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Title: Structural Characterization of Amyloid β17-42 Dimer by Potential of Mean Force Analysis: Insights from Molecular Dynamics Simulations

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Keywords: Potential of mean force, dimerization, amyloid, protein aggregation, Alzheimer's disease, hydrophobic region.

Abstract: Background: Recent experiments with Amyloid β1-42 peptide have indicated that the initial dimerization of Aβ1-42 monomers to form amyloid dimers stand out as a key event in the generation of toxic oligomers. However, the structural characterization of Aβ1-42 dimer at the atomistic level and the dimerization mechanism by which Aβ1-42 peptides co-aggregate still remains not clear.

Objective: In the present study, the process of Aβ17-42 peptide dimerization which is known to play an important role in the plaque formation in Alzheimer's disease was evaluated in terms of potential of mean force.

Methods: The Aβ17-42 dimer was constructed using PatchDock server. We have used molecular dynamics (MD) simulation with the umbrella sampling methodology to compute the Potential of Mean Force for the dimerization of Aβ17-42. The global minima structure at the minimum distance of separation was isolated from the calculated free energy profile and the interactions involved in the formation of the dimer structure were examined. Protein-protein interfaces and the residueresidue interactions vital for generation of the dimer complexes were also evaluated.

Results: The simulation results elucidated the interaction between the monomeric units to be governed primarily by the hydrophobic and hydrogen bonds. The resultant Aβ17-42 dimer was found to have an increased β-strands propensity at the hydrophobic regions encompassing the CHC region. Furthermore, specific hydrophobic residues were found to play a vital role in the formation of the dimer complex.

Conclusion: From the results we may therefore conclude hydrophobic region encompassing the CHC region to be crucial in dimerization process. The findings from this study provide detailed information for the complex process of early events of Aβ aggregation.

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In *silico* investigation on the inhibition of $A\beta_{42}$ aggregation by $A\beta_{40}$ peptide by potential of mean force study

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Recent experimental data revealed that small, soluble Amyloid beta ($A\beta_{42}$) oligomers, especially dimers impair synaptic plasticity and memory leading to Alzheimer's disease. Here, we have studied dimerization of $A\beta_{42}/A\beta_{42}$ homo-dimer and $A\beta_{40}/A\beta_{42}$ hetero-dimer in terms of free energy profile by all-atom simulations using the ff99SB force field. We have found that in the presence of $A\beta_{40}$ peptide, there exists a strong tendency to form a hetero-dimer with $A\beta_{42}$ peptide, suggesting that a possible co-oligomerization. Furthermore, we have investigated the effects of $A\beta_{40}$ on the $A\beta_{42}$ peptide. Our study also shows that in presence of $A\beta_{40}$, the beta-content of $A\beta_{42}$ monomer is reduced. Additionally, certain residues important for bending in $A\beta_{42}$ peptide attained an increased flexibility in the presence of $A\beta_{40}$. The salt-bridge destabilization also manifested the impact of $A\beta_{40}$ on $A\beta_{42}$ peptide as a whole. Based on this, one may expect that $A\beta_{40}$ inhibits the aggregation propensity of $A\beta_{42}$. Moreover, the binding free energy obtained by the molecular mechanics–Poisson–Boltzmann surface area method also revealed a strong affinity between the two isoforms thereby suggests that $A\beta_{40}$ binding induces conformational change in $A\beta_{42}$. Our results suggest that co-oligomerization of Aβ isoforms may play a substantial role in Alzheimer's disease.

Keywords: potential of mean force; aggregation inhibition; amyloid peptide; Alzheimer's disease

1. Introduction

Alzheimer's disease (AD) is an irreversible neuro-degenerative disease and its incurable conditions are associated with the misfolding and aggregation of native monomeric proteins (Dobson, 2003). The deposition of aggregated amyloid-β peptide (Aβ) in the grey matter of brain is a pathological hallmark of AD (Hardy & Selkoe, 2002). A transmembrane receptor, the amyloid precursor protein (APP), undergoes proteolytic cleavage in various locations to generate Aβ peptides of varying lengths, most commonly 40 and 42 residues ($A\beta_{1-40}$ and $A\beta_{1-42}$; Haass, 2004). The $A\beta_{1-42}$ isoform has an additional 2 amino acid residues Ile and Ala at its C terminal end making it more hydrophobic and more aggregation prone than $A\beta_{1-40}$ (Jarrett, Berger, & Lansbury, 1993; Meisl et al., 2014). Hence, despite the fact that the relative ratio of the $A\beta_{1-40}$ to $A\beta_{1-42}$ in cerebrospinal fluid (CSF) is approximately 9:1, amyloid plaques are enriched with more amount of $A\beta_{1-42}$ relative to $A\beta_{1-40}$ (Gravina et al., 1995; Iwatsubo et al., 1994). Studies have also shown that overproduction of $A\beta_{42}$ relative to $A\beta_{40}$ have been related to some early onset versions of AD (Scheuner et al., 1996). Moreover, the existing evidence indicates an increase in the ratio of $A\beta_{42}$ to $A\beta_{40}$ generation from the cleaved APP that correlates to the increase in toxicity both *in vitro* and *in vivo* (Citron et al., 1997; Dahlgren et al., 2002; Duff et al., 1996; Hellström-Lindahl, Viitanen, & Marutle, 2009; Kuperstein et al., 2010; Pauwels et al., 2012).

Chemical cross-linking studies have shown that although solid fibrillar deposits of Aβ accumulate in AD affected brains; the major cytotoxic effects causing the earliest onset of pathological events are consistent with smaller aggregates of Aβ oligomers (Bitan et al., 2003; Haass & Selkoe, 2007). Monomers such as $A\beta_{40}$ and $A\beta_{42}$ easily form dimers, trimers and tetramers in solution. Furthermore, $A\beta_{42}$ is also capable of forming pentamers and hexamers that self-associate to form protofibrils, and later mature fibrils. Experimental studies have reported these higher form oligomers to be the proximate neurotoxins in AD (Klein, Stine, & Teplow, 2004; Lesné et al., 2006). Due to the transient characteristic of the Aβ oligomers and low abundance of the oligomers, study on these oligomers is still a difficult task. Although, both the isoforms differ from each other with respect to their structures and toxicity, numerous studies on the mixtures of Aβ isoforms directed co-interaction of $A\beta_{40}$ and $A\beta_{42}$ during the aggregation process are being explored (Frost, Gorman, Yip, & Chakrabartty, 2003; Hasegawa, Yamaguchi, Omata, Gejyo, & Naiki, 1999; Kim et al.,

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LETTER TO THE EDITOR

Inhibition of $A\beta_{1–42}$ peptide aggregation using short ss-oligonucleotide as polyions: an *in silico* approach

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1. Introduction

Neurodegenerative disorders form a vast majority of neuropathophysiological afflictions that affect the global population annually. Of all the neurodegenerative disorders AD comprises 75% of all dementia cases affecting the elderly population globally. Like many other diseases such as Parkinson's disease and type II diabetes, the initial development of the AD has primarily been associated to the progressive accumulation and aggregation of the amyloid forming proteins characterized as amyloidogenesis (Jucker, 2012; Lyubchenko, 2015; Park, Yoon, Jang, Lee, & Shin, 2015). The mechanism of amyloidogenesis is not clear and therefore there remains poor treatment for AD. The manifestation of AD is generally attributed to the Aβ peptide which undergoes intrinsic disorientation followed by self-aggregation rapidly. The Aβ peptides of AD are by-products of the amyloid precursor protein metabolism containing the sequence 1–40 or 1–42 of Aβ. Numerous studies have confirmed that $A\beta_{1–42}$, in comparison to $A\beta_{1–40}$, aggregates in the cell exterior much more effortlessly, and its toxicity is stronger. Thus the $A\beta_{1–42}$ with the amino acid sequence DAEFR HDSGY EVHHQ KLVFF AEDVG SNKGA IIGLM VGGVV IA has been the one most strongly linked to AD (Mitternacht, Staneva, Härd, & Irbäck, 2010). Existing reports on the conformational studies on $A\beta_{1–42}$ peptide describe two distinct helical regions in the peptide encompassing residues 8–25 and 28–38 which are connected by a regular type I β-turn.

Experimental results have defined the structural details of pathogenic $A\beta_{1–42}$ fibril and confirmed the presence of β sheets and β strands. Recent studies have shown soluble form of oligomers, originating from the disordered monomers to be the primary pathogenic components responsible for synaptic change in AD rather than the mature amyloid fibrils. It is evident from various studies that these oligomers of Aβ1−40/1−42 are rich in antiparallel β-sheets. Knowing that most of the amyloid-associated ailments arise at the late phase, it has become a major health concern along with the increase in life span. Hence, a common therapeutic approach to block the early step of Aβ aggregation is of utmost importance.

Many studies using beta sheet breakers have been carried out to study their effects on the aggregation of the Aβ peptide. Small organofluorine molecules and light have been used to disassemble the preformed fibrils and the use of short amyloid fragments and other inhibitors for the inhibition of fibril formation has also been reported (Berhanu & Masunov, 2015; Ghosh et al., 2015; Kumar et al., 2015). Recent studies have also successfully described interactions between amyloid fibrils and polyions such as DNA and ATP (Abraham et al., 2014; Barrantes et al., 2012; Macedo et al., 2012; Udomprasert et al., 2014). The binding affinity between these inhibitors and the short peptide segments as well as the full-length $A\beta_{1–42}$ peptide is known to be crucial in determining the extent of interaction.

In the present study we have focused on inhibiting the beta sheet formation in the monomer of $A\beta_{1–42}$ peptide using ss-oligonucleotide. Furthermore, we also investigated the disassembly of the $A\beta_{17–42}$ dimer by distortion of the β-stranded structure with the help of 18-mer-ss-oligonucleotides. The ss-oligonucleotides used in the study forms complex with the peptide in the long time-scale MD simulations in explicit solvent and the associations have been described comprehensively. The choice of the 12 mer-ss-oligonucleotide as well as the 18 mer-ss-oligonucleotide were critically based on their inherent constitution of highly repetitive homogeneous segments (consisting of only dA:dT or dC:dG) as the presence of these segments were likely to manifest a strong electrostatic attraction between the oppositely charged DNA and the target protein sequence. Thus, in

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RESEARCH ARTICLE

Cross-Seeding Interaction Between Amyloid β and Tau Protein can Enhance Aggregation

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Abstract:

Background: The two pathological hallmarks associated with Alzheimer's disease (AD) include the accumulation of senile plaques and the generation of neurofibrillary tangles. Although it is a known fact that both amyloid beta (Aβ) and Tau exist together in mitochondria, to date, there is no reasonable explanation for the Aβ and Tau interaction in particular.

Objective: The cross-seeding interactions between Aβ and Tau were studied using the potential of mean force (PMF) analysis.

Methods: The Aβ- Aβ homo-dimer; Tau-Tau homo-dimer and Aβ-Tau hetero-dimer were constructed using molecular docking tools. We have used molecular dynamics (MD) simulation with the umbrella sampling methodology to examine the cross-sequence interactions between homo-dimers and the hetero-dimer by computing PMF using umbrella sampling methodology.

Results: We observed the global minimum and energy barrier to be quite higher for both the homo-dimers relative to the hetero-dimer, thus indicating that Aβ (25-35) has a high affinity to form dimer complex with Tau (273-284) monomer. We also observed a relatively higher range of interacting residues and interface area between the monomeric units in hetero-dimer (Aβ-Tau) whiles the homo-dimer (Aβ- Aβ) and (Tau-Tau) showed a less number of the same.

Conclusion: From the results we may therefore conclude that Aβ fragments can form complexes with the Tau monomers which consequently advance to form aggregates. This aggregation may be favored by interactions between the hydrophobic residues and charged residues present in both the fragments.

Venkata Satish Kumar Mattaparthi

Keywords: Amyloid beta, Tau, Potential of mean force, Aggregation, Alzheimer's disease.

1. INTRODUCTION

Alzheimer's disease (AD) is a devastating neurodegenerative disorder with a persistent progression [1]. The two pathological hallmark of AD are the aggregation of senile plaques [2] and progressive accumulation of neurofibrillary tangles (NFT) [3] by Tau [4]. Tau is expressed in adult human brain in six different isoforms consisting of two functional domains whose major role is to bind and stabilize the microtubules facilitating axonal transport. Hyper phosphorylation or deficiency in de-phosphorylation of Tau promotes the aggregation of Tau into NFTs [5-7]. Although mutations of Tau isoforms have been reported to induce neurodegenerative diseases, so far no distinct studies have showed the simultaneous appearance of senile plaques and NFTs in AD. It seems that both the aggregation process occurs independently of each other, as NFTs develop intracellularly whereas senile plaques develop extracellularly. Nevertheless, the senile plaques are the dominant because mutations in amyloid precursor protein (APP) which leads to production of senile plaques cause autosomal dominant AD. On the other hand, mutations in Tau promote autosomal Frontotemporal dementia but not AD. While a "loss of function" hypothesis [8] is often invoked to explain the role of Tau aggregation in AD, but does not address the role of Aβ in AD, or explain exactly how could Aβ interact with Tau?

According to the amyloid cascade hypothesis, Aβ peptide aggregation leads to AD which is β- rich oligomers with beta-strand structure that eventually forms fibrillar aggregates [9-15]. While this Aβ peptide is produced extracellularly, intracellular Aβ oligomers also exist. These oligomers are reported to interact with a variety of proteins including Tau. Recent studies have suggested acceleration of Tau NFT formation by the Aβ [16-18]. Interaction of Tau with Aβ oligomers could destabilize the microtubule integrity [19] and

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Investigations on the Structural Characteristics that Seed the Aggregation of Amyloid-β_{1-42} Peptide: Insights from Molecular Dynamics Simulations

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Abstract: Amyloid β-peptide ($A\beta_{1-42}$) is known to be the primary component of the amyloid plaques found in the brain of patients suffering from Alzheimer's disease (AD). Extensive research has been done in the past with respect to $A\beta_{1-42}$ peptide and its involvement in AD. However, the initial seed structure responsible for the $A\beta_{1-42}$ peptide aggregation and the mechanism remains unclear. This may be chiefly attributed to the poor understanding of the processes involved in the $A\beta_{1-42}$ peptide mis-folding and aggregation. Further, the flexibility and the aggregation propensity involved in the $A\beta_{1-42}$ monomer, challenges the experimental and theoretical techniques to characterize the seed structure that drives the mis-folding and the subsequent aberrant aggregation. In this study, we employed all atoms fully unrestrained folding molecular dynamics simulation to identify the probable seed structure of $A\beta_{1-42}$ peptide responsible for the aggregation. The initial linear structure of the $A\beta_{1-42}$ peptide for the simulation was built from its amino acid sequence using leap module of AMBER. From our molecular dynamics study, we followed the secondary structure development in $A\beta_{1-42}$ peptide starting from its initial linear structure to its folded 3D structure. We observed the $A\beta_{1-42}$ peptide to sample diverse conformations which are rich in β-sheets and are stabilized by hydrogen bonding and other non-bonding interactions. The findings of this study shall be helpful in understanding the initial stage of $A\beta_{1-42}$ peptide aggregation process and can be applicable for the development of therapeutics to cure AD at an early stage.

Keywords: Alzheimer's, Protein aggregation, AMBER, misfolding.

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1. INTRODUCTION

Alzheimer's disease (AD) is one of the most common forms of progressive irreversible dementia, troubling large numbers of elderly Worldwide [1]. Like other neurodegenerative diseases such as Parkinson's and Huntington's, AD is characterized pathologically by the accumulation of amyloids plaques [2]. Amyloids in general and $A\beta_{1-42}$ peptide in particular, are known for their ability to form a number of polymorphs [3]. The major constituent of amyloid plaque, the amyloid β peptide is derived from successive sequential cleavages of amyloid precursor protein (APP) by the β and γ secretases [4, 5]. Among the two isoforms of amyloid β peptide ($A\beta_{1-40}$ and $A\beta_{1-42}$), $A\beta_{1-42}$ is found to be predominant in the aggregation process. The sequences of the $A\beta_{1-40}$ and $A\beta_{1-42}$ peptide highlighting the hydrophobic residues are shown in Figure **1.A** and **1.B** respectively. Structural studies have shown that $A\beta_{1-40}$ peptide and $A\beta_{1-42}$ peptide oligomerize through distinct pathways and $A\beta_{1-42}$ peptide forms spherical paranuclei which further assembles to oligomers [6, 7]. It has been well studied and documented that in contrast to the $A\beta_{1-40}$ peptide the $A\beta_{1-42}$ exhibits a strong neurological toxicity [8, 9]. But till now the physiological and pathological characteristics of $A\beta_{1-42}$ peptide in AD is not well understood.

Some studies have reported soluble form of oligomers such as dimer [10], trimer [11] and 12-mer [12] of $A\beta_{1-42}$ peptide to be the primary pathogenic components responsible for synaptic changes in AD rather than the mature amyloid fibrils [13-19]. The mature amyloid fibrils can thermodynamically be considered as the most stable aggregation state by self-association which forms a cross structure that contains β sheets. Several studies have also shown that soluble $A\beta_{1-42}$ peptide contains substantial β-sheets [20-22]. Thus it is necessary to understand the molecular details of each species in the early stage of $A\beta_{1-42}$ peptide aggregation as this will be helpful for the rational design of therapeutics to prevent AD. Despite a high degree of sophistication, the experimental techniques have not yet been able to provide an atomistic level of insight into the initial conformational transitions of $A\beta_{1-42}$ peptide and the subsequent folding that leads to misfolded aggregation prone structures [23]. The approach of various computational studies [24-32] has thus been complimentary to study the initial conformational changes of $A\beta_{1-42}$ peptide and to identify its transient states.

The general approaches employed to investigate and elucidate the native conformations of target peptides include

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