ISSN Print: 2664-6188
ISSN Online: 2664-6196
Impact Factor: RJIF 5.35
IJCBB 2023; 5(2): 31-35
www.biochemistryjournal.net
Received: 23-06-2023
Accepted: 29-07-2023

Mohammadi Maahtaab
Student, Department of Medicine, Royal College of Surgeons, Dublin, Ireland

Corresponding Author:
Mohammadi Maahtaab
Student, Department of Medicine, Royal College of Surgeons, Dublin, Ireland

Role of protein misfolding in amyloidosis: A comprehensive narrative review

Mohammadi Maahtaab

DOI: https://doi.org/10.33545/26646188.2023.v5.i2a.47

Abstract
Amyloidosis consist of diseases characterised by the accumulation of misfolded proteins in various organs throughout the body. These misfolded protein aggregates, called amyloids, are formed due to overridden biochemical defences and defects in the normal protein folding processes.
This narrative literature review aims to provide a robust overview of the role of protein misfolding in amyloidosis, highlighting the biochemistry of molecular stages of amyloid formation, structural and molecular features of amyloid and their contribution to disease pathogenesis.
The review explores the fundamental process of protein folding and describes the factors that can lead to misfolding. By describing the structural changes that occur when proteins adopt abnormal conformations, the review delineates the formation of amyloid precursor proteins such as oligomers and protofilaments in the amyloid cascade. The review further elucidates the generic molecular and structural features of amyloid protein in its final aggregated state. It also explores the pathophysiology of cellular dysfunction and tissue damage in amyloidosis.
In a nutshell, protein misfolding plays a crucial role in the pathogenesis of amyloidosis by initiating a cascade of events that ultimately lead to tissue damage and functional impairment. Understanding the role of each precursor molecule in the protein misfolding cascade of amyloidosis is sacrosanct for the development of effective therapeutic interventions. Further research in this field can provide avenues for the development of novel treatments which can help potentially prevent or halt the progression of amyloid-related diseases.

Keywords: Amyloidosis, misfolded protein, amyloid formation

Introduction
Amyloidosis refers to a set of diseases that have in common the deposits of insoluble proteinaceous aggregates called ‘amyloids’ leading to myriad downstream cytotoxic effects including cell atrophy and death, both seen systemically, as well as localized in certain tissues, for example, the brain in Alzheimer’s disease. The diagnosis of amyloidosis is generically made histologically as the amyloid fibrils appear red/pink under normal light, and when stained with Congo red Stain, show a characteristic green birefringence under polarized light [1].
Advances in molecular imaging techniques have now allowed us to further elucidate the structural properties of amyloids fastidiously, which, in a nutshell, have demonstrated that amyloids are formed by specific precursor proteins, which in their unfolded state self-assemble into oligomers, then into protofilaments and finally into amyloid fibrils.
As a protein misfolding disease, studying the mechanism of protein aggregation from the very onset of protein misfolding to the formation of amyloids becomes sacrosanct, not only to understand the latter’s pathogenic behaviour but also to provide scope for therapeutic interventions.

Mechanism of Amyloid fibril formation
Protein folding and unfolded states
The cellular environment consists of an ensemble of processes that work toward maintaining protein homeostasis, especially in providing a conducive environment for the spontaneous folding of proteins to their globular states post-translation.

The folding of a protein into its native or tertiary state is dependent on the primary conformation of the polypeptide chain, which is the sequence in which the amino acids are bonded together. This dictates the type of main chain and side chain interaction within the sequence and thus, the tertiary conformation.

The propensity of polypeptide sequence to form tertiary structures (usually globular) is in order to attain local minima of Gibbs free energy (ΔG). According to the energy funnel theory, this corresponds to higher stability and lesser entropy, Δ S (i.e., lesser chaos), of the native or tertiary states, vis-à-vis the unfolded states of the polypeptide chain [2, 3].

However, despite mostly remaining an unfavourable process, certain polypeptide chains remain in their unfolded states owing to the following mechanisms and are thus prone to aggregation:

- Denaturation is one of the most significant causative agents of unfolding. Aberrant cellular physiochemical environments such as extreme pH, temperature, ROS etc. due to aging and other factors can destabilise protein structure and lead to unfolding. Point mutations in the Open Reading Frame also cause amyloid pathology by similar means; by destabilising the protein tertiary structure [3].
- At physiological concentrations, the global Gibbs free energy minima can be obtained by a sequence in its native state; however, when the concentration of the polypeptide chains surpasses a certain critical concentration, the Gibbs free energy minima or stability can be attained in its unfolded state thus making the latter thermodynamically favourable due to the energy barriers.
- Some polypeptide chains achieve their local free energy minima, or stability, without forming globular structures or by doing so only in specific sequences of the chain and are thus called ‘natively unfolded’ or partially disordered. These proteins are functional and have evolved to maintain high kinetic barriers, such as the presence of large polar groups, to avert aggregation to some extent.
- Specific proteins that involve large and complex folds can also transiently adopt an intermediate unfolded state before fully folding into their native states to achieve a local minimum of Gibbs free energy, i.e., for intermediate stability [2].
- Certain proteins, due to changes within the protein molecules (for instance, ligand binding), can alter protein dynamics and tend to unfold.

The presence of hydrophobic side chains, relatively shorter polypeptide chains, reduced positive charge and tendency to form beta-pleated sheets in polypeptides increases the risk of aggregation [4]. However, various evolutionary mechanisms have aided in surpassing these risk factors. For instance, aggregation-prone regions are either coupled with aggregation-resistant amino acids (the ‘gatekeepers’) or are sequestered on the inner side of the globular proteins. Moreover, protein life cycles are regulated by molecular chaperones that aid folding, as well as through degradation processes carried out via autophagy or the ubiquitin-proteasome systems [2].

If the concentration of unfolded proteins is higher than the critical concentration, it elicits the Unfolded Protein Response (UPR), whereby unfolded polypeptides accumulate within the endoplasmic reticulum causing ER stress. ER stress response proteins, however, could aid in reducing the stress on ER by downregulating protein synthesis. Aberration in the family of these proteins can lead to systemic amyloidosis [5].

Formation of oligomers

Oligomers are aggregates of prefibrillar intermediates formed transiently when the unfolded states of polypeptides exceed a certain critical concentration and self-assemble. Oligomers present in the on-pathways of aggregation and amyloid formation mainly consist of protofibrils. Unlike amyloid fibrils, protofibrils are soluble, relatively unstable and non-fibrillar. The protofibrils consist of beta-pleated sheets (secondary structure characterized by backbone hydrogen bonds between carbon and amino groups of the amino acid sequence in a side-by-side arrangement of polypeptide chain) arranged into roughly spherical species with approximately 20 polypeptide chains. This gives them a beaded appearance when visualised under AFM methods or TEM, with a diameter of 2-3nm in linear, curly, and annular aggregates. The protofibrils play a salient role in the onset and pathogenesis. Of amyloidosis [4, 6].

Similarly, denatured globular proteins also form intermediate oligomers called ‘native-like oligomers’ that retain certain folds/interactions present in their native/tertiary state while also forming structures morphologically similar to protofibrils and are rich in beta-pleated sheets [7].

Protofilaments and Amyloid formation

After the oligomer formation, protofilaments are formed. Amyloid fibrils comprise of the intertwining of long, unbranched protofilaments, which are formed by the stacking of oligomers. Each protofilament consists of a cross-beta structured core, consisting of stacked beta-pleated sheets stacked with each other and interacting mainly through main chain hydrogen bonding between NH and CO as well as through other hydrophobic and Vander Waal’s forces [8].

The aggregation mechanism is nucleated polymerization as it follows a typical sigmoidal reaction time course consisting of a lag phase and rapid growth phase. Post primary nucleation, the rate of formation of aggregates increases and the reaction becomes favourable as the primary nucleus confers stability to the fibrillar structure. Furthermore, added monomers have the propensity to aggregate and adopt a cross beta structure as the pre-existing aggregates act as templates, thus allowing the formation of larger aggregates (templation and seeding, respectively) [2, 4].

Further steps such as fragmentation and secondary nucleation aid in multiplying the number of protofilaments [2, 4].

Protofilaments twist around each other via lateral association, giving rise to amyloid fibrils in a helical morphology [8].

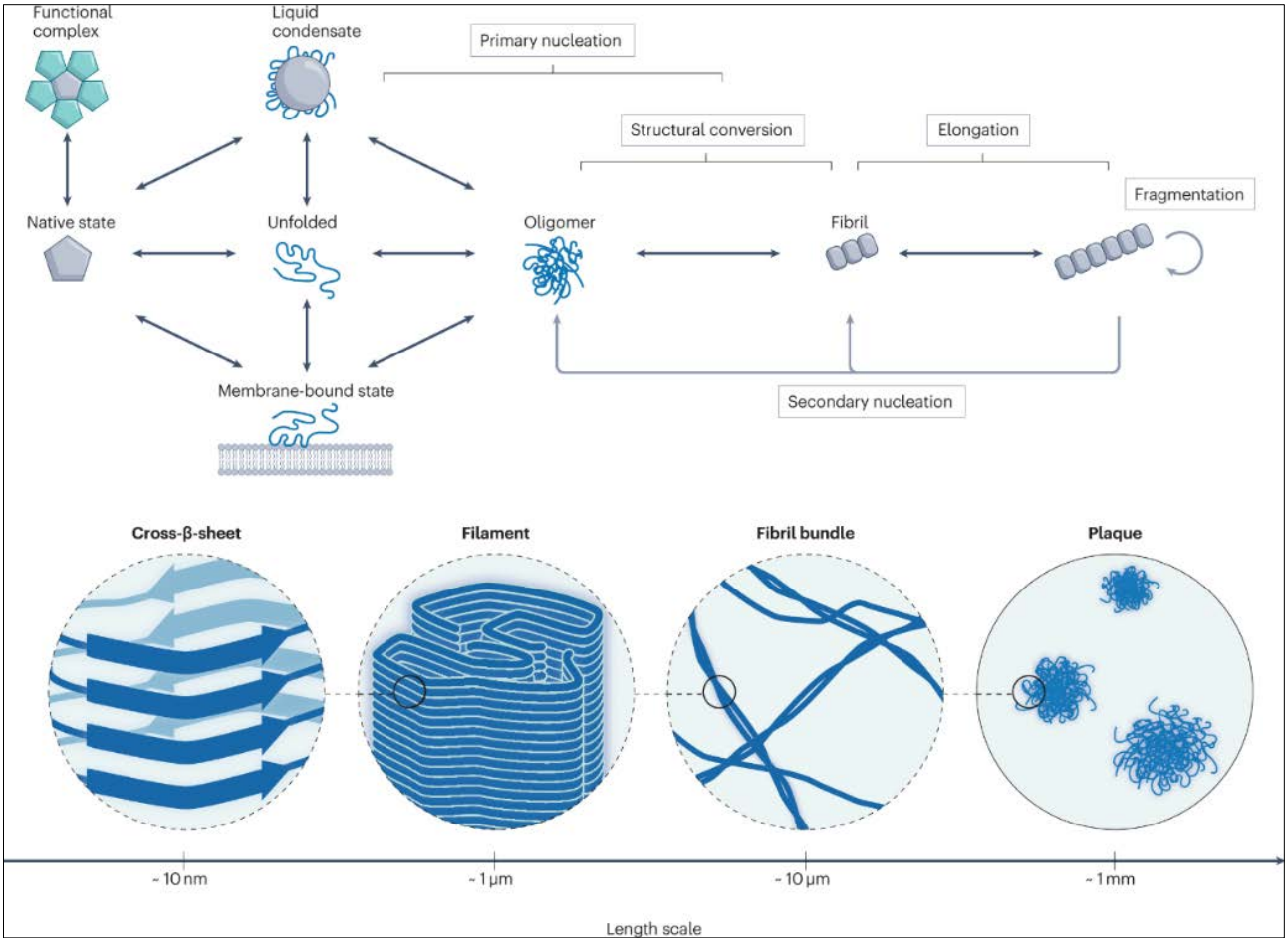

Fig 1: The schematic underlines the different precursor proteins to amyloids in their stages of formation. Protofilaments rapidly form amyloid aggregates through secondary nucleation and fragmentation. Image Source: [9]

Molecular and Structural features of Amyloid Fibrils

Molecular features

- The amyloid fibrils are high-order structures comprised of the cross-beta structure consisting of steric zippers (beta-pleated sheets) in different arrangements forming backbone hydrogen bonds between NH and CO as well as through hydrophobic and Vander Waal's forces [3].
- The beta sheets run perpendicular to the axis of the fibrils with an intersheet distance of approximately 10 Angstroms and an intrastrand distance of about 4.7 Angstroms [10, 11].
- The intertwined protofilaments form a number of interactions, including backbone hydrophobic and Vander Waals interaction, side-chain hydrogen bonds and salt bridges, as well as hydration shells [8].

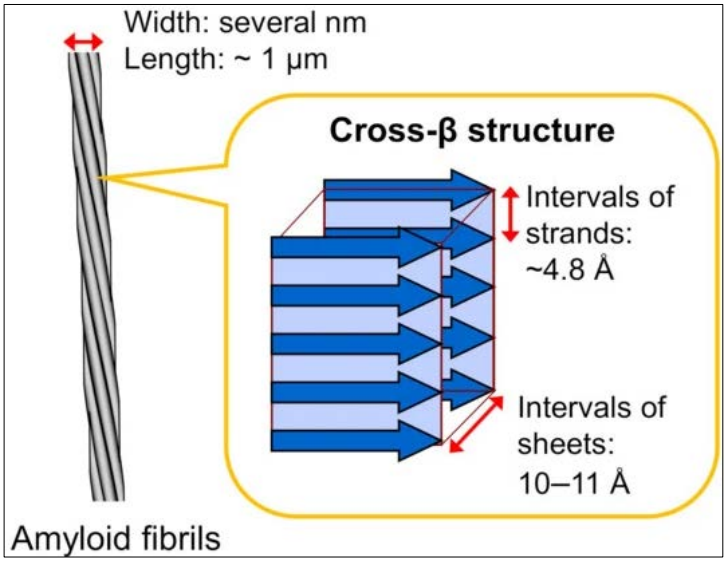

Fig 2: Image depicting the molecular features of each protofilament that intertwines to form an amyloid fibril. The steric zippers are the cross beta-pleated sheets that run perpendicular to the access of the fibrils and are intertwined together through various molecular interactions. Image Source: [12]

Structural features

- High tensile strength and elasticity due to the nature of the interactions within the fibrils [2, 4].
- Although the cross-beta structure of amyloids is ubiquitous, polymorphism exists due to the varied arrangement of polypeptide chains (steric zippers) with the amyloid fibrils [4].

Pathophysiology of Amyloidoses and Therapeutic Insights

As one can imagine, aberrant accumulation of assemblies of amyloid fibrils leads to protein metastasis, initiating a domino effect of cytotoxic effects once the cellular "housekeeping and defence" mechanisms are overwhelmed. However, the intermediate oligomers have also been shown to exert cytotoxic effects at a much earlier stage that could define disease progression.

Certain annular oligomers and protofibrils have the ability to insert themselves within lipid bilayers, thus disrupting membrane permeability. The aberration of ion gradient and resting potential leads to downstream ER stress, and Reactive Oxygen Species release that activates the caspases, ultimately leading to apoptosis. These reactions have been key in neurodegenerative disorder pathogenesis involving amyloid aggregates of precursor proteins such as alpha-synuclein and beta-amyloid that lead to Alzheimer's and Parkinson's disease, respectively. These oligomers also elicit an inflammatory immune response via the Mitogen-Activated Protein Kinase (MAPK) pathway [3].

Drug targets: The active involvement of these amyloid intermediates in disease pathogenesis has allowed for avenues for promising drug targets. For instance, Alzheimer-targeting drugs such as Aducanumab and gantenerumab primarily focus on oligomers, partly eliminating insoluble amyloid plaques [13, 14]. BAN2401 specifically aims at soluble protofibrils and ALZ-801 hinders oligomer formation [15].

The mature amyloid fibrils can also lead to a variety of downstream pathological effects such as chronic inflammation and cell death. These amyloid fibrils are highly resistant to degradation from proteases [2]. Furthermore, through mechanisms of fragmentation and secondary nucleation, the amyloid fibrils can spread intercellularly, eventually leading to the atrophy of tissues. Moreover, such largescale deposition and accumulation could lead to disruption in mundane neuromuscular body functions (for example, deglutition) and can thus lead to reduced Quality of life [3, 4]. Of Note, Tetracyclines, especially doxycycline, have shown to display anti-amyloid properties by blocking the nucleation of protofilaments and destabilizing the fibrillar aggregates and have thus been investigated as a promising drug lead for a variety of systemic amyloidosis [16, 17].

An Overview of a few different types of Amyloidoses

Table 1: The table highlights a few diseases classified into systemic and local amyloidosis. The precursor proteins are relatively short polypeptide chains and have natively unfolded structures or beta-pleated sheets, thus increasing their risk of formation of amyloids [2, 3, 4]

Protein precursor	Native Structure	Polypeptide length	Associated disease	Affected tissues
Systemic amyloidosis				
Ig light chains	Beta-pleated sheet	90	AL amyloidosis	Most tissues
Serum amyloid A1 protein fragments	Alpha helical	75-104	AA amyloidosis	Most tissues
Lysozyme amyloidosis	Alpha helical and beta-pleated sheets	130	Lysozyme amyloidosis	Liver kidney
Localized Amyloidosis				
Alpha-synuclein	Intrinsically disordered	140	Parkinson's disease	Brain
Amyloid-beta	Intrinsically disordered	37-43	Alzheimer's disease Cerebral amyloid angiopathy	Brain
Amylin	Intrinsically disordered	37	Diabetes mellitus type 2	Pancreas

Conclusion

The structured detail of amyloid fibrils that have come to the frontline, owing to the advancements in molecular imaging techniques, has opened a plethora of research avenues. Comprehension of these mechanisms can aid in identifying possible drug leads that can help treat and manage the debilitating effects of diseases grouped under amyloidosis. The elucidation of the molecular and structural features of amyloid fibrils, from the earliest stages of protein misfolding to the formation of insoluble aggregates, provides a critical foundation for understanding the pathophysiology and progression of various amyloidoses. The diverse drug targets that have come to light due to deep understanding of protein misfolding, ranging from oligomers to mature fibrils, offer a promising avenue for the development of effective treatments for amyloidosis-associated disorders. For instance, targeted therapy of modified antibodies can help establish protein homeostasis by acting as artificial molecular chaperones or can increase the stability of intrinsically disordered proteins. Thus, an in-depth understanding of misfolding mechanisms aids advancements in therapeutic interventions for amyloidoses. Further research that delves deeper into the intricacies of protein misfolding states and its aetiology can thus help improve patient outcomes for individuals affected by this debilitating condition.

References

1. Ferrier D. Biochemistry. 6th ed. Philadelphia: Wolters Kluwer; c2014.
2. Knowles TPJ, Vendruscolo M, Dobson CM. The amyloid state and its association with protein misfolding diseases. Nature Reviews Molecular Cell Biology [Internet]. 2014 May 23 [cited 2019 Nov 26];15(6):384-96. Available from: https://www.nature.com/articles/nrm3810
3. Herczenik E, Gebbink MFBG. Molecular and cellular aspects of protein misfolding and disease. The FASEB Journal. 2008 Feb 26;22(7):2115-33.
4. Chiti F, Dobson CM. Protein Misfolding, Functional Amyloid, and Human Disease. Annual Review of Biochemistry. 2006 Jun;75(1):333-66.
5. Chen JJ, Genereux JC, Wiseman RL. Endoplasmic reticulum quality control and systemic amyloid disease:

Impacting protein stability from the inside out. IUBMB Life. 2015 May 26;67(6):404-13.
6. Kumar S, Udgaonkar JB. Mechanisms of amyloid fibril formation by proteins. Current Science [Internet]. 2010 [cited 2022 Sep 17];98(5):639-56. Available from: http://www.jstor.org/stable/24111817
7. Chiti F, Dobson CM. Amyloid formation by globular proteins under native conditions. Nature Chemical Biology. 2008 Dec 16;5(1):15-22.
8. Taylor AIP, Staniforth RA. General Principles Underpinning Amyloid Structure. Frontiers in Neuroscience. 2022 Jun 2;16.
9. Michaels TCT, Qian D, Šarić A, Vendruscolo M, Linse S, Knowles TPJ. Amyloid formation as a protein phase transition [Internet]. Nature Publishing Group; c2023. [cited 2023 Oct 29]. Available from: https://www.nature.com/articles/s42254-023-00598-9
10. Sabaté R, Ventura S. Cross-β-sheet supersecondary structure in amyloid folds: techniques for detection and characterisation. Methods in Molecular Biology (Clifton, NJ) [Internet]. 2013 [cited 2022 Sep 17];932:237-57. Available from: https://pubmed.ncbi.nlm.nih.gov/22987357/
11. Serpell LC, Sunde M, Blake CCF. The molecular basis of amyloidosis. Cellular and Molecular Life Sciences. 1997;53(12):871.
12. Chatani E, Yuzu K, Ohhashi Y, Goto Y. Current understanding of the structure, stability and dynamic properties of amyloid fibrils [Internet]. Multidisciplinary Digital Publishing Institute; c2021. [cited 2023 Oct 29]. Available from: https://www.mdpi.com/1422-0067/22/9/4349
13. Shi M, Chu F, Zhu F, Zhu J. Impact of anti-amyloid-β monoclonal antibodies on the pathology and clinical profile of alzheimer's disease: A focus on aducanumab and Lecanemab [Internet]. Frontiers; c2022. [cited 2023 Oct 29]. Available from: https://www.frontiersin.org/articles/10.3389/fnagi.2022.870517/full
14. Bateman RJ, Cummings J, Schobel S, Salloway S, Vellas B, Boada M, *et al*. Gantenerumab: An anti-amyloid monoclonal antibody with potential disease-modifying effects in early alzheimer's disease - alzheimer's research & therapy [Internet]. BioMed Central; c2022. [cited 2023 Oct 29]. Available from: https://alzres.biomedcentral.com/articles/10.1186/s13195-022-01110-8
15. Tolar M, Abushakra S, Hey JA, Porsteinsson A, Sabbagh M. Aducanumab, gantenerumab, BAN2401, and ALZ-801-the first wave of amyloid-targeting drugs for alzheimer's disease with potential for near term approval [Internet]. U.S. National Library of Medicine; [cited 2023 Oct 29]. Available from: https://pubmed.ncbi.nlm.nih.gov/32787971/
16. Forloni G, Colombo L, Girola L, Tagliavini F Salmona M. Anti-amyloidogenic activity of tetracyclines: Studies *in vitro* [Internet]. U.S. National Library of Medicine; [cited 2023 Oct 29]. Available from: https://pubmed.ncbi.nlm.nih.gov/11163366/
17. Gautieri A, Beeg M, Gobbi M, Rigoldi F, Colombo L, Salmona M, *et al*. The anti-amyloidogenic action of doxycycline: A molecular dynamics study on the interaction with AB42 [Internet]. U.S. National Library of Medicine; c2019. [cited 2023 Oct 29]. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6769662/