AMYLOID DESIGNABLE PEPTIDE MATERIALS AND THEIR USE AS SCAFFOLDS FOR BIOMEDICAL AND ENVIRONMENTAL APPLICATIONS

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ABSTRACT

Fibrous amyloid structured aggregates are not only involved in misfolding and disease, but can also be exploited for the formation of novel functional amyloid biomaterials. In this study we took advantage of the unique self-assembling properties of the amyloigenic peptides GAIIG and GAITIG, associated with the Alzheimer's Aβ peptide and the adenovirus fiber shaft, accordingly. With the aid of computational methods, we applied suitably selected modifications at key positions, to tune the properties of amyloid forming peptides in order to discover novel functional biomaterials in two different applications. In the first application, our computational and experimental results suggest interaction of a designed beta-breaker peptide GAIPIG with Alzheimer's Aβ peptide, delaying the aggregation of the peptide Aβ1-40 in vitro and considering it as a potential inhibitor of amyloid formation. In the second application, we present novel amyloid biomaterials that are capable of binding and capturing cesium ions at neutral and low pH conditions, enabling their use as scaffolds for the removal of cesium ions from nuclear waste or blood.

INTRODUCTION

Amyloid self-assembly refers to the conversion of specific proteins and peptides from their native functional states into long unbranched fibers that are characterized by a cross-beta sheet quaternary structure. Amyloid formation has been associated with a range of human disorders, including Alzheimer's disease, prion and Parkinson's disease. Amyloid biomaterials have significantly advantageous properties, which among others include their easy fabrication, and the capacity to tune their properties by changes at their sequence level. Naturally occurring peptide sequences extracted from amyloid proteins or beta-sheet protein regions can self- assemble outside the context of the entire sequence into amyloid fibrils and can serve as scaffolds for novel biomaterials. Peptide sequences GAIIG and GAITIG are part of the amyloid-beta (Aβ) peptide linked to Alzheimer's disease, and the adenovirus fiber shaft, respectively. They can also independently self-assemble into amyloid fibrils, indicating that they may contribute to the initiation of amyloidosis of the full length proteins. Our previous experiments showed that the insertion of a proline between the two isoleucine residues (GAIPIG peptide)[2] results in the disruption of the amyloid formation. We aim to prove that the GAIPIG peptide due to its homology with the GAIIG peptide can interact and interfere by inhibiting the amyloid formation of the Aβ peptide due to its protruding proline.

This study could serve as the basis for structure -based design of potential inhibitors of amyloid formation.

The discovery of such amyloid peptide scaffolds can serve as a source of inspiration for the fabrication of amyloid materials with advanced properties, as the exposed residues can be modified accordingly depending on the desired application. One highly on demand application is the development of materials that can effectively remove potentially hazardous ions from the environment. As such is the removal of cesium ions from nuclear waste or blood. During nuclear reactor accidents or minor nuclear power station accidents, cesium, a key uranium fission product, can easily dissolve in water, posing a significant risk to human health. With its long half-life (approx. 30 years) and high activity, volatility, and solubility in water, cesium-137 can easily enter the food chain to inflict radiological harm to humans. To address this, we developed a computational protocol in which we introduced suitably selected mutations at the non-β-sheet forming exposed terminal residue positions of the longer sequence YATGAIIGNII. These amino acid motifs aim to mimic the binding abilities of experimentally resolved proteins that bind cesium ions.

EXPERIMENTAL PART

For the cesium capture experiments:

Amyloid materials synthesis. All peptides were custom-synthesized by WuXi AppTec upon our request ,with purity over 95%. Each peptide powder was dissolved in sterile double distilled water (pH 7) to a concentration of 12mg/ml and incubated at room temperature for 3 days.

Transmission Electron Microscopy (TEM). Fibrillar formation was confirmed by TEM observation. 8μλ of each sample was deposited on a copper grid with a formvar/carbon coating for 2 min. Washes followed with distilled water and the fibrils were further stained with uranyl acetate. Peptide samples after incubation with CsCl were not treated with uranyl acetate so the contrast observed is only attributed to the cesium ions. Specimens were examined in a JEOL JEM-2100 Transmission Electron Microscope at an accelerating voltage of 80 kV and 200kV.

Field Emission Scanning Electron Microscopy (FESEM) : 10μλ of each sample was deposited on a circular glass and was air dried. The sample was coated with 10nm Au before the observation. Experiments were performed using a JEOL JSM-7000F microscope operating at 15 kV.

Congo Red staining. Each potential fibrillar solution was mixed with the Congo Red assay solution (10mM Congo Red, 2.5mM NaOH in 50% ethanol) in a 6:1 ratio. A portion of the sample was deposited on a glass coverslip and it was subsequently examined with a Zeiss Stemi 2000-C microscope with and without the use of a crossed polarizer.

Cesium capture analysis with ICP-MS. The self assembled fibrils were collected by centrifugation at 13300 rpm for 30 min at 25^{o}C. They were subsequently resuspended in a cesium chloride aqueous solution(10ppm,pH 7) and also to an acidic cesium chloride solution (10mM boric acid solution, pH 4.5). They were incubated for 6 hours in room temperature. The fibrils were separated from the supernatant through a total of 4 centrifugation cycles at 13300 rpm for 30 min. As a last separation step, 0.22 μm nylon filters were used. The supernatant solution was collected and submitted for cesium elemental analysis. The analysis was conducted on a PerkinElmer NexION 300D ICP-MS instrument.

For the amyloid inhibition experiments

Thioflavin T assay

Synthetic lyophilized peptide Ab1–40 was dissolved in DMSO to a concentration of 100 μM. Each Ab1–40 aliquot was sonicated in ice cold water for 20 s to prevent preaggregation, and immediately diluted to a final concentration of 5μM with 10 mM phosphate-buffered saline containing 0,05%

sodium azide at pH 7.4. Another aliquot was immediately mixed with the GAIPIG stock solution (1 mM) to a final Ab1–40 concentration of 5 μM and GAIPIG concentration of 50 μM. A solution of the GAIPIG peptide at the same final concentration (50 μM) without Ab1–40 was also prepared.
The samples were incubated without agitation at 37 °C, and the fibrillogenesis rate was monitored by using ThT fluorescence analysis in a SPEX FluoroMax fluorimeter. Excitation and emission wavelengths were 450 nm and 480 nm accordingly.

RESULTS AND DISCUSSION

According to our MD simulations and subsequent computational and experimental analysis, the common sequence (GAIIG) present in the Alzheimer's alpha beta peptide and the V3 loop of HIV-1 gp120 can independently self-assemble into antiparallel off register β-sheets. Subsequently, it can be facilitated as a beta sheet core for the development of amyloid scaffolds. The homologous peptide GAIPIG, which contains the beta breaker aminoacid proline, was tested for its self assembly into amyloid fibrils in previous studies, which concluded that even after a long incubation period and in high concentrations, no fibrillar formation was observed.[2]
Moreover, the crystallographically resolved structure of GAIPIG revealed that the GAIPIG molecules interact with each other by forming an antiparallel dimer, reminiscent of a beta-sheet. That observations concluded that the proline residue of the GAIPIG peptides could prevent the formation of ordered b-sheets structures and disrupt the formation of extended beta sheet conformations.
We simulated the self assembly process of the Aβ1-42 peptide in the presence of the GAIPIG peptide. The results indicated that the GAIPIG peptide could bind to the GAIIG and KLVFFA fragments of the Aβ, potentially inhibiting amyloid formation. Experimentally, a 10 fold molar excess of GAIPIG led to an increase in the lag phase to a 25-30% reduction in fibril formation in the period of 7days. Therefore, GAIPIG likely inhibits the elongation through binding to the Aβ peptide in such a way that the proline side chains prevent the further formation of beta sheet interactions. (***Figure 1***)

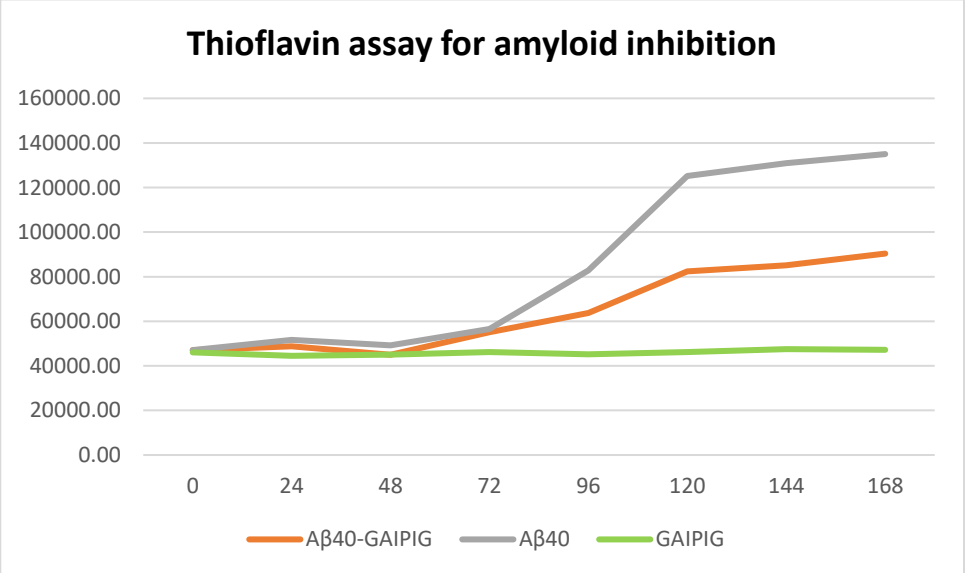


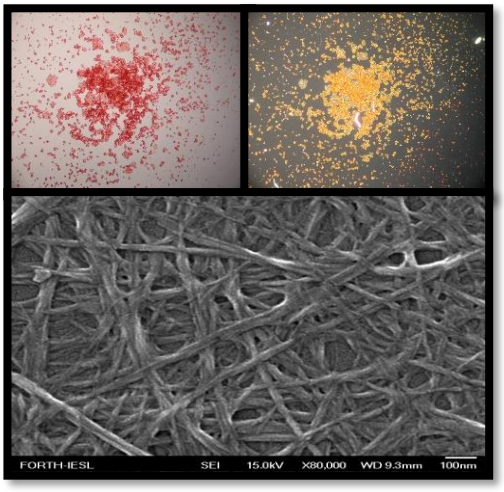
Figure 1*: Kinetics of the Ab1–40 peptide fibril formation (5 μM) as assessed by the Thioflavin-T binding assay in the absence (grey) or in the presence of 50 μM (orange) GAIPIG peptide. 50 μM of GAIPIG peptide alone(green) emitted no fluorescence after excitation at 482nm. The presence of the GAIPIG peptide hinders amyloid formation in a range of 25-30%.*

In another concept, the GAIIG amyloid core can be successfully exploited for the development of functional amyloid materials by inserting rationally designed insertions of amino acid residues outside the beta sheet core.

The computational protocol we developed was applied for the "on demand" design of amyloid materials with cesium capture capabilities. We have identified the sequences FQGAIIGFNE and FNGAIIGFQE as the most promising peptide scaffold for cesium ion binding. The scaffolds aim to mimic the binding ability and morphology of natural cesium capture proteins, while being able to self assemble in amyloid fibrils.

The optimal amyloid scaffolds were designed and validated concerning its amyloid forming ability and cesium capture capacity by computational methods. The experimental confirmation concerning the fiber formation ability was confirmed by facilitating Field Emission Scanning Electron Microscopy(FESEM) to observe the formation of the long unbranched fibrils and Congo Red staining to identify the amyloid nature of the fibrils due to the green/yellow birefridgence (***Figure 2***) under polarized light. To better simulate the nuclear wastewater environment the stability of the fibrils was validated under harsh conditions (pH:3, pH:12 and high temperatures) with the aforementioned methods. The morphology and the amyloid nature of the peptides are not disturbed.

Concerning scaffold's cesium capture capability, the fibrils were incubated with CsCl ions overnight and after several steps of centrifugation and washes, the fibrils were separated from the unbound cesium ions and analyzed with ICP-MS. Peptide FQGAIIGFNE demonstrated the highest cesium binding ability both in neutral and acidic environment at a percentage of 70% removed cesium ions, followed by the peptide FNGAIIGFQE with a binding capacity of 64%. Whereas, the control peptide AGKGAIIGFIK exhibited low-non specific binding at 18.1% of cesium ions removed. For additional qualitative confirmation, the fibrils were observed before and after incubation with cesium ions under Transmission electron microscopy (TEM). No negative staining was used on the samples incubated with cesium so the fibrils can be observed only due to the bound cesium ions which with its high atomic number(z=55) has increased electron density. (***Figure 3***)



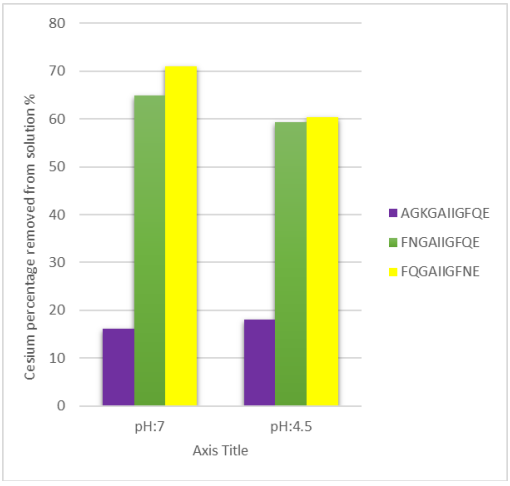


Figure 2. *FESEM picture of the self-assembled fibrils of the peptide FQGAIIGFNE after incubation in double distilled water for 3 days(bottom) The scale bar is 100 nm. Congo Red staining proves the formation of amyloid fibrils. Top(left) without and Top(right) with the use of a crossed polarizer.*

Figure 3. *Percentage (%) of cesium ions removed from a 10 ppm cesium chloride solution by the amyloid material formed by peptide FQGAIIGFNE (yellow), amyloid material formed by peptide FNGAIIGFQE (green), and fibrils formed by peptide AGKGAIIG- FIK (purple) under two different pH conditions, pH 4.5 and pH 7.*

CONCLUSIONS

Our computational and experimental research, concerning the amyloid aggregating sequence GAIIG, reveals that this amyloid building block has the potential to be further investigated as a potential core recognition sequence in Aβ Alzheimer's peptide. Moreover, our computational results suggested and our experimental results confirmed that upon co-incubation of the beta-breaker GAIPIG peptide with the Aβ1-40 peptide, an inhibition of 30% in amyloid elongation is observed in vitro. The X-ray diffraction results indicate that two molecules of the GAIPIG peptide interact to form an antiparallel dimer, but only as a reminiscent of a small beta-sheet. Upon interaction with the homologous GAIIG sequence unstable and not well-formed beta sheets are formed that could possibly be responsible for the reduction of the amyloid formation rate . The GAIPIG peptide cannot be envisaged as a potential therapeutic per se, due to potential degradation and stability issues in vivo. However, non-natural analogs and peptidomimetics structurally related to the parent peptide can be developed. Thus, the single-crystal structural information on the beta-breaker GAIPIG peptide could be exploited as a minimal framework for future structure-based design of Aβ inhibitors.

Regarding the cesium capture amyloid scaffold, we have developed and applied a computational protocol for the rational design of functional amyloid biomaterials with cesium capture capabilities. This protocol can substitute other time-consuming approaches based on the scientist's intuition that aim to create functional materials with specific properties. With the aid of molecular dynamics and by the acquired knowledge from previously resolved protein structures that can bind cesium, mutations were inserted in the amyloid scaffolds that have the GAIIG sequences as the beta sheet core. The self-assembly of our best peptides obtain the most energetically stable and favorable morphology that can simulate the binding pockets of natural cesium capture proteins. Additionally, negatively charged residues were strategically inserted into the non-beta regions to enhance the binding with the positively charged cesium ions. Finally, these scaffolds can self assemble into stable amyloid fibrils that render this biomaterial ideal for potential applications in the biomedical(tissue engineering, drug delivery), environmental (water treatment) and technological area.

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