How the amyloid-β peptide and membranes affect each other: An extensive simulation study

Chetan Poojari [a], Andreas Kukol [b], Birgit Strodel [a,c,*]

[a] *Research Centre Jülich, Institute of Complex Systems: Structural Biochemistry, 52425 Jülich, Germany*
[b] *University of Hertfordshire, School of Life Sciences, College Lane, Hatfield AL10 9AB, United Kingdom*
[c] *Institute of Theoretical and Computational Chemistry, Heinrich Heine University Düsseldorf, 40225 Düsseldorf, Germany*

ARTICLE INFO

ABSTRACT

The etiology of Alzheimer's disease is thought to be linked to interactions between amyloid-β (Aβ) and neural cell membranes, causing membrane disruption and increased ion conductance. The effects of Aβ on lipid behavior have been characterized experimentally, but structural and causal details are lacking. We used atomistic molecular dynamics simulations totaling over 6 μs in simulation time to investigate the behavior of $A\beta_{42}$ in zwitterionic and anionic lipid bilayers. We simulated transmembrane β-sheets (monomer and tetramer) resulting from a global optimization study and a helical structure obtained from an NMR study. In all simulations $A\beta_{42}$ remained embedded in the bilayer. It was found that the surface charge and the lipid tail type are determinants for transmembrane stability of $A\beta_{42}$ with zwitterionic surfaces and unsaturated lipids promoting stability. From the considered structures, the β-sheet tetramer is most stable as a result of interpeptide interactions. We performed an in-depth analysis of the translocation of water in the $A\beta_{42}$-bilayer systems. We observed that this process is generally fast (within a few nanoseconds) yet generally slower than in the peptide-free bilayers. It is mainly governed by the lipid type, simulation temperature and $A\beta_{42}$ conformation. The rate limiting step is the permeation through the hydrophobic core, where interactions between $A\beta_{42}$ and permeating H_2O molecules slow the translocation process. The β-sheet tetramer allows more water molecules to pass through the bilayer compared to monomeric Aβ, allowing us to conclude that the experimentally observed permeabilization of membranes must be due to membrane-bound Aβ oligomers, and not monomers.

© 2012 Elsevier B.V. All rights reserved.

1. Introduction

Alzheimer's disease (AD) is a neurodegenerative disorder associated with synaptic loss, abnormalities in functioning of neurons, neuronal cell death and extracellular accumulation of senile plaques composed of the neurotoxic amyloid-β peptide (Aβ) [1,2]. Aβ is derived from the amyloid precursor protein (APP), a type-1 membrane integral glycoprotein through sequential cleavage by β- and γ-secretases [3]. The major alloforms of Aβ are $A\beta_{40}$ and $A\beta_{42}$, which differ by the presence of two amino acids, I41 and A42 at the C-terminus of the latter. The more hydrophobic $A\beta_{42}$ is the prevalent alloform seen in amyloid plaques, and has a greater tendency to aggregate into fibrils and plaques [4,5]. There is appreciable evidence suggesting that Aβ exerts its cytotoxic effect by interacting with membranes of neurons and other cerebral cells, such as astrocytes, microglial and cerebral endothelial cells [6,7]. A potential pathway for Aβ toxicity lies in its ability to alter biophysical membrane properties [8–11]. Aβ aggregates cause membrane disruption and increased permeability, allowing excessive leakage of ions, particularly calcium ions [12]. This imbalance in calcium homeostasis promotes neuronal excitotoxicity [12,13]. $A\beta_{42}$ oligomers interact with lipid raft related ganglioside GM1, further accelerating the amyloidogenic processing of APP [14].

Various experimental studies investigating the interactions between Aβ and phospholipids have revealed that Aβ prefers to bind to negatively charged lipids compared to zwitterionic lipids [15–17]. It has been shown that the enhanced association of Aβ with anionic lipid membranes leads to the insertion of Aβ into the membrane [15–17] and induces the formation of β-sheets [15,17–19] and Aβ fibrils [19–21]. NMR spectroscopy studies on $A\beta_{40}$ in a membrane-mimicking environment concluded that the peptide is unstructured in the N-terminal region from residues 1–14 and that the C-terminal hydrophobic residues from 15 to 36 adopt an α-helical conformation with a kink at residues 25–27 [22]. This kink may be significant in membrane insertion and conformational rearrangements [22]. Coles et al. proposed three possible models corresponding to different Aβ insertion depths in the membrane based on structural findings for $A\beta_{40}$ [22]. The two experimentally determined insertion depths have K28 and V24, respectively, at the membrane–water interface [22,23]. A third proposed model is with K16 at the membrane–water interface, where the entire α-helical conformation adopted by $A\beta_{40}$ (residues 15–36) spans the plasma

* Corresponding author at: Research Centre Jülich, Institute of Complex Systems: Structural Biochemistry, 52425 Jülich, Germany. Tel.: +49 2461 613670.
E-mail address: b.strodel@fz-juelich.de (B. Strodel).

membrane [22]. A study on soluble and aggregated forms of $A\beta_{40}$ on rat cortical synaptic plasma membrane using small angle X-ray diffraction and fluorescence spectroscopy showed that the monomer penetrates into the hydrophobic core of the bilayer, whereas the aggregated form was found interacting with the phospholipid headgroups [24]. Similarly, soluble $A\beta_{42}$ was found to intercalate the membrane of giant unilamellar vesicles composed of 1-palmitoyl 2-oleoyl phosphatidylcholine (POPC) or POPC/sphingomyelin (SM)/cholesterol (Chol), altering permeability properties of the bilayer [25]. However, permeabilization of lipid bilayers can also be caused by soluble amyloid oligomers [26]. NMR, CD, fluorescence and monolayer studies on $A\beta_{42}$ inserted into a POPC/POPS (palmitoyl-oleoyl phosphatidylserine) bilayer showed reduction in membrane stability with an increase in membrane fluidity [27]. This study also indicated that $A\beta_{42}$ alone could destabilize the membrane integrity in absence of ions, and that the peptide adopts a β-sheet structure in the membrane with increase in β content when Cu^{2+} is added [27]. Further experimental work carried out on $A\beta_{40}$ inserted into a zwitterionic phosphatidylcholine bilayer revealed that the perturbation of the bilayer integrity is caused by short β-sheet assemblies embedded in the lipid bilayer [28]. Atomic force microscopy of $A\beta_{42}$ [29] and $A\beta_{40}$ [30] in reconstituted membranes revealed ion-channel-like structures, which are able to cause cellular ionic imbalance [30–34]. Lal and coworkers also demonstrated through biochemical analysis that $A\beta$ forms stable tetramers and hexamers in lipid membranes [29].

It was shown that theoretical approaches are needed as a complement to experimental studies probing the principles governing $A\beta_{42}$ aggregation and $A\beta$–membrane interactions [35,36]. Various computational studies of $A\beta$ interacting with lipids have been performed to gain structural information at an atomistic level [37–59]. An atomistic model of $A\beta$ channel structures developed by Nussinov and coworkers provided information about the $A\beta$ conformation in membranes and ion-channel activity [37,38]. In another study they found that the channels break into mobile β-sheet subunits, which enable toxic ionic flux [39]. Strodel and coworkers also proposed $A\beta$ pore models composed of tetrameric to hexameric $A\beta$ subunits, which are similar to the models suggested by Nussinov and coworkers [46]. In [47] the stability of transmembrane β-barrel structures, each composed of eight $A\beta$ fragments $A\beta_{25–35}$, was investigated. Molecular dynamics (MD) studies of $A\beta_{40}$ inserted in a dipalmitoyl phosphatidylcholine (DPPC) bilayer with the peptide positioned with either K28, V24 or K16 at the membrane–water interface showed that in either case the peptide remained partially embedded in the membrane [48]. Loss of α-helicity in favor of β-strands was observed when the peptide was inserted at K28 and V24, whereas with K16 at the interface α-helicity was retained. For the deeper insertion depths, water molecules were seen entering the hydrophobic core accumulating near the charged residues of the peptide within the bilayer. It has also been reported that $A\beta_{40}$ causes DPPC lipid headgroup disorder and reduces the membrane thickness around $A\beta$ [49]. In a recent study, Lemkul and Bevan explored the interactions between $A\beta_{40}$ and several pure and mixed model membranes, and lipid rafts, both with and without GM1 [50]. $A\beta_{40}$ remained inserted in the membranes without GM1, but in several instances exited the raft containing GM1 initiated through hydrogen bonding of $A\beta_{40}$ with GM1. Another study on $A\beta_{40}$ preinserted in a DPPC bilayer found the peptide exiting the membrane and adsorbing to its surface, with helix conformation being the major secondary structure observed in the membrane-adsorbed $A\beta$ structure [54]. In a recent MD simulation study, the self-assembly of $A\beta$ in a mixed DPPC/cholesterol bilayer was investigated, uncovering the formation of a short parallel β-sheet between two peptides [59].

In the present MD study, we report the behavior of $A\beta_{42}$ preinserted into zwitterionic POPC and DPPC bilayers, and anionic 1-palmitoyl 2-oleoyl phosphatidylglycerol (POPG) bilayers. Here, our focus is on membrane-spanning structures based on the observation that $A\beta$ can form pore-like structures in reconstituted membranes [30–34]. Due to conflicting experimental results as to whether $A\beta$ is in a helical or in a β-sheet conformation in a lipid bilayer, we considered both transmembrane conformations as starting structures for our MD simulations in order to investigate whether the secondary structure leads to different behavior of the membrane-inserted $A\beta_{42}$ peptide. We used a β-sheet structure (monomer and tetramer) obtained from a global optimization approach [46] and a helix structure from an NMR study in an apolar solvent [22]. During each of the 500 ns MD simulations, $A\beta_{42}$ remains embedded in the lipid bilayer. We discuss our results in terms of structural stability of $A\beta_{42}$ and its effects on membrane functionality.

2. Methods

2.1. Starting structures

The two initial membrane-spanning $A\beta_{42}$ structures are a β-sheet and a helical conformation. The transmembrane β-sheet was obtained from a study for the $A\beta_{42}$ monomer and small oligomers using a global optimization approach in an implicit membrane model [46]. In this structure, the more hydrophobic C-terminal region starting from residue 17 is fully inserted into the hydrophobic membrane core, forming an antiparallel β-sheet with two turn regions, the first ranging from residues 23 to 29 and the second one involving residues 37 and 38. The first turn is prominent in many $A\beta$ structures identified from experiment [60–63] and simulation [64–66]. However, each of these models predicts a distinct turn structure. Ma and Nussinov independently predicted that the $A\beta$ peptide amyloid adopts a U-turn β-strand–loop–β-strand motif [66], qualitatively agreeing with the Tycko et al. model [63]. Lührs et al. [62] presented a 3D structure of $A\beta_{17–42}$ fibrils with a U-turn bent β-sheet based on hydrogen/deuterium-exchange NMR data, which further validates the computational model of Ma and Nussinov [66] and is consistent with the experimental model of Petkova et al. [63]. All these models, including our β-hairpin structure [46] share the key structural features of the salt bridge between Asp23 and Lys28 and the intramolecular hydrophobic cluster between Leu17/Phe19 and Ile32/Leu34. We decided to use our β-hairpin model as starting structure as it also provides a structural model for the more hydrophilic residues 1–16, which form a β-hairpin outside the membrane [46]. We study this transmembrane β-sheet as monomer (denoted SHEET in the following) and tetramer as obtained in [46] (Fig. 1a and b). The α-helical starting structure was obtained from an NMR study of $A\beta_{40}$ in an apolar solvent (PDB ID: 1BA4) [22]. We extended the 40 residue peptide to $A\beta_{42}$ by adding the two hydrophobic residues I41 and A42 in a coil conformation. Our motivation behind this extension was to study the role of the extra I41 and A42 residues in peptide–lipid interactions and the resulting structural changes in the peptide and membrane. Previous studies revealed an increased stability provided by I41 and A42 to the antiparallel β-sheet when compared to $A\beta_{40}$ [67]. Furthermore, by using the same peptide we wanted to be able to compare our findings for the helical and β-sheet transmembrane structures. The helical structure was studied for two insertion depths: (i) with K16 (denoted HEL-16) and (ii) with D23 (denoted HEL-23) at the membrane–water interface (Fig. 1c and d). Note that this nomenclature refers to the initial condition only as no restraints are imposed on the peptide. This implies that during the MD simulations the peptide can experience secondary structure changes and/or transpositions within the lipid bilayers so that the final state of an MD run does not necessarily correspond to the initial notation.

All our simulations were carried out at physiological pH giving rise to charge -3 for $A\beta_{42}$ with His residues modeled uncharged, Asp and Glu negatively charged, and Lys and Arg assumed being protonated. Our choice of the protonation state for the ionizable residues was based on their pK_a values as a function of depth in the membrane [68]. At pH 7, Lys and Arg become deprotonated only when they are in close vicinity to the membrane center, while the pK_a values of Asp and Glu rise above 7 inside the membrane core. In our simulations $A\beta_{42}$ is positioned such that K16, E22, D23 and K28 are at the membrane–water interface. Therefore, we assumed positive charges for