MECHANISTIC INSIGHTS INTO THE EFFECT OF TREHALOSE ON STABILIZATION AND AGGREGATION OF PROTEINS

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by

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Submitted

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to the

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To My Parents

CERTIFICATE

This is to certify that the thesis entitled "**Mechanistic insights into the effect of trehalose on stabilization and aggregation of proteins",** being submitted by Ms. **Nidhi Katyal** 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. Nidhi Katyal 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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(Nidhi Katyal)

ABSTRACT

Amyloid fibrils are aberrantly associated with a plethora of pathological neurodegenerative diseases. This fibrillation process is believed to be initiated by partial misfolding of proteins and proceeds via oligomer formation. Thus, understanding the mechanism by which any known inhibitor stabilizes the native state of the protein and/or impedes the assembly process is imperative for the design of new therapeutic drugs. Amidst various additives, trehalose has emerged as a promising cosolvent with GRAS status and wide-ranging applications in preventing unfolding, stress intolerance etc. However, its mechanism of action still awaits an adequate description. Various theories proposed in literature have elements of contrasting features. Additionally, the literature is also deficit in the database that sheds light on its efficacy in impeding aggregation pathway and onto intrinsically disordered proteins. Thus, in this thesis entitled **'Mechanistic insights into the effect of trehalose on stabilization and aggregation of protein'**, we have delved into each of these aspects at a reasonable atomic level using molecular dynamics simulations. Experimental validation is also done in cases where experimental data were not available.

The thesis is composed of seven chapters. **Chapter 1** (Introduction) provides an overview of protein aggregation, its repercussions, strategies to curb the process with a detailed review of osmolytes and special emphasis on "Trehalose" and the apparent contradiction in the literature about its mechanism of action. The chapter finally discusses the origin of the problem in the context of this thesis and the outline of the present research problem. **Chapter 2** (Materials and Methodologies) describes the theory of molecular dynamics simulations, the details of procurement of chemicals, and methodologies used. **Chapter 3** (Scrutinizing trehalose's interaction with peptides of different nature) is aimed at discerning the plausible interaction motifs of trehalose and comparing its induced perturbation on

structural and dynamical properties of different solution species. The dissection of the interaction energy profiles of acidic, basic, neutral, hydrophilic and hydrophobic peptides with trehalose suggests a stronger preference of trehalose to interact with acidic amino acids. **Chapter 4** (Revisiting the conundrum of trehalose stabilization) focuses on the controversial dichotomy between direct and indirect interactions of trehalose with protein, and their relevance in driving the folding-unfolding equilibria. We propose that direct interactions mainly rule the mechanistic action of trehalose thereby providing relative stabilization to native state of protein rather than relative destabilization of the denatured state. **Chapter 5** (Inhibition of GNNQQNY prion peptide aggregation by trehalose: a mechanistic view) delves into the way trehalose inhibits the oligomerization phenomenon of "GNNQQNY" peptide fragment from the yeast prion protein SUP35. It interacts with the hydrophilic amino acids, delays the encounter of different peptides, and inhibits the interchain β-sheet formation. The expelled water molecules during the process have larger translational movement, suggesting entropy factor to play a role in the presence of trehalose. **Chapter 6** (Introspecting trehalose's intervention into the aggregation pathway of α-synuclein) provides insights into the way trehalose affects the fibrillation process of intrinsically disordered protein "α-synuclein", which is associated with the etiology of Parkinson's disease. It expedites onset of aggregation by stabilizing the aggregation-prone "extended" conformer of α-synuclein yet the total fibrils formed are still promisingly less since it accelerates the competing pathway toward the formation of amorphous aggregates. **Chapter 7** (Summary and Future Perspectives) presents the highlights of the study. In a nutshell, our findings have been instrumental in understanding the mechanistic role of trehalose at different stages of protein aggregation. The information might also be helpful in designing new therapeutics and to *a priori* predict trehalose's action on different proteins.

सार

एमिलॉयड फाइब्रिल, पैथोलॉजिकल न्यूरोडेजेनरेटिव बीमारियों के साथ काफी हद तक जुड़े हुए हैं। माना जाता है कि यह फाइब्रिलेशन प्रक्रिया प्रोटीन के आंशिक मिस्फोल्डिंग द्वारा शुरू की जाती है और ओलिगोमर गठन के माध्यम से आगे बढ़ती हैं। इस प्रकार, तंत्र को समझना जिसके द्वारा कोई भी ज्ञात अवरोधक किस प्रकार प्रोटीन की मूल स्थिति को स्थिर करता है और/या असेंबली प्रक्रिया में बाधा डालता है, नई चिकित्सीय दवाओं के डिजाइन के लिए अनिवार्य है। विभिन्न ऐडटिव के बीच, ट्रेहलोस GRAS स्थिति के साथ एक आशाजनक कोसोल्वेंट के रूप में उभरा है और इसके व्यापक अनुप्रयोग है जैसे प्रोटीन को खुलने न देना, तनाव असहिष्णुता को रोकना आदि। हालांकि, कार्रवाई की इसकी व्यवस्था अभी भी एक पर्याप्त विवरण का इंतजार कर रहा है। साहित्य में प्रस्तावित विभिन्न सिद्धांतों में विरोधाभासी विशेषताओं के तत्व हैं। इसके अतिरिक्त, साहित्य डेटाबेस में भी घाटा है जो एकत्रीकरण पथ को रोकने और आंतरिक रूप से विकृत प्रोटीन पर अपनी प्रभावकारिता पर प्रकाश डालता है। इस प्रकार, इस सिद्धांत में 'प्रोटीन के स्थिरीकरण और एकत्रीकरण पर ट्रेहलोस के प्रभाव में मैकेनिकल अंतर्दृष्टि' नामक थीसिस, हमने आणविक गतिशीलता सिमुलेशन का उपयोग करके उचित परमाणु स्तर पर इन पहलुओं में से प्रत्येक को हल किया है। प्रायोगिक सत्यापन भी उन मामलों में किया गया है जहां प्रयोगात्मक डेटा उपलब्ध नहीं था।

थीसिस सात अध्यायों से बना है। अध्याय 1 (परिचय) प्रोटीन एकत्रीकरण, इसके प्रतिक्रियाओं, ओस्मोलाइट्स की विस्तृत समीक्षा और "ट्रेहलोस" पर विशेष जोर और कार्रवाई के तंत्र के बारे में साहित्य में स्पष्ट विरोधाभास के साथ प्रक्रिया को रोकने के लिए रणनीतियों का एक सिंहावलोकन प्रदान करता है। अध्याय अंततः इस थीसिस के संदर्भ में समस्या की उत्पत्ति और वर्तमान शोध समस्या की रूपरेखा पर चर्चा करता है। अध्याय 2 (सामग्री और पद्धतियां) आण्विक गतिशीलता सिमुलेशन, रसायनों की खरीद के विवरण, और उपयोग की जाने वाली पद्धतियों के सिद्धांत का वर्णन करती हैं। अध्याय 3 (विभिन्न प्रकृति के पेप्टाइड्स के साथ ट्रेहलोस की बातचीत को जांचना) का उद्देश्य ट्रेहलोस के व्यावहारिक परस्पर क्रिया की प्रकृति को समझना और मिश्रण के विभिन

प्रजातियों के संरचनात्मक और गतिशील गुणों पर इसके प्रभाव की तुलना करना है। ट्रेहलोस के साथ अम्लीय, मूल, तटस्थ, हाइड्रोफिलिक और हाइड्रोफोबिक पेप्टाइड्स की इंटरैक्शन ऊर्जा प्रोफाइल का विच्छेदन अम्लीय एमिनो एसिड के साथ बातचीत करने के लिए ट्रेहलोस की एक मजबूत वरीयता का सुझाव देता है। अध्याय 4 (ट्रेहलोस स्थिरीकरण के पहेली की समीक्षा) प्रोटीन के साथ ट्रेहलोस के प्रत्यक्ष और अप्रत्यक्ष बातचीत के बीच विवादास्पद विरोधाभास पर केंद्रित है, और फोल्डिंग-अनफोल्डिंग एक्विलिब्रिअ ड्राइविंग में उनकी प्रासंगिकता। हम प्रस्ताव देते हैं कि प्रत्यक्ष अंतःक्रिया मुख्य रूप से ट्रेहलोस की यांत्रिक क्रिया पर शासन करती है जिससे डेनिचरड राज्य के सापेक्ष अस्थिरता के बजाय प्रोटीन की मूल स्थिति को सापेक्ष स्थिरीकरण प्रदान किया जाता है। अध्याय 5 (ट्रेहलोस द्वारा GNNQQNY प्रायन पेप्टाइड एकत्रीकरण का अवरोध: एक यांत्रिक दृश्य) ट्रेहलोस खमीर प्रिये प्रोटीन SUP35 से "GNNQQNY" पेप्टाइड खंड की ओलिगोमेरएजैशन घटना को रोकता है। यह हाइड्रोफिलिक एमिनो एसिड के साथ बातचीत करता है, विभिन्न पेप्टाइड्स के मुठभेड़ में देरी करता है, और इंटरचैन β-sheet गठन को रोकता है। प्रक्रिया के दौरान निष्कासित पानी के अणुओं में बड़े अनुवादक आंदोलन होते हैं, जो ट्रेहलोस की उपस्थिति में भूमिका निभाने के लिए एन्ट्रॉपी कारक का सुझाव देते हैं। अध्याय 6 (α-synuclein के एकत्रीकरण मार्ग में ट्रेहलोस के हस्तक्षेप को आत्मनिर्भर करने) अंतर्दृष्टि प्रदान करता है जिस तरह से ट्रेहलोस आंतरिक रूप से विकृत प्रोटीन "α-synuclein" की फाइब्रिलेशन प्रक्रिया को प्रभावित करता है, जो पार्किंसंस रोग की ईटियोलॉजी से जुड़ा हुआ है। यह α-synuclein के एकत्रीकरण-प्रवण "विस्तारित" अनुरूपता को स्थिर करके एकत्रीकरण की शुरुआत को तेज करता है, फिर भी गठित कुल फाइब्रिल अभी भी कम से कम कम हैं क्योंकि यह असंगत समेकन के गठन की दिशा में प्रतिस्पर्धी मार्ग को गति प्रदान करता है। अध्याय 7 (सारांश और भविष्य के दृष्टिकोण) अध्ययन की मुख्य विशेषताएं प्रस्तुत करते हैं। संक्षेप में, हमारे निष्कर्ष प्रोटीन एकत्रीकरण के विभिन्न चरणों में ट्रेहलोस की यांत्रिक भूमिका को समझने में महत्वपूर्ण भूमिका निभा चुके हैं। जानकारी नए चिकित्सकीय डिजाइन करने और पूर्व प्रोटीन पर ट्रेहलोस की कार्रवाई की भविष्यवाणी करने में भी मददगार हो सकती है।

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ALS	Amyotrophic lateral sclerosis
AD	Alzheimer's disease
ANS	1-anilinonaphthalene-8-sulphonate
APS	Ammonium persulfate
BEMD	Bias exchange metadynamics
CD	Circular dichroism
DSSP	Dictionary of secondary structure of proteins
DLS	Dynamic light scattering
ER	Endoplasmic reticulum
EGCG	Epigallocatechin gallate
FEL	Free energy landscape
FTIR	Fourier-transform infrared
GB	Generalized Born
GROMACS	GROningen machine for chemical simulation
GdnHCl	Guanidinium hydrochloride
GRAS	Generally regarded as safe
IDP	Intrinsically disordered protein
ITC	Isothermal titration calorimetry
IPTG	Isopropyl-β-D-1-thiogalactopyranosid
LINCS	Linear constraint solver
LJ	Lennard Jones
MD	Molecular dynamics
MSD	Mean square displacement
MM-PBSA	Molecular mechanics poisson Boltzmann surface area
NP	Nucleation polymerization
NMR	Nuclear magnetic resonance
NCC	Nucleated conformational conversion

PAGE	Polyacrylamide gel electrophoresis
PD	Parkinson’s disease
PBC	Periodic boundary conditions
PME	Particle mesh ewald
PCA	Principal component analysis
pdb	Protein data bank
QENS	Quasi-elastic neutron scattering
RMSD	Root mean square deviations
RMSF	Root mean square fluctuations
RDF	Radial distribution function
R_g	Radius of gyration
REMD	Replica exchange molecular dynamics
SOD	Superoxide dismutase
SDS	Sodium dodecyl sulphate
SPC	Simple point charge
SASA	Solvent accessible surface area
TMAO	Trimethylamine N-oxide
TIP	Transferable interaction potential
ThT	Thioflavin T
TEMED	Tetramethylethylenediamine
TEM	Transmission electron microscopy
TFE	Trifluoroethanol
UPS	Ubiquitin-proteasome system
UV	Ultraviolet
VMD	Visual molecular dynamics