SUPEROXIDE DISMUTASE 1 (SOD1) AND ITS MUTANTS: STUDY OF THEIR AGGREGATION PROPENSITY UNDER PHYSIOLOGICALLY RELEVANT CONDITIONS

MD. ASHHAR I. KHAN

DEPARTMENT OF CHEMISTRY

INDIAN INSTITUTE OF TECHNOLOGY DELHI

JANUARY 2016

SUPEROXIDE DISMUTASE 1 (SOD1) AND ITS MUTANTS: STUDY OF THEIR AGGREGATION PROPENSITY UNDER PHYSIOLOGICALLY RELEVANT CONDITIONS

by

MD. ASHHAR I. KHAN

Department of Chemistry

Submitted

in fulfillment of the requirements of the degree of

DOCTOR OF PHILOSOPHY

to the

INDIAN INSTITUTE OF TECHNOLOGY DELHI

January 2016

CERTIFICATE

This is to certify that the thesis entitled, "***Superoxide Dismutase 1 (SOD1) and its mutants: Study of their aggregation propensity under physiologically relevant conditions***", being submitted by Mr. **Mohammad Ashhar Iqbal Khan** to the **Indian Institute of Technology Delhi** for the award of the degree of **Doctor of Philosophy** in Chemistry is a record of bona fide research work carried out by him. Mr. Mohammad Ashhar Iqbal Khan 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.

(**Dr. Shashank Deep)**

Assoc. Professor, Department of Chemistry

Indian Institute of Technology Delhi

New Delhi-110016

LUND UNIVERSITY

Department of Biophysical Chemistry
Center for Molecular Protein Science
Mikael Akke, Professor
mikael.akke@bpc.lu.se

August 16, 2015

Indian Institute of Technology Delhi

Certificate

Mr. Mohammad Ashhar Iqbal Khan worked in my research group for a period of 22 months (August 2012–June 2014) under the Erasmus Mundus EMEA 2 scholarship programme. The work carried out by Mr. Khan is of high standard and clearly suitable for inclusion in a doctoral dissertation.

Mr. Khan is now submitting his thesis “Superoxide dismutase 1 (SOD1) and its mutants: Study of their aggregation propensity under physiologically relevant conditions” to the Indian Institute of Technology Delhi for the award of the degree of Doctor of Philosophy in Chemistry. The results contained in this dissertation have not been submitted in part or full for the award of any degree or diploma at Lund University.

Sincerely,

Mikael Akke

Postal address: Center for Molecular Protein Science, Biophysical Chemistry, Lund University, Box 124, SE-221 00 Lund, Sweden *Courier deliveries:* Getingevägen 60, SE-222 41 Lund; *Visiting address:* Sölvegatan 39, 223 62 Lund
Telephone: +46 (0)46 222 8247; *Fax:* +46 (0)46 222 4543; *E-mail:* mikael.akke@bpc.lu.se; *Website*: www.mps.lu.se

ACKNOWLEDGEMENTS

"*The truth is that the religious and the scientific processes, though involving different methods, are identical in their final aim. Both aim at reaching the most real*"

Muhammad Iqbal (Allama)

I thank the Almighty for blessing me with everything that I could have asked for.

My Ph.D. would have been a tough journey without the efforts and company of so many people to whom I am indebted, and owe a deep sense of gratitude. To begin with, I would like to acknowledge my Ph.D. supervisor Dr. Shashank Deep with immense gratitude and appreciation for his supervision and guidance. As a mentor, Dr. Shashank Deep, has brought out in himself the true meaning of a guide. I am deeply grateful to him for his inspiration, patience, continuous motivation, and oversight knowledge of my research work. His guidance helped me throughout my research tenure and writing of this thesis. I could not have speculated any better, of having an advisor for my research work than him.

I would also like to extend my deepest gratitude to my Mentor at Lund University, Sweden, Prof. Mikael Akke and co-Mentor Prof. Sara Linse. Research with them has given me the greatest opportunity to be part of their great scientific and non-scientific supervision which, I believe, very few would be lucky enough to get. In particular, Prof. Mikael for his support on and off the research and Prof. Sara for being an inspiration for

life. I am extremely thankful and indebted to them for sharing their expertise, their sincere and valuable guidance and encouragement extended to me. And I sincerely thanked them for their excellent mentorship and for making their lab an open and exciting place to work.

This work would have lost its sheen without the expertise and effort of all my collaborators over the years. I would like to convey my heartiest thank to Prof. Cecilia Emanuelsson for her generous support and guidance in chemical cross-linking experiments, to Prof. Karin Schillén for the valuable time and pain she took in conducting the DLS experiments and to Prof. Derek Logan for his help in the x-ray crystallography and Dr. Gunnel Karlsson for her assistance in TEM. I express my deepest appreciation to Prof. Sven Kjellström for his insightful discussions, feedback, support and encouragement and for giving an excellent company throughout my mass spectroscopic experiments.

I am grateful to the members of SRC for their thoughtful discussions and suggestions throughout my research. I express my humble thanks to past and the current Heads of Department and DRC chairpersons for their support. I am thankful to the Department of Chemistry and all its staff for providing an efficient infrastructure and working facilities for the research.

I would like to express my generous regards to all my colleagues and lab mates. I am thankful to my seniors Anurag sir, Komal ma'am and Unnati ma'am for their supportive nature. My heartfelt appreciation goes to Shahid sir who has always endeavored to be a strong supportive figure in all my odd and stressed situations. His

extraordinary tolerant and supportive nature has been a great pillar in my hard times. His constructive comments, warm encouragement and advices always inspires me to get going. I am thankful to Vinay, Preeti, Nidhi Kaur, Nidhi Katyal, Amrita, Brahma, Javed and Aritrika for their company and support in creating a wonderful environment in the lab. I would also thank my friends and colleagues- Ingemar, Michal, Olof, Zhiwei, Risto, Filip, Karin, Tanja, Xioating, Li, Raza, Sabeen, Shuji, Henry, Wael, Sumitha, Kalyani, Lavaniya Nadine, Saher, Martha, Birgitta and Adine at Lund University. Their everlasting support, advice and friendship have made my stay at Lund a very enjoyable experience. My special thanks and appreciation goes to Johan for inspiring me through his witty discussions to stretch myself over my limits, and for his never ending enthusiasm about life.

Being so close to the world of science and research would have never been so wonderful and intriguing, had it not been for Shivnetra's and Uli's company. Words alone cannot express their contribution to my thesis and to my personal realm. Their advices, both on and off research, have been priceless and instrumental to my success.

I thank everyone in the Dr. P.K. Chaudhary's lab for their support. Especially Ashima, Jayanta, Sanjib, Sandip and Saikat for the open and healthy discussion on scientific and non-scientific subjects and for all the lighter moments that we shared together.

Simple words of appreciation would be too less to express my feelings to all my family members for their love and patience. I really fall short of words to express how grateful I am for all of the sacrifices my parents have made to make me what I am today. Their prayer for me was what sustained me thus far. I owe and dedicate all my success to

each one of them especially my father who has been an inspiration and always incented me to strive towards my goal. My elder brother who stood as an ideal for me and was my strength and supportive of me all the time.

Many thanks to my friend Kalpana, Mohit, Prakash, Shilpi, Neha, Bikesh and Abhishek who always cheered me up and helped me stay to senses and sanity through these difficult years. My childhood friend Yusuf deserves much more than just words of appreciation. He has been an anchor of my strength and stability and a good listener with whom I have shared every rough and smooth moments, only to end with something constructive.

(Mohammad Ashhar Iqbal Khan)

ABSTRACT

The thesis entitled '***Superoxide Dismutase 1 (SOD1) and its mutants: Study of their aggregation propensity under physiologically relevant conditions***' is concerned with the understanding of the aggregation propensity of superoxide dismutase 1(SOD1) and its variants. Cu/Zn superoxide dismutase (SOD1) forms intracellular aggregates that are pathological indicators of amyotrophic lateral sclerosis (ALS) - a fatal neurodegenerative disorder that causes motor neuron degeneration in the cortex. Post translational modifications and protein aggregation inside cells occurs in a highly complex environment consisting of several salts, cosolutes and cellular surfaces, and a thorough understanding of the mechanism of aggregation of SOD1 from all such aspects, which are of profound relevance, is rigorously needed. The main aim of this work is to make a systematic study of SOD1 fibrillation by tuning some of the structural features and environmental factors including the contact surfaces which could impact its fibrillation behaviors, so as to have a better understanding on fibrillation mechanism of SOD1.

The thesis is composed of seven chapters. **Chapter 1** (***Introduction***) the main aim of this chapter is to provide an overview of the Cu/Zn superoxide dismutase (SOD1) and its involvement in the familial amyotrophic lateral sclerosis (fALS) disorder. A detail review of the current state of understanding about SOD1-mutation in ALS, their mechanism of toxicity and events that steer SOD1 aggregation is presented. **Chapter 2** (***Materials and Methodologies*)** deals with protein and chemicals procurement,

expression and purification as well as techniques used in the investigation. All the detail of expression and purification of labeled as well as unlabeled protein and experimental techniques are discussed in this chapter. **Chapter 3 (*Superoxide Dismutase forms amyloid fibrils under quiescent conditions: Intermolecular disulfide bonds and disulfide bond scrambling not required for its fibrillation*)** deals with the study and elucidation of the role of intrinsic disulfide bridge in the stability and aggregation of SOD1. To answer this elusive and controversial issue whether the intermolecular disulfide cross-linking or any abnormalities in the thiol-disulfide state of SOD1 are involved or critical in the SOD1 aggregation mechanism, we designed a cysteine free mutant ($SOD1^{NOCYS}$) that negates chances of the formation of any intermolecular-disulfide crossing in aggregates. The fibrillation kinetics of the mutant under various experimental conditions was followed and a comparison was made with the $SOD1^{PWT}$ kinetics to get a clear idea about the role of disulfide bridge in SOD1 aggregation. **Chapter 4 (*Tuning the structural features in SOD1 renders its aggregation propensity dependent on the contact surfaces*)** describes, in detail, the modulating role of contact surface in the aggregation of SOD1. A systematic study of SOD1 fibrillation on hydrophobic as well as hydrophilic surfaces is conducted to ascertain that tuning some of the structural features and environmental factors could lead to the differential behaviors on the two surfaces. Unfolding, masking of electrostatic interactions, presence or absence of functional loops, and/or intrinsic disulphide linkages and nature of contact surface acts in conjunction with each other, leading to a diverse range of aggregation propensities in SOD1. In **Chapter 5 (*Loss of conformational constrain in SOD1 lays the foundation***

for non-native interactions amongst the monomers*)** the kinetics, thermodynamics and structural aspects of the conformational exchange process during the early stage of SOD1 fibrillation was studied through a combination of CPMG relaxation dispersion profiles over the time, chemical cross-linking coupled to mass spectroscopy (CXMS), dynamic light scattering (DLS) and molecular dynamics (MD). Through CPMG relaxation dispersion profiles over the time, we measured millisecond time-scale exchange dynamics of SOD1 mutant ($SOD1^{NOCYS}$) that mimics the reduced state form. Subsequently, the size distribution of oligomers and protofibrils built up during the $SOD1^{NOCYS}$ aggregation process in NMR sample was determined by dynamic light scattering (DLS) experiments. The CXMS technique was employed for the determination of the initial interactions and the cross-talks between the SOD1 monomers during the early stage of its fibrillation. MD simulation was also conducted to validate the experimental results. **Chapter 6 (*Molecular description of the aggregates of structurally different SOD1 variants through limited proteolysis*)** deals with the information about structural segment(s) which forms the core of the SOD1 aggregates, formed under nascent conditions without shaking or stirring through limited proteolysis coupled with the MS/MS mass spectroscopy experiments. The gross morphologies of fibrils of SOD1 variants were also studied by TEM. **Chapter 7** (Conclusions and Future Perspectives*)** contains salient highlights of this work. In the nutshell, the findings of this work have thrown light on the various structural and environment factors that modulates the aggregation of SOD1. These studies will help in a better understanding of the complexity of aggregation mechanism of SOD1, and hence the ALS pathology.

CONTENTS

List of Figures

List of Tables

List of Abbreviations

SOD	Superoxide dismutase
SOD1	Superoxide dismutase 1
SOD1^{PWT}/ pwt SOD1	Pseudo wild-type Superoxide dismutase
SOD1^{NOCYS}	Superoxide dismutase without any cysteine residues
SOD1_ΔIV_ΔVII_C103S	Superoxide dismutase with truncated loop IV and VII
TCEP	Tris(2-carboxyethyl)phosphine)
ThT	Thioflavin-T dye
DLS	Dynamic light scattering
SDS-PAGE	Sodium dodecyl sulfate polyacrylamide gel electrophoresis
Non-TP	Non-treated plate (plain polystyrene plate) provide hydrophobic surface
TP	Treated plate (polyethylene glycol coated plate) provide hydrophilic surface
Gdn-HCl	Guanidine hydrochloride
GROMACS	GROningen Machine for chemical Simulation.
MD	Molecular Dynamics.
TEM	Transmission electron microscopy
pU/ p_U	Population of unfolded species.
TMD	Targeted molecular dynamics
ALS	Amyotrophic Lateral Sclerosis
BS3	Bis[sulfosuccinimidylsuberate]
fALS	Familial amyotrophic lateral sclerosis
sALS	Sporadic amyotrophic lateral sclerosis
EDTA	Ethylenediaminetetraacetic acid
α-CHCA	α -cyano-4-cinnamic acid
FPLC	Fast protein liquid chromatography
CPMG	Carr-Purcell-Meiboom-Gill (spin relaxation dispersion)
LC MALDI-TOF/TOF	Liquid Chromatography-Matrix Assisted Laser desorption ionization mass spectrometry Time of Flight
CXMS	Chemical cross-linking coupled with mass spectrometry
NMR	Nuclear magnetic resonance
UV	Ultra Violet
TIC	Total ion current
HPLC	High Performance Liquid Chromatography
wt	Wild-type