Amyloid Inhibitors

DOI: 10.1002/anie.201105706

Terminating the Amyloid Zipper by Design

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amyloid β-peptidescomputer chemistry · inhibitors · proteins · steric zippers

The aggregation of proteins in vivo to form cytotoxic oligomers and amyloid fibrils is linked to cell damage and the pathogenesis of a number of devastating cell-degenerative diseases including Alzheimer's disease (AD), Parkinson's disease, and type II diabetes.[1,2] Although specific disease-associated proteins or polypeptides are the key components of the amyloid deposits in each of the diseases, increasing evidence suggests that common mechanisms underlie self-assembly by these different proteins.[1,2] Devising compounds to interfere with or block these processes is a very important aim of biomedical research as it should contribute to both understanding the molecular mechanisms linking protein aggregation to disease and to developing therapeutic strategies in these yet incurable diseases. In the past two decades, several compounds including small organic molecules, antibodies, and designed peptides have been generated and shown to modulate or inhibit self-assembly and/or cell toxicity of amyloid polypeptides.[3,4] However, none of them has yet found therapeutic application.

One of the earliest developed chemical approaches to devise peptide-based inhibitors of amyloid formation and toxicity was exploiting the high self-association potential of specific short β-sheet- and amyloid-forming segments of the amyloid polypeptides.[3] In fact, non-amyloidogenic analogues of such sequences, often called "amyloid core" sequences, including short peptides containing "β-sheet breakers", non-natural amino acids, or *N*-methyl amino acids, have been shown to inhibit the fibrillogenesis and/or cell toxicity of the amyloid polypeptides.[3] However, generally applicable computational approaches for designing inhibitors of amyloid formation have not been available. Possible reasons include the lack of atomic level information on the structure of fibrils and cytotoxic assemblies, and in several cases the intrinsically disordered nature of the full-length amyloidogenic polypeptides. Such approaches could be used to generate large numbers of potential amyloid inhibitors, thus accelerating the development of therapeutics.

In their current work,[5] Eisenberg, D. Baker et al. now present a novel, likely general, and apparently highly effective structure-based computational design approach to devise peptide-based inhibitors of amyloid formation (Figure 1). The peptides generated by this approach were designed to target the ends of the fibrils and thus block their further elongation. Rosetta software was used and the "steric-zipper" motif served as a structural template.[6–8] The steric-zipper motif describes a structure consisting of a pair of tightly packed β sheets with complementary and interdigitating side chains that keep the two sheets together.[7,8] In previous studies, the Eisenberg group determined the fibril-like atomic structures of a number of steric zippers in microcrystals formed by short fibril-forming and identical segments of various amyloidogenic proteins; they suggested that steric zippers form the "spine" of amyloid fibrils and are their basic structural unit.[7,8] The inhibitor design concept in the present study features a tight interface between the steric zipper at the end of the fibril and the inhibitory peptide aimed at blocking further fibril elongation, and natural or nonnatural amino acids can be applied. The inhibitory peptide should attach at the top of the steric zipper, forming all backbone hydrogen bonds and with side chains capable of entering a maximum number of hydrogen-bonding and hydrophobic interactions across the β-sheet interface (Figure 1). The ability to block further addition of amyloid peptide β strands is conferred to the inhibitory peptide by the steric clashes between the side chains of specifically introduced residues with side chains of residues of the amyloid β sheet.

The crystal structure of the fibril-like steric zipper formed by the hexapeptide segment of tau protein VQIVYK (tau-(306–311)) was applied as a first template to design hexapeptides consisting exclusively of D-amino acids as tau fibrillization inhibitors. The intracellular formation of neurofibrillar tangles (NFTs) composed mainly of fibrillar aggregates of tau protein, and the extracellular deposition of amyloid plaques consisting of fibrillar aggregates of β-amyloid peptide (Aβ) are the two histopathological hallmarks of the brains of Alzheimer patients. The use of the structure of VQIVYK as a template was based on its known crucial role in tau self-assembly and on the hypothesis that this structure may also form in fibrils of full-length tau protein.[9]

Four of the designed peptides were identified as potential inhibitors and their effects on fibril formation of the template VQIVYK and of the two tau protein constructs K19 and K12 (roughly 130 and 150 residues, respectively) were then studied by using transmission electron microscopy (TEM) and the amyloid-specific thioflavin T assay. The hexapeptide D-TLKIVW was identified as the most potent inhibitor. In fact, D-TLKIVW inhibits, in a dose-dependent and sequence-specific manner, fibril formation by both VQIVYK and the

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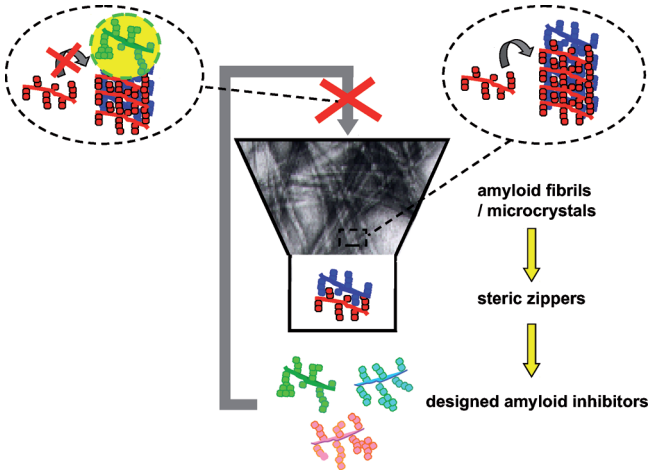

Figure 1. Design of peptide-based inhibitors of amyloid formation as described by Eisenberg et al.[5] The atomic structure of a short fibril- and microcrystal-forming segment of an amyloid-forming protein is determined by analysis of microcrystals. The identified steric-zipper structure (in the stem part of the funnel) is then applied as a template in the computational design of inhibitors of amyloid formation (lower part of figure, below the stem of the funnel). Inhibitors were designed to a) bind tightly to existing amyloid β sheets and b) block addition of further amyloid peptide β strands (inset, top right) through side-chain-mediated steric clashes between the inhibitor (green structure in yellow circle; inset, top left) and the amyloid peptide (inset, top left).

tau constructs. Importantly, the diastereomer, L-TLKIVW did not affect tau fibrillization, while D-TLKIVW had no effect on fibril formation of Aβ, suggesting that a specific interaction underlies the inhibitory effect of D-TLKIVW. Biophysical studies on its inhibitory effect, on fibril elongation rates, and on its interaction with fibrillar or soluble states of tau constructs suggested that D-TLKIVW interacts with amyloid fibril-like assemblies rather than with monomers, and a binding affinity in the low micromolar range was determined. Finally, TEM studies supported the suggestion that D-TLKIVW binds at the ends of fibrils, consistent with the design concept.

First evidence on the more general applicability of the presented inhibitor design approach was obtained by the design of an inhibitor of amyloid formation by PAP(248–286). PAP(248–286) is a proteolytic fragment of the semen protein prostatic acid phosphatase (PAP) consisting of residues 248–286 of PAP. PAP(248–286) is able to aggregate into amyloid fibrils that can capture human immunodeficiency virus (HIV) virions and dramatically enhance HIV infectivity by promoting their attachment to target cells.[10] The designed hexapeptide (abbreviated WW61) was based on the steric-zipper structure of GGVLVN, a segment of PAP(248–286), and consisted of natural and nonnatural L-amino acids. Biophysical studies showed that WW61 strongly delayed fibrillogenesis of PAP(248–286) in vitro, and its inhibitory effect was dose-dependent and sequence-specific. Most importantly, WW61 prevented PAP(248–286) fibril-mediated enhancement of HIV infection in a functional assay system. These results demonstrated that the inhibition of PAP(248–286) amyloid formation suppresses HIV infectivity, providing a lead for compounds preventing HIV infection.

Taken together, the results of the amyloid inhibitor design strategy described by Eisenberg et al. are very exciting because they demonstrate that it is possible to computationally design peptide-based amyloid inhibitors (and in the future possibly from other molecule classes as well) by applying as templates known structures of short fibril-forming segments of amyloidogenic proteins. In addition, the fact that the design of the inhibitors was based on steric-zipper structures provides strong evidence for the hypothesis that steric zippers are present in amyloid spines.[7,8] As the atomic level characterization of such steric zippers has become possible over the past few years, the application of this design approach to generate large numbers of potential inhibitors of amyloid formation by the various different disease-associated proteins appears feasible.[7,8] As these amyloid inhibitors are short peptides and can also contain nonnatural amino acids, they are promising candidates for the development of amyloid disease therapeutics, noninvasive amyloid diagnostics, and research tools for understanding pathogenic protein self-assembly. In this context, one very important question that needs to be addressed is: what is the effect of these new amyloid inhibitors on the formation of cytotoxic protein aggregates? Inhibition of amyloid formation by blocking the ends of protofibrillar assemblies or fibrils may result in decreased or increased cell toxicity owing to formation of lower or higher amounts of cytotoxic protein assemblies.

Whatever the outcome of these studies, the design approach offered by Eisenberg and colleagues paves the way to gaining a better understanding of basic mechanisms of pathogenic protein self-assembly and amyloidogenesis as well as to the discovery and development of novel compounds with biomedical and therapeutic value in amyloid diseases.

Received: August 12, 2011
Published online: November 4, 2011

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