amyloidosis in the intact gelsolin protein and its amyloidogenic variants has been attempted utilizing different physico-chemical parameters. Another focus of the study was to identify small molecule modulators against gelsolin amyloidosis. For this, library of bio-active compounds was created and computationally screened against the amyloidic stretch of gelsolin protein.

The identified compounds were tested for their effect on the in vitro amyloidosis. It is shown here that the amyloid intervention of AGel (gelsolin's core amyloidogenic stretch with D187N FAF mutation) by two natural compounds, curcumin and emetine follow two distinct pathways. Curcumin accelerates the amyloid fibril formation and reduces the formation of potentially toxic oligomeric intermediates. On the contrary, emetine totally inhibits fibrillogenesis in AGel and produces non-fibrillar and non-toxic aggregates. Although both the natural compounds modulate amyloid fibrillation differently, they show remarkable reduction in amyloid toxicity in HT 22 and PC 12 cells. Based on substantial experimental evidences from multiple spectroscopic probes, atomic force microscopy, transmission electron microscopy and cytotoxicity studies on neuronal (SHS5Y) and cancerous (HeLa) cell lines, it is found that targeting the toxic stretch in proteins could modulate amyloidogenesis and hence could be an alternate strategy for preventing amyloid toxicity. The results were also confirmed on the disease-associated amyloidogenic fragment of gelsolin (*f*AGel). The identified compounds were also nanoencapsulated for increasing their solubility and bio-availability and were found giving similar results.

CONTENTS