

**INSIGHTS INTO THE MECHANISM OF
AGGREGATION OF SUPEROXIDE
DISMUTASE 1 (SOD1) AND ITS MUTANTS IN
THE ABSENCE AND PRESENCE OF SMALL
MOLECULES**

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INSIGHTS INTO THE MECHANISM OF AGGREGATION OF SUPEROXIDE DISMUTASE 1 (SOD1) AND ITS MUTANTS IN THE ABSENCE AND PRESENCE OF SMALL MOLECULES

by

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Submitted

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Dedicated to

The Divine Light

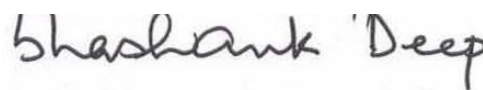
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All of Us

CERTIFICATE

This is to certify that the thesis entitled “**Insights into the mechanism of aggregation of Superoxide dismutase 1 and its mutants in the absence and presence of small molecules**”, being submitted by Ms. **Shilpa 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 her. Ms. Shilpa Sharma has worked under my guidance and supervision and has fulfilled the requirements for the submission of her 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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Shilpa Sharma

ABSTRACT

Neurodegenerative (ND) diseases are a group of progressive neurological disorders in which neurons in the brain and peripheral nervous system become dysfunctional with time and ultimately die, causing severe health issues. ND diseases affect millions of people and are the leading cause of weakness in humans; Amyotrophic Lateral Sclerosis (ALS) is one such ND disease. Aggregation of Superoxide dismutase 1 (SOD1) is associated with the pathogenesis of ALS. However, the aggregation mechanism of SOD1 is not fully understood, which is one reason we are still unable to find a cure for ALS. Targeting the steps forming toxic amyloid fibrils with small molecules has emerged as a successful strategy to treat ND diseases. This thesis entitled **“Insights into the mechanism of aggregation of Superoxide Dismutase 1 (SOD1) in the presence and absence of small molecules”** is aimed to understand the different steps involved in the formation of SOD1 aggregates and to find the small molecule inhibitor to prevent, delay or reverse this aggregation process.

The thesis has been comprised of eight chapters. In **Chapter 1** (Introduction), the phenomenon of protein folding, misfolding, protein aggregation, factors responsible for protein aggregation, and diseases related to protein aggregation was discussed in detail. Various kinetics theories were examined to understand the mechanism of protein aggregation, and strategies were mentioned for finding the inhibitors for its collection. A brief introduction of the neurodegenerative disease of interest, ALS, and the associated protein SOD1 is also provided. Further, the literature studies on the current state of understanding of SOD1 aggregation mechanism, aggregation inhibition strategies, and the potential of computer simulations in

studying these complex processes were elaborated. At the end of this chapter, the origin of the scientific problem, objectives designed for this thesis, and outline of the research work performed were discussed.

Chapter 2 (Materials and methodologies) lists the details of chemicals procured for protein aggregation-related experiments. The principles of using computational and experimental techniques have been discussed, along with the details of various performed analyses.

Chapters 3-7 present all the work done in this thesis. In **Chapter 3** (Mechanism of Aggregation of Superoxide Dismutase 1 (SOD1)), the aggregation mechanism of Superoxide dismutase 1 (SOD1) was explored employing ThT aggregation kinetics and size exclusion chromatography (SEC) experiments. Fitting the kinetics data to different models suggested that SOD1 follows a fragmentation-dependent mechanism. SEC experiments revealed that initial dimer to monomer conversion is fast compared to the total aggregation time, and aggregation proceeds expeditiously after the formation of a non-native dimer.

Chapter 4 (Computational screening criteria for selecting the aggregation inhibitors of SOD1 linked to ALS) was focused on understanding the effect of metal ions and intramolecular disulfide bonds on the structure and dynamics of SOD1 monomer through molecular dynamics simulations. Further, this chapter developed a computational strategy to predict the aggregation inhibitor of SOD1. The results revealed that removing metal ions destabilizes the protein structure incrementally and that eliminating disulfide bonds causes even more structural disruption. The study found that targeting different stages of amyloid fibril formation using polyphenols, as determined by MD simulations and experimental techniques, requires attention

not only to the binding energy of protein-inhibitor interaction but also to the stabilization of the SOD1 interface, formation of native and non-native dimers, elongation, and defibrillation of fibrils in the presence of inhibitors.

Chapter 5 (Myricetin acts as an aggregation inhibitor of SOD1) proposed myricetin as an excellent aggregation inhibitor of DTT-EDTA-induced SOD1 aggregation. We utilized the screening criterion proposed in Chapter 4, and myricetin was screened from a list of polyphenolic compounds. Our computational results suggested that myricetin binds strongly with apo disulfide reduced SOD1, stabilizes the protein interface, and binds moderately with native SOD1 dimer, Non-native SOD1 trimer, and corkscrew fibril. ThT aggregation kinetics, transmission electron microscopy, dynamic light scattering, and atomic force microscopy demonstrated that myricetin breaks preformed fibrils into shorter fibrils and forms less number of fibrils. Our seeding experiment and MD simulations show that myricetin inhibits fibril elongation, while our size exclusion chromatography experiment shed light on the depolymerization of preformed fibrils. The quenching mechanism and dissociation constant were predicted by fluorescence spectroscopy.

Chapter 6 (Dissecting the mechanism of oligomer interaction with two potent polyphenols) explored the mechanism of action of quercetin and curcumin on corkscrew SOD1 fibril, which are made up of eight chains (A-H), with each chain containing 11 amino acid residues from positions 28 to 38 of the full length SOD1 sequence. Molecular dynamics simulations were used to investigate the effects of these compounds on the fibrils. Quercetin was found to bind to a critical residue involved in the toxic amyloid formation, Trp 32, in chains D, E, and F, and

also caused the peptide chains G-H to break away from the rest of the oligomer. In contrast, curcumin is bound to the hydrophobic amino acids of chains B-H, stabilizing the fibril rather than destabilizing it. MM/PBSA analysis was carried out to predict the binding affinity of quercetin and curcumin with corkscrew SOD1 fibril. The simulation results are in accordance with previous experimental results which showed that only quercetin disrupts the pre-formed fibril, not curcumin.

Chapter 7 (Computational alanine scanning mutagenesis of the zinc-binding loop and electrostatic loops of SOD1) cantered to understand the role of each amino acid of zinc binding and electrostatic loops through virtual alanine scanning mutagenesis. It was observed that H71, R79, D125, and S134 are four critical residues that affect the protein structure and function significantly in fully metallated and disulfide bind intact dimer. Analysis revealed that observed mutations caused disordering in the loops due to the loss of native contacts and disruption of extended H-bond networks.

In brief, **Chapter 8** (Conclusion and future perspectives) provided an overview of the research. Essentially, our discoveries have been critical in comprehending the process of SOD1 aggregation and how employing small molecules to target various stages of the aggregation can be utilized to develop treatments for ALS associated with SOD1 aggregation.

सार

न्यूरोडीजेनेरेटिव (एनडी) रोग प्रगतिशील न्यूरोलॉजिकल विकारों का एक समूह है जिसमें मस्तिष्क और परिधीय तंत्रिका तंत्र में न्यूरोन्स समय के साथ खराब हो जाते हैं और अंततः मर जाते हैं, जिससे गंभीर स्वास्थ्य समस्याएं होती हैं। एनडी रोग लाखों लोगों को प्रभावित करते हैं और मनुष्यों में कमजोरी का प्रमुख कारण हैं; एमियोट्रोफिक लेटरल स्क्लेरोसिस (एएलएस) एक ऐसी एनडी बीमारी है। सुपरऑक्साइड डिसम्यूटेस 1 (SOD1) का एकत्रीकरण ALS के रोगजनन से जुड़ा है। हालाँकि, SOD1 का एकत्रीकरण तंत्र पूरी तरह से समझा नहीं गया है, जो एक कारण है कि हम अभी भी ALS का इलाज खोजने में असमर्थ हैं। छोटे अणुओं के साथ जहरीले अमाइलॉइड फाइब्रिल बनाने वाले कदमों को लक्षित करना एनडी रोगों के इलाज के लिए एक सफल रणनीति के रूप में उभरा है। "छोटे अणुओं की उपस्थिति और अनुपस्थिति में सुपरऑक्साइड डिसम्यूटेस 1 (SOD1) के एकत्रीकरण के तंत्र में अंतर्दृष्टि" शीर्षक वाली इस थीसिस का उद्देश्य SOD1 समुच्चय के निर्माण में शामिल विभिन्न चरणों को समझना और रोकने, देरी करने के लिए छोटे अणु अवरोधक का पता लगाना है। या इस एकत्रीकरण प्रक्रिया को उलट दें।

थीसिस को आठ अध्यायों में शामिल किया गया है। अध्याय 1 (परिचय) में प्रोटीन फोल्डिंग, मिस फोल्डिंग, प्रोटीन एग्रीगेशन, प्रोटीन एग्रीगेशन के लिए जिम्मेदार कारकों और प्रोटीन एग्रीगेशन से संबंधित बीमारियों की घटना पर विस्तार से चर्चा की गई थी। प्रोटीन एकत्रीकरण के तंत्र को समझने के लिए विभिन्न कैनेटीक्स सिद्धांतों की जांच की गई और इसके संग्रह के लिए अवरोधकों को खोजने के लिए रणनीतियों का उल्लेख किया गया। रुचि के न्यूरोडीजेनेरेटिव रोग, ALS, और संबद्ध प्रोटीन SOD1 का संक्षिप्त परिचय भी दिया गया है। इसके अलावा, SOD1 एकत्रीकरण तंत्र, एकत्रीकरण निषेध रणनीतियों और इन जटिल प्रक्रियाओं

का अध्ययन करने में कंप्यूटर सिमुलेशन की क्षमता की वर्तमान स्थिति पर साहित्य अध्ययन विस्तृत थे। इस अध्याय के अंत में वैज्ञानिक समस्या की उत्पत्ति, इस थीसिस के लिए निर्धारित उद्देश्यों और किए गए शोध कार्य की रूपरेखा पर चर्चा की गई। अध्याय 2 (सामग्री और कार्यप्रणाली) में प्रोटीन एकत्रीकरण से संबंधित प्रयोगों के लिए खरीदे गए रसायनों का विवरण सूचीबद्ध है। कम्प्यूटेशनल और प्रायोगिक तकनीकों का उपयोग करने के सिद्धांतों पर विभिन्न प्रदर्शन किए गए विश्लेषणों के विवरण के साथ चर्चा की गई।

अध्याय 3-7 इस थीसिस में किए गए सभी कार्यों को एकत्र करता है। अध्याय 3 (सुपरऑक्साइड डिसम्यूटेस 1 (SOD1) के एकत्रीकरण की क्रियाविधि) में, ThT एकत्रीकरण कैनेटीक्स और आकार अपवर्जन क्रोमैटोग्राफी (SEC) प्रयोगों को नियोजित करके सुपरऑक्साइड डिसम्यूटेज़ 1 (SOD1) के एकत्रीकरण तंत्र का पता लगाया गया। कैनेटीक्स डेटा को विभिन्न मॉडलों में फिट करने का सुझाव दिया गया है कि SOD1 एक विखंडन-निर्भर तंत्र का अनुसरण करता है। एसईसी प्रयोगों से पता चला है कि मोनोमर रूपांतरण के लिए प्रारंभिक डिमर कुल एकत्रीकरण समय की तुलना में तेज है, और गैर-देशी डिमर के गठन के बाद एकत्रीकरण तेजी से आगे बढ़ता है। अध्याय 4 (एएलएस से जुड़े SOD1 के एकत्रीकरण अवरोधकों के चयन के लिए कम्प्यूटेशनल स्क्रीनिंग मानदंड) आणविक गतिशीलता सिमुलेशन के माध्यम से SOD1 मोनोमर की संरचना और गतिशीलता पर धातु आयनों और इंट्रामोलेकुलर डाइसल्फ़ाइड बांड के प्रभाव को समझने पर केंद्रित था। इसके अलावा, इस अध्याय ने SOD1 के एकत्रीकरण अवरोधक की भविष्यवाणी करने के लिए एक कम्प्यूटेशनल रणनीति विकसित की। परिणामों से पता चला कि धातु के आयनों को हटाने से प्रोटीन संरचना तेजी से अस्थिर हो जाती है और यह कि डाइसल्फ़ाइड बॉन्ड को नष्ट करने से और भी अधिक संरचनात्मक व्यवधान होता है। अध्ययन में पाया गया कि एमडी सिमुलेशन और प्रयोगात्मक

तकनीकों द्वारा निर्धारित पॉलीफेनोल्स का उपयोग करके एमिलॉयड फाइब्रिल गठन के विभिन्न चरणों को लक्षित करने के लिए न केवल प्रोटीन-अवरोधक बातचीत की बाध्यकारी ऊर्जा पर ध्यान देने की आवश्यकता है बल्कि एसओडी 1 इंटरफ़ेस के स्थिरीकरण, देशी और के गठन पर भी ध्यान देने की आवश्यकता है। अवरोधकों की उपस्थिति में गैर-देशी डिमर, बढ़ाव और तंतुओं का डिफिब्रिलेशन।

अध्याय 5 (माइरिकेटिन SOD1 के एकत्रीकरण अवरोधक के रूप में कार्य करता है) ने Myricetin को DTT-EDTA-प्रेरित SOD1 एकत्रीकरण के एक उत्कृष्ट एकत्रीकरण अवरोधक के रूप में प्रस्तावित किया। हमने अध्याय 4 में प्रस्तावित स्क्रीनिंग मानदंड का उपयोग किया, और पॉलीफेनोलिक यौगिकों की सूची से माइरिकेटिन की जांच की गई। हमारे कम्प्यूटेशनल परिणामों ने सुझाव दिया कि माइरिकेटिन एपो डाइसल्फ़ाइड-कम SOD1 के साथ दृढ़ता से बांधता है, प्रोटीन इंटरफ़ेस को स्थिर करता है, और मूल SOD1 डिमर, गैर-देशी SOD1 ट्रिमर और कॉर्कस्कू फ़िब्रिल के साथ मध्यम रूप से बांधता है। ThT एकत्रीकरण कैनेटीक्स, ट्रांसमिशन इलेक्ट्रॉन माइक्रोस्कोपी, डायनेमिक लाइट स्कैटरिंग, और एटॉमिक फोर्स माइक्रोस्कोपी ने प्रदर्शित किया कि माइरिकेटिन पूर्वनिर्मित तंतुओं को छोटे तंतुओं में तोड़ता है और तंतुओं की कम संख्या बनाता है। हमारे सीडिंग प्रयोग और एमडी सिमुलेशन से पता चलता है कि माइरिकेटिन फाइब्रिल बढ़ाव को रोकता है, जबकि हमारे आकार अपवर्जन क्रोमेटोग्राफी प्रयोग पूर्वनिर्मित तंतुओं के अपचयन पर प्रकाश डालते हैं। प्रतिदीप्ति स्पेक्ट्रोस्कोपी द्वारा शमन तंत्र और पृथक्करण स्थिरांक की भविष्यवाणी की गई थी। अध्याय 6 (दो शक्तिशाली पॉलीफेनोल्स के साथ ओलिगोमेर इंटरैक्शन के तंत्र को विच्छेदित करना) सह पर केरसेटिन और करक्यूमिन की क्रिया के तंत्र का पता लगाया आरकेस्कू एसओडी1 फाइब्रिल, जो आठ श्रृंखलाओं (ए-एच) से बने होते हैं, जिसमें प्रत्येक श्रृंखला में पूर्ण लंबाई वाले एसओडी1 अनुक्रम के 28 से 38 पदों से 11 अमीनो एसिड अवशेष होते हैं। तंतुओं पर इन यौगिकों के

प्रभावों की जांच के लिए आणविक गतिकी सिमुलेशन का उपयोग किया गया था। केरसेटिन को जहरीले अमाइलॉइड गठन, टीआरपी 32, श्रृंखला डी, ई, और एफ में शामिल एक महत्वपूर्ण अवशेषों से बाँधने के लिए पाया गया था, और पेप्टाइड श्रृंखला जीएच को बाकी ओलिगोमर से अलग करने का कारण बना। इसके विपरीत, कर्क्यूमिन श्रृंखला बी-एच के हाइड्रोफोबिक अमीनो एसिड से बंधा होता है, जो फाइब्रिल को अस्थिर करने के बजाय स्थिर करता है। कॉर्कस्कू SOD1 फाइब्रिल के साथ केरसेटिन और कर्क्यूमिन के बंधन संबंध की भविष्यवाणी करने के लिए MM/PBSA विश्लेषण किया गया था। अध्याय 7 (जिंक-बाइंडिंग लूप और SOD1 के इलेक्ट्रोस्टैटिक लूप का कम्प्यूटेशनल एलेनिन स्कैनिंग म्यूटाजेनेसिस) वर्चुअल एलेनिन स्कैनिंग म्यूटाजेनेसिस के माध्यम से जिंक बाइंडिंग और इलेक्ट्रोस्टैटिक लूप के प्रत्येक अमीनो एसिड की भूमिका को समझने के लिए केंटर किया गया। यह देखा गया कि H71, R79, D125, और S134 चार महत्वपूर्ण अवशेष हैं जो प्रोटीन संरचना को प्रभावित करते हैं और पूरी तरह से मेटालेटेड और डाइसल्फ़ाइड बाइंड इंटेक्ट डिमर में महत्वपूर्ण रूप से कार्य करते हैं। विश्लेषण से पता चला है कि मूल संपर्कों के नुकसान और विस्तारित एच-बॉन्ड नेटवर्क के विघटन के कारण देखे गए म्यूटेशन लूप में विकार पैदा करते हैं।

संक्षेप में, अध्याय 8 (निष्कर्ष और भविष्य के दृष्टिकोण) ने शोध का अवलोकन प्रदान किया। अनिवार्य रूप से, हमारी खोजें SOD1 एकत्रीकरण की प्रक्रिया को समझने में महत्वपूर्ण रही हैं और एकत्रीकरण के विभिन्न चरणों को लक्षित करने के लिए छोटे अणुओं को कैसे नियोजित किया जा सकता है, इसका उपयोग SOD1 एकत्रीकरण से जुड़े ALS के लिए उपचार विकसित करने के लिए किया जा सकता है।

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ABBREVIATIONS

ALS	Amyotrophic Lateral Sclerosis
fALS	Familial Amyotrophic Lateral Sclerosis
sALS	Sporadic Amyotrophic Lateral Sclerosis
SOD1	Superoxide Dismutase 1
MD	Molecular Dynamics
ER	Endoplasmic Reticulum
FRET	Fluorescence Resonance Energy Transfer
PDB	Protein Data Bank
TARDBP	Transactive Response DNA-Binding Protein
FUS	Fused in Sarcoma
ROS	Reactive Oxygen Species
AD	Alzheimer's disease
UV	Ultra Violet
NMR	Nuclear Magnetic Resonance
CD	Circular Dichroism
ANS	1-anilinonaphthalene-8-sulphonate
SEC	Size Exclusion Chromatography
DLS	Dynamic Light Scattering

SDS	Sodium Dodecyl Sulphate
PAGE	Polyacrylamide Gel Electrophoresis
ThT	Thioflavin T
DTT	Dithiothreitol
EDTA	Ethylenediaminetetraacetic acid
BO	Born-Oppenheimer
PME	Particle Mesh Ewald
AMBER	Assisted Model Building and Energy Refinement
CHARMM	Chemistry at HARvard Molecular Mechanics
GROMOS	GRoningen MOlecular Simulation
OPLS	Optimized Potentials for Liquid Simulations
GAFF	General AMBER force field
TIP3P	Transferable Intermolecular Potential with 3 Points
SPC	Simple Point-Charge
LJ	Lennard-Jones
SD	Steepest Descent
CG	Conjugate Gradient
PBC	Periodic Boundary Condition
RMSD	Root Mean Square Deviation
R_g	Radius of Gyration

RMSF	Root Mean Square Fluctuation
FES	Free Energy Surface
SASA	Solvent Accessible Surface Area
DSSP	Dictionary of Secondary Structure of Protein
LB	Luria Broth
NTA	Nitrilotriacetic acid
WT	Wild Type
Que	Quercetin
Gens	Genistein
Gal	Galangin
PTM	Posttranslational Modifiactions
CNS	Central Nervous System
Myr	Myricetin
MM-PBSA	Molecular Mechanics Poisson-Boltzmann Surface Area