

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

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by

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Department of Chemistry

Submitted

in fulfillment of the requirements of the degree of

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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.

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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,

A handwritten signature in black ink, appearing to read 'Mikael Akke'.

Mikael Akke

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“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)

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(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.

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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/ pU	Population of unfolded species.
TMD	Targeted molecular dynamics
ALS	Amyotrophic Lateral Sclerosis
BS3	Bis[sulfosuccinimidyl]suberate]
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