

Protein Folding, Misfolding, Aggregation And Amyloid Formation: Mechanisms of A β Oligomer Mediated Toxicities

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ABSTRACT

Protein folding is one of the most perplexing problems in molecular biology. Protein folding is a complex process through which protein molecule acquires unique native structure which carry out specific biological function. However, recently it has been recognized that some proteins have no single well-defined tertiary structure. These proteins are termed intrinsically disordered protein (IDP) which are involved in regulation and signaling. In 1969, Cyrus Levinthal noted that, because of the very large number of degrees of freedom in an unfolded polypeptide chain, the protein molecule has an astronomical number of possible conformations. Hence, from one calculation, for 100 amino acids polypeptide chain, 10^{11} years will be required for protein to fold, which is an unrealistic time because in vivo protein folding occurs in seconds or minutes. This is known as Levinthal paradox. To overcome Levinthal paradox, several folding models have been proposed. This includes from classical nucleation-propagation model to folding funnel model. The in vitro and in vivo conditions of protein folding are not the same. This was particularly challenged by the discovery of molecular chaperones that assist in correct folding of protein and if protein still misfolds it is subjected to proteasomal degradation for the maintenance of cell homeostasis. Despite of cellular protein quality control proteins often misfold. This happens due to mutations, changes in environmental conditions and includes many more factors. These misfolded proteins give rise

to increase population of partially misfolded intermediates which have exposed hydrophobic residues that interact with complementary intermediates and consequently results in the formation of oligomers thereby proto-fibrils and fibrils. These fibrils are deposited in the brain and CNS leading to the manifestation of neurodegenerative diseases. Keeping above views in mind, in this review I have focused on, various folding models, folding in the cell, misfolding, aggregation and mechanism of A β fibril formation. Since A β oligomers are now considered as more toxic entities than fibrils. Hence, their mechanisms of toxicities also form the theme of the review.

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Key words: Protein folding; Protein misfolding; Protein aggregation; A β oligomers; Protein folding models

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Abbreviations

A β : Amyloid beta;
ATP: Adenosine Tri Phosphate;
CNS: Central Nervous System;
IDP: Intrinsically Disordered Protein;
NMR: Nuclear Magnetic Resonance;
UV-CD: Ultraviolet-Circular Dichroism.

INTRODUCTION

Protein folding is a complex process through which protein molecule acquires unique three-dimensional conformation that carry out specific biological function. However, recently it has been found that some proteins have no single unique tertiary structure. These proteins are termed intrinsically disordered protein (IDP)^[1]

which are involved in regulation and signaling. The type of native structure which a protein molecule adopts is specified in its amino acid sequence^[2]. There are several questions that are related to protein folding. For instance, why should polypeptide chain fold? The answer is eukaryotic cell cannot accommodate 3-4 million different polypeptides if all of them occur in an unfolded state. Besides, unfolded proteins will be subjected to enormous proteolytic degradation hazard. Formation of a crevice or active site in a protein molecule is inconceivable without protein folding. Protein folding may serve as a model for delineating molecular basis of protein-mediated morphogenesis of viruses, subcellular organelles and tissues, because the kind of forces that are believed to be involved in the self-assembly processes are the same as those that hold different segments of the polypeptide chain together in the native state^[3]. Protein folding has also applications in genome research, in the understanding of different pathologies and in the design of novel proteins with special function.

In 1969, Cyrus Levinthal noted that, because of the very large number of degrees of freedom in an unfolded polypeptide chain, the protein molecule has an astronomical number of possible conformations. Hence, for 100 amino acids polypeptide chain if we assume only two possible conformations for each residue, then there are 10^{30} possible conformations for the polypeptide chain. If only 10-11 second is required to convert one conformation into another, a random search of all conformations would require 10^{11} years, which is an unrealistic time because in vivo protein folding occurs in seconds or minutes. This is known as Levinthal paradox. To overcome Levinthal paradox, several folding models have been proposed. This includes from classical nucleation-propagation model, nucleation condensation model, stepwise sequential and hierarchical folding model, framework model, modular model, diffusion-collision model, hydrophobic collapse model, jigsaw puzzle model and folding funnel model. Currently, folding funnel model has replaced all other models of protein folding. The folding funnel model is represented in terms of energy landscape and describes both thermodynamic and kinetic aspects of the transformation of an ensemble of unfolded protein molecules to a predominantly native state. Various types of interactions are involved in protein folding including hydrophobic interaction, hydrogen bonding, van der Waal's interaction and electrostatic interactions. Research has shown that main chain hydrogen bond plays a key role in protein unfolding and folding^[4-6]. This is supported by the findings that hydrogen bond plays an important role in unfolding of β -catenin^[7]. Traditionally, disruption of hydrophobic interactions instead of hydrogen bonds has been thought to be the most important cause of protein denaturation.

The competition between productive folding and aggregation is a fundamental feature of folding in cells. When proteins misfold specialized proteins known as molecular chaperones assist in the refolding of misfolded proteins and if protein still persists in misfolded state it is subjected to proteasomal degradation for the maintenance of cell homeostasis. Despite of cellular protein quality control, proteins often misfold in the cell. This occurs because of dominant-negative mutations, from changes in environmental conditions (pH, temperature, protein concentration), error in posttranslational modifications, increase in the rate of degradation, error in trafficking, loss of binding partners and oxidative damage. All of these factors can act either independently of each other or simultaneously^[8]. Misfolded proteins are associated with many diseases (Table 1). A number of in vitro and in vivo experiments have lead to the conclusion that especially partially unfolded or misfolded intermediates are prone to aggregation, in particular at

high peptide concentrations^[9-13]. Besides this, natural mutations that decrease the net charge or increase the hydrophobicity and β -sheet propensity of a polypeptide chain can also result in the formation of partially misfolded intermediates. Such partially unfolded/misfolded intermediates are populated under denaturing conditions. Contrary to this belief, recent studies have shown that denaturation of IDP Osteopontin (OPN), lead to formation of extended, random coil-like conformation and stable, cooperatively many folded conformation^[14]. Further, these IDPs are associated with human diseases, including cancer, cardiovascular disease, amyloidoses, neurodegenerative diseases, and diabetes. According to Uversky hypothesis: interconnections among intrinsic disorder, cell signaling, and human diseases suggest that protein conformational diseases may occur not only from protein misfolding, but also from misidentification, missignaling, and unnatural or nonnative folding. Thus, reducing the capability to recognize proper binding partners thereby leading to the formation of aggregate^[15].

The intermediate including partially misfolded intermediates aggregate by interacting with complementary intermediate through exposed hydrophobic residues and form oligomers and consequently, protofibrils and fibrils. These intermediates do not cross polymerize or aggregate. These amyloid fibrils accumulate as amyloid deposits in the brain and central nervous system in Alzheimer's disease (AD), Prion disease, Parkinson's disease (PD) and Amylo lateral Sclerosis (ALS). Amyloid-like fibrils display many common features including a core cross- β -sheet structure in which continuous β -sheets are formed with β -strands running perpendicular to the long axis of the fibrils^[16]. These amyloid fibrils typically consist of 2-6 unbranched protofilaments of 2-5 nm in diameter which are associated laterally or twisted together to form fibrils of 4-13 nm diameter^[17-19]. These fibrillar aggregates bind dyes such as congo red and thioflavin-T and give rise to birefringence and fluorescence respectively.

Recently, Sambashivan and colleagues^[20] have proposed that fibrils contain native-like structure possessing biological activity based on the model of domain-swapped functional units of RNase. These fibrils contained native like carboxy-terminal β -strand and core domain. The spine of the fibril exists as twisted pair of interdigitated, antiparallel β -sheets formed by the Q10 insertions, suggesting that protein refolding is not required to create fibrils. In a similar vein, it was shown that at physiological pH, human pancreatitis-associated protein form fibrillar aggregates that contained native-like structure^[21] unlike fibrillar species which adopt cross-beta sheet structure. For transthyretin (TTR) the solvent accessibility of the fibrils were compared with the native TTR crystal structure and the result showed that TTR fibrils retained native-like structure^[22]. Thus, these studies suggest that amyloid-beta fibrils often possess native like structure.

Table 1 Protein misfolding diseases.

Disease	Protein	Location of folding
Huntington's disease	Huntingtin	Cytosol
Cystis fibrosis	Cystis fibrosis trans-membrane regulator	ER
Sickle cell anaemia	Haemoglobin	Cytosol
α 1-Antitrypsin deficiency	α 1-Antitrypsin	ER
Phenylketonuria	Phenylalanine hydroxylase	Cytosol
Tay-Sach disease	β -Hexoseaminidase	ER
Alzheimer's disease	Amyloid β -peptide/tau	ER
Parkinson's disease	α -Synuclein	Cytosol
Scrapie Creutzfeldt-Jakob disease	Prion protein	ER
Familial Amyloidoses	Transthyretin/lysozyme	ER
Catracts	Crystallin	Cytosol
Cancer	p53	Cytosol

Until the end of 1990s, studies have shown that the amyloid fibrils were the main toxic species in amyloid plaques. These findings were not validated until then. However, at the end of the 1990s the attention shifted to the cytotoxicity of amyloid fibril precursor: amyloid oligomers^[23]. This was confirmed by the severity of cognitive impairment in Alzheimer's disease which appears to better correlate with the levels of oligomeric species of A β rather than with the amount of fibrillar deposits^[24]. Therefore, amyloid oligomers are now considered as important key players of amyloid cytotoxicity. Later on, more amyloid oligomers were discovered and were implicated in the neurodegenerative diseases thus supporting amyloid oligomer as main culprit behind toxicity^[25]. Keeping above views in mind, in this review current knowledge of protein folding including various folding models and protein folding in the cell have been discussed. Moreover, the mechanism of amyloid fibril formation and mechanisms of A β oligomer mediated toxicities have also been discussed.

PROTEIN FOLDING MODELS

Several different folding models arising from theoretical considerations^[26], folding simulations, or experimental observations^[27], have been proposed to overcome Levinthal paradox. Among them, classical nucleation-propagation model suggests that helix-coil transition involves nucleation step followed by a rapid propagation, the limiting step being the nucleation process. More recently, a nucleation condensation model, different from the classical one, has been proposed by Fersht^[28]. According to this model, weak local nucleus are formed which is stabilized by long range interactions. The stepwise sequential and hierarchical folding model suggests that several stretches of secondary structures are formed and assemble at different levels following a unique route^[27,29]. In this model, the first event is nucleation consequently the secondary structures are formed that associate to generate supersecondary structures, then domains and eventually monomer. The framework model assumes that the secondary structure is formed in an early step of folding, followed by the formation of tertiary structure, emphasizing the role of short range interactions in directing the folding process^[30]. A modular model of folding was proposed based on the three-dimensional structures of proteins. This model assumes that not only domains, but also subdomains can serve as folding units which fold independently of each other forming structural modules that assemble to yield the native protein^[31,32]. The diffusion-collision model of folding^[33] suggests nucleation occurs simultaneously in different regions of the polypeptide chain generating microstructures which diffuse, associate and coalesce to form substructures with a native-like conformation that eventually give rise the native protein structure. The hydrophobic collapse model implies that in the first step, polypeptide chain collapses via long range hydrophobic interactions followed by the formation of secondary structure and consequently tertiary structure. Later, Dill and co-workers proposed that stretches of secondary structures are formed simultaneously with the hydrophobic collapse and consequently native protein structure is formed^[34]. The jigsaw puzzle model was introduced in 1985 by Harrison and Durbin^[35]. This model supports the existence of multiple folding routes to reach a single native state.

DETECTION AND CHARACTERIZATION OF INTERMEDIATES IN PROTEIN FOLDING

The unfolding-refolding transition under equilibrium has often been treated as a two-state mechanism. This implies that transition of the

native to the denatured state is an "all-or-none" process that involves only two conformational states, the native and denatured states which are significantly populated. Further, if at all any intermediate state exists; it exists transiently and poorly populated under equilibrium conditions. However, existence of intermediates has been shown from kinetic studies for most proteins even for proteins showing two-state mechanism. These experimental evidences prove the occurrence of intermediates in the folding pathway. The structural characterization of such intermediates is a prerequisite to solving the folding problem. Two major obstacles are encountered in characterizing these species: high cooperativity of the transition and rapidity of the process, especially in the early steps of protein folding. Nevertheless, using improved methods it is possible to detect intermediates, for instance during the refolding of disulfide-bridged proteins like lysozyme^[36] and bovine pancreatic trypsin inhibitor (BPTI)^[37,38]. An elegant method using differential chemical labeling has been elaborated by Ghéllis^[39] and applied to the refolding of elastase. In the past decades, substantial technological advances have been made to characterize intermediates, particularly by stopped-flow mixing devices coupled to circular dichroism, and NMR using rapid hydrogen-deuterium exchange associated with a mixing system allowing for the pulse labeling of transient species. This method is highly informative, yielding residue-specific information^[40-42]. Classical rapid mixing techniques such as stopped-flow, continuous flow and quenched-flow are limited to the millisecond time scale, thus preventing analysis of the early events occurring within the initial burst phase of protein folding. In spite of this, recently technical advances in kinetic studies have been made in characterizing these intermediate^[43]. For example, sub millisecond mixing techniques have been developed for studying the early steps of folding of cytochrome c.

Studies have shown that protein folding involves three common stages:

1. Initially, the unfolded protein collapses to more compact state containing substantial nonpolar surfaces and secondary structure. This species has little thermodynamic stability and encompasses an ensemble of conformations which are in dynamic equilibrium and may contain non-native structure. This stage occurs in less than 5 ms and, transition maybe noncooperative in nature.
2. The next phase involves further development of secondary and the beginnings of specific tertiary structure throughout the protein molecule showing measurable stability. In this step, subdomains are formed that are yet to be properly docked. Further, in these intermediate steps, substrate or ligand-binding sites are formed in protein molecules. For example, in α -lactalbumin, Ca²⁺-binding sites appear before completion of the native structure^[44]. The packing is not as tight as is ultimately found in the native conformation, suggesting that the side chains are in general more mobile. This stage, which may consist of more than single kinetic step and occurs in the 5-1,000 ms time range.
3. In the final steps of protein folding, precise ordering of the elements of secondary structure, the correct packing of the hydrophobic core, the correct domain pairing in multidomain proteins, the reshuffling of disulfide bonds, cis-trans proline isomerization occur before the formation of the native structure.

MOLTEN-GLOBULE, PRE-MOLTEN GLOBULE AND DRY-MOLTEN GLOBULE INTERMEDIATES

Kinetic refolding experiments *in vitro* as well as theoretical calculations suggest that protein folding is a sequential hierarchical

process^[45], with the existence of early stable species with a high content of secondary structures. These secondary structures were coined as molten globule by Ohgushi and Wada^[46]. The characteristic features of the ‘molten globule’ state are: (i) It contains extensive secondary structure; (ii) It has loose tertiary contacts without tight side-chain packing; (iii) It is less compact than the native state; (iv) It is more compact than the unfolded state^[47]; (v) It contains an accessible hydrophobic surface which binds hydrophobic dye aniline naphthalene sulfonate. Since the tertiary structure is not stabilized, therefore near UV-CD spectra is not detected. The formation of a molten globule as an early folding intermediate has been reported for several proteins including α -lactalbumin, carbonic anhydrase, β -lactamase, and the α - and β - subunits of tryptophan synthase, bovine growth hormone, and phosphoglycerate kinase^[48-50].

An intermediate state has been identified that precedes the molten globule state^[49,51]. This species is less compact than a molten globule, contains significant amount of secondary structure contents which are smaller than that of a molten globule, and displays hydrophobic regions accessible to a solvent. This intermediate state has been called a pre-molten globule by Jeng and Englander^[52] and has been observed during the cold denaturation of β -lactamase, carbonic anhydrase, and also during the refolding of several proteins^[53]. Since these transient intermediate states are formed within the dead-time of a stopped flow device. Thus, it is possible that their formation might be preceded by an earlier folding step.

Recently, dry molten globule intermediates have been discovered, which exists in expanded state lacking appreciable solvent in which side chains unlock and gain conformational entropy, while liquid-like van der Waals interactions persist. The dry molten globule does not bind hydrophobic dye aniline naphthalene sulfonate. Currently, research has shown that four different proteins form dry molten globules as the first step of unfolding, suggesting that such an intermediate may be commonplace in both folding and unfolding^[54]. Discovery of dry molten globule intermediates has major implications for future experimental work on the mechanism of protein folding.

THE ENERGY LANDSCAPE AND THE FOLDING FUNNEL MODEL

The folding funnel model has evolved from both experiment and theory through the use of simplified mechanical models that benefit from the concept of folding funnel introduced by Wolynes and co-workers^[55]. The model is represented in terms of an energy landscape and describes both thermodynamic and kinetic aspects of the protein folding. A folding funnel is a simplified 2D representation of the very high-dimensional conformational space that is accessible to the polypeptide backbone during folding^[56]. The broad top of funnel shows vast number of conformations present in the soluble denatured state, the narrow bottom of the funnel represents the unique native structure of the protein. The separation between the top and bottom of the funnel represents other energy contributions (chain enthalpy, solvent entropy and enthalpy) to each chain conformation. Starting from the ensemble of unfolded conformations the folding funnel allows several pathways that proceed to the global free energy minimum corresponding to the native structure. As the chain folds to lower energy conformations, it might also populate intermediate states along the sides of the funnel. These kinetic traps might hinder and/or promote the formation of the native structure depending on their depth, the barriers between the trap and the native conformation, and the rest of the funnel surface. According to the statistical mechanics, the number and depth of local kinetic traps

on the funnel landscape represent the degree of frustration of the polypeptide sequence^[57]. Current folding funnels cannot, however, account the behavior of most polypeptide chains under physiological conditions. Although the model starts with all possible initial conformations at the top of the funnel, they describe the folding behavior of only single polypeptide chain at infinite dilution. They do not consider intermolecular collisions between partially folded chains which is intrinsic feature of actual folding processes leading to self-association. Because misfolding is often associated with self-association, polymerization or aggregation, the funnel models cannot account for the aggregation behavior of many proteins^[58,59]. However, in folding funnel diagrams, an off-pathway aggregation reaction can be incorporated as second ‘aggregation’ funnel^[60]. Like intramolecular folding, in aggregation the association of two or more non-native protein molecules is largely driven by hydrophobic forces and primarily results in the formation of amorphous structures (Figure 1)^[61]. Alternatively, aggregation can lead to the formation of highly ordered, fibrillar aggregates called amyloid (Figure 1).

These results are restricted to a subset of proteins under physiological conditions. Thus, energy landscape metaphor provides a conceptual framework for understanding two-state and multistate kinetics, misfolding and aggregation process. Energy landscape ideas also have allowed successful development of protein structure prediction algorithms^[62].

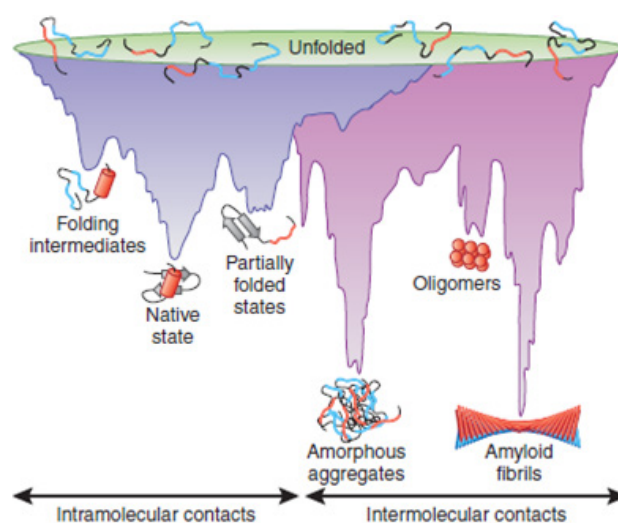


Figure 1 Energy landscape scheme of protein folding and aggregation. The purple surface shows the multitude of conformations ‘funneling’ to the native state via intramolecular contacts and the pink area shows the conformations moving toward amorphous aggregates or amyloid fibrils via intermolecular contacts. Both parts of the energy surface overlap. Cell-toxic oligomers may occur as off-pathway intermediates of amyloid fibril formation (reproduced with permission).

PROTEIN FOLDING IN THE CELL

The main rules that govern protein folding have been mainly deduced from *in vitro* studies. The *in vitro* refolding is considered as a good model to understand the mechanisms by which a nascent polypeptide chain acquires the three dimensional structure in the cell. However, the intracellular environment is highly crowded containing about 300-400 mg/mL of macromolecules^[63] which differs markedly from that of the test tube where low protein concentrations are used for carrying out protein folding-unfolding transition. Under these in

vitro and in vivo conditions, do the same mechanisms account for protein folding? This question has been particularly challenged by the discovery of molecular chaperones in 1987. These molecular chaperones are nanomachines that catalytically unfold misfolded and alternatively folded proteins^[64]. Molecular chaperones and their associated co-chaperones are essential in health and disease as they are key facilitators of protein folding, quality control and function. The HSP70 and its co-chaperones have been recognized as potent modulators of inclusion formation and cell survival in cellular and animal models of neurodegenerative disease. Now, it has also become evident that the HSP70 chaperone machine functions not only in folding, but also in proteasome mediated degradation of neurodegenerative disease. Thus, there has been a great deal of interest in the potential manipulation of molecular chaperones as a therapeutic approach for many neurodegenerations. Most recently, mutations in several HSP70 co-chaperones and putative co-chaperones have been identified as causing inherited neurodegenerative and cardiac disorders, directly linking the HSP70 chaperone system to human disease^[65]. The molecular chaperones GroEL/GroES also accelerate the refolding of a multidomain protein by modulating on-pathway intermediates^[66]. Now more than 20 protein families have been identified as molecular chaperones, the heat-shock protein Hsp 70 (Dnak in *Escherichia coli*), and Hsp40 (DnaJ in *E. coli*) show little or no specificity for the proteins they assist.

Molecular chaperones assist in the folding of protein by two different mechanisms. In the first mechanism, small chaperones bind transiently to small hydrophobic regions of nascent polypeptide chains thereby prevent aggregation and premature folding. This binding and release by some, but not all, small molecular chaperones is regulated in a complex ATP-dependent pathway. Contrarily, in the second mechanism large chaperones such as the GroEL-GroES system in prokaryotes or TriC in eukaryotes completely sequester the non-native proteins in a central cage. This cage is formed by the heptameric double ring of GroEL and is capped by GroES to prevent the premature release of the folding protein. This cage is large enough to accommodate protein molecules up to about 70kDa. The Figure 2 shows the GroEL reaction cycle^[67]. Briefly, the non-native protein binds to the apical domains of the upper ring of GroEL-GroES. Consequently, ATP and GroES bind to the ring and sequester the protein. The binding of GroES induces a large conformational change in GroEL and ATP hydrolysis induces a conformational change in the bottom ring allowing it to bind a misfolded protein. This promotes subsequent binding of ATP and GroES in the lower ring, disrupting the upper complex and ejecting GroES and releasing the protein. If the protein does not attain the native state, it is subjected to a new cycle. The hydrolysis is required in some cases for the release of the protein.

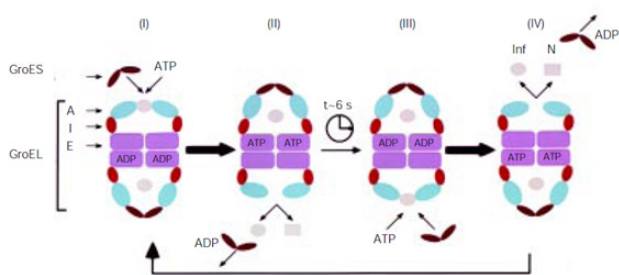


Figure 2 The GroES-GroEL cycle according to Wang and Weissman^[67]. Inf is the unfolded protein, N the folded one. A is the apical domain (in blue) which binds the unfolded protein and GroES; I is the intermediate domain (in red) and E is the equatorial domain (in magenta) which binds and hydrolyzes ATP (reproduced with permission).

Thus, molecular chaperones transiently associate with nascent misfolded proteins; therefore play an important role in preventing improper folding and aggregation. Infact they do not interact with native proteins. They bind non-native proteins through hydrophobic interactions. They do not carry code for directing a protein to adopt a structure different from that dictated by the amino acid sequence. Therefore, the role of molecular chaperones is to assist protein folding in vivo without violating the Anfinsen's postulate. They also increase the yield but not the rate of folding reactions; which implies they do not act as catalysts.

Other accessory molecules also play a helper role in the folding of proteins in vivo. For instance, protein disulfide isomerase, an abundant component of the lumen of the endoplasmic reticulum, catalyzes the formation of disulfide bonds in secretory proteins thereby accelerate the folding process. Another enzyme, peptidyl-prolyl-cis-trans isomerase, catalyzes the cis-trans isomerization of X-Pro peptide bonds. Consequently, it accelerate the folding process.

PROTEIN MISFOLDING, AGGREGATION AND AMYLOID FIBRIL FORMATION

In protein misfolding, protein molecule is converted into non-native state. These misfolded proteins are kinetically trapped in local energy minima. Misfolding generally occurs due to dominant-negative mutations, from changes in environmental conditions (pH temperature, protein concentration), error in posttranslational modifications (phosphorylation, advanced glycation, deamidation, etc.), increase in the rate of degradation, error in trafficking, loss of binding partners and oxidative damage^[68]. These factors can act either independently of each other or simultaneously^[81]. Misfolded protein or partially folded intermediates have large patches of contiguous surface hydrophobicity and therefore aggregate more readily than native and unfolded state which have hydrophobic amino acid located at the interior core of protein and lie scattered in the polypeptide chain respectively. These partially misfolded intermediates aggregate by interacting with complementary intermediate and consequently give rise to the formation of oligomers thereby proto-fibrils and fibrils. These proteinaceous fibril seeds can therefore serve as self-propagating agents for the instigation and progression of disease. The alzheimer's disease and other cerebral proteopathies seem to arise from the de novo misfolding and sustained corruption of endogenous proteins, whereas prion diseases can also be infectious in origin^[69].

Recently, several independent lines of studies on different proteins indicate that oligomers might be the most toxic species in the misfolding and aggregation pathway^[70-72]. This is validated by the findings that early aggregate of A β peptides, α synuclein^[73], transthyretin^[74] lead to the formation of AD, PD and ALS disease^[75,76,73]. Lack of a direct correlation between the fibrillar plaque density and the severity of the clinical symptoms in patients suffering with AD or PD further justify that early aggregates are more toxic entities^[77]. Furthermore, when transgenic mouse models were exposed to early aggregates disease-like phenotypes appeared in these mouse^[78].

Both amyloid oligomers and fibrils are formed via a variety of pathways including reversible association of native monomers, aggregation of conformationally altered monomer, aggregation of chemically modified product, nucleation-elongation polymerization and surface induced aggregation^[79]. Thus giving rise to diverse fibril structures or polymorphism^[80]. Additional polymorphisms arise when the same polypeptide chain occurs in a range of structurally different morphologies^[79]. Among these fibrillation pathways, nucleation-elongation polymerization is generally more accepted (Figure 3).

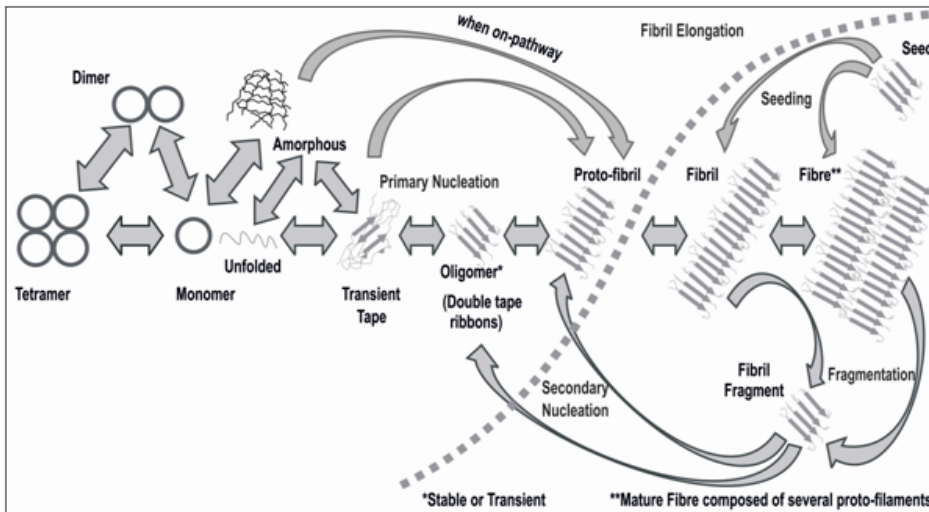


Figure 3 Amyloid aggregation pathway. Adapted from reference^[81].

Therefore in the following passage only this mechanism has been discussed. Briefly, in this mechanism the reaction rate depends on the protein concentration and can be accelerated by the addition of homologous pre-aggregated proteins^[81-86]. The amyloid aggregation occurs in three consecutive stages: (1) The first stage is thermodynamically disfavored and is known as lag phase where the soluble monomers associate to form nuclei; (2) The second step is exponential phases in which population of these transient nuclei species triggers the polymerization and fibril growth; (3) The third stage is saturation phase in which essentially all soluble species are converted into mature fibrils by associating laterally^[84,87].

The nucleation-elongation aggregation reaction was first described by Oosawa and Asakura^[88]. According to this model, the lag phase nuclei are in a very unfavorable thermodynamic equilibrium with native monomeric species^[89]. In nucleation-elongation aggregation reaction, the fibril mass is proportional to the square of the elapsed time consequently no lag phase exists. But actually the scenario is much more complex because nucleation step is catalyzed by pre-existing aggregates. Thus, from these pre-existing aggregates initial nuclei are formed, leading to the formation of critical number of aggregates and secondary nucleation pathway.

The second phase is a growth phase which consists of several steps and is thermodynamically driven^[84,87]. In the first step of growth phase, β -sheet oligomers are converted into non-fibrillar β -sheet assemblies or these oligomers are converted into large amorphous aggregates, which undergo structural rearrangement, first, to nonfibrillar β -sheet assemblies and finally to fibrils. In the last step, mature fibrils are formed usually by lateral association.

Formation of amyloid oligomers and fibril are significantly affected by macromolecular crowding. The major effects being those due to excluded volume and increased viscosity. This is validated by the findings that macromolecular crowding may lead to a dramatic acceleration in the rate of alpha-synuclein aggregation and formation of amyloid fibrils^[90].

Most recently the structures of human brain-derived A β fibrils from two patients have been studied^[91]. The structures of human brain-derived A β fibrils were compared with the structures of in vitro A β fibrils. Results have shown novel conformational features in Ab40 fibrils from patient I, for instance a twist in residues 19-23 occur that allows side chains of either F20 or E22 to be buried within the structure, a kink at G33 that allows side chains of I32 and

L34 to point in opposite directions and make contacts with different sets of A β 40 molecules, and a bend in glycine residues 37 and 38. Contrary to this, fibrils formed in vitro by A β 40 and A β 42 contain relatively simple strand-bend-strand conformations. The N-terminal is disordered in A β 40 and A β 42 whereas A β 40 fibrils from patient I showed structural order in this region. Analysis of the fibril structure from patients I and II showed differences in both peptide backbone conformation and interresidue interactions, but not overall symmetry. Thus, these data have lead to conclusion that fibrils in the brain may spread from a single nucleation site and that structural variations may correlate with variations in AD^[91].

MECHANISMS OF A β OLIGOMER MEDIATED TOXICITY AT A MOLECULAR LEVEL

The oligomeric species of A β is now considered more pathogenic than amyloid fibril. The A β oligomers play an important role in the pathogenesis of Alzheimer diseases. There are several mechanisms by which A β oligomer causes toxicity to neuron cell. For instance, increase in membrane conductance or leakage in the presence of small globulomers to large prefibrillar assemblies lead to toxicity to neuron cell^[92,93]. Studies have shown that formation of discrete ion channels or pores in the membrane is another mechanism that caused toxicities^[94-96]. Further, changes in the ratio of cholesterol to phospholipids in the membrane alter membrane fluidity and thereby favor aggregation of A β . The presence of rafts on the membrane may also influence aggregation of A β ^[97]. Thus, these data along with other reports have lead to "channel hypothesis"; implicating amyloid peptide channels are involved in ion deregulation leading to the manifestation of neurodegenerative diseases^[98,99].

Once A β channels are formed on neuronal membrane, disruption of calcium and other-ion homeostasis may take place resulting in the promotion of numerous degenerative processes, including free radical formation^[100] and phosphorylation of tau^[101], thereby accelerating neurodegeneration. The free radicals also induce membrane disruption; consequently, unregulated calcium influx is amplified which influences the production and processing of APP. Thus, a vicious cycle of neurodegeneration is initiated (Figure 4)^[102].

Contrary to the amyloid channel hypothesis, recent data suggest that homogeneous solutions of amyloid oligomers increase the conductance of artificial lipid bilayers that do not show channel-like properties.

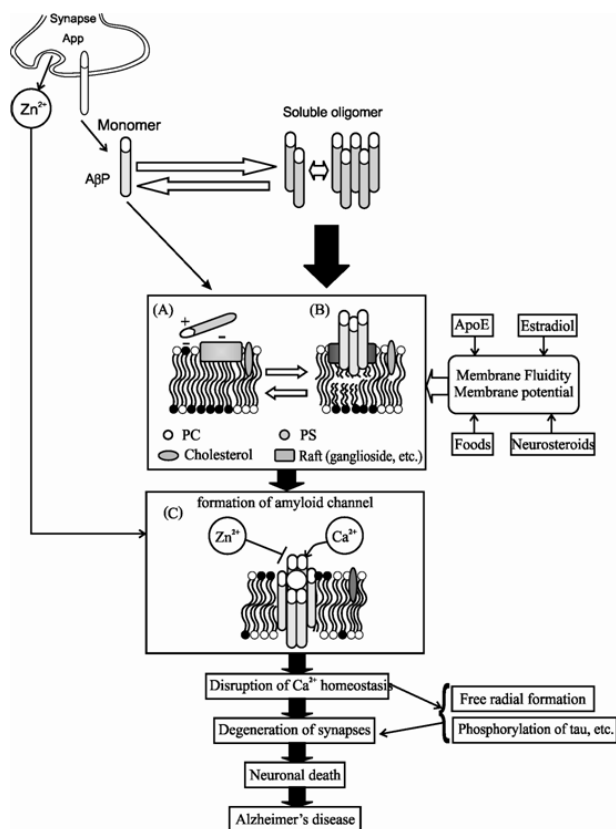


Figure 4 Membrane incorporation, amyloid channels formation and pathogenesis of Alzheimer's disease. Adapted from reference[102].

These oligomers enhanced ion mobility across the lipid bilayer^[103] by permeabilizing membrane and this is a common mechanism of pathogenesis in amyloid-related degenerative diseases^[70,104-118]. Interestingly, studies also suggest that membrane permeabilization caused by amyloid oligomers is due to defects in the lipid bilayer, rather than the formation of discrete proteinaceous pores^[118]. In accordance with this observation, Demuro et al. have demonstrated that amyloid oligomers lead to increase in Ca²⁺ levels, whereas equivalent concentrations of monomers or fibrils did not^[108]. These amyloid oligomers disrupt the integrity of both plasma and intracellular membranes in a channel independent^[108]. Thereby they increased the permeability of the plasma membrane and penetrate cells^[119] and disrupt intracellular membranes to cause leakage of sequestered Ca²⁺.

The extracellular Aβ oligomer also causes toxicity to neuron cells by binding to the cell-surface N-methyl-D-aspartate receptor (NMDAR)^[120] and other receptors resulting in synaptic dysfunction and neurodegeneration. Yamamoto and colleagues^[121] have shown that Aβ oligomers induce nerve growth factor (NGF) receptor-mediated neuronal death. Other reports on neuronal receptor-mediated toxicity mechanisms suggest that Aβ disturbs NMDAR-dependent long-term potentiation induction both in vivo and in vitro thereby causing neurodegeneration. Besides this, Aβ oligomer specifically inhibits several major signaling pathways downstream of NMDAR, including the Ca²⁺-dependent protein phosphatase calcineurin, Ca²⁺/calmodulin-dependent protein kinase II (CaMKII), protein phosphatase 1, and cAMP response element-binding protein (CREB)^[122].

Lastly, because these species are foreign to host therefore they are likely to trigger inflammatory and apoptotic responses in brain. This is supported by the findings that oligomers and fibrils form of beta-amyloid triggers inflammatory and apoptotic responses in human brain and Alzheimer's disease mouse model^[123-125].

CONCLUSIONS

Acquisition of the native three-dimensional structure of protein is one of the most fascinating areas of molecular biotechnology and biochemistry. Consequently, protein folding has been the subject of extensive investigation for the last five decades. To overcome Levinthal paradox several folding models have been discussed. Among them, folding funnel model has replaced all existing folding models. This model is represented in terms of an energy landscape and describes both thermodynamic and kinetic aspects of the transformation of an ensemble of unfolded protein molecules to a predominantly native state. According to this model, there are parallel micropathways, where each individual polypeptide chain follows its own route. Towards the bottom of the folding funnel, the number of protein conformations decreases as does the chain entropy. The second funnel shows the aggregation pathway to amorphous structure and to fibrillar state. Now oligomeric species is considered more toxic species than fibrils. The Aβ oligomeric species cause toxicities by several mechanisms including neuron membrane disruption through increase in membrane conductance or leakage in the presence of small globulomers to large prefibrillar assemblies, direct formation of ion channels and by binding to different cell-surface receptors. Thus, by inhibiting these toxic pathways will possibly lead to cure of devastating AD in future. This can be achieved by designing novel inhibitors for these toxic pathways.

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CONFLICT OF INTERESTS

The Author has no conflicts of interest to declare.

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