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Research paper

Inhibition of tau aggregation using a naturally-occurring cyclic peptide scaffold

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ABSTRACT

Disulfide-rich macrocyclic peptides are emerging as versatile scaffolds for the development of stable biochemical tools. This potential is due to the combination of their structural stability and range of bioactivities. Here, we explored the activity of these peptides on fibril growth of the hexapeptide Ac-VQIVYK-NH2 (AcPHF6), which is a tau-derived peptide that has been widely used to understand the pathological mechanism of numerous tauopathies, including Alzheimer's disease. Of the cyclic peptides tested, SFTI-1 and kB1 showed an inherent ability to inhibit AcPHF6 fibril formation. Using an end-capping strategy and combining it with a molecular grafting approach, we demonstrated that SFTI-1 could be used as a starting point to design more potent fibril inhibitors. We further identified chemical and structural features of SFTI-1 and its analogues that underpin their inhibitory activity. The ability to inhibit fibril growth using the strategy employed herein supports the 'steric zipper' model of AcPHF6 fibril formation and shows that naturally-occurring cyclic peptides have potential as drug leads or molecular probes for understanding fibril formation.

1. Introduction

Aggregation of the microtubule-associated protein tau is a pathological hallmark of a number of neurodegenerative diseases, including Alzheimer's disease, progressive supranuclear palsy, corticobasal degeneration, Pick's disease and Huntington's disease [1–3]. From studies characterizing the molecular basis of tau aggregation, it has been reported that specific polypeptide regions of tau are important for its aggregation by fibril formation. One widely-studied region is the hexapeptide Ac-VQIVYK-NH_2 (AcPHF6), derived from residues 306–311 of tau, which forms fibrils with biophysical properties similar to full-length tau fibrils [4,5]. This hexapeptide has been used as a model system for both the design of tau aggregation inhibitors and the elucidation of the molecular forces driving tau aggregation. The atomic structure of AcPHF6 fibrils has revealed that fibril formation involves hydrogen bond interactions in one direction and other non-covalent interactions (e.g. hydrophobic) in the orthogonal direction [6]. Interestingly, the structure of these fibrils contains a 'steric zipper' motif that is shared by the structures of segments from several other fibril-forming peptides and proteins [6,7], suggesting that a common strategy can be employed to inhibit fibril growth.

A novel strategy that has been exploited recently is to cap the ends of fibrils to prevent attachment of additional fibril components, thereby preventing fibril growth. Using this approach, inhibitors based on a cyclic β-sheet peptidomimetic peptide containing a Hao group were shown to be effective in inhibiting fibril growth of the tau segment and other amyloid-forming proteins [8–10]. In another study, a linear peptide inhibitor composed of only D-amino acids was designed to specifically inhibit AcPHF6 fibril formation [11]. In addition to validating the end-capping approach to fibril inhibition, these studies are interesting, particularly from a peptide drug design perspective, because they demonstrate two popular approaches to enhance the stability and bioavailability of peptides; namely, (a) using non-natural amino acids, and (b) backbone cyclization [12,13].

We are interested in peptides that have a cyclic backbone and are disulfide-rich, the combination of which grants them promising biopharmaceutical properties, such as high stability [14]. We have also demonstrated that these structural constraints underpin their remarkable stability in adverse chemical and enzymatic conditions [15], making them excellent scaffolds for stabilizing foreign peptide sequences through an approach called molecular grafting [14]. Many of the scaffolds that have been used for molecular grafting to

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date are derived from nature; for example, the peptide MCoTI-II (*Momordica cochinchinensis* trypsin inhibitor-II) has been re-engineered to antagonize intracellular p53 degradation [16] and inhibit serine proteases involved in inflammatory disease [17], and kalata B1 from *Oldenlandia affinis* has been re-engineered to become an orally-active bradykinin B1 receptor antagonist [18]. One of the smallest disulfide-rich scaffolds that has been used for molecular grafting is SFTI-1 (sunflower trypsin inhibitor-1) [19–23], which has been re-engineered for the treatment of cancer [20–25]. Compared to scaffolds containing unnatural amino acids, these nature-derived scaffolds have the benefit of being easily amenable to both chemical and recombinant methods for engineering and production [26–28].

Here, we explored the potential of naturally-occurring disulfide-rich templates as fibril inhibitors or chemical probes for understanding the fibril formation process, using the aggregation of AcPHF6 as the test system. We screened several naturally-occurring cyclic peptides to examine their effect on fibril growth. Focusing on the scaffold SFTI-1 (Fig. 1A), we used molecular grafting to design a series of inhibitors to cap the ends of fibrils (Fig. 1B) and subsequently studied their structure–activity relationships.

2. Results and discussion

2.1. Naturally-occurring cyclic peptides can have fibril inhibitory activity

We first validated that AcPHF6 could form fibrils by monitoring its aggregation over time using the widely-used thioflavin S fluorescence assay and experimental conditions as reported previously [8], as shown in Fig. 2A (left panel, black line). In agreement with previous studies [4,8], fluorescence intensity increased over time, confirming the growth and accumulation of fibrils, which was further validated using electron microscopy (Fig. 2A, right panel). Having confirmed that the thioflavin S fluorescence assay tracks the aggregation behavior of AcPHF6, we used it to screen for fibril inhibitory activity of our library of cyclic disulfide-rich peptides, including: SFTI-1, a potent inhibitor of trypsin [29]; BTD-2, a baboon θ-defensin known for its antimicrobial activity [30]; cVc1.1, a backbone cyclic analog of a toxin from *Conus victoreae* [31]; and kB1, a cyclotide originally discovered from *O. affinis*, which has a range of bioactivities, including anti-viral and anti-insecticidal activity [32].

By examining the effect of SFTI-1 on fibril growth over a range of SFTI-1:AcPHF6 ratios (1:1–1:32; Supplementary Fig. S1), we found that SFTI-1 exhibited a concentration-dependent reduction of fluorescence intensity (Fig. 2A, left panel), indicating that SFTI-1 was able to inhibit fibril formation. Furthermore, little to no effect on the fluorescence output was observed in the absence of AcPHF6 (Fig. 2A, left panel) and over a range of SFTI-1 concentrations (Supplementary Fig. S1), suggesting that SFTI-1 did not bind to thioflavin S and confirming the inhibitory activity of SFTI-1.

We then screened the other cyclic peptides which differ from SFTI-1 in amino acid content and disulfide bond configuration (Fig. 2B). The time-course data for various cyclic peptide:AcPHF6 ratios (Supplementary Fig. S1) and the fluorescence intensity at 60 min for these peptides (Fig. 2B) show that these peptides have a variable effect on fibril growth. Specifically, kB1 is a stronger inhibitor of fibril formation than SFTI-1, suggesting that the sequence or structure of kB1 makes it better able to bind to and/or disrupt AcPHF6 fibrils. On the other hand, cVc1.1 had essentially no effect on fibril growth. Interestingly, BTD-2 appeared to enhance fibril growth. Combined with the observation that BTD-2 by itself (i.e. in the absence of AcPHF6) exhibited an increased in fluorescence over time (Supplementary Fig. S1), we speculate that BTD-2 can form fibrils and promote fibril growth; indeed, it has been recently proposed that β-sheet antimicrobial peptides like BTD-2 can form amyloid-like fibrils [33]. Although cVc1.1 and BTD-2 did not inhibit fibril growth, the promising result observed for both SFTI-1 and kB1 suggests that a wider screen of naturally-occurring cyclic peptides

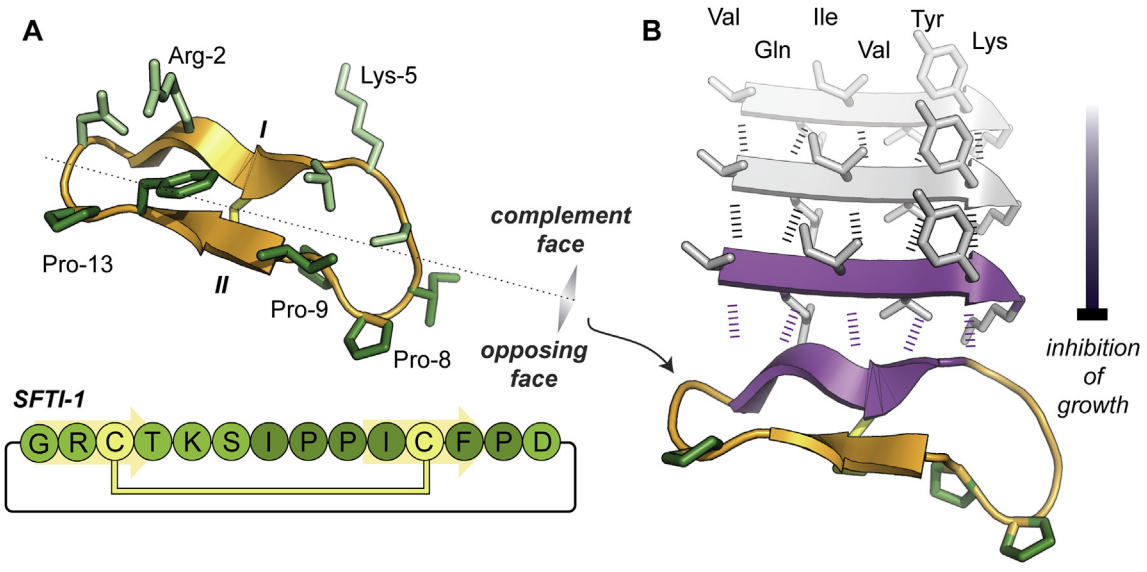

Fig. 1. Cyclic peptides as templates for the design of fibril formation inhibitors. (A) The structure and sequence of sunflower trypsin inhibitor-1 (SFTI-1), one of the smallest known cyclic disulfide-rich peptides is depicted. Selected residues are labeled, as are the two Cys residues (I and II) involved in the disulfide bond. The backbone is colored orange and the side chains colored green, except for the disulfide bond, which is colored yellow. A dotted line marks the two 'faces' of SFTI-1, which can be used to design fibril formation inhibitors. The strategy of fibril formation inhibitor design is illustrated in (B). Specifically, SFTI-1 can be modified to include: (i) a 'complement face' (shown in purple) that recognizes the end of AcPHF6 fibrils (also shown in purple) through hydrogen bond and other non-covalent interactions; and (ii) an opposing face that prevents binding of additional peptides (Pro residues in green are highlighted because they are not hydrogen bond donors and will inhibit hydrogen bond formation), thereby inhibiting fibril growth. Modification of SFTI-1 by replacing a segment of its sequence with a foreign sequence (shown in purple) is an approach that we refer to as molecular grafting. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).