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Serendipitous inhibition of A β and rV λ 6Wil amyloid fibril growth by bi-functional peptides

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Background

Amyloidosis is characterized by the aggregation of partially misfolded proteins or peptides as fibrils that deposit in organs and tissues resulting in dysfunction and death. We have previously identified a series of amyloid-targeting, synthetic, polybasic peptides, such as peptide p5, that specifically interact with numerous forms of synthetic amyloid fibrils and human amyloid extracts *in vitro* as well as systemic inflammation-associated (AA) amyloid deposits *in vivo* [1].

Although there has been great progress in the development of amyloid-targeting immunotherapeutic agents designed to selectively bind amyloid and facilitate their removal [2], at present, there are no amyloid-directed therapeutics that successfully prevent the growth or extension of amyloid fibrils. Indeed, the process of fibril growth post nucleation is widely considered to be kinetically and thermodynamically remarkably favorable and, as such, potentially unstoppable. However, decreasing the rate of fibrillogenesis or amyloid growth is an important, clinically relevant, intervention point and would represent another treatment option for patients with amyloidosis.

To address this, we have developed novel, bi-functional amyloid binding peptides originally designed to enhance the circulating lifetime of peptide p5 by the addition of albumin-binding peptide sequences. Using a battery of *in vitro* fibrillogenesis assays, certain peptides have been shown, serendipitously, to inhibit fibril formation of two amyloidogenic precursors: A β (1–40) and a recombinant human λ 6 variable domain derived from patient Wil(rV λ 6Wil). Data from molecular dynamics simulations, using a model of A β amyloid fibril as the substrate [3], suggest that these peptides may serve as inhibitors of fibril elongation by “capping” the fibril end, a site of potential precursor protein recruitment.

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Declaration of interest

The authors report no conflicts of interest.

Materials and methods

Crude preparations of bifunctional peptide p52 and A β (1–40) were purchased from Anaspec (Fremont, CA). The peptides were further purified by reverse phase HPLC and the integrity confirmed by mass spectrometry. Recombinant V λ 6Wil was generated in *E. coli* and purified from the periplasmic space using standard procedures [4]. Fibrillogenesis assays were performed in phosphate-buffered saline (150 mM NaCl, pH 7.5) using 5 μ M rV λ 6Wil or 7.5 μ M A β (1–40) in the presence of peptides at a 10:1, 1:1, or 1:10 amyloid precursor to peptide molar ratio. The formation of fibrils was monitored by measuring the fluorescence emission of thioflavin T at 490 nm (excitation = 450 nm) over a 24 h period. Molecular dynamics simulations over 20 ns was used to study the interaction of peptide p52 and a model of A β amyloid fibril (PDB# 1BEG), as previously described [5] using the Titan supercomputer at Oak Ridge National Laboratory.

Results

Bifunctional peptide p52 at 10-fold molar excess, or equimolar in certain cases, was able to delay the fibrillogenesis of both A β (1–40) and rV λ 6Wil proteins as evidenced by an increase in the lag time (the time of the initial ThT signal increase) by at least 4-fold, from ~1 to 2 h to more than 8 h. In the case of rV λ 6Wil fibrillogenesis, there was a dose-dependent effect on the inhibition of fibrillogenesis, where even a 10:1 ratio of λ 6 to p52 increased the lag time from 2 to 3 h. At higher p52 concentrations (5 μ M and 50 μ M), the lag time was extended to >7 h (Figure 1A). Notably, non-bifunctional peptides had no effect on the lag time of fibrillogenesis.

Peptides that were effective at inhibiting A β (1–40) were also inhibitors of rV λ 6Wil, suggesting that there may be shared mechanisms of fibril formation. The molecular dynamics simulations suggested that the bifunctional peptide may act as an inhibitor of fibrillogenesis by capping the end of the fibril. We hypothesize that such an interaction during fibrillogenesis may prevent recruitment of other precursor proteins and, thereby, prevent fibril growth (Figure 1B).

Discussion

Preventing fibrillogenesis is an important clinical goal that would complement current management of patients with amyloidosis. Our preliminary data indicate that bifunctional peptides, based on the structure of peptide p5, have the ability to inhibit fibril formation of multiple fibril types. Computer modeling and molecular dynamics simulations of the peptide–A β fibril interaction indicate that the peptide may inhibit by “capping” the growing end of the fibril (Figure 1). Based on these preliminary positive observations, we anticipate that these peptides may yield a novel therapeutic reagent for the inhibition of amyloid fibril growth.

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Figure 1. Inhibition of diverse amyloid fibrils by bifunctional peptide p52. (A) Dose-dependent inhibition of rVλ6WII fibrillogenesis *in vitro*. The lag time for fibrillogenesis of 5 μM Vλ6 was extended from 2 to 3 h by the addition of 0.5 μM peptide p52. (B) Molecular dynamics simulation of peptide p52 “capping” a model fibril composed of Aβ peptides.