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Pseudo-peptide amyloid-β blocking inhibitors: molecular dynamics and single molecule force spectroscopy study☆

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ABSTRACT

By combining MD simulations and AFS experimental technique, we demonstrated a powerful approach for rational design and single molecule testing of novel inhibitor molecules which can block amyloid-amyloid binding – the first step of toxic amyloid oligomer formation. We designed and tested novel pseudo-peptide amyloid-β (Aβ) inhibitors that bind to the Aβ peptide and effectively prevent amyloid-amyloid binding. First, molecular dynamics (MD) simulations have provided information on the structures and binding characteristics of the designed pseudo-peptides targeting amyloid fragment Aβ (13 − 23). The binding affinities between the inhibitor and Aβ as well as the inhibitor to itself have been estimated using Umbrella Sampling calculations. Atomic Force Spectroscopy (AFS) was used to experimentally test several proposed inhibitors in their ability to block amyloid-amyloid binding – the first step of toxic amyloid oligomer formation. The experimental AFS data are in a good agreement with theoretical MD calculations and demonstrate that three proposed pseudo-peptides bind to amyloid fragment with different affinities and all effectively prevent Aβ-Aβ binding in similar way. We propose that the designed pseudo-peptides can be used as potential drug candidates to prevent Aβ toxicity in Alzheimer's disease.

1. Introduction

Alzheimer's disease (AD) is a neurodegenerative disorder that leads to progressive cognitive impairment, memory loss, and eventually death. Recent prevalence estimates indicate that 36 million individuals are living with AD worldwide costing an estimated $608 billion annually, worldwide, and growing [1,2]. The societal impacts and the effects on quality of life for both patients and caregivers are devastating. With no therapeutics that modify disease progression [3,4], new drug candidates that are rationally designed to interfere with disease mechanisms are urgently required.

AD is a complex disease, with many suggested mechanisms and contributing factors, central to these mechanisms is the definitive amyloid-β (Aβ) pathology [5–9]. According to the amyloid cascade hypothesis Aβ is a key contributor to neural degeneration [8]. Increasing data suggests that small soluble oligomers are a major source of neurotoxicity [10–13]. One prospective strategy to prevent neurodegeneration is to block the formation of toxic Aβ oligomers. The common classes of drugs being explored that target Aβ are small non-specific molecules (e.g., tramiprosate and flurizan) [14,15], or very large specific monoclonal antibodies (MAbs) including Bapineuzumab and Solanezumab [16,17]; to date these interventions have failed in or before Phase III clinical trials [16–20]. Recently, Solanezumab has been shown to delay disease progression in a subset of mild and prodromal AD patients but failed to meet primary endpoint targets in expanded phase 3 clinical trials and was discontinued [18,19]. Another MAb, Aducanumab has been shown to lower Aβ aggregates with concomitant diminishing of the cognition decline in pre-dementia and mild AD patients and is currently in phase 3 clinical trials [20]. These studies suggest that targeting Aβ, especially in early and pre-AD patients, may be a viable preventative strategy.

Small molecules lack target specificity due to their small size and lack of recognition elements while larger MAbs have other significant drug design challenges but seem to have shown the most promise in

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recent years [21]. MAbs are large molecules, that are designed to bind Aβ and recruit microglial cells for uptake and phagocytosis, and therefore have the capacity to prevent aggregation and facilitate clearance of Aβ [22]; however, caution must be applied to their design as they have been shown to activate a dangerous immune response while the large size of MAbs make their delivery into the brain a challenge [23–25]. The limitations that impede the use of small molecules and large MAbs may not apply with rationally designed highly specific peptide Aβ aggregation inhibitors. Peptide therapeutics in general are quickly gaining a foothold in the market due to their high specificity and the ease at which they can be modified, while the limitations associated with peptide therapies are now possible to overcome [26]. As such therapeutic peptides that target Aβ and prevent aggregation represent an avenue for exploration.

Aβ oligomers have an internal anti-parallel β-sheet structure [12]. Blocking β-sheet formation with compounds specifically targeted to Aβ is a rational strategy for the prevention of neurotoxicity [27]. It is believed that certain parts of Aβ have higher self-assembling probability making these regions key targets for preventing aggregation. One of the distinguished sections is Aβ16–20 (KLVFF) [28,29], which is often referred to as the main recognition site because of its high affinity to its self. The KLVFF recognition region has been shown to be the smallest section of Aβ that will aggregate [29]. Hence, many research groups have targeted this section for finding an appropriate inhibitor for Aβ aggregation [30,31,32,33,34,35,36,37]. Oligomerization is reported to take place in an anti-parallel β-sheet fashion, for the full length Aβ [38], and Aβ11–25 [39]. His13 and His14 have been reported to have implications in metal binding and the concomitant metal cytotoxicity theory of Aβ [40,41,42]. The amino acids Glu22 and Asp23 have been reported to be important in the aggregation mechanism of the Aβ peptide [43,44,45,46,47]. One hypothesis is that lys28 can form a salt-bridge with either Glu22 or Asp23 and induce a turn in the structure of the peptide [46]. As KLVFF is in the vicinity of these two regions, it is convenient to target all these three sections together. For brevity, in this work the section His13 to Asp23 of Aβ ($His^{13}HisGln^{15}LysLeuValPhePhe^{20}AlaGluAsp^{23}$) is denoted R, the main recognition site.

In Rauk's group the focus has been this particular section, with four classes of pseudo-peptides designed to bind to Aβ with high affinity: SGA, SGB, SGC, SGD [48,49]. The classification of the pseudo-peptides are as follows: SGA and SGB groups bind to R as an antiparallel β-sheet, and the SGC and SGD bind in a parallel mode; SGA and SGC are all L-amino acids, and SGB and SGD are all D-amino acids. All of these pseudo-peptides contain the unnatural amino acids; diaminobutyric acid (daba), Ornithine (Orn), *N*-methylleucine (Me)Leu, *N*-methylphenylalanine (Me)Phe, and beta-alanine (Bala). The *N*-methylated groups aid in preventing aggregation from the other edge of the pseudo-peptides, as has been reported by others [30,34,37,50,51]. When complexes with Aβ form, the *N*-methylated groups on one edge of the SG pseudo-peptides prevent intermolecular hydrogen bonding with the amine backbone, and as a result, further aggregation from that edge should, in theory, be halted. The small size and use of synthetic amino acids (*N*-methylated residues, ornithine etc.) in SG inhibitors aid in evasion of the immune system and prevent proteolytic degradation compared to standard amino acid residues [52]. The present study examines the inhibitory effect of two of these classes, SGA and SGC.

Detailed MD simulations and the energy analysis of R with itself and one monomer of the peptides in the SGA class, with R was reported earlier by our research group [53]. In this work, we compare the theoretical interaction energy of three more inhibitors (Myr-SGA1, SGA3 and SGC1) with R and the respective homodimers. A myristyl group (Myr) was added to the pseudo-peptide with the expectation that it would increase transport across the blood brain barrier [54].

SGA1 = *N*-Acetyl-Daba1-Orn2-(Me)Leu3-Phe4-(Me)Phe5-Leu6-Pro7-Bala8

MyrSGA1 = *N*-Myristyl-Daba1-Orn2-(Me)Leu3-Phe4-(Me)Phe5-Leu6-Pro7-Bala8

SGA3 = *N*-Acetyl-Daba1-Orn2-(Me)Leu3-Phe4-(Me)Phe5-Phe6-Ala7-Glu8-NH_2

SGC1 = *N*-Acetyl-Glu1-Leu2-(Me)Phe3-Phe4-(Me)Phe5-Leu6-Orn7-Daba8- NH_2

In Leonenko's research group, our previous work has shown that single molecule force spectroscopy (SMFS), an operational mode of atomic force spectroscopy (AFM), can be used to test the binding of single amyloid monomers [55]. This technique has demonstrated to be useful for testing amyloid aggregation inhibitors [56], as well as the effects of metal ions, on the binding forces between individual amyloid-amyloid peptides [57]. Previously, we showed that a rationally designed inhibitor (SGA1), predicted to be a potential pseudo-peptide candidate from MD simulations in Rauk's lab, demonstrated the ability to inhibit dimerization of Aβ using this SMFS platform [56]. Here we build on previous work testing several more Aβ peptide inhibitors (SGA3, SGC1 and MyrSGA1) showing that these peptide inhibitors are capable of preventing the dimerization of two individual Aβ monomers supporting our hypothesis.

These two separate but complementary analyses of SG pseudo-peptide inhibitors are reported here, first the molecular dynamics simulations, and second the single molecule testing of SG inhibitors to prevent individual Aβ dimerization. Although complementary, they serve different purposes: MD simulations allow for the theoretical determination of inhibitor binding affinity for Aβ whereas SMFS serves as the next step, to experimentally determine how inhibitors affect amyloid dimerization. Overall, we show that these pseudo-peptide inhibitors are potential candidates for targeting the amyloid aggregation pathway as a preventative AD treatment, which could be explored in further pre-clinical studies.

2. Materials and methods

2.1. Molecular dynamics simulations and steered MD

The ligand monomers were docked into the R monomers by Hex software 6.3 [58,59,60,61]. In order to study the self-assembly of the monomers, the MyrSGA-MyrSGA1 and SGC1-SGC1 homodimers were generated by Hex, as well. The computational data on R monomer, SGA1, and SGA3, and their self-dimers have previously been published [53,62]. Relevant details are repeated below for completeness.

All the calculations were performed by GROMACS 4.0.7 or 4.6.5 software [63]. The GROMOS96 53a5 force field was chosen for the simulations [64]. The energy analysis is done by the GROMACS software. The VMD software was used for visualization [65].

2.1.1. The structural search of monomers and dimers

Each monomer or dimer was put in a cubic box with the dimension of 6x6x6 nm^3. The system was solvated by the simple point charge water model (SPC) [66]. The systems were neutralized by either Na^+ or Cl^- ions. Initially a 10,000 step steepest descent energy minimization was performed. Moreover, to remove any high energy interactions within the water model, a 100 ps position-restrained MD simulation on the peptides was performed. Next, the MD equilibration was performed by having the position restraining removed. The Isothermal-isobaric (NPT) ensemble was chosen for the MD equilibration with the time step of 2 fs. The monomers were equilibrated for a range of 50 to 200 ns, depending on their flexibility. The dimers and complexes were equilibrated for 200 ns. For long range electrostatic interactions, the Particle mesh Ewald summation was chosen, with the Fourier spacing of 0.12 nm. The LINCS algorithm was used to constrain all the bonds. The Nose-Hoover temperature coupling [67,68] was used to set the temperature at 310 K. Additionally, the pressure was kept at 1 bar with the Parrinello-Rahman pressure coupling [69,70]. The cluster analysis, as well as the RMSD calculations, was used as a guide to find the local minimum energy structure. By the former, the most representative