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Review

How our bodies fight amyloidosis: Effects of physiological factors on pathogenic aggregation of amyloidogenic proteins

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ABSTRACT

The process of protein aggregation from soluble amyloidogenic proteins to insoluble amyloid fibrils plays significant roles in the onset of over 30 human amyloidogenic diseases, such as Prion disease, Alzheimer's disease and type 2 *diabetes mellitus*. Amyloid deposits are commonly found in patients suffered from amyloidosis; however, such deposits are rarely seen in healthy individuals, which may be largely attributed to the self-regulation *in vivo*. A vast number of physiological factors have been demonstrated to directly affect the process of amyloid formation *in vivo*. In this review, physiological factors that influence amyloidosis, including biological factors (chaperones, natural antibodies, enzymes, lipids and saccharides) and physicochemical factors (metal ions, pH environment, crowding and pressure, etc.), together with the mechanisms underlying these proteostasis effects, are summarized.

Introduction

Pathological amyloids are composed of misfolded proteins, which are the main causes of over 30 human amyloidogenic diseases (Table 1). A common hallmark of these diseases is the existence of misfolded amyloids, or even amyloid plaques, which is a final morphology of pathological aggregations [1]. In healthy individuals however, such amyloid deposits are rarely found, suggesting the existence of self-defending mechanisms by our body against pathological protein aggregations (see Table 2).

A consensus has been reached that the aggregation of amyloidogenic proteins triggers some lethal effects, such as ubiquitin proteasome system failure, lipid membrane permeabilization, oxidative stress, endoplasmic reticulum stress and mitochondrial dysfunction [2–4]. To prevent or to survive from such threatening circumstances, cells employ multiple natural physiological factors to regulate or reverse these damages (Fig. 1). In this review, currently known physiological factors that involved in self-defense against misfolded amyloidogenic proteins, including both biological and physicochemical factors, are summarized, and their possible mechanisms are also discussed.

Amyloid aggregation

A classic pathological aggregation usually starts with the unfolding or misfolding of native proteins, which results in conformational transition and loss of biological functions. *In vivo*, misfolded proteins sometimes bring lethal effects [5,6]. Take PrP^C, a pathological protein in Prion disease, for instance, the normal form of PrP^C is non-amyloidogenic and proteinase K sensitive with high content of α-helix under physiological conditions; however, under abnormal circumstances, PrP^C converts into PrP^{Sc}, which becomes resistant to protease and contains a β-sheet rich structure, and more importantly, is highly prone to aggregation [7].

Subsequently, misfolded monomers assemble to form oligomeric nuclei, which act as a core to recruit monomers to further grow into larger oligomers. It has been generally accepted that the transient oligomer species contribute most to cytotoxicity *in vivo* during aggregation. For example, oligomers of human islet amyloid polypeptide (hIAPP[1]), an amyloidogenic protein contrib-

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[1] *Abbreviations used:* α_2M, α_2-Macroglobulin; AD, Alzheimer's disease; Aβ, amyloid beta; ALS, amyotrophic lateral sclerosis; APP, amyloid precursor protein; ApoE, apolipoprotein E; ApoA-I, apolipoproteins A-I; BBB, blood–brain barrier; CJD, Creutzfeldt–Jakob disease; CSF, cerebral spinal fluid; DPPIV, dipeptydil peptidase IV; GAGs, glycosaminoglycans; H_2O_2, hydrogen peroxide; hIAPP, human islet amyloid polypeptide; HD, Huntington's disease; HHP, high hydrostatic pressure; HMW, high molecular weights; Hsp, heat shock proteins; Htt, Huntington; IDE, insulin-degrading enzyme; NAC, non-Aβ component; NO, nitric oxide; PD, Parkinson's disease; PGs, proteoglycans; PolyQ, polyglutamine; PC, prohormone convertases; SAP, serum amyloid P component; SOD1, superoxide dismutase 1; SUV, small unilamellar vesicles; T2DM, type 2 *diabetes mellitus*, TTR, transthyretin.

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uted to the onset of type 2 *diabetes mellitus*, are considered to be responsible for membrane permeabilization of pancreatic β-cells to induce cytotoxicity, which leads to apoptosis and eventually diabetes [8]. Oligomers of intraneuronal amyloid beta (Aβ), the pathological protein in Alzheimer's disease (AD), have also been shown to cause cell death by inducing ER stress in hippocampal neurons of a transgenic mouse expressing amyloid precursor protein (APP) [9]. Similar to Aβ, α-synuclein, another amyloidogenic protein whose aggregation is considered as the cause of Parkinson disease (PD), were found on mitochondrial membrane in oligomeric states, which may result in the release of cytochrome C and the subsequent activation of the apoptosis cascade [10]. In addition to the widely acknowledged toxic oligomeric species, the reactive oxygen species generated during aggregation has also been suggested to contribute to cytotoxicity [11].

Finally, oligomers are further assembled into insoluble mature fibrils [12] (Fig. 2). Since aggregation is not a synchronized process, and there are also different physiological clearance rates for different species; therefore, monomers, oligomers and fibrils co-exist. The preexisting fibrils could be further fragmentized into small pieces which act as new oligomeric nuclei to amplify aggregation, such a new nuclei forming process is also known as "secondary nucleation" and has been identified in α-synuclein and Aβ [13,14]. Noteworthy, it is also reported that the "secondary nucleation" process is evitable when the protein concentration is greater than a critical fibril mass concentration [14].

Factors affect self-defense

Biological factors

Chaperones

Molecular chaperones are ubiquitous in mammals, which prevent newly synthesized polypeptide chains from aggregating into nonfunctional structures. Molecular chaperones could be classified into intracellular or extracellular chaperones. Mounting studies have shown that heat shock proteins (Hsp) are the major intracellular molecular chaperones with a property of preventing amyloid aggregation in amyloidogenic diseases. Extracellular chaperones in body fluids have also been shown to modulate the aggregation of amyloidogenic proteins. Presently known chaperones against amyloid formation are summarized in Fig. 2.

Intracellular chaperones.

Hsp70. The 70 kilodalton heat shock protein (Hsp70) is one of the most studied Hsps, which plays essential roles in protein biogenesis, transport and degradation. Furthermore, Hsp70 also assists in protein folding, protects cells from stress, and is also strictly associated with pathological aggregation of amyloidogenic proteins [15–17]. The structure of Hsp70 consists of two functional domains: an N-terminal ATPase domain (1–385) and a C-terminal substrate binding domain (386–640). The C-terminal region can be further divided into two sub-domains: a substrate binding subdomain (386–543) and a C-terminal lid sub-domain (544–640) [18].

The beneficial effects of Hsp70 are mostly studied in neurodegenerative diseases, such as Parkinson disease (PD), Huntington's disease (HD) and Alzheimer disease (AD) [19–22]. The ability of Hsp70 in regulating proteins aggregation and reducing the toxicity at molecular level has been confirmed as *via* interacting with oligomeric intermediates and promoting the degradation of amyloidogenic proteins [23].

Hsp70 can directly interact with pathological proteins to block aggregation. It has been reported that Hsp70 inhibited α-synuclein fibril formation by interacting with multiple early stage intermediates, such as by binding with aggregates smaller than nuclei in size to impede nuclei formation, and by binding with nuclei to retard fibril elongation [24]. It was proposed that the hydrophobic stretches formed during aggregation may be the target for Hsp70 to recognize *via* its C-terminal substrate binding domain (386–640), especially the lid subdomain (544–640) [24]. Moreover, by using fluorescence lifetime imaging to monitor the conformation of oligomeric species of α-synuclein, Klucken and coworkers found that Hsp70 can unpack the N- and C-terminus of α-synuclein to stabilize monomers in an open conformation [23]. Such conformation decreases the potential interaction between non-Aβ component (NAC) domains, and suppresses toxic amyloidogenic aggregation [23].

It was also suggested that Hsp70 facilitated the degradation of mutant Huntingdon (Htt) protein, which causes HD [25,26]. Since the mutant Htt is characterized as repeated abnormal polyglutamine (polyQ) expansion in the N-terminal, mutant Htt is also known as polyQ. In a *Drosophila* model of spinobulbar muscular atrophy, activated Hsp70 facilitated the clearance of misfolded and/or damaged polyQ by ubiquitination and polyglutamine androgen receptor clearance [26]. It has been demonstrated that Hsp70 enhanced the degradation of mutant polyQ through chaperone-mediated autophagy [25]. However, how Hsp70 targets mutant/misfolded polyQ rather than the native ones remains unclear.

Hsp40. Hsp40, also known as chaperone DnaJ, is a 40 kD co-chaperone of Hsp70. It has been shown that Hsp40 can inhibit the aggregation of multiple amyloidogenic proteins both *in vivo* and *in vitro* [27,28]. Hsp40 has a J domain composed of 70 amino acids, which is responsible for interacting with Hsp70 to facilitate the binding of client proteins to Hsp70 [29]. Hsp40 serves as a modulator to upregulate the activity of Hsp70 by enhancing its ATPase activity *in vivo* [30]. It is currently accepted that Hsp40 suppresses the aggregation of pathogenic proteins *via* enhancing the activity of Hsp70 and direct interaction with pathogenic proteins.

It was shown in a *Drosophila* model for PD that Hsp40 suppresses neurotoxicity induced by α-synuclein, through augmenting the activity of Hsp70 [22]. It was also proven that Hsp40 and Hsp70 partitioned misfolded monomeric conformations by folding them into native state and attenuating the formation of oligomers, which is the most toxic form [31].

Hsp90. Hsp90 is a common chaperone protein with a molecular weight of 90 kD. The biological functions of Hsp90 in normal cells include promoting folding of natural proteins, aiding in protein degradation and facilitating cell signaling [32].

Studies on the anti-aggregation property of Hsp90 have been focused on neurodegenerative diseases. Elevated Hsp90 expression has been observed in the brains of both AD and PD patients compared with those in healthy subjects [33,34]. It was also reported that Hsp90 is colocalized with intracellular α-synuclein and Aβ plaques in the brains of AD and PD patients, respectively [34,35].

The mechanisms of anti-aggregation effects of Hsp90 include direct interference in the aggregation of amyloidogenic proteins and other biological pathways. It was shown that Hsp90 could bind with monomeric α-synuclein at a low micromolar concentration [36]. The binding region of Hsp90 to α-synuclein is identical to the region critical for the self-assemble of α-synuclein, which creates a competitive inhibition that suppresses aggregation [36]. Furthermore, it was also reported that Hsp90 could bind with soluble α-synuclein oligomers instead of monomers to form stable, non-toxic complexes to avoid aggregating into fibrils [37]. Another report showed that Hsp90 could suppress the aggregation of Aβ at substoichiometric concentrations with interaction between monomeric and oligomeric forms of Aβ and Hsp90 [38]. Additionally, by another mechanism, it was also evident that Hsp90 could