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New mimetic peptides inhibitors of Aβ aggregation. Molecular guidance for rational drug design

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ABSTRACT

A new series of mimetic peptides possessing a significant Aβ aggregation modulating effect was reported here. These compounds were obtained based on a molecular modelling study which allowed us to perform a structural-based virtual selection. Monitoring Aβ aggregation by thioflavin T fluorescence and transmission electron microscopy revealed that fibril formation was significantly decreased upon prolonged incubation in presence of the active compounds. Dot blot analysis suggested a decrease of soluble oligomers strongly associated with cognitive decline in Alzheimer's disease. For the molecular dynamics simulations, we used an $A\beta_{42}$ pentameric model where the compounds were docked using a blind docking technique. To analyze the dynamic behaviour of the complexes, extensive molecular dynamics simulations were carried out in explicit water. We also measured parameters or descriptors that allowed us to quantify the effect of these compounds as potential inhibitors of Aβ aggregation. Thus, significant alterations in the structure of our $A\beta_{42}$ protofibril model were identified. Among others we observed the destruction of the regular helical twist, the loss of a stabilizing salt bridge and the loss of a stabilizing hydrophobic interaction in the β1 region. Our results may be helpful in the structural identification and understanding of the minimum structural requirements for these molecules and might provide a guide in the design of new aggregation modulating ligands.

1. Introduction

Alzheimer's disease (AD) is a devastating neurodegenerative disease that affects approximately 35 million people worldwide [1]. With the increase in human life span, AD will continue to afflict ever-increasing numbers of the elderly, augmenting an already serious health problem. Current AD drugs are targeting just the symptomatic mechanisms with limited benefit. In the absence of effective drugs, the incidence of AD is expected to rise rapidly over the coming years. Thus, the discovery of disease-modifying therapeutics that can slow or ultimately halt disease progression is paramount. Currently, several therapeutic approaches targeting amyloidogenic proteins are under development, which include reducing the expression level of the amyloidogenic protein, increasing the clearance rates of misfolded amyloidogenic proteins, increasing the stability of properly folded amyloidogenic proteins, and inhibiting the self-assembly of amyloidogenic proteins into oligomers and fibrils [2].

The most widely accepted theory regarding the etiology of AD is known as the “amyloid cascade hypothesis”, which features the

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amyloid β-protein (Aβ) as the central pathological agent. Amyloid is a generic term used to refer to protein aggregates adopting a cross-β-sheet structure [3]. Several studies have shown that the aggregation and fibril formation of Aβ involve a change in conformation, from an intrinsically disordered peptide to insoluble fibrils [4]. Experimental data have revealed an underlying superstructure of anti-parallel, intra-molecular β-strands stabilized predominantly by backbone hydrogen bonds and salt bridges and parallel, inter-molecular β-sheets stabilized predominantly by backbone hydrogen bonds and hydrophobic interactions [5–8]. A network of inter and intra-molecular hydrogen bonds maintains the stability of the fibril once it has formed [9–12].

Aβ is a short peptide, ranging in length from 37 to 43 residues, with its most common alloforms being 40 and 42 residues in length. The aggregation and deposition of Aβ in neural tissue is believed to be linked to neuronal cell death and loss of cognitive function seen in patients with AD [13]. Although the characteristic lesion of AD are large plaques, consisting of fibrilar Aβ, the most toxic forms of Aβ are generally believed to be soluble oligomers [14,15].

Extensive studies exploring the mechanism of Aβ misfolding and aggregation have been ongoing for several decades [16–18] leading to the identification of a variety of inhibitors targeting self-assemble of Aβ. These compounds can be divided into three general categories: small molecules, short peptides and antibodies [2]. Two types of small molecule inhibitors for amyloidogenic proteins have been identified so far: polyphenols and non-polyphenols. Polyphenols comprise a large group of aromatic compounds containing one or more phenolic hydroxyl groups including epigallocatechin gallate, curcumin, wine-related polyphenols, coffee derived polyphenols and oleuropein. These groups may competitively interact with aromatic residues in amyloidogenic proteins, be sandwiched between them, prevent the π–π interaction, and block the amyloid formation [19]. This process is explained in the "π-stacking" theory, in which the aromatic residues of amyloidogenic proteins interact with each other *via* π-stacking [20]. Non-polyphenol inhibitors include small molecules like alkaloids, flavonoids, glycosides and phenazines. Most of these inhibitors contain one or several aromatic rings, which are key factors to the inhibitory effect according to the "π-stacking" theory mentioned above. Aside from the compounds described above, other small molecule inhibitors of Aβ aggregation have been modeled based on the histological dyes used to characterize amyloid both *in vitro* and *in vivo*. This class of compounds includes sulfonated dyes like congo red, chrysamine G, and thioflavin S. Congo red is capable of binding to various Aβ species ranging from monomers to mature fibrils and it has been shown to interrupt the normal aggregation of Aβ peptides, thereby reducing toxicity. MD simulations revealed that congo red may play a role in interrupting sheet-to-sheet stacking and blocking strand-to-sheet extension [21].

With respect to peptide compounds, a pioneering effort by Ghanta et al., in 1996 demonstrated the possibility to use small peptides related to protein self-recognition regions as a viable strategy for the development of new Aβ antiaggregants [22]. Tjernberg and coworkers synthesized a series of short peptides derived from Aβ and found that KLVFF (Aβ 16–20) is one of the nucleation sites in Aβ [23]. They further observed that KLVFF can bind to full-length Aβ and prevent its self-assembly into β-sheet-rich amyloid fibrils. These results indicated that peptide based self-recognition regions may inhibit the self-assembly of amyloidogenic proteins by acting as β-sheet breakers. Subsequent work expanded upon these original findings [24,25]. Soto and coworkers designed and synthesized β-sheet breaker peptides (BSB) based on the 17–20 region of Aβ. These BSB inhibited and disassembled amyloid fibrils *in vitro* and, prevented Aβ induced neurotoxicity in cells [26,27]. Moreover, treatment with these peptides inhibited neuronal death, brain inflammation and memory impairment *in vivo* [28]. These peptides showed low toxicity, low immunogenicity, high solubility and reasonably high brain uptake. Unfortunately, their biodisponibility was very poor as they were rapidly degradable and had low permeability to cross biological barriers imposing serious limitations for their therapeutic use [29]. The BSB affinity for Aβ oligomers is significantly improved by appending polar groups to the peptide scaffold. Autiero and co-workers [30] combined polar groups with the peptide portion LPFFD, the well-known BSB Soto's peptide. These compounds were able to efficiently interact with the protofibril ends preventing fibril growth.

Chen et al. reported small mimetic peptides with similar ability to inhibit and reverse Aβ misfolding and aggregation, but with potentially better drug-like properties [31]. Among these molecules, BSBM6 (**1**) (Fig. 1) possessed a significant antiaggregant effect and appeared as one of the best candidates for further studies. Chen et al. suggested that this compound interferes with electrostatic interactions during aggregation and destabilizes the Aβ fibril internal hydrogen bond network that is necessary to maintain the β-sheet structure, by forming strong hydrogen bonds with the Aβ subunits [31]. Although this hypothesis seems reasonable, it should be noted that such assumptions have been made on the basis of simple docking analysis. Therefore, several questions regarding the molecular mechanism of these compounds at a sub-molecular level remain unanswered. The main limitation in using docking to identify binding modes of antiaggregating compounds against Aβ is the rigidity of the target structure. If an antiaggregating small molecule alters the structure of Aβ, such changes cannot be reflected during docking, and thus a structure candidate will be identified largely based on rigid interactions. Another challenge associated with docking is the scoring and refinement of docked poses. This particular hurdle is not unique to the study of Aβ, but it affects all docking studies even those involving well-characterized receptors [32–34].

Molecular Dynamics (MD) simulations provide a molecular-level view of a system as it evolves over time [35], making them particularly useful in studying Aβ and its interactions with small molecules. Since Aβ adopts a variety of structures along the aggregation pathway [36], MD simulations can be used to examine how these different structures may interact with small molecules that may serve as therapeutic antiaggregating compounds. Over the past few years, a growing number of theoretical studies have been conducted to analyze the interactions of Aβ with anti-aggregating molecules. Liu and co-workers conducted simulations of polyphenol (−)-epigallocatechin-3-gallate binding to $A\beta_{42}$ [37]. Raman and co-workers [38] and Takeda and co-workers [39,40] have reported the interactions of naproxen and ibuprofen with Aβ fibrils using simulations employing implicit solvents. Lemkul and co-workers conducted simulations of binding of the flavonoid morin to a model of $A\beta_{42}$ protofibrils [41] and more recently examined the binding of this molecule to monomers and dimers of full-length $A\beta_{40}$ and $A\beta_{42}$ [42]. Attanasio and co-workers used MD simulations and NMR experiments to study the effect of carnosine on the inhibition of $A\beta_{42}$ aggregation by perturbing the H-bond network near the central hydrophobic core [43]. Bruce and co-workers [44] used MD simulations to compare the mode of interaction of an active (LPFFD) and inactive (LHFFD) β-sheet breaker peptide with an Aβ fibril structure from solid-state NMR studies. All these efforts have shown several strengths and limitations of current techniques. Lemkul and Bevan reported a comprehensive review about the role of molecular simulations in the development of inhibitors of Aβ peptide aggregation, which is a very complete and concise contribution on this topic [45]. Among their conclusions,