Protein Aggregation in Neurodegenerative Diseases

Subjects: Neurosciences

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Here we summarize the heterogeneity of structures that are produced from intrinsically disordered protein domains and highlight the routes that lead to the formation of physiological liquid droplets as well as pathogenic aggregates. The most common proteins found in aggregates in neurodegenerative diseases and their structural variability will be addressed.
We will further evaluate the clinical relevance and future applications of the study of the structural heterogeneity of protein aggregates, which may aid the understanding of the phenotypic diversity observed in neurodegenerative disorders.

Keywords: intrinsic disorder ; phase separation ; protein aggregation ; neurodegenerative disease ; prion protein ; alpha synuclein ; TDP-43 ; tau ; amyloid beta

1. Introduction

The correct function of living organisms depends on the concerted effort of a network of thousands of proteins [1][2][3], which are required to assume a defined structure to exert their function. However, intrinsically disordered regions (IDRs), found in most eukaryotic proteins [4][5][6], appear to show no preference for chaperone binding [7]. Thus, proteins mostly or entirely composed of IDRs, defined as intrinsically disordered proteins (IDPs), may escape the classical folding pathway and maintain a metastable and plastic state. A subset of IDRs termed low complexity domains (LCDs), found in up to 1.2% of the protein-coding human genes [8], is gaining attention due to its presence in aggregated state in most neurodegenerative disorders.

In this review, we will first recapitulate how intrinsic disorder determines the formation of a variety of structures and produces different states of the matter, owning both physiological and pathological features. Next, we will provide evidence of structural variability observed in the proteins most commonly associated with neurodegeneration, specifically the prion protein (PrP), amyloid β-peptides (Aβ), tau, α-synuclein and TAR DNA binding protein 43 (TDP-43). As a caveat, we will focus on the wild-type sequence of these proteins, as most neurodegenerative diseases are sporadic in nature [9]. The clinical relevance and future applications of the study of the structural variability of protein aggregates will be addressed, as it may aid the understanding of the phenotypic diversity observed in human neurodegenerative disorders.

2. Intrinsic Disorder

The presence of IDRs appears to correlate with the complexity of the living organism [10][11], with half of eukaryotic proteins expected to possess at least one IDR in their sequence [4][5][12]. A plethora of biological functions are associated with IDRs [5][6][13], spanning from stress response [14] to high-order assembly [15] to RNA metabolism [16][17][18]. A further level of variability resides in the ability of IDRs to assume non-native states of globular proteins such as the molten-globule or the coiled-coil state [5][6][19][20]. Being metastable and displaying weak and transient interactions [21][22], IDRs may change their structure in time, fluctuating among different low energetic levels.

Intrinsic disorder is encoded by the composition of the sequence rather than the exact string of amino acids [23][24]. Low mean hydrophobicity and high net charge have been reported to increase the disorder and reduce the solubility by reducing compaction and increasing electrostatic repulsion [24][25][26].

Therefore, it is apparent that the lack of structural arrangement may be achieved through different compositions and features, ultimately converging in the ability to coalesce into higher order assemblies. Overall, low complexity offers another angle to achieve the preservation of the unfolded state, increasing the probability of forming β-structures and allowing for the formation of homomeric and heteromeric assemblies. Moreover, polymerization into supramolecular structures may differ in the state of the matter, implying different routes of aggregation.

3. PrP: The Protein That Started It All

Historically, the concept of the self-propagation and infectivity of a protein was postulated for the scrapie agent, a factor causing neurodegeneration in sheep[27]. The scrapie pathology showed remarkably similar features both to Creutzfeldt–Jakob Disease (CJD) and Kuru. The former is a rare neurodegenerative disease while the latter was an endemic neurodegeneration affecting the Fore tribe in the highlands of Papua New Guinea linked to their cannibalistic rituals[28]. Both Kuru and CJD were shown to be transmissibleto chimpanzees and hamsters[29]. The term “prion” (proteinaceous infectious particle, PrP) was proposed for the scrapie pathology as sole causative agent of the disease. Biochemically, the prion derived from scrapie infected hamsters showed an unusual resistance to non-denaturing detergents and to proteinase K,resulting in 27–30 kDa bands after electrophoresis. the now widely accepted “protein only” hypothesis was proposed for the self-replication of the PrP[30]: its propagation requires the formation of a homotypic complex between the two molecules[31], causing the conversion of the native cellular form (PrP-C) into the pathogenic conformation. Outside of Kuru and CJD, other human pathologies termed transmissible spongiform encephalopathies have been linked to the misfolding of PrP[32], namely the variant CJD (the “mad cow” disease)[33], Gerstmann–Sträussler-Scheinker disease[34], fatal familial insomnia[35] and variably protease-sensitive prionopathy[36].
Overall, the classical studies conducted to delve into the peculiar properties of PrP paved the way for the central role of protein misfolding and transmission in neurodegenerative diseases.

4. The Curious Case of Aβ

The Aβ peptides are small amino acidic chains derived from the proteolytic process of the larger amyloid precursor protein (APP), whose aggregation into plaques represents the major hallmark of Alzheimer’s disease along with tau-derived neurofibrillary tangles [37]. Human APP can be processed through two different routes, defined as non-amyloidogenic and amyloidogenic pathways [38]. In the non-amyloidogenic processing, the enzyme α-secretase cleaves the extracellular domain, producing a short soluble N-terminal fragment and leaving a membrane-bound 83 kDa fragment [38][39][40].

Although molecular dynamics simulation showed that Aβ peptides are IDPs [41], transient secondary structures have been reported for various fragments. Raman and infrared spectroscopy studies showed that peptides containing residues 1–28 fold in a polyproline-II α-helical structure that transition into β-sheet upon binding to phospholipid bilayers [42]. Likewise, Aβ fragments of various sizes were shown by NMR to display very different conformational states (see ref. Overall, NMR structural studies [43] showed that Aβ peptides may populate multiple conformational levels ranging from α-helices to β-sheets, with rapid transitions among different structural features.

Beyond dimerization, the path of Aβ peptides toward aggregation involves a widely heterogeneous population of oligomers, spanning from low molecular weight assemblies such as dimers and tetramers to midrange aggregates, protofibrils and fibrils [44]. Characterization of oligomers through different experimental approaches showed high structural variability, from discoidal shapes devoid of β structures to antiparallel β-turn-β motif to collapsed coil [38][45].

The extreme variability of Aβ structures at the low scale of monomers and oligomers mirrors the diverse landscape of high order aggregates and plaques found in human brains. Fibrillar and dense cored plaques often associate with dystrophic neurites and reactive astrocytes and microglia, forming a unity termed neuritic plaque [46]. Furthermore, Aβ plaques are found in healthy subjects as a normal consequence of aging with a frequency comparable to those found in AD [47]. Recently, prion protein oligomers have been associated with rapid onset forms of AD [48].

The simultaneous presence of Aβ deposits in various neurodegenerative diseases, along with the presence of Aβ plaques in healthy individuals and the lack of neurodegeneration associated with Aβ alone, may suggest a “chaperoning” role for Aβ fragments in assisting neurodegeneration, exacerbating the pathologic phenotype regardless of the main proteinaceous aggregating species. Overall, neurodegenerative diseases are multi-faceted pathologies in which IDPs interact with one another and with the environment in a narrow equilibrium between functionality and uncontrolled aggregation.

5. Protein Quality Control

As already mentioned [7], to prevent the accumulation of potentially pathogenic aggregates, neural cells make use of a series of chaperones capable of recognizing misfolded proteins by means of the exposed hydrophobic portions, thus guiding their correct folding. Proteostasis or homeostasis of proteins, through the protein quality control system (PQC),

requires the prompt degradation and eventual recycling of aggregates and misfolded proteins. PQC includes several proteolytic systems, including ubiquitin-proteasome system (UPS), chaperone-mediated autophagy (CMA), and macroautophagy [49].

The UPS is the system responsible for the degradation of most of the misfolded proteins. These are conjugated with ubiquitin, then deubiquitinated, linearized and introduced into the proteasome, which degrades them into smaller peptides [50]. It is important to underline that the proteasome is particularly vulnerable to protein aggregates; in fact, the passage channel of this structure has a very small diameter (just over 10–12 angstroms) and this does not allow the digestion of aggregates that are difficult to linearize. Proteotoxicity resulting from decreased UPS activity could represent potential damage to neurons [51][52][53].

The degradation system by CMA is able to act on misfolded cytosolic protein without interfering with normal molecules. Target proteins of CMA include aggregates showing a specific degradation signal, the KFERQ sequence and substrates generated by post-translational modifications. These substrates may be entrusted to the CMA-mediated degradation system in lysosomes by interaction of the chaperone (mainly Hsp70 family) with the lysosome membrane molecule LAMP2A[54].

When aggregates show resistance to both the CMA and the UPS, autophagy comes into play. In the proteostasis of post mitotic neurons the role of autophagy is of fundamental importance. Efficacy of autophagy clearance has been shown to play an important role for neuronal homeostasis and maintenance. Moreover, several studies have shown that a number of signaling molecules responsible for regulating neuronal activity are localized in membrane lipid rafts [55], for example, neuroglobin, which is found in the lipid raft and is involved in neuronal survival mechanisms [56].

As a consequence, alterations in lipid rafts' components have been hypothesized to contribute to the loss of neural function and potentially to the cell death/cell survival or autophagy balance associated with neurodegeneration. In particular, lipid rafts at mitochondria associated membrane (MAM) level are structures involved in a number of key metabolic functions, shown to be altered in neurodegenerations such as AD, PD and ALS [57][58]. In addition, disruption of mitochondrial dynamics by the knocking down of strategic molecules associated to MAM's lipid rafts including MFN2, GD3 or ERLIN1 significantly prevented autophagosome biogenesis and maturation [59]. In light of this evidence, a dysregulation during autophagosome maturation might drive the accumulation of protein aggregates and increase neurodegeneration

It is possible to direct misfolded proteins prone to aggregation to the autophagic mechanism for lysosomal degradation thanks to the involvement of molecules that function as adapters, such as p62 and NBR1 [60][61]. After the accumulation of non-degradable autophagic cargoes, the chaperone molecules residing in the ER and participating in this signaling chain are arginylated and, via the N-terminal arginine residue, bind to the ZZ domain of p62 in the cytosol. Once bound, p62 undergoes a conformational modification that induces its polymerization and the interaction with LC3-II, a molecule anchored on the membrane of autophagosomes [62]. The autophagosome thus begins its load and, once completed, fuses with the lysosome to form the autolysosome, for the degradation by lysosomal hydrolases of both, load and p62.

The failure of the PQC system to remove misfolded proteins in the nervous system is the biochemical process behind most neurodegenerative diseases. During aging, deterioration in the PQC systems causes the failure of protein degradation, which may result in the accumulation of misfolded proteins. The successive modifications of structure and aggregation represent in many cases hallmarks of neurodegenerative diseases.

In light of this, it is clear that molecules involved in the clearance of misfolded proteins could represent new pharmacological targets, for example, by controlling the activation of CMA chaperones and adapters, as well as using autophagy inducers, which could be included in future therapeutic strategies for the improvement of neurodegenerative diseases.

6. Clinical Outlook and Concluding Remarks

Protein aggregation is an emerging concept in biology. Accumulating knowledge is suggesting that a convergent evolution has positively selected IDRs. Living organisms exploited the intrinsic property of IDRs to form amyloids to their advantage, incorporating it as a key signaling mechanism. Functional amyloids have indeed been documented throughout evolution, from yeasts to mammals[63]. Intriguingly, amyloid species of proteins associated with neurodegeneration may, in principle, serve a physiological role as well. For instance, proteinase K-resistant PrP was found in response to chronic morphine withdrawal in rats[64], whereas reversible, hyperphosphorylated tau tangles were found in brains of hibernating mammals[65]. The dysregulation of a physiological function associated with the aggregated state may thus represent the mechanism behind the abundance of IDR sequences found in proteins associated with neurodegenerative diseases.

The understanding of the link between structural plasticity of proteins and neurodegeneration has led researchers to investigate this phenomenon as both a diagnostic and therapeutic tool. Particularly, the exploitation of the prion-like seeded conversion mechanism, produced outstanding results in the early and differential diagnosis of neurodegenerative diseases. Protein misfolding cyclic amplification and real-time quaking-induced conversion assays have been applied to an increasing number of pathologies, starting with prion-related pathologies[66] and expanding to synucleinopathies [67],tauopathies and AD[68] and TDP-43-related pathologies[69], yielding a very high diagnostic accuracy from ex vivo human samples.

As we are just beginning to understand the phenomenon of phase separation and protein aggregation, the direction of the molecular pathogenetic study, an expression of basic science, seems right. This approach makes predictable a future in which these devastating diseases may be early and accurately diagnosed, so that personalized and disease-modifying therapies could slow down the insurgence of pathologies.

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