Biophysical Chemistry 182 (2013) 30–43

Contents lists available at ScienceDirect

Biophysical Chemistry

journal homepage: http://www.elsevier.com/locate/biophyschem

The amyloid-cell membrane system. The interplay between the biophysical features of oligomers/fibrils and cell membrane defines amyloid toxicity

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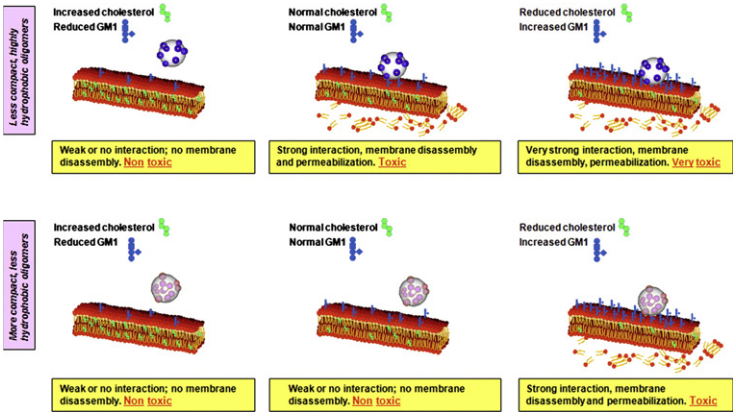
HIGHLIGHTS

- Amyloid aggregation and aggregate cytotoxicity are generic properties of any polypeptide chain.
- A close relation between amyloid biophysical features and cytotoxicity does exist.
- Biological surfaces are important inductors of protein misfolding/aggregation and sites of amyloid interaction.
- A close relation between cell membrane biophysical features and amyloid cytotoxicity does exist.
- Amyloid cytotoxicity appears as an emerging property depending on the biophysical features of the amyloid-membrane system.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 9 May 2013
Received in revised form 3 June 2013
Accepted 3 June 2013
Available online 11 June 2013

Keywords:
Amyloid
Protein misfolding
Protein aggregation
Amyloid toxicity
Amyloid oligomers

ABSTRACT

Amyloid cytotoxicity, structure and polymorphisms are themes of increasing importance. Present knowledge considers any peptide/protein able to undergo misfolding and aggregation generating intrinsically cytotoxic amyloids. It also describes growth and structure of amyloid fibrils and their possible disassembly, whereas reduced information is available on oligomer structure. Recent research has highlighted the importance of the environmental conditions as determinants of the amyloid polymorphisms and cytotoxicity. Another body of evidence describes chemical or biological surfaces as key sites of protein misfolding and aggregation or of interaction with amyloids and the resulting biochemical modifications inducing cell functional/viability impairment. In particular, the membrane lipid composition appears to modulate cell response to toxic amyloids, thus contributing to explain the variable vulnerability to the same amyloids of different cell types. Finally, a recent view describes amyloid toxicity as an emerging property dependent on a complex interplay between the biophysical features of early aggregates and the interacting cell membranes taken as a whole system.

1. Introduction

A broad range of human diseases arises from the failure of a specific peptide or protein to adopt, or remain in, its native functional

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http://dx.doi.org/10.1016/j.bpc.2013.06.003

conformational state. These pathological conditions are generally referred to as protein misfolding diseases. The largest group of misfolding diseases is represented by amyloidoses [1], that are characterized by the presence of deposits of fibrillar aggregates found as intracellular inclusions or extracellular plaques whose main constituent is a specific peptide or protein, different in the varying disorders [2]. These diseases include a range of sporadic, familial or transmissible degenerative conditions, some of which affect the brain and the central nervous system (e.g. Alzheimer's and Creutzfeldt–Jakob diseases), while others involve peripheral tissues and organs such as the liver, heart and spleen (e.g. systemic amyloidoses and type II diabetes) [3]. In addition, there are other diseases (Parkinson's and Huntington's diseases) characterized by the presence of intracellular, rather than extracellular, deposits localized in the cytoplasm, or less frequently in the nucleus, in the form of specific inclusions known as aggresomes, Lewi bodies, or others. The various peptides and proteins associated with amyloid diseases have no obvious similarities in size, amino acid composition, sequence or structure. The features of the soluble forms of the proteins involved in the amyloidoses are varied, ranging from intact globular proteins to largely unstructured peptide molecules, but the aggregated forms have many common characteristics [4]. In particular, the amyloid fibrils into which they convert are very similar in their morphology and internal structure, sharing a common β sheet-rich motif [3]. Such structure confers to the amyloid fibril specific biophysical features and a variety of tinctorial properties, notably staining with thioflavin T (ThT) and Congo red (CR) and is considered the main structural hallmark of the amyloid aggregates [1].

Until the end of 1990s the data available and the genetics of amyloid diseases supported a quite general consensus that the amyloid fibrils were the main toxic species in amyloid plaques, even though no mechanistic data supporting fibril cytotoxicity had been clearly reported. Such a scenario provided a theoretical frame to understand the molecular basis of amyloid diseases and stimulated the exploration of therapeutic approaches mainly focused at hindering amyloid fibril growth and deposition. However, at the end of the 1990s the attention shifted to the cytotoxicity of amyloid fibril precursors, notably amyloid oligomers and protofibrils [5]. Indeed, the severity of cognitive impairment in Alzheimer's disease (AD), the most severe form of senile dementia associated with the presence, in the brain parenchyma, of amyloid-like deposits of the Aβ peptides, appears to correlate with the levels of aggregates of low-molecular-weight species of Aβ rather than with the amount of fibrillar deposits [6]. The appearance of pre-fibrillar aggregates in tissues precedes the expression of the clinical phenotype thus explaining the lack of relationship found in most cases between extent of amyloid deposits and severity of the clinical symptoms [7]. In addition, transgenic mice show deficits in cognitive impairment, cell function, and synaptic plasticity before the accumulation of significant quantities of amyloid plaques [8]. At the present, the pivotal role of amyloid oligomers as key players of amyloid cytotoxicity is widely recognized and the amyloid cascade hypothesis, initially stated to explain AD, has subsequently been extended to cover other neurodegenerative diseases with amyloid deposits [9]. The cytotoxicity of pre-fibrillar amyloid assemblies has been confirmed for all proteins and peptides associated with amyloid diseases, including Aβ peptides, α-synuclein, amylin, β2-microglobulin, transthyretin and others [10–18]. A further step forward was done in 2002, when data were reported suggesting that cytotoxicity is a generic property of amyloid oligomers, which is associated with a shared "toxic" fold [19,20]. These data have led to propose that the pre-fibrillar assemblies share basic structural features that, at least in most cases, seem to underlie common biochemical mechanisms of cytotoxicity. This new view shifted the target of pharmacological research aimed at finding molecules useful to prevent cell/tissue impairment in amyloid diseases from counteracting fibril growth to hindering the appearance of amyloid oligomers.

The toxicity of early oligomers appears to result from their intrinsic ability to impair fundamental cellular processes most often upon their interaction with cell membranes and subsequent disassembly of the lipid bilayer. It has been hypothesized that pre-fibrillar aggregates impair cell function because they expose on their surface an array of groups that are normally hidden in globular proteins or dispersed in unfolded peptides or proteins. The exposed regions can be rich in hydrophobic groups able to stick onto, and to penetrate inside, the cell membrane. Actually, pre-fibrillar assemblies have been shown to interact with synthetic phospholipid bilayers and with cell membranes, possibly destabilizing them and impairing the function of specific membrane-bound proteins [21–23]. The data on aggregate interaction with the cell membrane underscore a key role of the resulting free Ca^{2+} level alterations with subsequent intracellular redox status modifications, suggesting a mechanism of cell death possibly shared among pre-fibrillar aggregates of most peptides and proteins [19,21,23–25].

On the other hand, peptides and proteins can interact with, and be actively recruited by, biological membranes, thus modifying their conformational states which result in non-native, aggregation-prone conformations [26]. Such a view has led to the proposal that surfaces can catalyze amyloid aggregate nucleation and growth by a mechanism that can be different from that observed in the bulk solution (reviewed in [27,28]) (see below). Moreover, the biochemical and biophysical features of the cell membrane can affect the conformation, distribution and proteolytic processing of membrane proteins involved in neurodegenerative conditions such as AD or prion diseases. In addition, the protein/peptide interaction with the cell surface, particularly with cholesterol and ganglioside-rich areas such as lipid rafts, is considered an important requirement for cytotoxicity (reviewed in [28–30]). This review will focus the role of biological surfaces, notably phospholipid bilayers, as key players of protein destabilization and aggregation as well as of aggregate recruitment. The importance of the interplay between membrane and oligomer physicochemical features modulating amyloid cytotoxicity in the membrane–oligomer complex will be also discussed.

2. Structural features of amyloid aggregates

The observation that proteins associated with amyloid diseases, thought displaying in their soluble native state a very different nature, can generate similar fibrillar forms, encouraged the proposal that there are strong relationships between the intrinsic structure of the amyloid fibrils and in the mechanism by which they are formed [31]. Actually, the ability of polypeptide chains to form amyloid structures is not restricted to the relatively small number of proteins associated with recognized clinical disorders, but it now seems to be a generic feature of polypeptide chains [31,32]. The core structure of the fibrils seems to be stabilized primarily by interactions, particularly hydrogen bonds, involving the polypeptide main chain. Because the main chain is common to all polypeptides, this observation explains why fibrils formed from polypeptides of very different sequence bear significant similarities [4,32]. The fibrils can be imaged in vitro using transmission electron microscopy (TEM) or atomic force microscopy (AFM). By these techniques it has been shown that amyloid fibrils usually consist of a number (typically 2–6) of protofilaments, each about 2–5 nm in diameter, that are often twisted around each other to form supercoiled rope-like structures typically 7–13 nm in width [4,33] or that associate laterally to form long ribbons that are 2–5 nm thick and up to 30 nm wide [34,35]. Circular dichroism, Fourier transform infra-red spectroscopy and solid-state NMR and X-ray fiber diffraction data have shown that in each individual protofilament the protein or peptide molecules are arranged so that the polypeptide chain forms β-strands that stack in register and run perpendicular to the long axis of the fibril to generate what is described as a cross-β structure [4]. Usually, each strand of in register β-sheets makes its full complement of hydrogen bonds with the strands above and below it in the fibril. However, it has been recently reported an out of register amyloid fibril structure, in which each antiparallel pair of strands is out of register with neighboring pairs by six