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Review

Natural product-based amyloid inhibitors

Paul Velander [a], Ling Wu [a], Frances Henderson [a], Shijun Zhang [b], David R. Bevan [a,c,d], Bin Xu [a,c,d,e,*]

[a] Department of Biochemistry, Virginia Polytechnic Institute & State University, Blacksburg, VA 24061, USA
[b] Department of Medicinal Chemistry, School of Pharmacy, Virginia Commonwealth University, Richmond, VA 23298, USA
[c] Center for Drug Discovery, Virginia Polytechnic Institute & State University, Blacksburg, VA 24061, USA
[d] School of Neuroscience, Virginia Polytechnic Institute & State University, Blacksburg, VA 24061, USA
[e] Translational Obesity Research Center, Virginia Polytechnic Institute & State University, Blacksburg, VA 24061, USA

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ABSTRACT

Many chronic human diseases, including multiple neurodegenerative diseases, are associated with deleterious protein aggregates, also called protein amyloids. One common therapeutic strategy is to develop protein aggregation inhibitors that can slow down, prevent, or remodel toxic amyloids. Natural products are a major class of amyloid inhibitors, and several dozens of natural product-based amyloid inhibitors have been identified and characterized in recent years. These plant- or microorganism-extracted compounds have shown significant therapeutic potential from *in vitro* studies as well as *in vivo* animal tests. Despite the technical challenges of intrinsic disordered or partially unfolded amyloid proteins that are less amenable to characterizations by structural biology, a significant amount of research has been performed, yielding biochemical and pharmacological insights into how inhibitors function. This review aims to summarize recent progress in natural product-based amyloid inhibitors and to analyze their mechanisms of inhibition *in vitro*. Major classes of natural product inhibitors and how they were identified are described. Our analyses comprehensively address the molecular interactions between the inhibitors and relevant amyloidogenic proteins. These interactions are delineated at molecular and atomic levels, which include covalent, non-covalent, and metal-mediated mechanisms. *In vivo* animal studies and clinical trials have been summarized as an extension. To enhance natural product bioavailability *in vivo*, emerging work using nanocarriers for delivery has also been described. Finally, issues and challenges as well as future development of such inhibitors are envisioned.

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* Corresponding author at: Department of Biochemistry and Center for Drug Discovery, Virginia Polytechnic Institute & State University, 105 Engel Hall, Blacksburg, VA 24061, USA.
E-mail address: binxu@vt.edu (B. Xu).

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1. Introduction

Amyloidosis is associated with the largest class of protein misfolding diseases that includes a broad spectrum of neurological, metabolic and aging related diseases including Alzheimer's disease (AD), prion disease, Parkinson's disease, and type 2 diabetes (T2D). The pathological hallmarks of amyloidosis are structurally conserved intracellular and extracellular insoluble proteinaceous deposits termed amyloid fibrils [1–3]. Protein amyloid aggregation proceeds through a nucleation dependent process wherein monomeric and oligomeric aggregates form "seeds" that initiate an aggregation cascade that results in equilibrium between mature amyloid fibrils and their small precursor aggregates. Mature amyloid fibrils are comprised of several unbranched protofilament segments, which are in turn made up of β-sheet rich protein structures. These structures stack upon one another, forming the conserved amyloid "cross beta spine", characterized by individual β-strand units being positioned perpendicular to the long axis of the protofilament [2]. Even though certain physiochemical properties conferred by amino acid sequence such as hydrophobicity, charge and β-sheet propensity can affect amyloidogenicity of natively unfolded proteins, extensive literature suggests that amyloid formation is facilitated by backbone interactions [2]. Over the last two decades, increasing evidence indicates that the primary pathological amyloid species are non-fibrillar precursor aggregates that range from unstructured oligomers (as small as dimers) to β-sheet rich aggregates termed protofibrils (as small as 20-mers) [2,4–9].

Generic mechanisms of amyloid induced cytotoxicity include cell membrane damage, organelle dysfunction, and impaired proteostasis that can ultimately lead to cell death [10–13]. Protein amyloid specific pathologies can also arise due to the cellular and physiological processes that are perturbed in specific tissues as well as the unique consequences linked to losing the native function of the aggregating proteins. For example, microtubule dysfunction as well as increased insulin resistance and reduced β-cell mass are manifestations of specific amyloid pathologies present in tauopathies and T2D, respectively. Currently amyloidosis can be classified based on if the amyloid deposits are localized or systemic and if the underlying pathologies are neuropathic. Using these criteria, Dobson and colleagues delineated amyloid diseases into three categories: neurodegenerative, non-neuropathic systemic and non-neuropathic localized amyloidosis [1,2]. Over fifty human protein misfolding diseases and their associated proteins and peptides have been described, including several physiologically important peptide hormones such as insulin [14] and amylin [15].

Due to a rapidly aging population and the modern sedentary lifestyle, we are witnessing rapidly growing numbers of people with chronic human diseases, including protein amyloid diseases. AD, for which currently there are no known cures, is reaching epidemic proportions. Progress towards managing protein misfolding diseases in general has been hampered by the failure to develop any effective disease-modifying drugs. This is in part due to our very limited mechanistic understanding of amyloidogenic protein – drug/small molecule interaction. Identification of effective therapeutic inhibitors is challenging because of intrinsic structural disorder of many protein targets of amyloid assembly. In this review we will primarily focus on natural product based amyloid inhibitors (Fig. 1) and in-depth analysis of their mechanisms of inhibition.

2. Drug discovery strategies against amyloidosis

There are multiple therapeutic strategies to identify disease-modifying agents against protein amyloidosis (for a recent review, see [16]). For natural compound identification, one source of information comes from epidemiological studies that suggest preventative effects against dementia, AD, or diabetes may be associated with the diets containing high intake of flavonoids and polyphenolic compounds [17]: The Mediterranean diet, featuring by a high intake of vegetables, fruits, cereals, and olive oil, was reported to be associated with reduced risk for AD and mild cognitive impairment in multiethnic community studies in New York [18,19]. Several cohort studies suggested that moderate intake of red wine (containing resveratrol) was associated with a reduction in risk of dementia, AD, or cognitive decline [20,21]. Curcumin, found in yellow curry spice turmeric in traditional Southeast Asian diets, and EGCG and myricetin, polyphenolic compounds present in green tea, have been associated with cognitive health [17]. However, the protective effects of diet as a whole are not the same with the specific effects of a single compound. How diet-specific natural compounds may provide healthy effects are not well known. Nevertheless, information from these epidemiological sources as well as information reported by alternative and complementary medicine led to testable hypotheses and experimental efforts that successfully identified numerous natural compound amyloid inhibitors [22–25].

One of the current strategies aimed at identifying therapeutic lead compounds for amyloidosis focuses on inhibiting amyloid aggregation by (i) inhibiting toxic amyloid formation and/or stabilizing its native form from aggregating and (ii) remodeling or degrading toxic amyloid oligomers and/or insoluble fibrils. Various approaches have been used. A variety of platforms, including *in vitro* ([26] and cell based approaches [27] have been used in a semi-to-high throughput capacity to screen for small molecules that prevent or modulate amyloid aggregation. One selection criterion used to choose the library of compounds for screening emphasizes the overall quantity and diversity of compounds rather than any specific underlying physicochemical features [26]. For instance, Chen and colleagues developed a high throughput small molecule microarray assay capable of identifying amyloid inhibitors by assessing binding affinity with amyloid β-peptide with ~11,000 different small molecule leads per array slide. Activities were assessed from a range of synthetic and natural compounds as well as compounds derived from diversity-oriented synthesis. Several high-resolution crystal structures of fragment sequences of amyloidogenic proteins [28,29] in concert with atomic structural analysis on small molecules that bind these structures [30–32] have revealed a variety of molecular scaffolds that either inhibit or modulate amyloid formation. These structures, some of which have been proposed as potential pharmacophores [30] that can presumably target the generic cross beta spine architecture common to all amyloids, are currently being used for structure-based drug design efforts. For example, Eisenberg's group, utilizing Orange G, an amyloid binding dye, developed a high throughput screening platform that utilized iterative computational and