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Protein Misfolding, Aggregation and Diseases

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ABSTRACT

Proteins are the most abundant macromolecules in living system. They are polymers consisting of 20 different kinds of amino acids. Each protein folds into a unique three-dimensional structure defined by its amino acid sequence. The biological function of a protein is determined by its three dimensional structure. Various factors affect protein folding,. among these factors; protein structure, post transcriptional modification, mutation and the chemical environment within the cell are the major ones. Whenever there is uproar in these factors protein misfolding occurs. Protein misfoding leads to the formation of amyloid fibrils which are extracellular proteinaceous deposits found in patients suffering from any of the amyloid diseases. A broad range of human diseases arises from the failure of a specific peptide or protein to adopt, or remain in, its native functional conformation state. The pathology of such diseases underlies basically due to the formation of insoluble fiblary aggregate that resists proteolytic degradation. Such disease includes prion disease, Alzheimer's disease, Parkinson's disease and Huntington's diseases. Despite the crowded cellular environment which increases chance of a newly formed protein to misfold, protein misfolding is abridged due to molecular chaperon. Molecular chaperon are proteins which aids the proper folding of a newly formed proteins by forming a non-covalent interaction. Heat sock proteins are one form of molecular chaperons.

INTRODUCTION

1. Protein misfolding

Proteins are the most abundant molecules in biology next to water. Human beings contain almost 100,000 different types of protein and they stimulate or control virtually every chemical process on which lives depend. Different proteins are distinguished by a different order of amino acids or primary structure. There are polymeric sequences of typically 300 such building blocks. Following their biosynthesis, the majority of proteins must be converted into tightly folded compact structures in order to function. As many of these structures are astoundingly complicated, the fact that folding is usually extremely efficient is a remarkable testament to the power of evolutionary biology. It is sobering to recognize that, because there are 20 different naturally occurring amino acids found in proteins, the total possible number of different proteins with the average size of those in our bodies is much greater than the number of atoms in the universe. Natural proteins are therefore a very extraordinary group of molecules in fact. Their properties are not typical of random sequences, but have been selected through evolutionary pressure to have specific characteristics. Which gives them the ability to fold to a unique structure and hence to generate enormous selectivity and diversity in their functions is a particularly important one. (Dobson, 2004)

Protein misfolding and aggregation is one of the most exciting new frontiers in protein chemistry as well as in molecular medicine. The current interest in this topic arises from several considerations; it is thought that the knowledge of the molecular basis of protein misfolding and aggregation may help to explain the physicochemical features of protein folding; it is also expected to shed light on the molecular and biochemical basis of a number of pathological conditions of dramatic social impact such as Alzheimer's and Parkinson's diseases, type 2 diabetes, cystic fibrosis, some forms of emphysema and others. The common characteristic of such degenerative diseases is the presence, in the affected tissues and organs, of proteinaceous deposits that, in most cases, are believed to represent the main causative agents of the clinical symptoms (Stefani, 2004).

One of the defining characteristics of a living system is the ability of even the most obscure component molecular structures to self-assemble with precision and fidelity. Revealing the

mechanisms through which such processes take place is one of the many challenges of modern science. The folding of proteins into their compact three-dimensional structures is the most fundamental and universal example of biological self-assembly; understanding this complex process will therefore provide a unique insight into the way in which evolutionary selection has influenced the properties of a molecular system for functional advantage. The wide variety of highly specific structures that result from protein folding and that bring key functional groups into close proximity has enabled living systems to develop astonishing diversity and selectivity in their underlying chemical processes. In addition to generating biological activity, however, we now know that folding is coupled to many other biological processes, including the trafficking of molecules to specific cellular locations and the regulation of cellular growth and differentiation. In addition, only correctly folded proteins have long-term stability in jam-packed biological environments and are able to work together selectively with their natural partners. It is therefore not startling that the malfunction of proteins to fold correctly, or to linger correctly folded, is the starting point of a wide variety of pathological conditions (Dobson, 2003).

2. Fundamental mechanism of protein folding

Proteins are on average 100 to 500 amino acids in length. To reduce the magnitude of the folding crisis, larger proteins are divided into units (domains) that fold separately. The folding process is thermodynamically driven by the hydrophobic effect, basically the tendency of the water-rejecting (hydrophobic) amino acids to interact with one another and form a hydrophobic core while the water-loving (hydrophilic) amino acids remain at the surface. As a result, the expanded protein chain rapidly collapses into a globular structure. This significantly reduces the available conformational space (Figure 2.1). Rearrangement steps within the globule finally give rise to the correct amino acid packing that usually corresponds to the most stable, biologically active state. Small proteins can complete this task within milliseconds, whereas large ones may take seconds (Hartl, 2010).

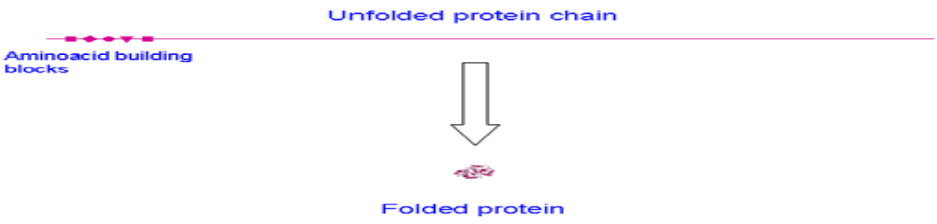

Figure 2.1: protein folding (Hartl, 2010)

Considering the complexity of the folding process, it is not too surprising that things can go wrong. The main problem is that not-yet folded proteins are sticky, because their hydrophobic amino acids are not yet covered completely. As a result, they may clump together into intractable aggregates (Figure 2.2)

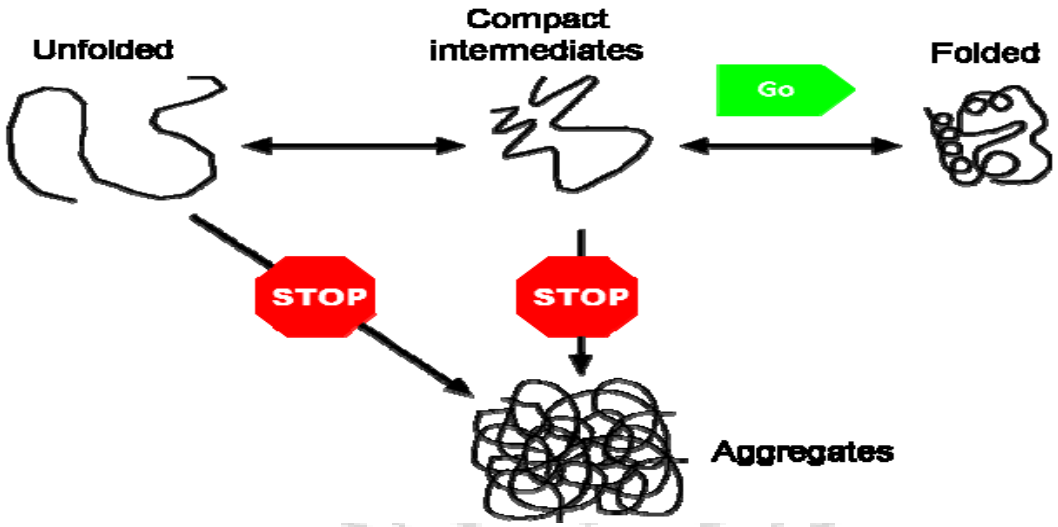

Figure 2.2: aggregation as side reaction of protein folding (Hartl, 2010)

The propensity to aggregate is strongly enhanced due to the 'crowding' of the cellular environment. The interior of a cell is an extraordinarily complex environment in which proteins and other macromolecules are present at a concentration of 300–400 mg/ml (Ellis and Minton, 2003). It is now acknowledged that within the cells of living organisms there are large numbers of supporting factors that help in the folding process, including folding catalysts and molecular chaperones (Gething and Sambrook, 1992). Molecular chaperones were discovered in 1980s and led to the understanding of how proteins fold in the cell. Molecular chaperones are themselves proteins and, help other protein chains to fold proficiently, mostly by preventing aggregation. They achieve this by Regulated cycles of chaperone binding and release then result in suppression of aggregation and productive folding (Hartl, 2010). Numerous classes of chaperones exist and many of them are so-called stress proteins or heat-shock proteins, i.e. therir production in the cell is increased stress condition, in which proteins may unfold and give rise to aggregation. It is now known that these factors function as part of a complex network of protein quality control (Balch et al., 2008).

The mechanism by which a polypeptide chain folds to a specific three-dimensional protein structure has not been clear till recent time (Karplus, 1997). There is considerable evidence that the native state of a protein corresponds to the structure that is most stable under physiological conditions. Nevertheless, the total number of possible conformations of a polypeptide chain is so large that it would take a very long time to find this particular structure by means of a systematic search of all conformational space. Recent experimental and theoretical studies have, however, provided a resolution of this apparent paradox. It is now evident that the folding process does not involve a sequence of mandatory steps between specific partially folded states, but rather a stochastic search of the many conformations accessible to a polypeptide chain. The conceptual basis of such a mechanism is shown in (Figure 2.3) In essence, the intrinsic fluctuations in the conformation of an incompletely folded polypeptide chain enable even residues at very different positions in the amino acid sequence to come into contact with one other. Because the correct (native-like) interactions between different residues are on average more stable than the incorrect (nonnative) ones, such a search mechanism is in principle able to find the lowest energy structure. It is evident that this process is extremely efficient for those special sequences that have been selected during evolution to fold to globular structures, and indeed only a very small number of all possible conformations needs be sampled during the search process (Dobson, 2004).

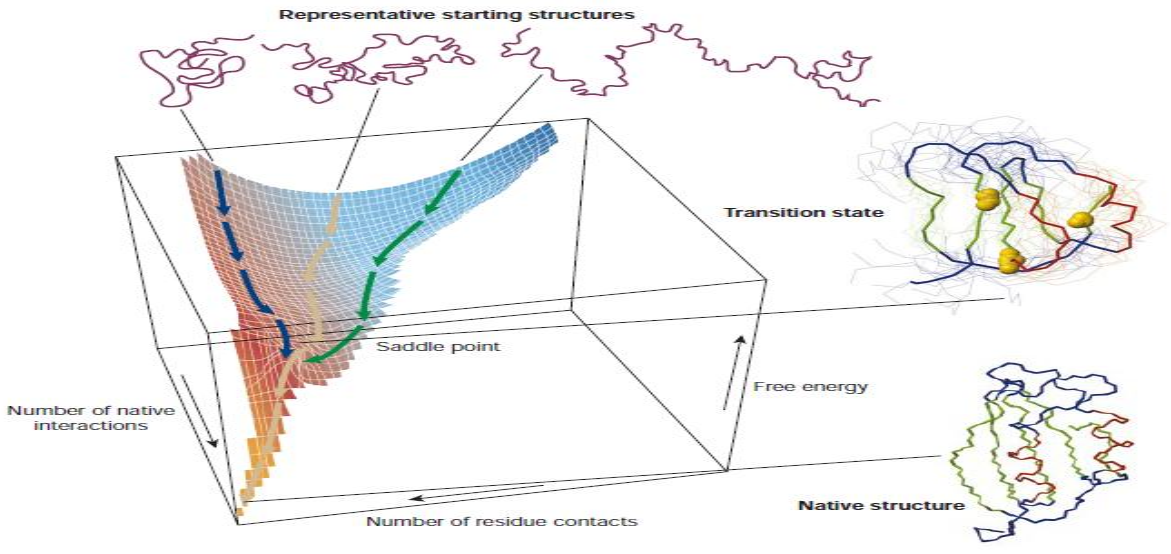

Figure 2.3: Schematic energy landscape for protein folding (Dobson, 2004)

Further details of how such a mechanism is able to generate a unique fold have emerged from a range of theoretical studies, predominantly involving computer simulation techniques (Shea and Brooks, 2001). Once the correct topology has been achieved, the native structure will then almost perpetually be generated during the final stages of folding. On the other hand, if these key interactions are not formed, the protein cannot fold to a stable globular structure; this mechanism therefore acts also as a 'quality control' process by which misfolding can generally be avoided (Dobson et al., 2001).

3. The determinants of protein folds

3.1. Protein structures

The first significant factor is protein structure. Particularly, primary and secondary structures of a protein are two of the most vital factors for physical and chemical features. Encoded information in amino acid sequence of a protein determines the three dimensional structure. Position and number of different characteristic amino acid residues in primary structure may lead to an increase or a decrease in aggregation behavior. In most case the number of hydrophobic amino acids in proteins is proportional to tendency of aggregation. Secondary structures of proteins involve in protein misfolding as well as stability. Proteins often fold locally into stable structures that comprise α-helix and β-sheet (Tutar et al., 2013).

3.2. Mutations

Mutations also has a key role in protein aggregation and they may significantly alter solubility, stability, and aggregation tendency of proteins .Thermally stable proteins may alter its stability even with a point mutation in its structure. For example, a human lysozyme I56T and D67H mutants greatly decreases the lysozyme stability and as a result the lysozyme aggregates easily upon heating. Further aggregation cause amyloid fibrils and these fibrils are deposited in tissues and are associated with neurodegenerative diseases (Tutar et al., 2013). Lately, scientists have been suggested a new protein for understanding of ALS (amyotrophic lateral sclerosis), AD, cystic fibrosis (CF) and frontotemporal lobar degeneration (FTLD) mechanisms. The transactive response DNA binding protein 43 (TDP-43) is expressed by all mammalian tissues, conformational changes in this protein cause aggregation and loss of function. TDP-43 has been shown to bind to DNA and mRNA and participate in regulation of transcription and translation.

TDP-43 has a glycine rich C-terminal tail and mutation occurs from this region. Consequently, TDP-43 is converted to aggregated form which is accumulated in tissues (Dickson et al., 2011).

3.3. Post-translational modifications

After a protein is synthesized, the posttranslational modifications (PTM) of amino acids may increase the diversity of proteins by additional functional groups (acetate, phosphate, various proteins etc.) and structural changes. In particular, phosphorylation plays a significantrole in neurodegenerative diseases. It is also known that, occurrence of AD is associated with tauopathy due to aggregation of the tau protein. In brain, tau protein is found in neurons and it can be phosphorylated with kinase enzymes. Thus, aberrant tau aggregates are formed and they can be accumulated in neurons, thereby their toxic effects are caused neuronal loss and synaptic alteration (Buee et al.,2000 and Martin et al., 2011).

Among the post- translational modification glycosylation is an important PTM for protein stability and aggregation potential. Forexample human prion protein has two potential *N*-glycosylation sites (Asn181 and Asn197). However, in prion pathology, conversion of PrPc to PrPSc occurs easily if the PrPc is glycosylated. In AD patients, hyperglycosylated tau protein is found in brains (Rudd et al., 2002 and Rudd et al., 2001).

3.4. Oxidative stress

Oxidative stress leads to protein oxidation which is a biomarker for many neurodegenerative diseases. In particular, free radicals and ROS (reactive oxygen species) cause protein oxidation. A multiplicity of oxidants can be occurred in normal aerobic metabolism. Also, lack of antioxidants, excess of oxygen and lipid and metal ions can generate free radicals. The oxidation of proteins extremely depends on their amino acid compositions. Generally; lysine, histidine, arginine, methionine, cysteine, phenylalanine, tryptophan, threonine, glutamic acid, and proline residues incline oxidation. Some proteins have metal binding regions on its structure. Metal ions such as copper, zinc, and iron, are capable of redox reactions and electrons are transferred from ions to oxidizing compounds. Therefore, toxic free radicals are formed and proteins can be converted into aggregation forms or proteins can be aggregated by conformational changes (Tutar et al., 2013).

3.5. Protein concentration

Protein-protein interactions and intermolecular interactions (especially interactions among hydrophobic amino acids) may generate aberrant protein structures. Some misfolded protein aggregates can be constituted neurodegenerative diseases above a certain concentration. Moreover, proteins are refolded at low concentrations spontaneously. For example, lysozyme and immunoglobulin G refold itself at low protein concentration however, refolding yield decreases with increasing protein concentration. Therefore, the optimum spontaneous protein concentration range is accepted as 10-50 μg/ml (Tutar et al., 2013).

3.6. pH

Environmental pH is to be critical for protein aggregation due to changes in net charge on protein. Protonation state of ionizable sites of protein and positive net charge are increased in acidic conditions. Especially, organization of salt bridges is changed in parallel with composed new secondary structures (Tutar et al., 2013). In prion diseases, acidic pH facilitates generation of PrPSc. At low pH, PrPc gains β-sheet structures and shows aggregation tendency (Demarco and Daggett, 2005).

4.Protein folding and misfolding in the cell

In a cell, proteins are synthesized on ribosomes from the genetic information encoded in the cellular DNA. Folding in vivo is in some cases co-translational; that is, it is initiated before the completion of protein synthesis, where the nascent chain is still attached to the ribosome. Other proteins, however, undergo the major part of their folding in the cytoplasm after release from the ribosome, whereas yet others fold in specific compartments, such as mitochondria or the endoplasmic reticulum (ER), after trafficking and translocation through membranes. Molecular chaperones often work in tandem to ensure that the various stages in the folding of such systems are all completed efficiently. Many of the details of the functions of molecular chaperones have been determined from studies of their effects on folding in vitro. The best characterized of the chaperones studied in this manner is the bacterial complex involving GroEL, a member of the family of ‘chaperonins’, and its ‘co-chaperone’ GroES. Many aspects of the sophisticated mechanism through which this coupled system functions are now well understood. Of particular

interest is GroEL, and other members of this class of molecular chaperone, contains a cavity in which incompletely folded polypeptide chains can enter and undergo the final steps in the formation of their native structures while sequestered and protected from the outside world (Dobson, 2004).

In eukaryotic systems, most of the proteins that are synthesized in a cell are intended for secretion to the extracellular environment. These proteins are transported into the ER, where folding takes place before secretion through the Golgi apparatus. The ER contains a wide range of molecular chaperones and folding catalysts, and in addition the proteins that fold here must satisfy a 'quality-control' check before being exported (Figure 4.1) Such a process is particularly important because there seem to be few molecular chaperones outside the cell, although one (clusterin), at least, has recently been discovered. This quality-control mechanism involves a remarkable series of glycosylation and deglycosylation reactions that enables correctly folded proteins to be distinguished from misfolded ones. The importance of these regulatory systems is underlined by recent experiments that suggest that a large fraction of all polypeptide chains synthesized in a cell fail to pass this test and are targeted for degradation. Like the 'heat shock response' in the cytoplasm, the 'unfolded protein response' in the ER is also stimulated (upregulated) during stress and, as we shall see below, is strongly linked to the avoidance of misfolding diseases. Folding and unfolding are the ultimate ways of generating and abolishing specific types of cellular activity. In addition, processes as apparently diverse as translocation across membranes, trafficking, secretion, the immune response and regulation of the cell cycle are directly dependent on folding and unfolding events. Either failure to fold correctly, or to remain correctly folded, will therefore give rise to the malfunctioning of living systems which will result disease (Dobson, 2004).

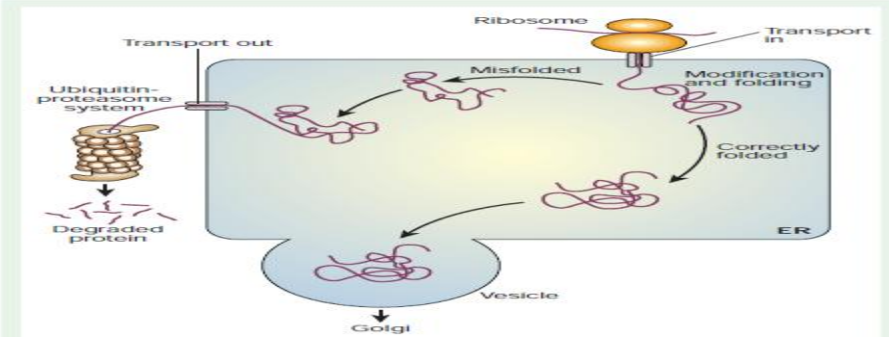

Figure 4.1: regulation of protein folding in ER (Dobson, 2004).

5. The dark side of the protein world

Recent data released have showed new features of protein misfolding and aggregation. Initially, it appears that protein aggregation may be a generic property of polypeptide chains possibly linked to their common peptide backbone that does not depend on specific amino acid sequences. Besides, it has been shown that even the toxic effects of protein aggregates, mainly in their pre-fibrillar organization; result from common structural features rather than from specific sequences of side chains. These data direct to hypothesize that every polypeptide chain, in itself, possesses a previously unsuspected concealed dark side leading it to transform into a generic toxin to cells in the presence of appropriate destabilizing conditions. This new outlook of protein biology underscores the key importance, in protein evolution, of the negative selection against molecules with significant tendency to aggregate as well as, in biological evolution, of the development of the complex molecular machineries intended at hindering the appearance of misfolded proteins and their toxic early aggregates (Stefani, 2004).

A large number of neurodegenerative diseases in humans result from protein misfolding and aggregation. Protein misfolding is believed to be the primary cause of Alzheimer's disease, Parkinson's disease, Huntington's disease, Creutzfeldt–Jakob disease, cystic fibrosis, Gaucher's disease and many other degenerative and neurodegenerative disorders. Cellular molecular chaperones, which are ubiquitous, stress-induced proteins, and newly found chemical and pharmacological chaperones have been found to be effective in preventing misfolding of different disease-causing proteins, essentially reducing the severity of several neurodegenerative disorders and many other protein-misfolding diseases (Chaudhuri and Pau, 2006). The common seal of such degenerative diseases is the presence, in the affected tissues and organs, of proteinaceous deposits that, in most cases, are believed to represent the main causative agents of the clinical symptoms. A group of roughly 20 protein deposition diseases, usually referred to as amyloidoses, are characterized by the presence of deposits of fibrillar aggregates found as intracellular inclusions or extracellular plaques (amyloid) whose main constituent is a specific peptide or protein, different in the varying diseases **(Table 5.1)** Despite the structural and chemical differences of the polypeptide chains aggregating into amyloid, amyloid fibrils are surprisingly similar in their appearance and structural features (increased content of beta structure) and tinctorial properties (binding of dyes such as thioflavin T and Congo red). In

addition to amyloidoses, other protein misfolding diseases with deposition of protein aggregates which are not amyloid in nature have been known for a long time. Serine protease inhibitors such as α1-antitrypsin, antithrombin and plasminogen activator inhibitor 1 may be destabilized by specific mutations. As a consequence, the exposed mobile reactive loop inserts, as an extra beta-strand, into the beta-sheet of another identical molecule; when propagated, these structural modifications lead to the formation of protein chains aggregating into amyloid, amyloid fibrils are surprisingly similar in their appearance and structural features (increased content of beta structure) and tinctorialproperties (binding of dyes such as thioflavin T and Congo red) (Stefani, 2004).

Table 5. 1: A summary of the main amyloidoses and the proteins or peptides involved (Stefani, 2004).

Alzheimer's disease	*Aβ peptides (1–40, 1–41, 1–42, 1–43); Tau*
Spongiform encephalopathies	*Prion protein (full-length or fragments)*
Parkinson's disease	*α-synuclein (wild type or mutant)*
Fronto-temporal dementias	*Tau (wild type or mutant)*
Familial Danish dementia	*ADan peptide*
Familial British dementia	*ABri peptide*
Hereditary cerebral haemorrhage with amyloidoses	*Cystatin C (minus a 10-residue fragment); Aβ peptides*
Amyotrophic lateral sclerosis	*Superoxide dismutase (wild type or mutant)*
Dentatorubro-pallido-Luysian atrophy	*Atrophin 1 (polyQ expansion)*
Huntington disease	*Huntingtin (polyQ expansion)*
Cerebellar ataxias	*Ataxins (polyQ expansion)*
Kennedy disease	*Androgen receptor (polyQ expansion)*
Spino cerebellar ataxia 17	*TATA box-binding protein (polyQ expansion)*
Primary systemic amyloidosis	Ig light chains (full-length or fragments)
Secondary systemic amyloidosis	Serum amyloid A (fragments)
Familial Mediterranean fever	Serum amyloid A (fragments)
Senile systemic amyloidosis	Transthyretin (wild-type or fragments thereof)
Familial amyloidotic polyneuropathy I	Transthyretin (over 45 variants or fragments thereof)
Hemodialysis-related amyloidosis	β2-microglobulin
Familial amyloid polyneuropathy III	Apolipoprotein A-1 (fragments)
Finnish hereditary systemic amyloidosis	Gelsolin (fragments of the mutant protein)
Type II diabetes	Pro-islet amyloid polypeptide (fragments)
Medullary carcinoma of the thyroid	Procalcitonin (full-length or fragment)
Atrial amyloidosis	Atrial natriuretic factor
Lysozyme systemic amyloidosis	Lysozyme (full-length, mutant)
Insulin-related amyloid	Insulin (full-length)
Fibrinogen α-chain amyloidosis	Fibrinogen (α-chain variants and fragments)

In addition to amyloidoses, other protein misfolding diseases with deposition of protein aggregates which are not amyloid in nature have been known for a long time. Serine protease inhibitors such as α1-antitrypsin, antithrombin and plasminogen activator inhibitor 1 may be destabilized by specific mutations. As a consequence, the exposed mobile reactive loop inserts, as an extra beta-strand, into the beta-sheet of another identical molecule; when propagated, these structural modifications lead to the formation of protein polymers that are retained intracellularly, leading to cell impairment by a loss of function (the lack of active protein) and a toxic gain of function (the cytotoxicity of protein aggregates). In α1-antitrypsin deficiency, 1 out of over 70 α1-antitrypsin mutants aggregate into the endoplasmic reticulum of hepatocytes leading to liver disease. In addition, the lack of active protein leaves lung parenchyma unprotected against the enzyme neutrophil elastase leading to early-onset emphysema (Lomas and Carrell, 2002).

Protein misfolding leads to protein aggregation and accumulation of these aggregates is implicated as the main reason of neurodegenerative diseases. In brain, some native proteins (prion, tau, β-amyloid, α-synuclein, and huntington) undergo conformational changes through genetic and environmental factors. Therefore, secondary structures of protein convert from α-helix/random coil to β-sheet (Tutar et al., 2013).

Table 5.2: Properties of neurodegenerative diseases (Tutar et al., 2013).

Neurodegenerative Diseases	Respective Proteins	Mechanism	Conformation in Aggregates	Inclusion
Prion Diseases	Prion	Protein aggregation	β-sheet	Spongiosis
Alzheimer's Diseases	Tau β-amyloid	Protein aggregation	β-sheet	Neurofibrillary Tangles Senile Plaques
Parkinson's Diseases	α-Synuclein	Protein aggregation	β-sheet	Lewy Bodies
Huntington's Diseases	Huntington	Protein aggregation	β-sheet	Huntington Inclusion

6. Protein-misfolding diseases

Protein misfolding and its pathogenic consequences have become an important issue I the last 20 years. According to the prion researcher Susan Lindquist, 'protein misfolding could be involved in up to half of all human diseases' (Bradbury,2003). Protein misfolding is also responsible for many cancers which are medated by p53, which are also the result of incorrect protein folding. Many cancers and other protein-misfolding disorders are caused by mutations in proteins (Table 6.1) that are key regulators of growth and differentiation. Structural changes in a few proteins subsequently lead to aggregated masses, which occasionally result in neurotoxicity and cell death (Chaudhuri and Pau, 2006). Misfolded proteins turn into neurotoxic (e.g. prion protein in mad cow disease; MCD) because of protein aggregation is an inevitable consequence of a cellular existence and these aggregates are oligomeric complexes of non-native conformers that arise from intermolecular interactions among structured and kinetically trapped intermediates in the protein folding or assembly pathway. Protein aggregates can be either structured (e.g. amyloid) or amorphous. In both case, they are insoluble and metabolically stable in the physiological environment. For various diseases associated with protein misfolding, one or more proteins are converted from the native structure to an aggregated mass, which is commonly called an 'amyloid'. The net accumulation of toxic protein aggregates in the cell depends on the stability, compactness and hydrophobic exposure of the aggregates, as well as on the rate of protein synthesis in the cell. The accumulation of toxic aggregates in the cell depends on chaperone expression and protease activities (Chaudhuri and Pau, 2006).

Table 6.1: Mutation observed in different disease causing proteins (Chaudhuri and Pau, 2006).

Disease	Proteins affected	Mutations/mutated gene
CF	CFTR	ΔF508
α-Antitrypsin deficiency	α-Antitrypsin	D342K
NDI	Aquaporin-2/V2asopressin	T126M, A147T, R187C
		R187C/Δ62–64, L59P, L83Q,
	1	Y128S, S16L, A294P, P322H, R337X
Fabry	α-Galactosidase A	R301Q, Q279E
Cancer	p-53	R175, G245, R248, R249,
		R273 and R282
PD	α-synuclein	A53T, A30P
AD	Amyloid precursor protein	AD 1, AD 2, AD 3, AD 4 Tau, preselinin 1 and 2,
α-		α-macroglobulin
HD	Huntingtin	HD
SCA	Ataxin	SCA

Neurodegenerative disorders that are chronic and progressive are characterized by the selective and symmetrical loss of neurons in motor, sensory or cognitive systems. The most common feature of all the neurodegenerative disorders is the occurrence of brain lesions, formed by the intra- or extracellular accumulation of misfolded, aggregated or ubiquitinated proteins. Proteins associated with some neurodegenerative diseases like AD, PD and HD, are tau ⁄ b-amyloid (Ab), a-synuclein and huntingtin, respectively (Chaudhuri and Pau, 2006).

The beginning of aggregation may be facilitated by any factor resulting in a rise of the concentration of the amyloidogenic precursors such as a shift of the equilibrium from correctly folded and partially folded molecules towards the latter or an increase of the expression level of the affected protein and hence its whole equilibrium population comprising partially folded molecules (Figure 6.1) (Stefani, 2004).

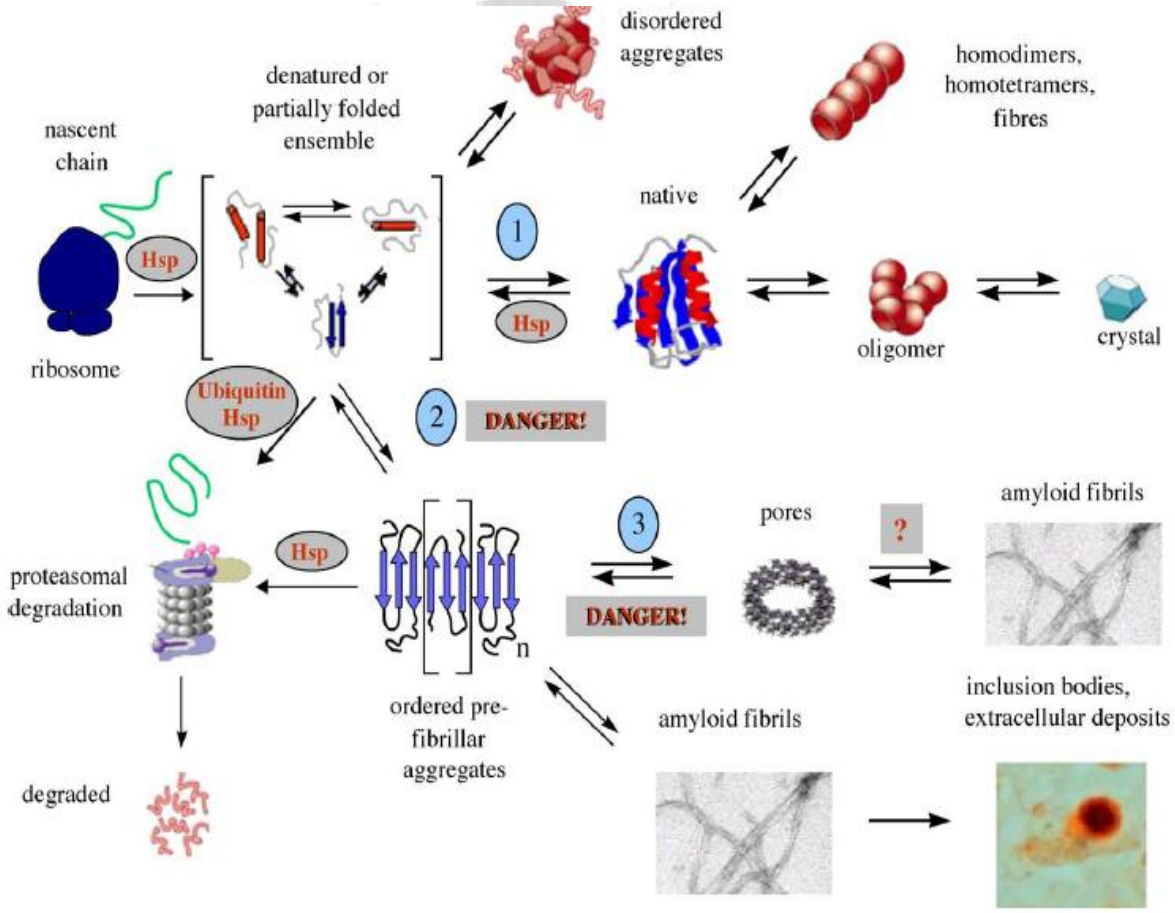

Figure 6.1: Different path ways of amyloide protein deposits formation (Stefani, 2004).

Despite the difference in the initial process of different diseases, a common trend is that during the formation of aggregates, a-helical domains disappear, leading to an increase of b-sheet-dominated secondary structure (Figure 6.2) lately, many other physiological disorders have been recognized as being caused by the formation of protein aggregation, which subsequently forms a plaque-like structure containing a large number of amyloid fibrils, these are polymerized to cross b-sheet structures with the b-strands arranged perpendicular to the long axis of the fiber (Chaudhuri and Pau, 2006)

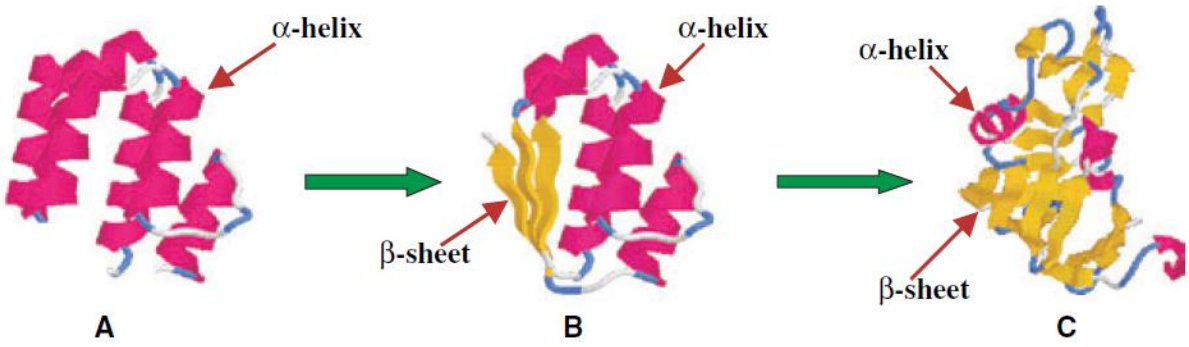

Figure 6.2: Convertion a-helical structures to b-pleated sheet during amyloid formation

(A) Native polypeptide chain composed of mainly a-helical secondary structure,

(B) Misfolding causes conversion of a-helicalstructure to b-pleated sheets and

(C) Final misfolded structure of polypeptide chain contains mostly b-pleated sheets (Chaudhuri and Pau, 2006)

The fibrillar structures that are characteristic of many of the aggregates have very related morphologies (long, unbranched and often twisted structures a few nm in diameter) and a characteristic "cross-beta" X-ray fibre diffraction pattern. **(Figure 6.3)**. Fibrils having the essential characteristics of ex vivo deposits can be reproduced in vitro from the component proteins under appropriate conditions, showing that they can self-assemble without the need for other components (Dobson, 2004).

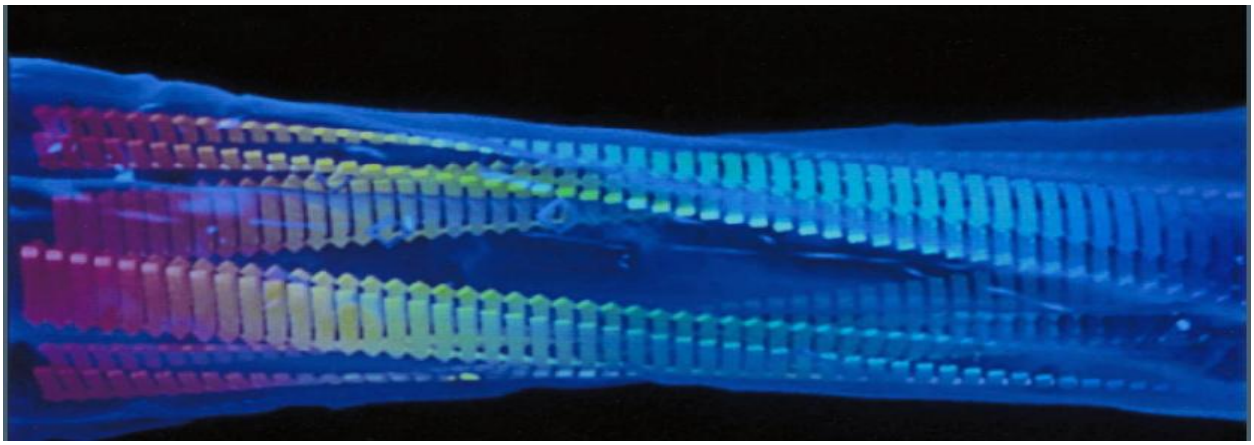

Figure 6.3: A molecular model of an amyloid fibril (Dobson, 2004).

a. Prion diseases

There are different groups of prion diseases, including Creutzfeldt-Jakob (CJD), fatal familial insomnia (FFI), Kuru, Gerstmann-Sträussler-Scheinker syndrome (GSS) are seen in humans, and in similar fashion scrapie, bovine spongiform encephalopathy (mad cow disease), chronic wasting diseases (CWD), transmissible mink encephalopathy (TME), feline spongiform encephalopathy (FSE) diseases are observed in animals. All of these diseases give similar neurological symptoms such as dysmnesia, depression, sense disturbances, and psychosis (Tutar et al., 2013).

In 1982, the term prion was coined by Stanley Prusiner and co-workers from "proteinaceous infectious particle". Prion protein is found in two different forms: a cellular form of prion protein (PrPc) and scrapie isoform of prion protein (PrPSc). Properly folded form is denoted as PrPc while misfolded form is denoted as PrPSc (Mehrpour and Codogno, 2010). The PrPc is an α-helix-rich glycoprotein that is approximately 250 amino acids in length. It is encoded by the prion protein gene (Prpn) which is located on chromosome 20. PrPc is commonly found on neuronal cell membrane by a glycosyl phosphatidylinositol (GPI). However it is also expressed on other cells such as leukocytes and dendritic cells. PrPc has been assumed a variety of functions including cell adhesion, intracellular signaling, copper metabolism, and protective antioxidant activity. PrPc is highly conserved protein among mammals during evolution (Fig 6.1.1). When we examine the primary structure of the protein, PrPc consist of a signal peptide (1-22), five octapeptide repeats (PHGGGWGQ) (51-91), a highly conserved hydrophobic domain

(106-126), and a GPI (glyco-sylphosphatidylinositol) anchor. Furthermore, PrPc contains two N-linked glycosylation sites (181Asn-Ile-Thr and 197Asn-Phe-Thr). Thus, they get dynamic and flexible properties and the glycan covers prevent intermolecular and intramolecular interactions.His96 and His111 are found in metal binding domains of PrPc and they compose coordination sites with metal ions (Cu+2, Zn+2, Mn+2, Ni+2). A disulfide bond between Cys179 and Cys214 play a significant role for proper folding of PrPc (Tutar et al., 2013).

Figure 6.1.1: Multiple protein sequence alignment of prion proteins (Tutar et al., 2013).

PrPSc can be defined as an infectious isoform of PrPc and causes fatal prion diseases. PrPSc is formed by misfolding of PrPc with a lost in α-helical content. PrPSc has same amino acid sequence with PrPc, but their secondary, tertiary, and quarternary structures are different. Approximately, PrPc includes 3% β-structure and 47% α-helix structure, but nonetheless PrPSc is composed of 43% β-structure and 30% α-helix structure . It becomes non-soluble and resists to proteolytic degradation with conformational changes, whereas PrPc is soluble and protease

sensitive. This insoluble protein accumulates in brain and causes a variety of prion diseases in human and animals (Tutar et al., 2013).

In the case of infectious prion disease, the infectious scrapie protein (PrPSc) drives the conversion of cellular PrPC into disease-causing PrPSc (fig 6.1.2) (Chaudhuri and Pau, 2006).

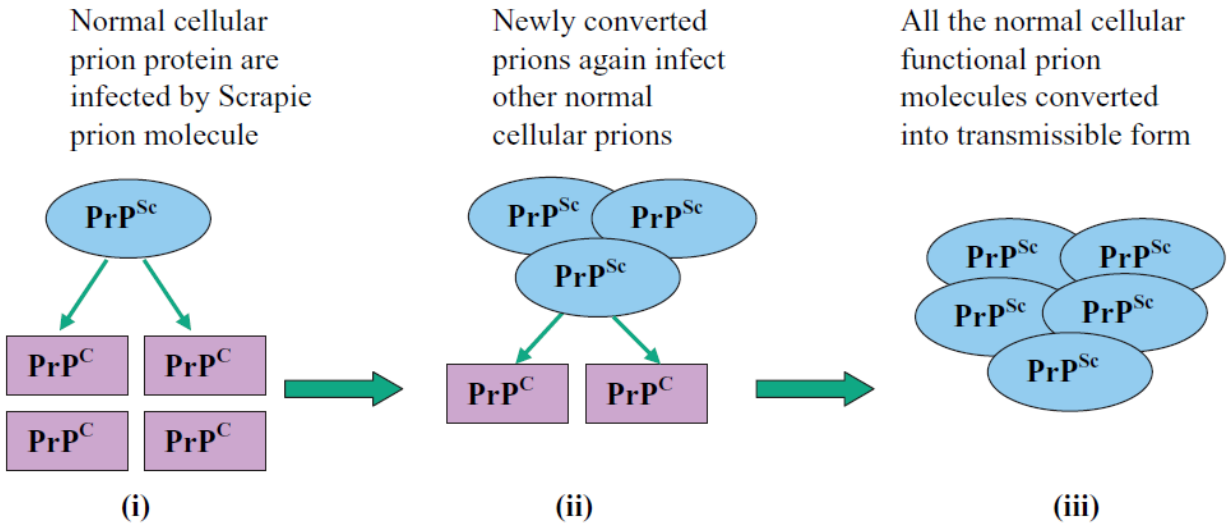

Figure 6.1.2: Propagation of PrPSc

(i)Transmissible isoform of one prion protein molecule infects other normal cellular prion molecules.

(ii) Infection causes induction in conformation of normal prions that converts them to transmissible prion molecules, which again start infecting other normal prion molecules.

(iii) All the cellular normal prions are transformed into disease causing scrapie prion proteins (Chaudhuri and Pau, 2006).

Preceding studies suggested a variety of mechanisms for explaining prion pathology. Oxidative stress and lipid peroxidation are the major factors in prion diseases. In central nervous systems, a variety of oxidative stresses including high level of oxygen and lipid, metal ions, and inadequate antioxidants produce free radicals. It has been showen that in PrPSc infected mice, superoxide anion (O2 -) is extremely increased in brain. As a result, high levels of heme oxygenase-1 and malondialdehyde are observed as oxidative stress markers in brain. In addition Cytochrome c oxidase is a large transmembrane protein in mitochondria and it shows antioxidant activity inmitochondria. The level of lipid peroxidation is increased while Cytochrome c oxidase

activities are reduced in scrapie infected animal models. It is knowen that Phospholipase D catalyzes the hydrolysis of phosphatidylcholine to generate choline and phosphatidic acid, and its expression level is induced by H2O2. According to the studies, activity of phospholipase D is increased in the brains of scrapie infected animals. As a result, PrPc transforms into PrPSc because of the formation of these free radicals (Tutar et al., 2013). Also Cu2+ions play a critical role in prion diseases. PrPc has five conserved octapeptide repeats (PHGGGWGQ) which have an affinity for Cu2+ ions in contrast, affinity of other metal ions (Mg2+, Mn2+, Ni2+ etc.) is weak or nonexistent. The binding of Cu2+ provides formation of protease resistant form: PrPSc. It is also suggested that, PrPc protects cells from harmful redox activities. Especially, in copper-rich environment, PrPc acts as a "copper buffer" that means it inhibits toxic effects of Cu2+ ions for central nervous system and helps maintaining neurons in high level of cupper ions. Thus, redox damage of PrPc has been involved in prion diseases. In brief, copper can convert the cellular prion protein into a protease-resistant species with conformational changes (Markus et al., 2014).

Glycosylation is one factor that increase stability during post translational modification in proteins. PrPc contains two potential glycosylation sites which are Asn181 and Asn197 in human PrPc. Generally, binding of carbohydrates can protect protein surface from proteases and undesired protein-protein interactions. Moreover, N-glycosylated prion protein is anchored to the lipid membranes via GPI. Interaction of PrPc with membrane lipid layers play a significant role in conversion of PrPc to PrPSc. PrPc is localized in cholesterol and sphingomyelin rich area on cell surface (known as lipid raft). PrPc is bound to lipid membranes through its GPI anchor. While leaving PrPc from the membranes by catalysis of phosphatidylinositol phospholipase C (PIPLC), PrPSc show resistance to PIPLC. When binding of PrPc to the lipid membranes, PrPc is degraded or converted into PrPSc form (Tutar et al., 2013).

b. Alzheimer's disease

Alzheimer's disease (AD) is the most frequent type of neurodegenerative disorder in the world. AD results from accumulation of aberrant folded tau protein and beta-amyloid protein (Aβ protein) in brain. In 1906, AD was first described by psychiatrist and pathologist Dr. Alois Alzheimer and then the disease was named with his surname till now, there are no effective treatment methods for AD, but some nuclear medicine applications (MRI and PET) are applied

in diagnosis of AD. Dramatically, symptoms of AD rise with increasing age and the first sign is memory lapse. Cellular and molecular mechanisms of AD are not well understood yet. Researchers have been reported that AD is associated with genetic and environmental factors and life-style (Tutar et al., 2013).

AD is a progressive degenerative disease of the brain in the elderly which clouds memory and causes impaired behavior. The neuropathological features of this overwhelming disease are the extracellular deposition of Aβ and neurofibrilary tangles (NFT) in the brain. A central process of AD is the cleavage of a 42 amino acid β-amyloid peptide from an otherwise normal membrane precursor protein. The main protein is a membrane protein called amyloid precursor protein, which after being cleaved by β-secretase produces a β-amyloid precursor peptide fragment; this is further cleaved by another protease β-secretase to produce Aβ-42 instead of Aβ-40, which is amyloidogenic. It is thought that cellular degradation of Aβ-42 is the normal fate of this peptide fragment when produced in small amounts under normal conditions, however, in some lesser known conditions it forms extracellular aggregates and subsequently generates amyloid plaques. Studies have reported that impairment of the UPS may be involved in this disorder. An increase in neurotoxicity has been generated by dimer and oligomer formation (Fig 6.2.1) of the Aβ fragment. NFTs are aggregations of the microtubular protein tau, which are found to be hyperphosphorylated in the neuronal cells of AD patients. Although, tau polymer formation is a hallmark of other degenerative disorders, such as corticobasal degeneration, progressive supranuclear palsy and pick disease, all differ from AD in that they lack Aβ plaque deposition (Chaudhuri and Pau, 2006).

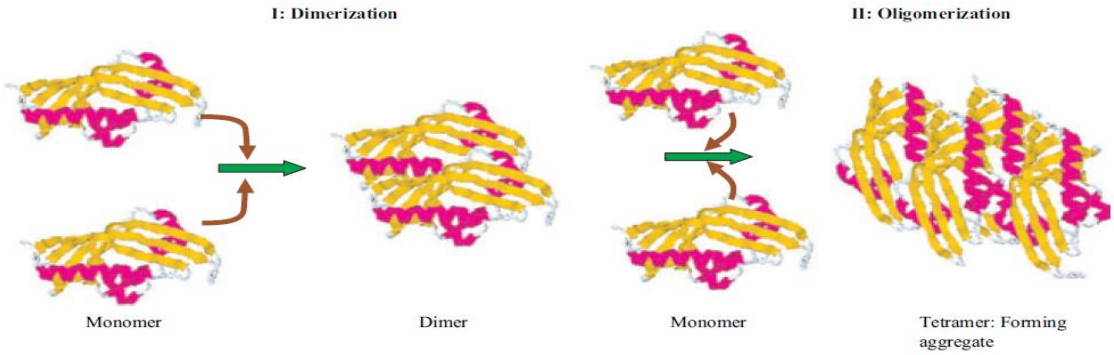

Figure 6.2.1: Protein oligomerization. Misfolded monomers forming aggregate through intermolecular hydrogen bonding interaction leading to b-sheet formation (Chaudhuri and Pau, 2006).

A microtubule associated protein, Tau, is a biggest component of AD. In 1975, tau proteins were first discovered by Marc Kirschner in Princeton University. Tau was derived from "**t**ubulin associated unit" as a term. It is highly expressed in brain, but other organs such as lung, hearth, and kidney have trace amounts. The human tau gene is located on chromosome 17q21 and contains 16 exons. Among these exons of the tau gene, exon 2, 3, and 10 are alternatively spliced and these exons allow six combinations (2-3-10-; 2+3-10-; 2+3+10-; 2-3-10+; 2+3-10+; 2+3+10+). Thus, human brain contains six isoforms of tau proteins which range from 352 to 441 amino acids length and approximate molecular weights are between 60 and 70 kDa (Fig. 6.2.2) (Tutar et al., 2013).

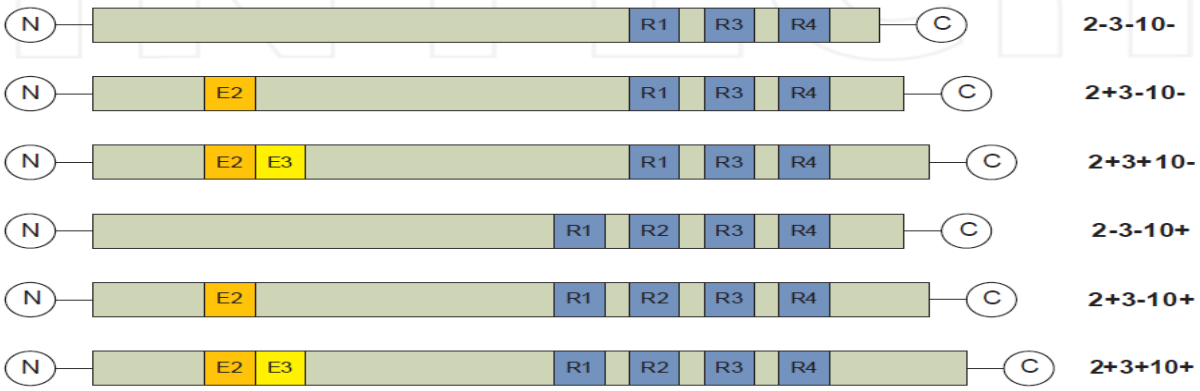

Figure 6.2.2: Schematic representation of six human tau isoforms (Tutar et al., 2013).

Tau protein has four main regions in its primary structures. Acidic region is located in the N-terminal part and it is encoded by exon 2 and exon 3. Prolin-rich region located in the middle of the protein is encoded by exon 7 and exon 9 and contains several PXXP motifs which can interact with tyrosine kinase. Prolin-rich region works together with acidic region, therefore these two regions are called projection domain which interacts with neural plasma membrane and cytoskeletal elements. Tau protein has three to four highly conserved repeats in the C-terminal part for binding to microtubules. Therefore, these repetitive regions are called microtubule binding domains (MBDs) which is encoded by exons 9-12. 275VQJINK280 and 306VQJVYK311are conserved hexapeptides which are located at the beginning of the second and third MBDs. These peptides involve in the generation of β-sheet structure during tauopathy.

Microtubules are major proteins of the cytoskeleton. They have hollow and cylindrical structure and participate in intracellular transport, protection cell structure, and continuity of cell viability.

The main function of the tau protein is to stabilize microtubules with binding to microtubules and to other proteins . To perform these functions, tau proteins must be phosphorylated at normal level. However, if tau protein hyperphosphorylate, its biological activity can be lost. Moreover, hyperphosphorylation causes conformational changes and aggregation of tau proteins. Other post-translational modifications such as glycosylation,glycation, polyamination, and nitration may play essential roles in AD (Tutar et al., 2013).

Thus in Alzheimer's disease there is, accumulation of either a small protein fragment called Aβ or tau protein in in the hippocampus, disturbing the complex neural networks of this brain region, resulting in cell death and loss of memory function (Hartl, 2010).

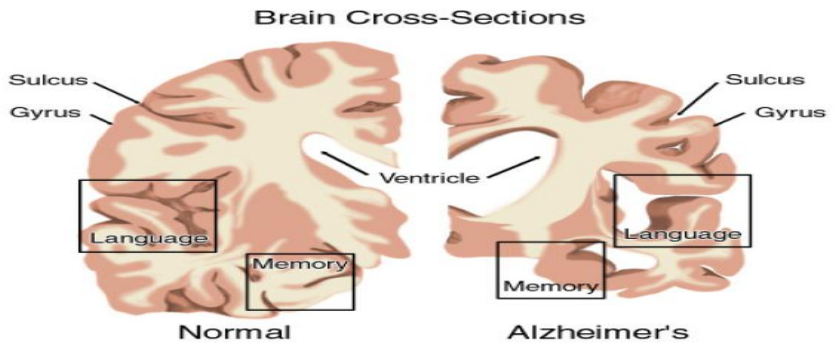

Figure 6.2.3: Cross sections of normal and Alzheimer's disease (AD) brain (Hartl, 2010).

It has been showen that these aggregates are highly ordered fibrils that are known as amyloid (Fig 6.2.4). They are generated from smaller, less ordered protein clumps, so-called soluble oligomers, which many believe are the most toxic misfolded protein species (Hartl, 2010).

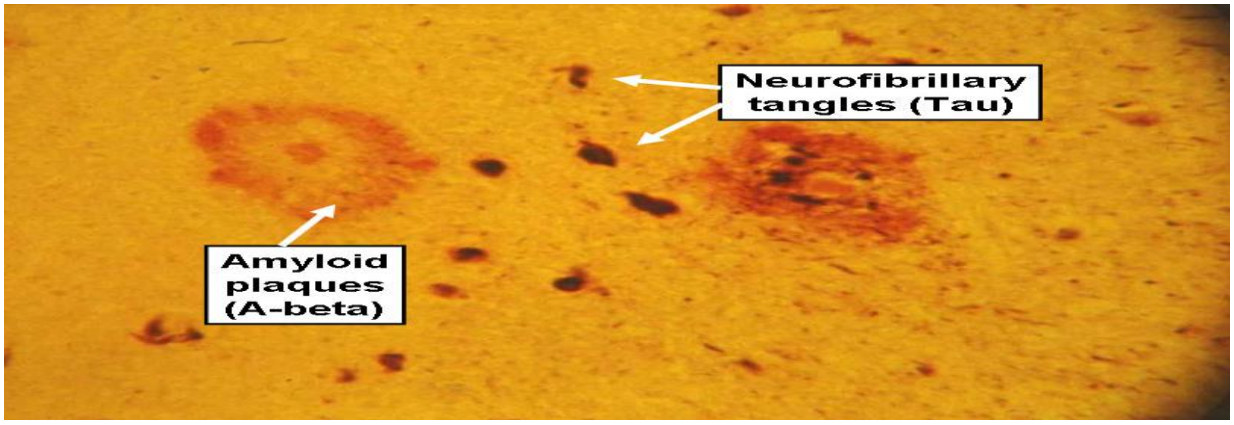

Figure 6.2.4: Microscopic image of brain tissue form an Alzheimer disease (AD) patient (Hartl, 2010).

Structurally in normal brain, Aβ proteins contain mixture of β-sheet and random coil structures. However, a number of β-sheet structures are increased at high protein concentration. Aβ protein constitutes SPs which are important markers in AD. The formation of SPs is a problem of protein folding because of misfolded and aggregated form of Aβ accumulates and shows toxicity in brain.

Genetically, three genes: *APP*, *PS1*, and, *PS2* are associated with AD. More than 50 different mutations in the APP gene can cause AD. The most frequent APP mutation is a single mutation in the APP at position 717. As a result of this single mutation, a valine residue is replaced by an isoleucine, or phenylalanine, or glycine. *APP* mutations lead to an increased amount of the Aβ proteins which are deposited in neuritic plaques (Tutar et al., 2013).

c. Parkinson's diseases

Parkinson's disease (PD) is neurodegenerative movement disorder of the central nervous systems. The occurrence of an illness is characterized by accumulation of misfolded α-synuclein protein in brain. Generally; anxiety, tremor, rigidity, depression, bradykinesia, and postural abnormalities are the most common symptoms in Parkinson's disease (Dauer and Przedborski, 2003). Natively unfolded α-syncline (α-Syn) is a 14 kDa and highly conserved protein that localize different regions of the brain. The name of protein was preferred as "α-synuclein" because of it shows synaptic and nuclear localization. α-Syn regulates dopamine neurotransmission by modulation of vesicular dopamine storage. It interacts with tubulin and can function like tau protein. Also, α-Syn shows a molecular chaperon activity in folding of SNARE (soluble N-ethylmaleimide-sensitive-factor attachment protein receptor) proteins. Natively, α-Syn is an unfolded protein, but obtains its conformation with biological interactions. In cytoplasm, α-Syn is a soluble and in an unfolded state, but it can be found in α- helical conformation for binding to lipid membranes. Also, α-Syn can be in the form of β- sheet for composing Lewy bodies. At present, biological role and pathogenic processes of Lewy bodies are still unclear. As far as we know, Lewy bodies are aberrant protein aggregates and their deposits cause PD. Lewy bodies are localized not only in PD brains, but also in other neurodegenerative disorders such as AD brains. According to the electron microscopy images, Lewy bodies are 8-30 μm which are consisted of approximately 10 nm amyloidogenic fibrils such as fibrillary α- Syn and neurofilaments. Lewy bodies contain a variety of proteins including

α-Syn, neurofilaments, ubiquitinated proteins, and heat shock proteins (Hsp70 and Hsp90) (McNaught and Olanow, 2006). It is known that, α-Syn is natively unfolded as well as predominantly non-phosphorylated in vivo. In PD brains, α-Syn is found to be phosphorylated at Ser87 and Ser129 in aggregates. These serine residues are phosphorylated with casein kinase 1 (CK1) and casein kinase 2 (CK2). Several studies reported that accumulation of phosphorylated α-Syn was observed in animal models of synucleinopathies. Therefore, this post translational modification has a pathological role in fibrillation of α-Syn (Tutar et al., 2013).

d. Huntington's diseases

Huntington's disease (HD) is a genetic neurodegenerative disorder and the disease is caused by autosomal dominant inheritance. In 1872, HD was first described as a genetic disease by Dr. George Huntington. Involuntary muscle contractions, movement, and mental disorders are progressed in HD. The disease is inherited as an autosomal dominant and effects brain and nervous systems. Huntington protein undergoes conformational changes with mutation and it shows aggregation tendency (Ross, 2010).

Huntington (Htt) is a large size protein of 350 kDa that is generally composed of 3144 amino acids. Normal protein is highly expressed in peripheral tissues and brain, and it is involved in endocytosis, cytosketal functions, vesicle trafficking, cellular signal transduction, and membrane recycling. In brains, Htt protein leads to cell damage and toxicity through deposition of misfolded aggregate form of Htt protein. The gene for HD is located on the tip of chromosome 4, and is called the *IT-15 gene*. This part of DNA contains cytosine-adenine-guanine (CAG) repeats which are called trinucleotide repeats. Number of trinucleotide repeats is determined as risk of HD development. The CAG repeats are translated into polyglutamine (polyQ) residues which are located in the N-terminal region. Htt proteins interact with a variety of peptides including huntington associated protein 1 (HAP1), huntington interacting protein 1 and 2 (HIP1 and HIP2) and huntington yeast partners A, B, and C (HYPA, HYPB, and HYPC). These peptides are functioned in cell signaling, transport, and transcription processes. In HD, the neuropathology is characterized with accumulation of Htt protein aggregates. HD is caused by a number of CAG repeats in the gene. To date, many theories have been suggested in HD, but functions of CAG repeats and mechanisms of HD cannot be understood yet. However, common opinion is long CAG repeats (polyQ) are the most important promoter for toxicity of Htt protein aggregates. The

polyQ region starts at residue 18 and the number of glutamine residues are the most important marker in HD. Surprisingly, 40 or more CAG repeats are always generated neuropathy, while 35 or few er CAG repeats are never generated neuropathy. However, in childhood, CAG repeats from 27 to 35 can develop neuropathy (Tutar et al., 2013)..

In 1994, Max Perutz put forward a "polar zipper" hypothesis about HD pathology. In this model, many polar glutamine residues can generate anti parallel β-sheet structures with hydrogen bonds. Therefore, aggregation tendency of Htt protein is increased and this state lead to cell death (Perutz, 1994).

7. Prevention of neurodegenerative diseases

Neurodegenerative diseases have been discussed on the basis of protein aggregation and misfolding. In healthy organisms, a variety of mechanisms work efficiently for prevention of preteopathies. Molecular chaperones are known to be critical for protein folding processes and neurodegenerative diseases. Heat shock proteins (Hsps) are well known molecular chaperons in living organisms (Tutar et al., 2013).

Large multidomain proteins have been found to form a misfolded structure and aggregated mass during in vitro refolding. The cellular environment is crowded with proteins and other macromolecules, and so the chance of a newly synthesized unfolded protein forming aggregates is greater in vivo than in vitro. Cellular molecular chaperones are proteins that change this equation by selectively recognizing and binding to the exposed hydrophobic surfaces of a non-native protein via non-covalent interactions, thus inhibitingirreversible aggregation of those proteins in vivo and in vitro(Chaudhuri and Pau, 2006).

Hsps are highly conserved proteins among living organisms. Hsps are an important class of molecular chaperons and they are located in different parts of the cells such as endoplasmic reticulum, cytosol, and mitochondria. Mainly, Hsps are related with formation of proper protein conformation, and also prevention protein aggregation, misfolding, and oligomerization. Proteins can be exposed a number of cellular and environmental factors including high temperature, inflammation, growth factors, oxidative stress etc. which can cause misfolding and protein aggregation. Overexpression of Hsps has been observed under these stress conditions. Several studies have focused on the neuroprotective role of Hsps. Therefore, the expression levels of

Hsps are decreased and misfolded protein accumulation can be occurred in brain. Generally, Hsps are divided into six groups on the basis of molecular mass. In this section; Hsp40, Hsp60, Hsp70, Hsp90, Hsp100, and small Hsp (sHsp) have been examined and characterized for association with neurodegenerative diseases and protein folding processes (Tutar et al., 2013). Hsp70 is a highly conserved protein in all living organisms. It makes complex with unfolded or partially denatured proteins. Hsp70 has two functional domains: ATPase domain and substrate binding domain (SBD). The operation of these domains is controlled with hydrolysis of ATP. ATPase domain binds to ATP and hydrolyzes it to ADP. This energy drives the protein folding function of the Hsp70. Similarly, Hsp70 binding to misfolded peptides, increases the ATP hydrolysis. Also, Hsp70 interacts with Hsp40 and Hsp90 to perform protein folding process. Hsp70 serves a neuroprotective role in all of neurodegenerative diseases. Auluck and co-workers indicated that, expression of Hsp70 reduced α-Syn aggregation,accumulation, and toxicity in PD animal models (Luo and Chen, 2007). Hsp40 is expressed in variety of organisms in different isoforms. It associates with unfolded polypeptides and prevents protein aggregation. Hsp40 can be found in a cell in three different types. All types of Hsp40 contain highly conserved J domain which interacts with Hsp70 ATPase domain. Hsp100 participates in counteraction of protein aggregation with Hsp70 and Hsp40. This hexameric 100 kDa protein has substrate and ATP binding regions. Large protein aggregates are broken by Hsp100 and formed small aggregates are carried forward by Hsp70-Hsp40 cHsp90 is a highly expressed cellular molecular chaperon and also stabilizes certain proteins and aids protein degradation. Hsp90 is a dimeric protein which has a highly conserved N-terminal domain and a C-terminal domain. Hsp90 is one of the main cytosolic molecular chaperons which is activated with Hsp40 and Hsp70omplex. Hsp60 is a heptameric 60 kDa protein which is located particularly in mitochondria. Hsp60 works with Hsp70 coordinately for protein folding. Furthermore, it plays key roles in mitochondrial protein transport, replication and transmission of mitochondrial DNA, and apoptosis. For actin and tubulin, Hsp60 is a specific chaperon which is decreased in AD. In AD effected neurons, aggregated and misfolded tau protein is increased in contrast with expression of Hsp60 is decreased. sHsp has a molecular mass between 12 and 30 kDa. As a molecular chaperone, sHsp are located at different compartments in the cell and they can protect protein structures and activities. Also, overexpression of sHsp have reported in many studies. In HD, the expression level of Hsp27 is increased and it prevents polyglutamine induced toxicity in neurons.

Furthermore, Hsp27 reduces α-syn-induced toxicity in PD patient brain. The other sHsps including Hsp10, Hsp12, Hsp20, Hsp26 are associated with protein folding diseases (Tutar et al., 2013).

CONCLUSION

It has been shown that the correct folding of polypeptide chain can be disturbed due to many factors including gene mutation which is common in many neurodegenerative diseases or a native protein can be convereted into misfolded conformation like in the case of prion disease. The final fate of these misfolded proteins is different in various disorders. In some case the misfoded proteins interats with each other to form an insoluble aggregate which eventually become a neurotoxic and in another case the proteosome pathway might be affected so that there will be an aggragation of the misfolded protein and there won't be secretion of the protein in ER. In general there are hundreds of diseases including Alzheimer's disease, Huntington's disease prion diseases, Parkinson's disease, multiple sclerosis (MS), amyotrophic lateral sclerosis (ALS) and others hallmarked by by progressive nervous system dysfunction. Millions of people are affected by these diseases worldwide. Although there are effective ways to diagnose these disease the proper treatment hasn't been found yet. The innovation of advanced analytical techniques such as variety of IR (infrared spectroscopy), NMR (nuclear magnetic resonance),CD (circular dichroism), calorimeters, and electron microscopy have been used for detection of aggregation process. However the detail molecular mechanism of most of the disease is not clear yet. Thus understanding the problem of complex protein folding and cellular quality control provides research opportunities in many areas of the life sciences in the future.

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