STUDY OF DIALYSIS-RELATED B2-MICROGLOBULIN AMYLOID FIBRILLATION AND ITS INHIBITION

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by

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Dedicating to my dear family, my teachers, and my friends

for their everlasting love and support.

CERTIFICATE

This is to certify that the thesis entitled **"Study of dialysis-related β2-microglobulin amyloid fibrillation and its inhibition"** being submitted by **Mr. Devanshu Mehta** to the **Kusuma School of Biological Sciences, Indian Institute of Technology Delhi** for the award of the degree of **"Doctor of Philosophy"** is a record of the bonafide research work carried out by him, prepared under my supervision, in conformity with the rules and regulations of the 'Indian Institute of Technology Delhi'. The research report and the results present in the thesis have not been submitted to any other University or Institute for the award of any other degree or diploma.

Date:

Place:

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"Hope begins in the dark, the stubborn hope that if you just show up and try to do the right thing, the dawn will come. You wait and watch and work: you don't give up."

– Anne Lamott

When I joined my Ph. D., I had a very different view of a Ph.D. research. I had not, at all, anticipated the extreme limits to which the physical, emotional, and mental capabilities of an individual can get appraised during this time. I believe HOPE was, has been and still is one of my closest companions and my strongest source of motivation. Being here in this moment makes me feel proud to know that I have contributed, albeit an extremely humble, yet a portion of my research to the science. Ph.D. has been an extensive, involving, and evolving journey. With time and energy that I invested through all these years, I believe that now I stand relatively wiser, more composed, and more patient than before. And this all would not have been possible if I had not gotten the trust, love, support, and guidance from some very important people around me.

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Devanshu Mehta

Abstract

In the present work, the *in vitro* process of β2 microglobulin (β2m) amyloid fibrillation and the molecular insights into its inhibition orchestrated by a rationally designed peptidomimetic have been investigated. β2m protein is involved in dialysis-related amyloidosis (DRA), a protein misfolding disorder that affects renal failure patients when subjected to prolonged hemodialysis. DRA involves the accumulation of long fibrillar aggregates (called amyloids) of β2m specifically into the musculoskeletal system of the patients. The deposition of β2m amyloids results in the inflammation of joints and bones, followed by their degradation causing chronic pain. Treatment methods currently available to manage DRA include surgery to remove amyloid deposits, medications for symptomatic relief, improved dialysis columns for β2m removal from the patient's serum, and kidney replacement therapy. However, these methods are plagued with the high cost and low success and only provide palliative care to the patients. With no drug-based treatment method currently available for managing DRA, our study explores the potential of a rationally designed peptidomimetic against *in vitro* process of β2m amyloid fibrillation.

Peptidomimetics are a class of molecules that may constitute a molecular scaffold intrinsically employed in their structure to provide them a specific overall structure. Such specific conformation can help a peptidomimetic mimic the PPI interfaces of species which are typically generated during the amyloid fibrillation process and help interfere with amyloid-competent intermolecular associations (Haridas *et al.*, 2012; Pelay-Gimeno *et al.*, 2015; Srivastava *et al.*, 2017; Goyal *et al.*, 2017; Singh *et al.*, 2021). The $B(LVI)_2$ peptidomimetic investigated in this study constitutes a centrally located, hydrophobic bispidine moiety to which hydrophobic amino acids leucine (L), valine (V), and isoleucine (I) have been attached on both sides. The bispidine scaffold provides $B(LVI)_2$ an overall structure that mimics the PPI interfaces typically observed during amyloid fibrillation. With its intrinsic hydrophobic nature and a β-strand conformation, $B(LVI)_2$ was hypothesized to interfere with hydrophobic, amyloid-competent, β-sheet driven intermolecular associations among β2m amyloid precursor species to inhibit the formation of long and ordered amyloid-like fibrils.

This thesis investigates and introduces a peptidomimetic-based approach that may help develop future therapeutics against DRA and amyloidogenesis in general.

The first part of this study explores the effect of $B(LVI)_2$ against *in vitro* developed process of β2m amyloid fibrillation. Thioflavin-T, SDS-PAGE, and transmission electron microscopy analysis show that $B(LVI)_2$ significantly reduces the yield and extends the time required to generate long and straight amyloid-like β2m fibrils in a dose-dependent manner. β2m is observed to develop into morphologically distinct short and straight amyloid-like fibril species in the presence of $B(LVI)_2$, indicative of an altered route from typical β2m amyloid fibrillation. The second part of this study delves into the molecular mechanism of $B(LVI)_2$-effected inhibition of β2m amyloid fibrillation. In the absence of $B(LVI)_2$, circular dichroism data shows that β2m species undergo typical β-sheet-rich amyloid-competent conformational rearrangements. However, in the presence of $B(LVI)_2$, β2m species exist in a non-amyloidogenic, non-β-sheet containing conformation indicative of β2m incompetence to undergo required conformational rearrangements for generation of control-like long and ordered amyloid-like fibrils. $B(LVI)_2$ is further observed using dynamic light scattering to interfere with the amyloid-competent intermolecular associations among β2m species. Our study demonstrates that the long and straight β2m amyloid-like fibrils are cytotoxic to human synovium cell lines, while the morphologically distinct $B(LVI)_2$-induced non-β-sheet containing soluble, non-fibrillar β2m species and β-sheet rich, insoluble, fibrillar β2m species are found to be non-cytotoxic. $B(LVI)_2$ peptidomimetic is non-cytotoxic at lower concentrations (250 μM) which, as per DRA relevant serum β2m levels, may prove promising for future study under *in vivo* setup.

Overall, this thesis reports the anti-fibrillation property of a novel rationally designed peptidomimetic '$B(LVI)_2$' against dialysis-related β2m amyloid fibrillation, which may help bring a potential shift in therapeutic approaches against DRA.

सार

वर्तमान कार्य में, β2 माइक्रोग्लोबुलिन (β2m) अमाइलॉइड फ़िब्रिलेशन की इन विट्रो प्रक्रिया और तर्कसंगत रूप से डिज़ाइन किए गए पेप्टिडोमिमेटिक द्वारा ऑर्केस्ट्रेटेड इसके निषेध में आणविक अंतर्दृष्टि की जांच की गई है। β2m प्रोटीन डायलिसिस से संबंधित अमाइलॉइडोसिस (DRA) में शामिल है, एक प्रोटीन मिसफॉल्डिंग विकार जो लंबे समय तक हेमोडायलिसिस के अधीन होने पर गुर्दे की विफलता के रोगियों को प्रभावित करता है। डीआरए में विशेष रूप से रोगियों के मस्कुलोस्केलेटल सिस्टम में β2m के लंबे तंतुमय समुच्चय (अमाइलॉइड कहा जाता है) का संचय शामिल है। β2m अमाइलॉइड के जमाव के परिणामस्वरूप जोड़ों और हड्डियों में सूजन हो जाती है, जिसके बाद उनका क्षरण होता है जिससे पुराना दर्द होता है। डीआरए को प्रबंधित करने के लिए वर्तमान में उपलब्ध उपचार विधियों में एमिलॉइड जमा को हटाने के लिए सर्जरी, रोगसूचक राहत के लिए दवाएं, रोगी के सीरम से β2m हटाने के लिए बेहतर डायलिसिस कॉलम और किडनी रिप्लेसमेंट थेरेपी शामिल हैं। हालांकि, ये विधियां उच्च लागत और कम सफलता से ग्रस्त हैं और केवल रोगियों को उपशामक देखभाल प्रदान करती हैं। डीआरए के प्रबंधन के लिए वर्तमान में कोई दवा-आधारित उपचार पद्धति उपलब्ध नहीं होने के कारण, हमारा अध्ययन β2m अमाइलॉइड फ़िब्रिलेशन की इन विट्रो प्रक्रिया के विरुद्ध तर्कसंगत रूप से डिज़ाइन किए गए पेप्टिडोमिमेटिक की क्षमता की पड़ताल करता है।

पेप्टिडोमिमेटिक्स अणुओं का एक वर्ग है जो एक विशिष्ट समग्र संरचना प्रदान करने के लिए उनकी संरचना में आंतरिक रूप से नियोजित आणविक पाड़ का गठन कर सकता है। इस तरह की विशिष्ट रचना एक पेप्टिडोमिमेटिक प्रजातियों के पीपीआई इंटरफेस की नकल करने में मदद कर सकती है जो आम तौर पर एमिलॉयड फाइब्रिलेशन प्रक्रिया के दौरान उत्पन्न होती हैं और एमिलॉयड-सक्षम इंटरमॉलिक्युलर एसोसिएशन (हरिदास एट अल।, 2012; पेले-गिमेनो एट अल।, 2015; श्रीवास्तव एट) के साथ हस्तक्षेप करने में मदद करती हैं। अल।, 2017; गोयल एट अल।, 2017; सिंह एट अल।, 2021)। इस अध्ययन में जांच की गई $(बीएलवीआई)_2$ पेप्टिडोमिमेटिक एक केंद्रीय रूप से स्थित, हाइड्रोफोबिक बिस्पिडीन की मात्रा का गठन करती है, जिसमें हाइड्रोफोबिक अमीनो एसिड ल्यूसीन (एल), वेलिन (वी), और आइसोल्यूसीन (आई) दोनों तरफ से जुड़े होते हैं। बिस्पिडीन मचान $(बीएलवीआई)_2$ को एक समग्र संरचना प्रदान करता है जो आम तौर पर एमिलॉयड फाइब्रिलेशन के दौरान देखे गए पीपीआई

इंटरफेस की नकल करता है। इसकी आंतरिक हाइड्रोफोबिक प्रकृति और एक β-स्ट्रैंड संरचना के साथ, $(बीएलवीआई)_2$ को हाइड्रोफोबिक, एमिलॉयड-सक्षम, β-शीट संचालित इंटरमोलेक्यूलर एसोसिएशन के साथ β2m एमिलॉयड अग्रदूत प्रजातियों के बीच हस्तक्षेप करने के लिए परिकल्पित किया गया था ताकि लंबे और आदेशित एमिलॉयड के गठन को रोका जा सके।

यह थीसिस एक पेप्टिडोमिमेटिक-आधारित दृष्टिकोण की जांच और परिचय देता है जो सामान्य रूप से डीआरए और एमाइलॉयडोजेनेसिस के खिलाफ भविष्य के चिकित्सीय विकसित करने में मदद कर सकता है।

इस अध्ययन का पहला भाग β2m अमाइलॉइड फाइब्रिलेशन की इन विट्रो विकसित प्रक्रिया के खिलाफ $(बीएलवीआई)_2$ के प्रभाव की पड़ताल करता है। थियोफ्लेविन-टी, एसडीएस-पेज, और ट्रांसमिशन इलेक्ट्रॉन माइक्रोस्कोपी विश्लेषण से पता चलता है कि $(बीएलवीआई)_2$ उपज को काफी कम कर देता है और खुराक पर निर्भर तरीके से लंबे और सीधे एमिलॉयड-जैसे β2m फाइब्रिल उत्पन्न करने के लिए आवश्यक समय बढ़ाता है। β2m को $(बीएलवीआई)_2$ की उपस्थिति में रूपात्मक रूप से अलग छोटी और सीधी अमाइलॉइड जैसी तंतु प्रजातियों में विकसित होने के लिए मनाया जाता है, जो विशिष्ट β2m अमाइलॉइड फ़िब्रिलेशन से परिवर्तित मार्ग का संकेत है। इस अध्ययन का दूसरा भाग $(बीएलवीआई)_2$ के आणविक तंत्र में β2m एमाइलॉयड फाइब्रिलेशन के 2-प्रभावित निषेध पर चर्चा करता है। $(बीएलवीआई)_2$ की अनुपस्थिति में, परिपत्र द्वैतवाद डेटा से पता चलता है कि m2m प्रजातियां विशिष्ट β-शीट-समृद्ध अमाइलॉइड-सक्षम गठनात्मक पुनर्व्यवस्था से गुजरती हैं। हालांकि, $(बीएलवीआई)_2$ की उपस्थिति में, β2m प्रजातियां एक गैर-एमिलॉयडोजेनिक, गैर-बीटा-शीट में मौजूद होती हैं, जिसमें β2m अक्षमता का संकेत होता है, जो नियंत्रण-जैसे लंबे और ऑर्डर किए गए एमाइलॉयड-जैसे फाइब्रिल की पीढ़ी के लिए आवश्यक गठनात्मक पुनर्व्यवस्था से गुजरना पड़ता है। . $(बीएलवीआई)_2$ को आगे β2m प्रजातियों के बीच अमाइलॉइड-सक्षम अंतर-आणविक संघों के साथ हस्तक्षेप करने के लिए गतिशील प्रकाश प्रकीर्णन का उपयोग करके देखा जाता है। हमारे अध्ययन से पता चलता है कि लंबे और सीधे β2m अमाइलॉइड जैसे तंतु मानव सिनोवियम सेल लाइनों के लिए साइटोटोक्सिक हैं, जबकि रूपात्मक रूप से अलग $(बीएलवीआई)_2$-प्रेरित गैर-β-शीट जिसमें घुलनशील, गैर-फाइब्रिलर β2m प्रजातियां और β-शीट समृद्ध हैं। , अघुलनशील, तंतुमय β2m प्रजातियां गैर-साइटोटॉक्सिक पाई जाती हैं। $(बीएलवीआई)_2$ पेप्टिडोमिमेटिक कम सांद्रता (250 माइक्रोन) पर गैर-

साइटोटॉक्सिक है, जो डीआरए प्रासंगिक सीरम β2m स्तरों के अनुसार, विवो सेटअप के तहत भविष्य के अध्ययन के लिए आशाजनक साबित हो सकता है।

कुल मिलाकर, यह थीसिस डायलिसिस से संबंधित β2m एमाइलॉयड फाइब्रिलेशन के खिलाफ तर्कसंगत रूप से डिजाइन किए गए पेप्टिडोमिमेटिक (बीएलवीआई)$_2$' की एंटी-फाइब्रिलेशन संपत्ति की रिपोर्ट करती है, जो डीआरए के खिलाफ चिकित्सीय दृष्टिकोण में संभावित बदलाव लाने में मदद कर सकती है।

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Abbreviations and Symbols

α	Alpha
β	Beta
~	Approximately
µ	Micro
µg	Microgram
µL	Microlitre
µm	Micrometer
µM	Micromolar
3D	Three-dimensional
%	Percent
℃	Degree Celcius
λ	Wavelength
λ_{max}	Fluorescence emission maxima
a.a	amino acid
Å	Angstrom
A_{280}	Absorbance measured at 280 nm
A_{260}	Absorbance measured at 260 nm
ANS	8-anilino-1-naphthalene-sulfonic acid
APS	Ammonium persulphate
β2m	Beta 2 microglobulin
$B(LVI)_2$	Bispidine peptidomimetic analogue
CD	Circular dichroism
CR	Congo red
CV	Column volumes
DLS	Dynamic light scattering
DMEM	Dulbecco's Modified Eagle's Medium
DMSO	Dimethylsulfoxide
DNA	Deoxyribonucleic Acid

DRA	Dialysis related amyloidosis
DTT	1, 4-Dithiothreitol
EDTA	Ethylene diamine tetra acetic acid
EtBr	Ethidium bromide
g	Gram
h	Hours
Hβ2m	His-tagged Beta 2 microglobulin
IPTG	Isopropyl- β- D-1-thiogalactopyranoside
kb	Kilobase pair
kDa	KiloDalton
LA	Luria Bertani agar
LB	Luria Bertani broth
M	Molar
mg	Milligram
min	Minute
mM	Millimolar
mol. wt.	Molecular weight
MRE	Molar residue ellipticity
MTT	MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide)
NDP	Nucleation dependent polymerization
Ni-NTA	Nickel-nitrilotriacetic acid
nm	Nanometer
$O.D._{600}$	Optical density (Absorbance) measured at 600 nm
O/N	Overnight
PAGE	Polyacrylamide gel electrophoresis
PBS	Phosphate Buffered Saline
PCR	Polymerase chain reaction
PDB	Protein data bank
PMSF	Phenyl methyl sulfonyl fluoride
PPIs	Protein-protein interactions
PTM	Post-translational modifications

rpm	Revolutions per min
RT	Room temperature
SDS	Sodium dodecyl sulphate
SW-982	Human synovium cells
s	Second
TAE	Tris-acetate-EDTA
TEM	Transmission electron microscopy
TEMED	N, N, N’, N’-Tetramethylethylenediamine
ThT	Thioflavin-T
Tris	Tris(hydroxymethyl)aminomethane
UV	Ultra-violet
Wt.	Wild type