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Minireview

Protein misfolding and disease; protein refolding and therapy

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Abstract Diverse human disorders, including several neurodegenerative diseases and systemic amyloidosis, are thought to arise from the misfolding and aggregation of an underlying protein. Recent findings strongly support this hypothesis and have increased our understanding of the molecular mechanism of protein conformational disorders. Many questions are still pending, but the data overall suggest that correction of protein misfolding constitutes a viable therapeutic strategy for conformational diseases.

Key words: Protein conformational disorders; Amyloid; Protein misfolding; Therapy; β-Sheet breaker peptides

1. Introduction

The biological function of a protein depends on its tridimensional structure, which is determined by its amino acid sequence during the process of protein folding. In the last few years, diverse diseases have been shown to arise from protein misfolding and are now grouped together under the name of protein conformational disorders (PCDs) [1–5]. This group includes Alzheimer's disease (AD), transmissible spongiform encephalopathies (TSEs), serpin-deficiency disorders, haemolytic anemia, Huntington disease (HD), cystic fibrosis, diabetes type II, amyotrophic lateral sclerosis (ALS), Parkinson disease (PD), dialysis-related amyloidosis and more than 15 other less well-known diseases (Table 1).

The hallmark event in PCD is a change in the secondary and/or tertiary structure of a normal protein without alteration of the primary structure. The conformational change may promote the disease by either gain of a toxic activity or by the lack of biological function of the natively folded protein [3,5] (Fig. 1). There is no evident sequence or structural homology among the proteins implicated in PCD. However, the striking feature of these proteins is their inherent ability to adopt at least two different stable conformations [5]. In most of PCDs the misfolded protein is rich in β-sheet conformation [4,5]. β-Sheets are one of the prevalent, repetitive secondary structures in folded proteins and are formed of alternating peptide pleated strands linked by hydrogen bonding between the NH and CO groups of the peptide bond. While in α-helices the hydrogen bonds are between groups within the same strand, in β-sheets the bonds are between one strand and another. Since the second β-strand can come from a different region of the same protein or from a different molecule, formation of β-sheets is usually stabilized by protein oligomerization or aggregation. Indeed, in most PCDs the misfolded protein self-associates and becomes deposited in amyloid-like aggregates in diverse organs, inducing tissue damage and organ dysfunction [2] (Fig. 1).

2. Role of protein misfolding and aggregation in disease

Neuropathologic and genetic studies as well as the development of transgenic animal models have provided strong evidences for the involvement of protein misfolding in disease. Almost 100 years ago, the neuropathologist Alois Alzheimer described for the first time the typical aggregates in the brain parenchyma of demented people [6]. We now know that these aggregates, called amyloid plaques, are composed of protein fibrils. With the exception of cystic fibrosis and some forms of TSE, the end point of protein misfolding in PCD is aberrant protein aggregation and accumulation as amyloid-like deposits in diverse organs [2,3,7–9]. The correlation and co-localization of protein aggregates with degenerating tissue and disease symptoms is a strong indication of the involvement of amyloid deposition in the pathogenesis of PCD [7–9]. Moreover, protein deposits have become a typical signature of PCD and their presence is used for definitive diagnosis [10,11]. However, it is still a matter of controversy whether the deposits of aggregated protein are the culprit of the disease or an inseparable epiphenomenon [5,12–14].

Another important piece of evidence for the role of protein misfolding in disease comes from genetic studies [2,15–19]. Most PCDs have both an inherited and sporadic origin. Interestingly, mutations in the genes encoding the protein component of fibrillar aggregates are genetically associated with inherited forms of the disease. The familial forms usually have an earlier onset and higher severity than sporadic cases. In the familial cases, a mutation may destabilize the normal protein folding, favoring the misfolding and aggregation of the protein. Mutations in the respective fibrillar proteins have been associated with familial forms of many diseases, including AD, TSE, HD and related polyglutamine disorders, PD, amyloid polyneuropathy, cardiac amyloidosis, visceral amyloidosis, cerebral hemorrhage with amyloidosis of the Dutch and Icelandic type, cerebral amyloidosis of the British and Dannish type, thromboembolic disease, angioedema, emphysema, sickle cell anemia and ALS [2,15–18].

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Studies with transgenic animal models have been useful in understanding the contribution of the misfolded protein in disease pathogenesis [19–25]. Several pathological features of diverse PCDs have been induced in animals by incorporation of the human mutated gene for the protein undergoing misfolding. Transgenic mice that overexpress high levels of human amyloid precursor protein containing diverse mutations progressively develop many of the pathological hallmarks of AD, including cerebral amyloid deposits, neuritic dystrophy, astrogliosis, neuronal loss and cognitive and behavioral alterations [19,23,26,27]. ALS pathology has been produced in mice by overexpressing the human mutated superoxide dismutase (SOD) gene [19,28]. Some of these mice develop motor neuron dysfunction similar to ALS patients, and typical pathological alterations, including the presence of hyaline inclusion bodies in degenerating axons, muscle atrophy and wasting, astrocytes damage and extensive loss of large myelinated axons of motor neuronal cells. Recently, it was reported that transgenic mice expressing the wild-type human α-synuclein gene developed several of the clinico-pathological features of PD, including accumulation of Lewy bodies in neurons of the neocortex, hippocampus and substantia nigra, loss of dopaminergic terminals in the basal ganglia and associated motor impairments [29]. Transgenic mice containing the exon 1 of the human huntingtin and carrying 115–156 CAG repeat expansions develop pronounced neuronal intranuclear inclusions, containing the proteins huntingtin and ubiquitin, prior to developing a neurological phenotype [30]. The cerebral abnormalities were strikingly similar to those observed in HD patients. In addition, these mice develop a progressive neurological dysfunction with a movement disorder and weight loss similar to HD [31]. One of the first transgenic models showing a neurodegenerative process similar to a human disease was made by overexpression of the human mutated prion protein (PrP) gene [25,32]. Spontaneous neurologic disease with spongiform degeneration developed and these abnormalities have been transmitted to non-transgenic mice by inoculation of the sick brain homogenate. Finally, a transgenic mouse model with high rates of expression of human islet amyloid polypeptide (IAPP) spontaneously developed diabetes mellitus by 8 weeks of age, which was associated with selective β-cell death and impaired insulin secretion [24]. Small intra- and extracellular IAPP aggregates were present in islets of transgenic mice during the development of diabetes mellitus.

The generation of animal models showing clinical and pathological features similar to the disease by expressing the human protein involved in abnormal folding and aggregation strongly supports a critical role for protein misfolding and polymerization in the disease. However, temporal studies of the appearance of disease-like features in some of the transgenic models have shown that significant tissue damage and clinical symptoms appear before detection of protein aggregates [24,26,33]. These findings suggest that a misfolded soluble intermediate, not yet deposited in the tissue, could be the real culprit of the PCD pathogenesis [5,12–14]. In this scenario, the formation of large protein aggregates deposited in the tissue could even be considered a protective event that allows the deposition and isolation of the toxic abnormally folded proteins.

Table 1
List of some diseases that have been classified in the group of PCDs [2–5]

Protein involved	Diseases
Amyloid-β	AD
α-Synuclein	PD
Amylin	Diabetes type 2
SOD	ALS
β2-Microglobulin	Haemodialysis-related amyloidosis
Amyloid-A	Reactive amyloidosis
CFTR protein	Cystic fibrosis
Hemoglobin	Sickle cell anemia
Huntingtin	HD
PrP	Creutzfeldt–Jakob disease and related disorders
Ten other proteins	Systemic and cerebral heriditary amyloidosis

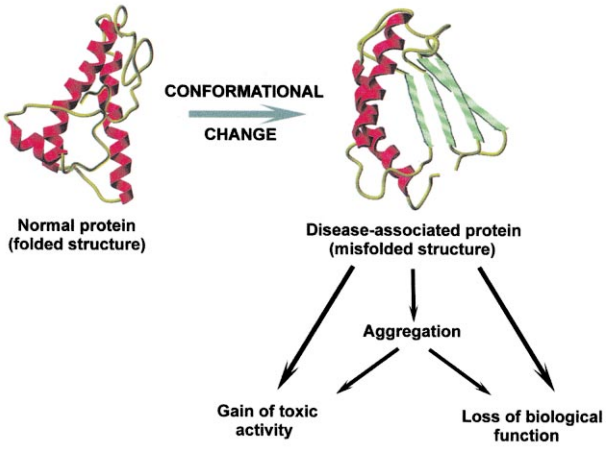

Fig. 1. Protein misfolding and disease. A conformational change in a normal protein seems to be the hallmark event in a group of diverse diseases. Protein misfolding may be associated to disease by either the absence of biological activity of the folded protein or by a gain of toxic activity by the misfolded protein. Aggregation of the misfolded protein may also contribute to the disease pathogenesis.

3. Protein misfolding and aggregation: which comes first?

Protein misfolding is dependent upon conformational changes, which could be induced, stabilized or independent of protein oligomerization. The starting point in PCD is the natural protein folded in the native and active conformation which is usually a mixture of α-helical and random structure, and the end point is the same protein aggregated and adopting a β-pleated sheet conformation. It is unclear at present whether the misfolding triggers protein aggregation or rather protein oligomerization induces the conformational changes (Fig. 2). The latter is not a purely academic debate, but it is very relevant for the design of effective therapeutic strategies.

Based on kinetic modeling of protein aggregation, it has been proposed that the critical event in PCD is the formation of protein oligomers that act as seeds to induce protein misfolding [34–36] (Fig. 2A). In this model, misfolding occurs as a consequence of protein aggregation (polymerization hypothesis), which follows a crystallization-like process dependent upon nucleus formation [34,35]. A nucleation-dependent polymerization process is characterized by a slow lag phase in which a series of unfavorable interactions occur to form an oligomeric nucleus that rapidly grows to form larger polymers [35] (Fig. 2A). The lag phase can be minimized or removed by

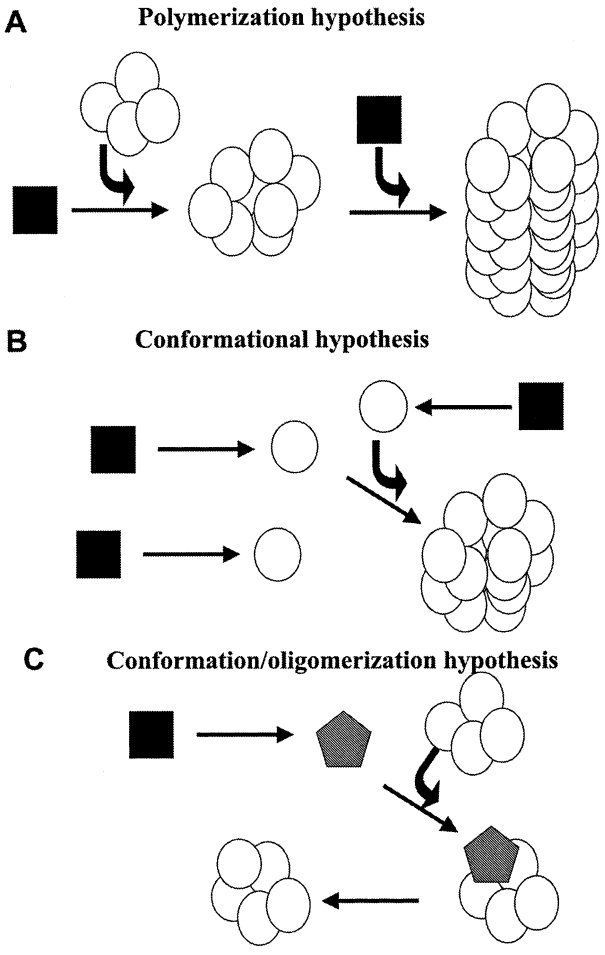

Fig. 2. Models for the molecular mechanism of protein misfolding and aggregation. Three different hypotheses have been proposed to describe the relationship between conformational changes and aggregation. In the polymerization hypothesis (A), aggregation induces the protein conformational changes, while in the conformational hypothesis (B), protein misfolding is independent of aggregation, which is a non-necessary end point of conformational changes. The conformation/oligomerization model (C) represents an intermediate view in which slight conformational changes trigger oligomerization that is essential for the stabilization of protein misfolding. Square represents the folded native conformation, circles the disease-associated conformer and pentagon corresponds to an unstable conformational intermediate.

addition of pre-formed nucleus or seeds. This hypothesis has a precedent in normal protein polymerization processes, such as microtubule formation.

The alternative model is that the underlying protein is stable in both the folded and misfolded forms in solution [3,37–40] (conformational hypothesis). This hypothesis proposes that spontaneous or induced conformational changes result in the formation of the misfolded protein that may or may not aggregate (Fig. 2B). In this model, the formation of amyloid is a non-necessary end point of the conformational change, which can be an accompanying consequence rather than a direct cause of the disease [5,37,40]. A central issue in the conformational hypothesis is the identification of the factors inducing the protein structural changes. Over the last few years, several factors have been described to play such a role [40,41], including mutations that destabilize the folded structure, modification on the environmental conditions (pH, oxidative stress, metal ions) and the activity of certain proteins collectively named pathological chaperones (apolipoprotein E, amyloid P component, protein X).

An intermediate view (Fig. 2C) is that slight conformational changes result in the formation of an amyloidogenic intermediate, which is unstable in an aqueous environment because of exposure of hydrophobic segments to the solvent (conformation/oligomerization hypothesis) [2,5,42,43]. This unstable intermediate is stabilized by intermolecular interactions with other molecules forming small β-sheet oligomers, which by further growth produce amyloid fibrils [2,42,43]. In this model the conversion of the folded protein into the pathological form is triggered by structural changes, but complete misfolding is dependent upon oligomerization. The presence of some degree of conformational changes prior to the formation of aggregates has been demonstrated for diverse proteins including transthyretin, serpins, amyloid-β and PrP [2,4,5,42].

The three models explain in variable degree most of the experimental results. However, it appears that the conformation/oligomerization hypothesis is the most comprehensive and accepted model of protein misfolding and aggregation.

4. Correcting protein misfolding as a novel therapeutic approach

Considering that protein misfolding and aggregation are central in the pathogenesis of PCD, a therapy directed to the cause of the disease should aim to inhibit and/or reverse the conformational changes that result in the formation of the pathological protein conformer.

Assuming that protein misfolding is triggered by conformational changes stabilized by protein oligomerization, an interesting strategy would be to preclude the stabilization of the misfolding or better to destabilize the monomeric intermediate and the early β-sheet oligomers. We have postulated that short synthetic peptides containing the self-recognition motif of the protein and engineered to destabilize the abnormal conformation might be useful to correct protein misfolding [4,12,43]. These peptides called synthetic mini-chaperones are designed to be similar to the sequence of the protein region responsible for self-association and contain residues that specifically favor or disfavor a particular structural motif [4,43]. Considering that in most PDCs the misfolded protein is rich in β-sheet structure, we have focused mainly on the design of peptides to prevent and to reverse β-sheet formation (β-sheet breaker peptides) [43].

β-Sheet breaker peptides have, so far, been designed for blocking the conformational changes and aggregation undergone by both Aβ and PrP [4,43–45]. We have reported that 11- and 5-residue β-sheet breaker peptides, homologous to the central hydrophobic region of Aβ, inhibited and dissolved amyloid aggregates in vitro and in animal models of AD [44,46]. Furthermore, the 5-amino acid peptide prevented neuronal damage induced by amyloid both in cell culture and in a disease animal model [44,46]. Based on the same concept and using the PrP sequence 114–122 as a template, we have also produced β-sheet breaker peptides for the treatment of TSE [45]. Several in vitro, cell culture and in vivo assays were used to test the activity of the peptides and the results clearly indicate that it is possible not only to prevent the $PrP^{c} \rightarrow PrP^{sc}$ conversion, but more interestingly to reverse the infectious

PrP^{sc} conformer to a biochemical and structural state similar to PrP^{c} [45].

These results together support the notion that synthetic β-sheet breaker peptides might be useful in destabilizing the β-sheet rich abnormal conformation, inducing its conversion into the normal form. Synthetic mini-chaperone peptides do not need to be restricted to breaking β-sheets [4,43]. Peptides can also be engineered to act as β-sheet promoters, α-helical breakers, β-turn promoters or even to induce a desired conformation in unstructured protein fragments. The principles to manipulate protein conformation may provide a general platform technology to design drugs for the treatment of PDC. Moreover, our findings suggesting that protein conformation can indeed be specifically altered open a new approach for modifying phenotypic characteristics by modulating experimentally the structure of a selected protein. Therefore, the discovery of the principles for generating synthetic mini-chaperones could be useful as a novel therapeutic approach for disorders where the function of a protein needs to be modified.

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