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Model of Alzheimer's disease amyloid-β peptide based on a RNA binding protein

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Abstract

Although Alzheimer's Aβ peptide has been shown to form β-sheet structure, a high-resolution molecular structure is still unavailable to date. A search for a sequence neighbor using $A\beta_{10-42}$ as the query in the Protein Data-Bank (PDB) revealed that an RNA binding protein, AF-Sm1 from *Archaeoglobus fulgidus* (PDB entry: 1i4k chain Z), shared 36% identical residues. Using AF-Sm1 as a template, we built a molecular model of $A\beta_{10-42}$ by applying comparative modeling methods. The model of $A\beta_{10-42}$ contains an antiparallel β-sheet formed by residues 16–23 and 32–41. Hydrophobic surface constituted by residues 17–20 (LVFF) separates distinctly charged regions. Residues that interact with RNA in the AF-Sm1 crystal structure were found to be conserved in Aβ. Using a native gel we demonstrate for the first time that RNA can interact with Aβ and selectively retard the formation of fibrils or higher-order oligomers. We hypothesize that in a similar fashion to AF-Sm1, RNA interacts with Aβ in the β-hairpin (β-turn-β) structure and prevents fibril formation.

Keywords: Comparative modeling; Oligomers; Alzheimer's disease; Amyloid; RNA binding protein

The processing of the amyloid precursor protein (APP) is a key event in the pathogenesis of Alzheimer's disease (AD). To date, the amyloid cascade hypothesis is the most accepted as an etiologic theory of AD [1,2]. It stipulates that senile plaques, which consist mostly of aggregated fibrillar amyloid β peptides ($A\beta_{1-40}$, $A\beta_{1-42}$ or $A\beta_{1-43}$), are at the origin of AD. This hypothesis has been supported by various in vitro and in vivo studies using cellular and animal models of AD [3–7]. However, the amount of Aβ deposited in senile plaques correlates poorly with the severity of dementia whereas soluble forms of the peptides appear to correlate well [8]. Recently, soluble oligomeric species of Aβ have been shown to be profoundly toxic compared to other Aβ species [9,10]. The exact structural characteristics of the different forms of Aβ are still in debate [11–14]. Experimental structures of monomeric Aβ peptides determined in solution using NMR techniques suggest a helical content [15]. However, electron microscopy, CD spectral studies, and solid-state NMR studies have shown that amyloid β peptides contain β-sheet structure in the oligomeric or aggregated fibrillar forms [16,17]. Furthermore, such studies along with molecular modeling have suggested that Aβ forms a characteristic "cross-β" structure in which several antiparallel or parallel β-sheets align perpendicularly to the fibril axis to form a twisted β helical arrangement [12,16,18]. Numerous simulations have revealed that like prion protein, Aβ can undergo conformational transition from helical to β structure while forming aggregates of fibrillar Aβ depending upon the environment [19–21]. Currently, a high-resolution molecular structure of Aβ in the β-sheet form is not available. Hence, a structural model of Aβ in

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β-sheet form that occurs in oligomers or fibrils will aid in the development of drugs and may provide clues regarding Aβ toxicity.

It has been shown that two sequences that share identical residues or have similar physicochemical property profiles at corresponding positions adopt similar structures [22]. Thus, it is possible to model the 3-D structure of unknown proteins by analogy to known structures of homologous proteins [23,24]. Comparative modeling techniques have been successfully applied to model several proteins for which structure was not known. For instance, such techniques were used to model a multimer of the anti-apoptotic protein Bcl-2, decay accelerating factor (DAF or CD55), DNA repair protein Neil-2 [25–28]. Using sequence searches in the Protein Data-Bank (PDB, http://www.rcsb.org) and physicochemical profile matching, we found that $A\beta_{1-42}$ shares high similarity with the β-sheet region of an RNA binding protein AF-Sm1. We applied homology modeling techniques and docking methods to obtain a molecular model of Aβ in β-sheet form. The model of Aβ consists of a core β-sheet region formed by two β-strands connected through a turn. Regions of distinct charge separated by a rich hydrophobic region occur on the surface of the Aβ model. We also show that, from a rigid-body docking study, two Aβ molecules can be arranged in an antiparallel fashion with favorable conformational energy. We found that the RNA binding domain of the AF-Sm1 ribonucleoprotein (RNP domain) was conserved in the Aβ structure and using our model we found that these sequences can adopt similar structures. Our observations led us to further ask whether there was any evidence that RNA could interact with Aβ. Here we demonstrate for the first time that incubation of Aβ with RNA alters the rate of formation of higher molecular weight oligomers and fibrils thereby suggesting interaction.

Materials and methods

Molecular model of Aβ. The $A\beta_{1-42}$ sequence was obtained from SWISSPROT (entry: P05067 residues 672–713, release 44.2) database. A FASTA [29] search for a suitable template within the PDB was performed. We excluded hits that corresponded to Aβ sequences which are mostly present in helical monomeric form. The top scoring sequences for similarity with $A\beta_{1-42}$ were Dr hemagglutinin (PDB code: 1ut1 chain F) [30] and an RNA binding protein Sm-Like Protein (AF-Sm1) from *Archaeoglobus fulgidus* (PDB code: 1i4k chain Z) [31,32]. The Dr hemagglutinin showed an homology to the C-terminal region of Aβ (residues 18–38) sharing 32% identical residues, whereas AF-Sm1 showed a global similarity with an alignment score of 25 bits (z-value of 118) and 36% identity with $A\beta_{1-42}$ sequence. The sequence alignment of AF-Sm1 and $A\beta_{1-42}$ is shown in Fig. 1. In our final model, residues 1–9 of Aβ were excluded as they did not form a rigid structure and were predicted to be structurally disordered [33–35]. Upon detailed structural analysis of Dr hemagglutinin, we found that residues 83–87 form an antiparallel β-sheet with residues 112–116 in the C-terminal region, which corresponds to residues 2–6 in Aβ that is shown to be unstructured. So we based our model on AF-Sm1 rather than Dr hemagglutinin. Hydrophobicity [36] and β-strand propensity [37] physicochemical profiles for Aβ sequence and AF-Sm1 were derived using the ProtScale tool available at Expasy Proteomics Server (http://us.expasy.org/ProtScale). The average physicochemical property value was calculated by averaging a sliding window of five residues along the sequence using a linear weight variation model. The profiles for two physicochemical properties are shown in Fig. 2. Geometric constraints were extracted using 1i4k chain Z as the template for structurally equivalent regions identified from the alignment by the EXDIS program (part of modeling suite MPACK) [25,27,28,38]. The dihedral constraints at the start and the end of the fragments along the inserted residues were relaxed by setting ϕ and ψ angles at 180°. Upper and lower distance constraints were allowed a threshold value of 0.25 Å to the actual value. Geometric constraints were progressively applied to the Aβ sequence using DIAMOD in MPACK and the final structure was energy minimized using FANTOM [39]. The final energy of the minimized structure had a Lennard potential of −132 kcal/mol. There was no conformational violation observed in the final model (Fig. 3).

Rigid body docking of two molecules of Aβ. Two molecules of Aβ were docked using AutoDock 3.0 [40]. One of the molecules was treated as stationery and the other was treated as a rigid ligand. We defined 80 grid points in X, Y, and Z directions with a grid spacing of 0.831Å. The grid box encompassed the static molecule and rigid ligand sufficiently to allow the ligand to survey the surface of the static molecule without falling out of the grid box. Kollmann charges and polar hydrogens were inserted using the AutoDockTool (http://www.scripps.edu/mb/olson/index.html). The torsion angles between bonds were set rigid for the ligand, allowing no rotatable bonds during docking. A random location was set for the initial position of the ligand. A Lamarckian genetic algorithm procedure was used to identify the optimal docking conformation. The number of individual conformations in the population was set to 50 with a maximum number of generations to 27,000. The models were rank ordered according to their docking energy. The member with the lowest docked energy (−3.04 kcal/mol) was noted.

Aβ aggregation studies in the presence of RNA. In order to determine whether RNA can binds to Aβ, we tested the ability of RNA to interfere with aggregation of the $A\beta_{1-42}$ peptide. Various ligands that bind to Aβ are all known to interfere with its aggregation (Congo Red, Thioflavin T, β-sheet breaker peptides, VEGF, etc.) [8,13,41]. As this peptide is known to adopt various conformations during storage, we identified a uniform monomeric starting configuration by pre-treating the peptide with 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP). $A\beta_{1-42}$

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Aß      DAEFRHD-SGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVVIA
1I4K:Z GREFRGTLDGYDIHMNLVLLDAEEI--QNGEVVR-KVGSVVIR
         ***     **::* : ::: **::   : * ::    **:***
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Fig. 1. Sequence alignment of human $A\beta_{10-42}$ with AF-Sm1 protein (PDB code: 1i4k). The alignment showed 36% identity and Smith–Waterman score of 61. The template used for modeling was identified (Z-score of 118) using a FASTA search in the PDB. Identical residues are indicated by '*' and similar residues by ':'.