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Molecular Interactions of Alzheimer Amyloid- β Oligomer with Neutral and Negatively Charged Lipid Bilayers

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Abstract

Interaction of p3 ($A\beta_{17-42}$) peptides with cell membrane is crucial for the understanding of amyloid toxicity associated with Alzheimer's disease (AD). Such p3-membrane interactions are considered to induce the disruption of membrane permeability and integrity, but the exact mechanisms of how p3 aggregates, particularly small p3 oligomers, induce receptor-independent membrane disruption are not yet completely understood. Here, we investigate the adsorption, orientation, and surface interaction of the p3 pentamer with lipid bilayers composed of both pure zwitterionic POPC (palmitoyl-oleyl-phosphatidylcholine) and mixed anionic POPC/POPG (palmitoyl-oleyl-phosphatidylglycerol) (3:1) lipids using explicit-solvent molecular dynamics (MD) simulations. MD simulation results show that the p3 pentamer has much stronger interactions with mixed POPC/POPG lipids than pure POPC lipids, consistent with experimental observation that $A\beta$ adsorption and fibrilization are enhanced on anionic lipid bilayers. Although electrostatic interactions are main attractive forces to drive the p3 to adsorb on the bilayer surface, the adsorption of the p3 pentamer on the lipid bilayer with a preferential C-terminal β -strands facing toward the bilayer surface is a net outcome of different competitions between p3 peptides-lipid bilayer and ions-p3-bilayer interactions. More importantly, Ca^{2+} ions are found to form ionic bridges to associate negatively charged residues of p3 with anionic headgroups of the lipid bilayer, resulting in $A\beta-Ca^{2+}-PO_4^-$ complexes. Intensive Ca^{2+} bound to lipid bilayer and Ca^{2+} ionic bridges may lead to the alternation of Ca^{2+} hemostasis responsible for neuronal dysfunction and death. This work provides insights into the mutual structure, dynamics, and interactions of both $A\beta$ peptides and lipid bilayer at the atomic level, which expand our understanding of the complex behavior of amyloid-induced membrane disruption.

Introduction

Alzheimer's disease (AD) is the most common age-related and progressive neurodegenerative disorder. The pathological hallmarks of AD are typically characterized by the coexistence of the extracellular senile plaques of amyloid- β ($A\beta$) and the intracellular neurofibrillary tangles of tau protein in the human brain^{1,2}. The major component of the senile plaques is insoluble $A\beta$ fibrillar aggregates containing a signature structure of in-register cross β -sheets, although soluble $A\beta$ oligomers are often regarded as more cytotoxic species than fibrillar aggregates^{3,4}. However, little is known about the structure of soluble

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oligomeric intermediates because of their transient nature, small sizes, and heterogeneous morphologies^{5, 6}.

In the amyloidogenic pathway, full-length A β _{1-40/42} is produced by proteolytic cleavage of the transmembrane amyloid precursor protein (APP) by β - (at position 1) and γ -secretases (at position 40 or 42)⁷⁻¹⁰. In the nonamyloidogenic pathway, N-terminal truncated A β _{11/17-40/42} (known as p3) and A β ₉₋₄₂ (known as N9) are produced by β' / γ -secretases and α / γ -secretases, respectively. A number of *in vitro* and *in vivo* studies have shown that introduction of the full-length A β , p3, or N9 peptides to cultured cells and living tissues can induce the damage of their functions and viability^{11, 12}. Moreover, other A β fragments such as A β ₂₅₋₃₅, A β ₁₋₂₈, and A β ₂₉₋₄₀ have also been found to have similar amyloidogenic properties to full-length A β by forming morphologically similar amyloid fibrils, inducing neurodegeneration and cellular apoptosis, and impairing learning and memory functions^{13, 14}. The exact role of the full-length or fragmental A β peptides in the pathogenesis of AD remains unclear, but it is generally believed that the formation of A β oligomers and the interactions of these A β oligomers with neuronal membranes are mainly responsible for such catastrophic events in AD.

Two general mechanisms have been proposed to explain amyloid-oligomer-mediated toxicity. First, A β oligomers can bind to a number of membrane receptors (i.e. insulin receptor, frizzled receptor, nerve growth factor receptor, and N-methyl-D-aspartate receptor) to interfere with their synaptic functions^{15, 16}. Unlike receptor-induced toxicity, misfolded A β oligomers can also directly interact with cell membranes to physically disrupt the permeability and integrity of cell membranes¹⁷. A number of experimental studies have shown that A β peptides can insert into the cell membrane to form receptor-independent pores¹⁸⁻²², followed by ions (particular Ca²⁺ ions) and water transport through the pores, thus causing unbalanced ionic homeostasis. Along similar lines, various ion-permeable pores formed by hIAPP peptides^{20, 23, 24} and the K3 peptides derived from β ₂-microglobulin^{25, 26} have been visualized and characterized by atomic force microscopy (AFM), electrophysiology, and cell calcium images. Another possible mechanism of membrane permeability increase is frustration of the tight lipid packing by thinning or curving the membrane, which makes it easier for small solutes to find a pathway through the membrane²⁷⁻²⁹. Different experimental observations may also suggest the co-existence of multiple A β -induced membrane-disruption mechanisms^{30, 31}, depending on different conditions in cell lines^{32, 33}, peptide preparation and concentrations^{34, 35}, and solvent conditions³⁶⁻³⁹.

Complementing to experimental studies, extensive molecular dynamics (MD) simulations have been performed to study the structure, dynamics, and membrane interactions of A β aggregates from monomers to oligomers with lipid bilayers using all-atom or coarse-grained models in explicit or implicit water. Nussinov and coworkers have simulated a series of A β pores of different pore topologies formed by different A β fragments (i.e. p3, N9, and full-length A β) in DOPC (dioleoylphosphatidylcholine), DOPS (dioleoylphosphatidylserine), and POPE (palmitoyl-oleoylphosphatidylethanolamine) bilayers^{18, 34-37, 22, 40-43}. The pore shapes, morphologies, and dimensions correlate well with the doughnut-like images observed by AFM and EM²⁰. More importantly, these modelled A β pores displayed a strong Ca²⁺ selection over other cations and anions, consistent with planar lipid bilayer current recordings and cell calcium imaging^{22, 44}. Chang et al.⁴⁵ modelled β -barrel-like pore structures formed by A β ₂₅₋₃₅ fragments in the negatively charged POPG (palmitoyl-oleoylphosphatidylglycerol) bilayer, which strongly perturbs local membrane structure and water leakage. Charles et al.⁴⁶⁻⁴⁸ computationally studied the interactions of full-length A β ₁₋₄₂ monomers and dimers with zwitterionic DPPC (dipalmitoylphosphatidylcholine) and negatively charged DOPS bilayers. They observed

that the DOPS bilayer promotes strong A β -A β interactions to facilitate the formation of higher-order aggregates, while the DPPC favors strong A β -lipid interactions to promote A β adsorption. Lemkul et al.^{49–51} systematically investigated the insertion of α -helical A β ₄₀ monomers into different POPC (1-palmitoyl-2-oleoylphosphatidylcholine), POPS (1-palmitoyl-2-oleoylphosphatidylserine), and mixed POPC/POPE (1-palmitoyl-2-oleoyl-sn-glycero-3-phosphoethanolamine) bilayers, as well as the effects of ionic strength, pH, and temperature on the structural changes of both A β and lipid bilayers. They found that that, regardless of the lipid composition, the A β ₄₀ monomer anchors itself to the membrane via C-terminal residues 29–40, inducing the disorder of surrounding lipids and decreasing the bilayer thickness. Tofoleanu et al.^{52, 53} recently reported that a preformed N9 protofilaments tended to penetrate into the head group region of a POPE bilayer via hydrophobic C-terminal residues, which lead to local membrane-thinning effects. The major driving forces to associate the N9 protofilaments with lipid bilayer were identified as hydrogen bonds and electrostatic interactions, consistent with the driven forces for facilitating the adsorption of A β monomer on different lipid bilayers as reported by Lemkul et al.^{49–51}. Our previous MD simulations showed that increased cholesterol level in the POPC bilayer from 0% to 20% and 40% promoted A β -membrane interactions and A β _{1–42} monomer adsorption on the membrane, which may facilitate subsequent A β aggregation and membrane insertion⁵⁴. However, Qiu et al.⁵⁵ reported that the cholesterol in the PC bilayer actually acts as a protective role to prevent A β -induced membrane disruption and membrane-induced β -sheet formation. Both simulations reveal different aspects of the role of cholesterol in modulating the membrane interaction of A β monomer, suggesting that enhanced A β adsorption may not necessarily induce further membrane disruption, based on the observation that cholesterol indeed increases lipid order in both simulations.

Although some discrepancies exist between different simulations and between simulations and experiments, presumably because of the usage of different force fields, simulation models and setups, peptide conformations, and biased sampling techniques, these computational studies still provide significant and different atomic insights into the monomeric and oligomeric structures in membrane and their membrane disruption effects. Additionally, some contradicting data also reflect complex interplays between highly polymorphic structures and dynamic kinetics of A β and heterogeneous membrane mimics with different sizes, shapes, and chemistries can all result in different outcomes.

Apart from those simulations of A β pores, most of current simulations focus on the study of the pre-insertion or the early adsorption of A β monomers on the lipid bilayer, although A β oligomers are thought to be more toxic species. Our previous studies have determined a number of A β oligomers with different structural morphologies including linears^{56, 57}, annulars⁵⁸, triangulars⁵⁹, and globulomers⁶⁰, but their disruptive ability to cell membrane remain unclear. In this work, we employed molecular modelling and all-atom explicit-water MD simulations to investigate the adsorption, orientation, and membrane interactions of a p3 pentamer (~20 kDa) on zwitterionic POPC bilayer and mixed anionic POPC/POPG (molar ratio of 3:1) bilayer. The A β -induced membrane disruption and membrane-induced A β conformation changes were also examined. The p3 peptide was selected because atomic structures of p3 fibrils have been determined by quenched hydrogen/deuterium-exchange NMR⁶¹, and available structures are amenable to MD simulations. Although the p3 peptide was initially thought to be nonamyloidogenic, recent experimental evidence has shown that the p3 peptides can form β -sheet-rich amyloid fibrils^{62, 63}, selectively activate caspase-8 and caspase-3 to induce neuronal apoptosis⁶⁴, and exist in neuritic plaques in the brain of Down syndrome (DS) patients⁶⁵. The p3 pentamer was used because A β ₄₂ peptides preferred to aggregate into pentamer and hexamer units at the early assembly of A β ₄₂ oligomerization^{66, 67}. Since initial orientation of the p3 pentamer relative to the lipid bilayer is important for the adsorption, conformational change, aggregation, and membrane

interactions of A β on the lipid bilayer, three typical A β orientations relative to the bilayer were considered separately: the hydrophobic C-terminal β -strands, the hydrophilic N-terminal β -strands, or both N-/C-terminal β -strands of the bottom peptide face to the bilayer. Simulation results reveal that the p3 pentamer exhibits different adsorption scenarios at the interface of lipid bilayers with different lipid compositions. The p3 pentamer interacts more strongly with and locates more closely to anionic POPC/POPG lipids than zwitterionic POPC lipids, resulting from competitive interactions among p3, lipids, and interfacial water molecules and counter ions. Both POPC and POPC/POPG bilayers stabilize the U-shaped conformation of the p3 pentamer for subsequent peptide association and elongation. Our simulation results are in good agreement with experimental observations that mixed anionic lipids significantly increase p3 aggregation rate and induce a rapid structural conversion of p3 from an unstructured conformation to a β -sheet structure. This work expands our fundamental understanding of how p3-lipid interactions induce mutual structural perturbation in both p3 peptides and lipid bilayers at the atomic level, which is useful for future development of therapeutic agents and approaches to effectively disrupt A β adsorption, aggregation, and surface interaction on the membrane.

Materials and Methods

p3 pentamer

Initial coordinate of the p3 monomer (A β_{17-42}) was extracted from the quenched hydrogen/deuterium-exchange NMR structures of A β_{17-42} fibrils in solution (PDB code: 2BEG)⁶¹. As shown in Figure 1, the p3 monomer adopted a U-bend β -hairpin structure, consisting of a negatively charged N-terminal β -strand (residue V17-S26) and hydrophobic C-terminal β -strand (residue I31-A42), connected by a U-bend turn spanning residues N26-A31. An intrapeptide salt bridge between D23 and K28 was formed near the U-turn region to stabilize the β -hairpin structure. A p3 pentamer was used because A β_{42} peptides preferred to aggregate into pentamer and hexamer units at the early assembly of A β_{42} oligomerization^{66, 67}. The p3 pentamer was constructed by stacking five p3 monomers on top of each other in a parallel and register manner with an initial peptide-peptide separation distance of ~ 4.7 Å, yielding a total net charge of $-5e$ for the p3 pentamer.

p3 pentamer-lipid bilayer systems

Two lipid bilayers of pure zwitterionic POPC and mixed anionic POPC/POPG (molar ratio of 3:1) were used to examine the effect of lipid compositions on the p3-lipid interactions. The initial POPC and mixed POPC/POPG bilayers were constructed using the CHARMM-GUI membrane builder (www.charmm-gui.org)^{68, 69}, as reported in our previous works^{24, 54}. The initial sizes of POPC and POPC/POPG bilayer were ~ 90 Å $\times$ 90 Å in the xy plane, which is large enough to accommodate the p3 pentamer with a minimal distance of 15 Å between any edge of a bilayer and p3 atoms. The POPC bilayer consists of 119 lipids in each top and bottom leaflet (i.e. total $2 \times 119 = 238$ lipids), while the POPC/POPG bilayer with a molar ratio of 3:1 consists of 93 POPC lipids and 31 POPG lipids in each top and bottom leaflet (total $2 \times (93 + 31) = 248$ lipids).

Considering the amphiphilic nature and the U-shaped conformation of p3 and the surface chemistry of the lipid bilayer, adsorption of the p3 pentamer on the lipid bilayer strongly depends on its initial orientation relative to the bilayer. However, all-atom explicit-solvent MD simulation generally prevents significant peptide rotation within the nanosecond time scale. To overcome this limitation, three typical p3 orientations relative to the lipid bilayer were simulated: the hydrophobic C-terminal β -strands facing to the bilayer, the hydrophilic N-terminal β -strands facing to the bilayer, and both N-/C-terminal β -strands of the bottom peptide in contact with the bilayer. All other possible A β orientations upon adsorption

should be in between these three typical orientations. Since we are not probing a complete adsorption procedure of the p3 pentamer diffusing from bulk solution to the bilayer surface, the p3 pentamer was initially placed at ~ 5.0 Å above the bilayer surface to mimic pre-adsorbed state with three typical orientations. This moderate distance allows the peptides to freely adjust their orientations on the lipid surface. All p3-lipid systems were fully solvated with a TIP3P water box and any water molecule within 2.4 Å of A β or lipids was deleted. Counter ions of KCl, NaCl, and CaCl₂ at the same concentration of 35 mM were added to neutralize the system and to achieve a total concentration of ~ 140 mM, close to the physiological ionic strength of 100–150 mM at pH 7.4. Each system consisted of a p3 pentamer, a lipid bilayer, explicit water molecules and counter ions, up to total $\sim 10,000$ atoms.

MD simulation protocol

A series of dynamic cycles were performed to equilibrate the systems from a pre-equilibrium stage to a production-run stage. In the pre-equilibrium stage, the harmonic position restraints on peptides and lipids were gradually removed to optimize peptide-lipid and peptide-water interactions. The entire pre-equilibrium runs were 5 ns to generate the starting configurations for the production runs. In the production stage, all simulations were performed using the NPAT (constant number of atoms, pressure, surface area, and temperature) ensemble at 310 K. The system pressure of 1 atm was maintained by the Langevin piston with a decay period of 100 fs and a damping time of 50 fs, while the temperature of 310 K, above the gel-liquid crystal phase transition temperature ~ 270 K of the POPC membrane⁷⁰, was controlled by a Langevin thermostat with a damping coefficient of 5 ps⁻¹. The surface area in the *xy* plane was kept as a constant while the volume was allowed to change in the *z* direction. Van der Waals (VDW) interactions were calculated using a switch function with a twin-range cutoff at 12 and 14 Å, while long-range electrostatic interactions were calculated by a particle-mesh Ewald sum with a grid size of ~ 1 Å and a real-space cutoff of 14 Å. The RATTLE algorithm was applied to constrain all covalent bonds involving hydrogen atoms. A velocity Verlet integrator was used with a 2 fs time step. Periodic boundary conditions were applied to all directions. All MD simulations were performed using the NAMD software⁷¹ with CHARMM27 force field⁷². Each p3-lipid system was independently repeated for three times to assess simulation reproducibility using the same A β coordinates, different lipid coordinates randomly selected from the lipid library, and different initial velocities for all atoms. MD trajectories were saved every 2 ps for analysis. A summary of the simulation systems is listed in Table 1.

Results and Discussion

For clarity and convenience, the p3-bilayer systems are denoted by the initial orientation of p3 pentamer relative to the bilayer (i.e. C, N, and U represent C-terminal β -strands, N-terminal β -strands, and U-bend residues facing toward the bilayer), followed by the type of lipid bilayer (i.e. CG represents for the mixed POPC/POPG bilayer, while PC for the pure POPC bilayer). For example, C_CG indicates that p3 pentamer initially places above the POPC/POPG bilayer via the C-terminal β -strands facing to the bilayer surface. Details of simulation systems are summarized in Table 1.

Adsorption and orientation of p3 pentamer on lipid bilayers

Figure 2 shows the initial and final MD snapshots of the p3 pentamer on the POPC and POPC/POPG bilayers with three different initial orientations. For the A β -POPC systems, regardless of the initial p3 orientations, the p3 pentamer consistently loses most of the initial contacts with the zwitterionic POPC lipids. It can be seen clearly that as the hydrophobic C-terminal β -strands initially orientated towards the lipid surface (i.e. the C_PC system), the

p3 pentamer completely fled away from the POPC surface within 32 ns. In the case of N-terminal β -strands facing towards the lipid surface (i.e. the N_PC system), the p3 pentamer gradually changed its orientation by lifting its N-terminal residues, leading to an almost vertical orientation of $\sim 90^\circ$ relative to the lipid bilayer, with a few contacts between the N/C tail residues and the lipids. Along the similar observation, with an unbiased p3 orientation (i.e. the U_PC system), the p3 pentamer also fails to establish steady and strong interactions with the POPC bilayer within 80 ns. In the presence of the zwitterionic POPC bilayer, it appears that the peptides are not likely to adsorb on the surface due to the lack of favorable peptide-lipid interactions. On the other hand, for the A β -POPC/POPG systems, different adsorption scenarios were observed. The p3 pentamer with the initial C-terminal or U-turn orientation remained to be adsorbed at the water-lipid interface throughout the 80-ns simulations. A similar tilted orientation toward the bilayer surface was observed, in which the C-terminal residues tends to make close contacts with the anionic lipid headgroups. However, starting with the N-terminal orientation, the p3 pentamer loses most of the initial peptide-lipid contacts and moves away from the anionic lipid bilayer, similar to the observation in the N_PC case. Comparing all simulation systems reveals that the p3 pentamer prefers to stay on the anionic POPC/POPG surface with respect to the zwitterionic POPC surface, indicating that electrostatic forces help to aid the adsorption of the p3 pentamer on the lipid bilayer. Upon accommodation of p3 pentamer on the anionic bilayer surface, the p3 pentamer adopts a favorable topological orientation, where the C-terminal hydrophobic residues tend to make more contacts with the lipid headgroups. Our results are in accordance with numerous experimental observations that the p3 filaments do not adsorb on the DOPC bilayers^{62, 73, 74}.

To better quantify the adsorption and desorption behavior of the p3 pentamer on different lipid bilayers, three geometric parameters are defined to characterize and distinguish the orientation and position of the p3 pentamer relative to different bilayer surfaces (Figure 3a). ϕ is defined by an angle between a vector along N-terminal β -strands and the bilayer normal (z axis), which is used to characterize the extent of the tilt of N-terminal β -strands relative to the bilayer. ψ is defined by an angle between a vector pointing from Ala22 in the first peptide chain to Ala22 in the last peptide chain and the bilayer normal, which is used to characterize the tilt of the U-bend orientation. The distance (D) between the mass center of the p3 pentamer and the average position of phosphate atoms of the upper leaflets is used to measure the relative position of the p3 pentamer above lipid bilayer. Since the p3 pentamer in the C_PC system quickly disassociated from the POPC surface, the analysis of the geometric parameters for the C_PC system becomes unnecessary and thus will not be discussed. For the other two POPC-involved simulation systems, the adsorption geometry as characterized by ϕ , ψ , and D suffered from large fluctuation, confirming that the p3 pentamer does not prefer to stay on the zwitterionic POPC bilayer surface. Unlike the weak dependence of initial p3 orientations on the p3-POPC association, comparing geometric parameters among three POPC/POPG systems clearly shows the orientation dependence of the p3 pentamer on its association with the anionic bilayer surface. In both simulations of the p3 pentamer with hydrophobic C-terminal residues (C CG system) or U-bend residues (U CG system) directing toward the bilayer surface, the p3 pentamer was able to remain on the POPC/POPG surface, as evidenced by the steady intermolecular distances (12 Å for the C CG system and 13 Å for the U CG system) (Fig. 3b), as well as ϕ angles ($\sim 70^\circ$ for the C CG system and 90° for the U CG system) (Fig. 3c). A large change of ψ angle from 0° to 60° in the U CG system indicates that the pentamer orient its hydrophobic C-terminal residues toward the lipid surface, leading to similar p3 orientation as observed in the C CG system (Fig. 3d). Taken together, it was shown from our MD simulations that the p3 pentamer prefers to stay on the anionic POPC/POPG surface, rather than on the zwitterionic POPC surface. Upon adsorption, the p3 pentamer has a tendency to orient itself parallel to

the membrane so that the hydrophobic C-terminal residues make favorable interactions with the anionic lipid headgroups.

Conformational dynamics of p3 pentamer on lipid bilayers

The p3 pentamer has two β -sheets, and each β -sheet is formed by five in-register C-terminal and N-terminal β -strands associated by backbone hydrogen bonds and sidechain interactions. Intra-peptide Asp23-Lys28 salt-bridges and inter-peptide Asn27 ladders provide additional stabilizing forces to maintain the U-shaped conformation and peptide-peptide association. In all simulations of the p3-bilayer systems, the p3 pentamer remained quite stable during the 80-ns simulation, despite undergoing certain degrees of β -strand twists to optimize sidechain interactions. Figure 4 shows the root-mean-square deviations (RMSDs) of the p3 pentamer compared to the starting structure during the course of simulations. It appears that the membrane-bound p3 pentamers (5.9 Å for C_CG, 5.9 Å for U_CG, and 4.0 Å for N_CG) had relatively smaller RMSDs than the membrane-disassociated p3 pentamers (6.1 Å for N_PC and 8.1 Å for U_PC). Upon drifting away from the lipid surface, the p3 pentamer experienced similar structural dynamics in the bulk solution. As expected, the weakly-membrane-bound p3 pentamers had relative larger structural flexibility (RMSF) than the strongly-membrane-bound p3 pentamers (data not shown). The RMSF profiles also show that the edge residues near the C- and N-terminus were more flexible than other residues. Combined RMSD and RMSF results suggest that the lipid surface enables surface confinement effect to restraint large conformational change of the p3 pentamer. A similar conformational trend of the p3 pentamer on the lipid bilayer was also obtained from other trajectories with different starting velocities and coordinates. *Additionally, due to the relative intact of U-bend conformation of A β , internal cavities of 6–7 Å formed by consecutive U-turns were hydrated by confined water molecules, which may in turn stabilize interior Asp23-Lys28 salt bridges. Similar hydrated U-turn cavity was also observed in other A β oligomers^{75, 76}. However, Buchete et al.⁷⁵ found that as finite A β oligomers grow into infinite A β fibrils, the desolvation of the U-turn cavity occurs.*

Few experimental data about the structural and dynamic information of A β oligomers was available in literature, particularly for those low-ordered A β oligomers that are likely to serve as nucleating seeds for facilitating other oligomer and fibril formation. Ono et al.⁷⁷ for the first time used photo-induced cross-linking technique to prepare and stabilize highly purified low-order A β oligomers from dimer to tetramer with 94.3–99.6% purities. These highly purified and structural stable A β _{1–40} oligomers enable to accurately examine the structure and toxicity of the oligomers to be examined. They found a direct structure-toxicity correlation, i.e. both toxicity and secondary β -structure increase as oligomer order, namely tetramer>trimer>dimer>monomer. Tofoleanu et al.⁷⁸ simulated a double-layered A β _{9–40} octamer formed by two tetramers on the POPE bilayer. They reported that membrane-bound tetramers had good conformational stability with the RMSDs of ~8–9. These tetramers also have a well-preserved β -sheet content and can preferentially interact with the lipids via their hydrophobic C-terminal residues. These results are in accordance with our simulation results. A β monomers adopted unstructured conformations on DPPC or DOPS bilayer⁴⁷. Combination of these results from literature and our work, at a first approximation, seems to imply that the presence of lipid bilayer will promote surface-peptide and peptide-peptide interactions, both contributing to the structural transition to produce the β -sheet structure. Along similar line, our previous computational and experimental studies of the p3 peptide transition from monomer to hexamer on the self-assembled monolayers (SAMs) terminated with different functional groups^{79–82} have also revealed similar trends; starting with the unstructured p3 monomer, the β -structure population was rapidly increased from 20–41% in dimer to 60–68% in trimer, and then slowly increased to 65–75% in tetramer, 67–76% in pentamer, and 68–79% in hexamer. These results suggest that the the peptide adsorption at

the lipid membrane is driven by hydrophobic and electrostatic interactions, while the interpeptide interactions facilitate oligomerization via hydrophobic and hydrophilic interactions. The stable and well-preserved p3 pentamer could serve as a nucleating seed for facilitating peptide aggregation into longer and thicker fibrils via peptide elongation and lateral association.

Structure and dynamics of lipid bilayers

The structure and dynamics of lipid bilayers play an important role in regulating the function and activity of cell membranes. Numerous studies have reported that A β peptides can either insert into the cell membranes to form ion-permeable pores or induce membrane thinning^{34, 83–85}. In this study, it is not likely for the p3 pentamer to insert into the bilayer to form a pore-like structure within nanosecond timescale, because of high energy penalty^{40, 41}. Instead, we aim to investigate the membrane thinning effect by characterizing

the surface order parameter of the lipid bilayer using
$$S_x = \sum_{\langle i,j \rangle} \left[1 - \frac{(z_j^x - z_i^x)^2}{r_{ij}^2} \right]^{\frac{1}{2}}$$
 where x represents either phosphate atoms of the POPC/POPG lipids or nitrogen atoms of the POPC lipids; r_{ij} is the distance between two phosphate atoms of POPC/POPG lipids or between two nitrogen atoms of the POPC lipids; z_i^x and z_j^x are the heights of the selected atoms of i^{th} and j^{th} residues along the bilayer normal, respectively. The S_x actually reflects the degree of flatness of the bilayer surface, where S_x of 1 indicates a perfect smooth surface, while S_x of 0 indicates a bent or very rough surface. Figure 5 compares the averaged S_x values between the lipids of the upper leaflet (namely $S_{\text{P-UP}}$ or $S_{\text{N-UP}}$) and at the partial lipids of the upper leaflet within 12 Å of the p3 pentamer (namely $S_{\text{P-Local}}$ or $S_{\text{N-Local}}$). It can be seen that the overall $S_{\text{P-UP}}$, ranging from 0.95 to 0.96, and $S_{\text{N-UP}}$, ranging from 0.91 to 0.93, were comparable to the corresponding local $S_{\text{P-Local}}$ and $S_{\text{N-Local}}$ values. No statistical difference in S_x between POPC and POPC/POPG bilayers was observed, suggesting that no obvious thinning effect was observed for both POPC and POPC/POPG bilayers through the 80-ns simulations. It should be also noted that if actual membrane thinning effect does occur, it will likely occur on a substantially longer timescale, which is beyond the typical nanosecond timescale in all-atom MD simulations. It is also possible that the S_x is not a sensitive parameter to capture the membrane thinning effect. Thus, the limited time scales accessible to the MD simulations prevented a clear conclusion as to whether or not the p3 pentamer indeed induces the membrane thinning effect.

On the other hand, the average positions of lipid groups can also illustrate how lipids are distributed in the bilayer in response to the pentamer adsorption, and can be used to characterize the ordering of lipid bilayer related to the disruption of the lipid bilayer. Figure 6 shows the position probability distribution functions for three different function groups of PO_4 , $\text{N}(\text{CH}_3)_3$, and CH_3 of POPC lipids, two groups of PO_4 and CH_3 of POPG lipids, and water as a function of the distance from the center of lipid bilayer. For the p3-POPC systems, the more symmetric distributions of the lipid headgroups of PO_4^- and the tailgroups of CH_3 indicate that there are no disturbances in the lipid arrangement (Fig. 6a–b). However, for the p3-POPC/POPG systems, the asymmetric distributions of CH_3 , $\text{N}(\text{CH}_3)_3$, and PO_4 groups suggest relatively large disturbances in the lipid arrangement (Fig. 6c–e). This indicates that addition of anionic POPG lipids into zwitterionic POPC lipids does not significantly change the surface flatness or roughness as evidenced by the order parameters of S_{P} and S_{N} , but indeed alters lipid order and structures.

Interactions of p3 pentamer with lipid bilayers

The interaction energy between the p3 pentamer and the lipid bilayer is calculated in order to understand the dominant forces that drive the peptide adsorption and conformational

change on the lipid bilayer. Figure 7 shows the probability distributions of total interaction energy between A β and the lipid bilayer. Considering that our simulations used different lipid compositions and peptide orientations, the p3-lipid interactions displayed a distinct distribution curve for each case, which reflects different p3 adsorption and desorption behaviors in response to different lipid compositions and peptide orientations. Consistent with visual inspection of the MD trajectories, starting with U-bend orientation, the p3 pentamer had almost no interaction with the POPC lipids due to the p3 desorption off the bilayer surface. With an initial N-terminal orientation, the p3 pentamer was bound to both pure and mixed lipid bilayers. Thus we observed peaks in the distribution curves at -1210 kcal/mol for the pure POPC bilayer and at ~400 kcal/mol for the mixed POPC/POPG bilayer. Although the p3 pentamer had a much higher binding interaction with the POPC bilayer than with the POPC/POPG bilayer, binding probability of the p3-POPC interaction was much less than that of the p3-POPC/POPG interaction. For the other two p3-POPC/POPG systems containing the C-terminal and U-bend orientations, the p3 pentamer had strong interactions with anionic lipids, since the peaks in the distribution curves were located at -1024 and -1063 kcal/mol, respectively. On the other hand, comparison of different distribution curves also reveals that the p3 pentamer interacted more strongly with lipids in the anionic lipid bilayers than those in the zwitterionic lipid bilayer. *The residue-lipid interactions are highly heterogeneous, strongly depending on A β orientation and lipid composition. However, several charged residues of Glu22, Asp23, and Lys28, as well as both C-/N-termini, are found to have strong interactions with the bilayers. The negatively charged residues may act as a hook to establish initial binding/adsorption of A β to the charged headgroups via direct contacts with N(CH₃)₃⁺ headgroups or the formation of salt bridges with phosphate headgroups by cations in the lipids.*

To further distinguish energy contributions from each type of lipids for the mixed POPC/POPG systems and from VDW and electrostatic interactions for both bilayers, the energy distribution of the peptide interaction with lipids was separated into POPC and POPG parts, and each part was further separated into VDW and electrostatic contributions (Table 2). It can be seen clearly that regardless of pure or mixed lipid bilayer, electrostatic interaction was the main force to govern the peptide adsorption. Our simulation results are in good accordance with a number of experimental observations showing that electrostatic interaction plays an important role in controlling A β adsorption and conformation at the lipid bilayers^{36, 85–89}. For the mixed POPC/POPG bilayer systems, the overall interaction energy of the p3 peptides/POPC lipids system was comparable to that of the p3 peptides/POPG lipids system. This fact indicates that although the incorporation of anionic POPG lipids into the zwitterionic POPC lipids can indeed promote favorable peptide-bilayer interactions, and energy contributions from POPC and POPG parts appeared to be equally important. It can be expected that the presence of anionic lipids provides the collaborative attraction to bring the peptides close to the surface. In addition, the adsorption of A β peptides on the bilayer surface can also be affected by water molecules and counter ions at the bilayer interface, and the competitive interplay of intermolecular interactions among A β -bilayer, interfacial water-bilayer, and ion-A β -bilayer controls the net outcome of A β adsorption on the surface.

Ca²⁺ ion bridges enhance the association of p3 pentamer with lipid bilayer

The energy analysis of each p3 residue interacting with the lipid surface showed that in most of the simulations, the residues that have the stronger interaction with the lipids (<-50kcal/mol), surprisingly, were all negatively charged residues of Glu22 and Asp23 and the charged N/C-terminal residues. In the POPC/POPG systems (i.e. C_CG and U_CG), at the first glance, strong repulsive interactions between the negatively charged residues and anionic lipids appear to contradict with the observation that the p3 peptide was adsorbed on the

POPC/POPG bilayer. Therefore it is reasonable to consider whether cations would play a role. Figure 8 shows the probability distribution function of Ca^{2+} , Na^+ , and K^+ as a function of the z coordinate along the bilayer normal. In all simulation systems, the cation distributions were highly heterogeneous, strongly depending on the A β adsorption status and the lipid composition. On the other hand, we also observed that Ca^{2+} ions displayed a strong binding tendency to PO_4^{3-} headgroups over other Na^+ and K^+ cations. The highly populated Ca^{2+} binding sites were indicated by two relatively broad peaks at z of +15 and -15 Å near the top and bottom bilayer leaflets. Moreover, comparison of cation distribution between POPC and POPC/POPG bilayers clearly reveals that as compared to pure POPC bilayers, the existence of POPG promotes more cations, particularly for K^+ and Na^+ , to be incorporated into the deeper interior of POPC/POPG bilayers, as indicated by the closer peak locations near to the center of lipid bilayers. *For all POPC and POPC/POPG bilayer systems, the lipids favored interaction with the cations in a decreased order of $\text{Ca}^{2+} > \text{Na}^+ > \text{K}^+$. Ca^{2+} had stronger interactions with lipids than Na^+ and K^+ , presumably because Ca^{2+} has two positive charges.*

Strong lipid interactions with Ca^{2+} can block the binding sites, further preventing other cations from getting into the bilayer surface. More interestingly, a close-up examination of the MD trajectories showed that during the p3 adsorption, a number of adsorbed Ca^{2+} ions formed ionic bridges to associate negatively charged residues of p3 with anionic headgroups of the lipid bilayer, resulting in the formation of a A β - Ca^{2+} - PO_4^- complexes. Once these A β - Ca^{2+} - PO_4^- complexes were formed, these Ca^{2+} bridges remained stable over 70% time occupancy for the last 20-ns simulations based on the geometrical criteria of both p3 and PO_4^- groups being within 4 Å of Ca^{2+} ions. Figure 9 shows the representative snapshots of Ca^{2+} bridges binding to both negative residues of Glu22 and Asp23 and phosphate oxygen in the CP_CG and UP_CG systems. The presence of Ca^{2+} bridges not only enables the p3 pentamer to gain significantly favorable electrostatic interactions with the lipid bilayer as shown in Figure 7 and Table 2, but also allows the p3 pentamer to adopt a preferential orientation for accommodating their charged residues to interact with the headgroups of the lipid bilayers upon adsorption. Consistently, our previous MD simulations of A β monomer interacting with the POPC bilayer with different cholesterol contents also revealed similar Ca^{2+} bridges to associate the A β monomer with the lipid bilayer⁵⁴. Although complete membrane thinning or membrane disruption induced by A β peptides was not observed within our MD timescale, it is still expected that strong Ca^{2+} bridges between A β and lipid bilayer might lead to the alternation of Ca^{2+} homeostasis responsible for neuronal dysfunction and death⁹⁰. Overall, the p3 peptide may aggregate differently on cell membrane than on the zwitterionic bilayer and anionic bilayer. For the zwitterionic POPC bilayer, no preferred orientation and strong adsorption of A β were observed, thus A β is likely to translocate to other positions of the cell membrane (e.g., cholesterol-rich region and membrane proteins) to trigger aggregation and neurodegeneration. For the anionic POPC/POPG bilayer, A β might be directly deposited on cell membrane to form polymorphic oligomers and fibrils.

Conclusions

The molecular mechanisms of A β toxicity underlying the interactions of A β peptides with cell membranes are still unclear. In this work, using the NMR-based, U-shaped, β -strand-turn- β -strand A β 17-42 conformation, we have carried out explicit-solvent MD simulations to investigate the adsorption behaviors of the p3 pentamer on different lipid environments (i.e. zwitterionic POPC and anionic POPC/POPG mixed bilayers), with particular attention to the effects of the initial orientation of the p3 pentamer and the surface chemistry of lipid bilayers on the mutual structural and dynamic changes in lipid-induced p3 adsorption, orientation, and structure, as well as p3-induced lipid thinning, orderings, and structure.

Simulation results showed that interaction of the p3 peptides with the membrane strongly depended on its lipid composition. As compared to pure POPC bilayer, the p3 pentamer exhibited a great tendency to interact strongly with and be adsorbed on anionic POPC/POPG bilayers via a preferential C-terminal β -strands orientation toward the bilayer surface. On the other hand, regardless of initial p3 orientations and lipid compositions, electrostatic interactions are the major forces governing A β -lipid interactions. Upon adsorption, the overall β -hairpin structure and peptide aggregation state of the p3 pentamer were well preserved, implying that the p3 pentamer could act as a nucleating seed to engage with other A β monomers or oligomers to grow into high-ordered protofibrils and fibrils. Moreover, the adsorption, orientation, and conformation of the p3 pentamer on the lipid bilayer were a net outcome of competitive interplays among the p3 peptides, the surrounding lipids, and interfacial ions. It was found that the preferential binding of Ca²⁺ ions to the lipid bilayer promoted the formation of ion bridges to facilitate the association of A β with the anionic bilayer. The simulation protocol used in this work including the length of simulation is a typical choice for simulations of peptides interacting with the lipid bilayer. Within the 80-ns MD simulations, neither A β -induced membrane thinning nor the lipid-induced p3 unfold was observed, which may require an even much longer timescale to occur. Further investigation of various A β oligomers in different lipid environments at a much longer time using the coarse-grained models or steered MD simulations are being conducted to better understand the complex behavior of A β -induced membrane disruption. This work provides a platform for model setup and the atomic-level understanding of A β peptides at lipid interface.

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Figure 1.

(A) Amino acid sequence of the p3 peptide, namely A β _{17–42}. (B) 3D atomic structure of the p3 pentamer; each p3 peptide is labelled as A, B, C, D, and E. Color identity: hydrophobic residues (white), positively charged residues (blue), negatively charged residues (red), and polar residues (green).

Figure 2.

Initial and final MD snapshots of the p3 pentamer on the pure POPC bilayer (left panel) and the mixed POPC/POPG bilayer (right panel). Three different initial orientations of the p3 pentamer relative to the lipid bilayer are C-terminal β -strands (a,d), U-turn (b,e), and N-terminal β -strands (c,f) facing to the bilayer surface. The averaged distance between the mass center of the p3 pentamer and the averaged phosphate atoms of the upper lipid leaflet is labelled.

Figure 3.

Geometrical parameters to quantify the adsorption and orientation of the p3 pentamer on the lipid bilayer. (a) Schematic illustration of ϕ and ψ angles of the p3 pentamer relative to the lipid bilayer. (b) Time series of p3 displacement from the average position of phosphate atoms of the upper lipid leaflet. Time series of (c) ϕ and (d) ψ angles of the p3 pentamer relative to the lipid bilayer.

Figure 4.
Backbone RMSDs of the p3 pentamer on lipid bilayers and in bulk solution.

Figure 5. Surface order parameter of the lipid bilayers that is used to measure the flatness or roughness of the bilayer surface. P-UP and N-UP represent surface order parameters for all lipids of the upper lipid leaflet, while P-Local and N-local represent surface order parameter for partial lipids of the upper leaflet within 12 Å of the p3 pentamer.

Figure 6. Probability distribution functions for PO_4^- , NH_3 , and CH_3 groups of POPC lipids, PO_4^- and CH_3 groups of POPG lipids, and water molecules in (a) U_PC, (b) N_PC, (c) C_CG, (d) U_CG, and (e) N_CG.

Figure 7. Probability of total nonbonded interactions between the p3 pentamer and (a) POPC bilayers or (b) POPC/POPG bilayers.

Figure 8. Probability distribution functions for Ca^{2+} (black line), Na^{+} (red line), and K^{+} (green line) as a function of the z distance from the center of lipid bilayer for (a) U_PC, (b) N_PC, (c) C_CG, (d) U_CG, and (e) N_CG.

Figure 9. Representative snapshots of the Ca^{2+} bridges (purple sphere) binding to both negatively charged residues (red color) of Glu22 and Asp23 and phosphate headgroups in (a) C.CG and (b) U.CG systems. Other cations of Na^+ (pink) and K^+ (cyan) also participate in p3-membrane association.

Table 1 Simulation details of the A β -lipid systems.

Model	Orientation	Lipid Component	Water Box	A β peptide / lipid ratio
CP_PC	C-terminal	pure POPC	91 $\times$ 91 $\times$ 123	5/238
UP_PC	U-turn	pure POPC	91 $\times$ 91 $\times$ 123	5/238
NP_PC	N-terminal	pure POPC	91 $\times$ 91 $\times$ 123	5/238
CP_CG	C-terminal	POPC/POPG=3:1	91 $\times$ 91 $\times$ 123	5/248
UP_CG	U-turn	POPC/POPG=3:1	91 $\times$ 91 $\times$ 123	5/248
NP_CG	N-terminal	POPC/POPG=3:1	91 $\times$ 91 $\times$ 123	5/248

Table 2

The mean nonbonded interaction energy between the p3 pentamer and the lipid bilayer for all six systems.

Model	Aβ-lipid Bilayer Interaction Energy (kcal/mol)		POPC		POPG	
	VDW(kcal/mol)	Elec (kcal/mol)	VDW (kcal/mol)	Elec (kcal/mol)	VDW (kcal/mol)	Elec (kcal/mol)
C_PC	/	/	/	/	/	/
U_PC	-7.46	-6.16	-1.30	-6.16	/	/
N_PC	-1147.24	-1155.24	8.50	-1155.24	/	/
C_CG	-1157.27	-347.13	-26.73	-347.13	4.10	-400.79
U_CG	-1102.55	-546.87	-12.64	-546.87	-0.515	-542.53
N_CG	-373.85	-290.02	-19.63	-290.02	-7.16	-57.05