Biochimie 95 (2013) 12–19

Contents lists available at SciVerse ScienceDirect

Biochimie

journal homepage: www.elsevier.com/locate/biochi

Mini-review

What does make an amyloid toxic: Morphology, structure or interaction with membrane?

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ARTICLE INFO

Article history:
Received 11 May 2012
Accepted 10 July 2012
Available online 20 July 2012

Keywords:
Amyloid
Toxicity
Membrane
Antiparallel β-sheet
Oligomers

ABSTRACT

The toxicity of amyloids is a subject under intense scrutiny. Many studies link this toxicity to the existence of various intermediate structures prior to the fiber formation and/or their specific interaction with membranes. Membranes can also be a catalyst of amyloidogenesis and the composition or the charge of membrane lipids may be of particular importance. Despite intensive research in the field, such intermediates are not yet fully characterized probably because of the lack of adapted methods for their analyses, and the mechanisms of interaction with the membrane are far to be understood. The purpose of this mini-review is to highlight some *in vitro* characteristics that seem to be convergent to explain the toxicity observed for some amyloids. Based on a comparison between the behavior of a model non-toxic amyloid (the Prion Forming Domain of HET-s) and its toxic mutant (M8), we could establish that short oligomers and/or fibers assembled in antiparallel β-sheets strongly interact with membrane leading to its disruption. Many recent evidences are in favor of the formation of antiparallel toxic oligomers assembled in β-helices able to form pores. We may also propose a new model of amyloid interaction with membranes by a "raft-like" mode of insertion that could explain important destabilization of membranes and thus amyloid toxicity.

1. Introduction

In 1854, the German laboratory physician Rudolph Virchow, while studying abnormal deposit within brain slice, coined the word amyloid [1]. He discovered that the corpora amylacea stained pale blue on treatment with iodine, and violet upon the subsequent addition of sulfuric acid. These staining properties are specific for cellulose and he concluded that the substance underlying the deposit was cellulose and gave it the name "amyloid", derived from the Latin amylum and the Greek amylon which mean "look like starch" (at this time cellulose and starch were supposed to be similar). Afterwards, it was demonstrated that proteins are indeed the structural basis of amyloids. Obviously, these proteins were called amyloid proteins. This lack of precision on the language still persists and the word amyloid is used to describe a quaternary structural organization, but also the proteins that fold into this shape.

1.1. Amyloid structure

The amyloid shape is based on stacking of β-sheets. This organization corresponds to a molecular arrangement of proteins into fibrils characterized by a cross-β X-ray diffraction pattern [2]. The pattern reveals that the fibrils contain a common cross-β spine with β-sheets parallel to the fibril axis, and their extended protein strands perpendicular to the axis. This pattern consists of an X-ray reflection at ~4.7 Å resolution (meridional) along the fibril direction, and another X-ray reflection at 8–11 Å resolution (equatorial) perpendicular to the fibril direction, and dependent on the size of the side chains. In other words, the distance between the two sheets is 8–11 Å and the distance between two strands in the same sheet is ~4.7 Å [2]. These amyloid fibrils share common properties as for example resistance to proteolysis. This resistance may participate to amyloid toxicity by poisoning the degradation machinery and particularly by inhibiting the proteasome [3]. The amyloid fibrils can be specifically stained by dyes such as thioflavine T, ANS or Congo red, with which they display additional birefringence under polarized light.

The formation of this fibrillar structure starts with a nucleation-dependent polymerization characterized by a lag phase in which

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http://dx.doi.org/10.1016/j.biochi.2012.07.011

nucleation occurs, and a rapid sigmoidal growth phase [4,5]. The lag phase can be partly or entirely bypassed by the addition of seeds [4]. In the case of amyloid fibrils, seeds are usually fragments of preformed fibrils. The formation of nucleus echoes the presence of oligomeric species that can be found "on pathway" or "off pathway" for fibril formation.

1.2. Amyloid proteins

Many human diseases are associated with protein aggregation and amyloid formation. More than 40 different proteins which are not related by their sequences, their secondary and or tertiary structures, sub-cellular localization, level of expression, transcriptional control or other "classical" biological link are involved in pathogenic process. In addition to this broad group, other proteins with no link to protein deposition diseases have been found to form, under particular conditions *in vitro*, amyloid fibrils leading to the idea that such aggregation could be a general property of polypeptide backbone and not of specific amino-acid side chains [6]. *In vivo,* the living organisms can also take advantage of amyloid formation for many mechanisms ranging from storage of peptides hormones in the brain [7] to epigenetic inheritance of adaptative traits in yeast [8]. They are called "functional" amyloids and could be more widespread in nature than initially through.

1.3. Amyloid and toxicity

In human, amyloid formation is often associated with diseases commonly referred to as protein misfolding diseases, aggregation diseases, proteopathies, or, more specifically, amyloid diseases or amyloidoses. Amyloid structure is also associated with an unusual class of transmissible pathogenic agent known as prion and found in transmissible spongiform encephalopathies. Interestingly, in most of these pathologies, mutations in genes encoding the amyloid proteins can increase the propensity to develop the disease. The presence of huge aggregates is correlated with the disease and for a long time led to the hypothesis that these aggregates would be toxic by themselves. This hypothesis may still explain the deleterious effect of amyloids for most systemic amyloidoses and for the amyloid associated with the cerebral vessels (e.g. cerebral amyloid angiopathy). This paradigm has progressively shifted for the other amyloid diseases. In Alzheimer's disease, it is now widely admitted that β-amyloid (Aβ) deposits are not directly correlated with the pathology whereas the presence of soluble Aβ species appear to be related with the severity of the dementia [9]. *In vitro*, the toxicity of amyloid proteins depends on their level of aggregation. When added to cell culture, monomeric or highly aggregated amyloid proteins do not affect cellular viability whereas oligomeric species lead to its decrease [10,11]. The physicochemical properties of the toxic oligomeric species are not well understood, and may also depend on the primary sequence of the amyloid proteins. But toxicity of various soluble oligomers may be inhibited by specific structural antibodies, which means that toxic oligomers may share a common structure [10]. Surprisingly, even "non-toxic" amyloids such as those formed by Ure2p in yeast (that leads to the prion phenotype [URE3]) can *in vitro* enter vertebrate cells and induce apoptosis [12]. These observations led us to begin a study that aimed to determine the differences between toxic and non-toxic amyloids *in vivo*. Our goal was to develop *in vitro* and *in vivo* approaches allowing to select toxic species after random mutagenesis and to analyze their behavior at the molecular level.

While numerous studies focused on pathological aspects, the origin of the amyloid toxicity remains unclear. Some authors shed lights on toxic intermediates that may or may not be "on pathway" for fiber assembly, demonstrating that monomers, oligomers, annular or short/quiescent fibrils could be the toxic species. Interestingly, it has been also proposed that these species would be toxic by their capacity to interact and disrupt the biological membranes. Such deleterious interactions may involve different mechanisms such as detergent, carpeting effects or pore formation. It is now widely accepted that amyloidogenesis itself is also influenced by membranes. Membranes can catalyse the amyloidogenesis and the composition or the charge of membrane lipids may be of particular importance, as for example the presence of cholesterol, gangliosides or lipid rafts. Here we present an overview of the few common parameters that can be responsible of the amyloid toxicity, as specific morphology, particular structures of the amyloid, and interaction with membrane comparing our results of model amyloid toxicity with the data reported in the literature for others amyloids such as Aβ, IAPP or α-synuclein, proteins involve in severe diseases.

2. Can the morphology or the structure modulate the toxicity?

The amyloid aggregation pathway is a multistep process, and many *in vitro* studies have highlighted the role of particular intermediates in the cellular toxicity of various amyloid diseases. It seems now established that the monomers and the mature fibers are not the toxic forms. Then, we focus our interest to determine if some specific morphology or structure can be predictive of the toxicity of the different forms involved in the amyloidogenesis.

2.1. Auto-assembling and morphology

In the last few years, we developed in the baker's yeast *Saccharomyces cerevisiae* a model of amyloid toxicity. Our model is based on the generation of toxic mutants of the HET-s prion of *Posdospora anserina* [13,14]. HET-s fulfills in the fungus the beneficial function of heterokaryon incompatibility, which leads to a process of localized cell death to avoid the fusion of incompatible hyphaes [15]. The amyloid part of this prion is the C-terminal domain HET-$s_{(218–289)}$ [wild-type (WT)], which is one of the most structurally studied amyloid with a fiber in a characteristic parallel-β-sheet solenoid structure [16]. When expressed in *S. cerevisiae*, this prion domain is not toxic, and we generated by random mutagenesis a collection of toxic mutants [13]. The most toxic one, called the M8 mutant (M8), possesses 10 mutations distributed all along the primary amino-acid sequence, and greatly differs from the WT domain both biochemically and structurally (Fig. 1A). *In vivo*, when WT is expressed coupled to GFP, large ring-type aggregates are observed into the yeast cells, which are obviously not detrimental to their host. When M8 is expressed, the yeast cells display a strong slow growth phenotype (Fig. 1B), which is also correlated with the presence of dot-type aggregates into the cells (Fig. 1C). *In vitro*, mature WT amyloid exhibits μM-long fibers, whereas the toxic M8 presents usually very short unbranched fibers, as observed on transmission electronic microscopy (Fig. 1 D) [17]. M8 shows all the characteristics of an amyloid protein, but has definitely a different morphology. From comparative ATR-FTIR experiments, M8 is organized as an antiparallel β-sheet structure, whereas WT is self-assembled in parallel-β-sheets as already demonstrated by solid-state NMR [16]. At this stage, we could observe that the morphology *in vivo* was in agreement with the microscopic observations *in vitro* [13,17]. But toxicity of amyloids is very often related to the species different of the mature fibers. Assembly of proteins or peptides into mature amyloid fibers is a multistep process initiated by conformational changes, during which intermediate aggregation states such as oligomers, protofibrils and filaments are formed. Many studies revealed that the