THE JOURNAL OF PHYSICAL CHEMISTRY Letters

pubs.acs.org/JPCL

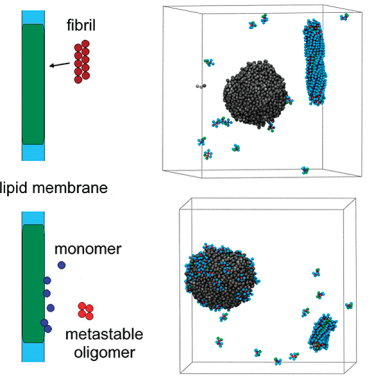
Soluble Protofibrils as Metastable Intermediates in Simulations of Amyloid Fibril Degradation Induced by Lipid Vesicles

Ran Friedman, Riccardo Pellarin, and Amedeo Caflisch*

Department of Biochemistry, University of Zürich, Winterthurerstrasse 190, 8057 Zürich, Switzerland

ABSTRACT Amyloid fibril disaggregation has been observed recently upon incubation with lipid vesicles, challenging the view of fibrils as end states of the aggregation process in vivo. Here, we follow fibril disaggregation in the presence of lipid vesicles by means of molecular dynamics simulations, using simplified models of peptides and lipids. The simulation results show that disaggregation is driven by an entropy increase and yields soluble protofibrillar intermediates. These intermediates are different from the metastable oligomers observed during fibril formation, and their stability depends on the morphology of the parent fibril.

SECTION Biophysical Chemistry



A variety of proteins assemble into cross β-sheet structures (amyloid fibrils), mostly under disease-related or nonphysiological conditions.[1,2] Lipid membranes are able to catalyze fibril formation for a variety of peptides.[3] Concomitantly, fibril growth on the membrane surface can damage the lipid bilayer. For example, leakage from lipid vesicles is observed during islet amyloid polypeptide fibril aggregation but not when the vesicles are incubated with preformed fibrils,[4] indicating that the growth of fibrils on the membrane is harmful to the cells, unlike the fibrils themselves, which are considered harmless.[5] Recently, this view has been questioned by a study in which soluble toxic oligomeric species were observed upon Aβ_{42} fibril degradation induced by lipid vesicles.[6]

Experimental characterization of forward (on-pathway) or backward oligomers is challenging due to their transient nature and to the mixture of monomeric, oligomeric, and fibrillar species that may be present at the same time. It is possible to conduct single-molecule experiments but not single-oligomer or single-fibril experiments. This gap can be filled in by molecular dynamics simulations.[7] Simplified models of peptides are particularly useful for this aim because atomistic models are typically limited to time scales of 0.1−1 μs, which is many orders of magnitude shorter than the aggregation process. For this reason, we have developed a phenomenological two-state model of amphipathic aggregation-prone peptides to study amyloid aggregation.[8] In simulations of the simplified peptides and a simple model of vesicle-forming lipids, it was observed that differences in the kinetics of fibril formation originate from different aggregation propensities of the peptides,[9] a finding that has been confirmed by experiments afterward.[10]

Here, we follow fibril disaggregation in the presence of a lipid vesicle by means of molecular dynamics simulations, using phenomenological models of peptides and lipids. Our aim is to study fibril degradation due to interactions with lipid vesicles. We assume that the ability of lipid vesicles (particularly those made of brain lipid extract) to induce fibril degradation is mainly due to relative attraction between the peptides and the lipids. This assumption is based on experimental evidence that GM1-containing lipid vesicles slow down or prevent the formation of α-synuclein fibrils.[11] GM1 vesicles strongly bind amyloidogenic peptides such as α-synuclein and have also led to fibril disaggregation.[6] While it is not possible in vitro to produce vesicles made only of GM1 (or to separate out the effects of specific lipids from vesicles prepared from a mixture of lipids extracted from the brain), the explicit treatment of individual intermolecular interactions in the simulations allows one to modify only the relative strength of peptide/lipid interactions without affecting the internal energy landscape of the peptides or the lipids. Details about the peptide and lipid models are given in the Methods section and in refs 8 and 9. Briefly, the simplified peptide has a single degree of freedom with two energy minima corresponding to amyloid-competent and amyloid-protected states, and the peptides can fibrillate only in the former conformation. It is important to mention that our simple model of an amphipathic aggregation-prone peptide was developed to reflect the phenomenology of fibril formation experiments and is not meant to be compared to any particular (poly)peptide sequence. As an example, while the

Received Date: November 27, 2009
Accepted Date: December 16, 2009

DOI: 10.1021/jz900318t | *J. Phys. Chem. Lett.* **2010**, *1*, 471–474

overall topology of the fibrils obtained in the simulations resemble the one observed by experimental means (e.g., twist along the fibrillar axis and multifilament composition[8]), the former lacks atomistic detail and therefore cannot emulate the details of side chain packing.

The simplified lipids (three-bead model[9]) spontaneously assemble into spherical, unilamellar bilayer vesicles, which can interact with the peptides. The peptide/lipid interactions are modeled by a Lennard-Jones effective potential. The affinity of the membrane for the peptides is approximated by a coupling parameter, λ, to enable the simulation of membranes with different affinities toward the peptides and lipids. In the present simulations, the relative strength of peptide/lipid van der Waals interactions results in adsorption of ~80% of the peptides to the vesicle surface in runs started from monodispersed peptides (compared to 50% in ref 9). Note that the peptides still have higher affinity for the fibril than for the lipid vesicle, as indicated by the more favorable constant of association to the former (see below). Furthermore, it is important to underline that the aforementioned change of λ is not a tuning of the parameter with the purpose of emulating an a priori defined behavior, for example, predefined pathways and kinetics of disaggregation. More specifically, the rich kinetics and multiple pathways of fibril disaggregation observed in the simulations and, in particular, the presence of metastable oligomers and their dependence on the morphology of the parent fibril (see below) are definitively not the result of a "what one puts in, one gets out" strategy.

The fibril disaggregation simulations are initiated by placing a vesicle of about 1000 lipids and a fibril in the same simulation box and at a separation at which there is no interaction between the fibril and vesicle (Figure 1A). The fibril consists of about 100 peptides arranged in four protofilaments (abbreviated as 4PF). It originates from a previous simulation without the vesicle, at the end of which isolated mature fibrils were in dynamic equilibrium with peptide monomers that bind and unbind at fibril edges.[8,12] In the presence of the vesicle, the peptide monomers attach to its surface, and in a first phase, the fibril shrinks along its axis according to first-order kinetics (Figure S1, Supporting Information). In this way, the fibril progressively degrades to a diffusible oligomer that preserves a four-protofilament morphology and twist (Figure 1B). The oligomer is a metastable state, whose stability depends on the morphology of the fibril from which it originates (see below and Figure 2, top). The total potential energy of the system increases during disaggregation (Figure 3), which indicates that fibril disaggregation is driven by the entropy gain due to the larger number of monodispersed peptides in the bulk and on the vesicle. Note that the peptides retain most of their conformational freedom when bound to lipid bilayers,[9] in agreement with experimental data.[13]

The stabilization of the oligomeric species during the fibril degradation simulations is due to the concomitant increase of monomeric peptides in the bulk (Figure S2, Supporting Information). The oligomers observed during vesicle-induced defibrillation (termed backward oligomers hereafter) consist of about 40 peptides arranged in a protofibrillar morphology. They are stable for 1−10 μs under the simulation conditions. Conversely, in simulations of fibril formation in the absence of a lipid vesicle, disordered oligomers of about 18 peptides and a half-life of only 0.2 μs appear on-pathway,[14] indicating that the mechanisms of aggregation (without a vesicle) and disaggregation (in the presence of a lipid vesicle) are distinct. In order to assess whether the formation of oligomers is due to the presence of the vesicle, fibril disaggregation was also induced by sufficiently diluting the solution, thereby driving the equilibrium between fibrils and monomers toward the monomeric species. Simulating the fibrils in a box of 30-fold larger volume leads to rapid disaggregation (Figure 2, bottom and Figure S3, Supporting Information). No disaggregation intermediates are observed in this case, indicating that the

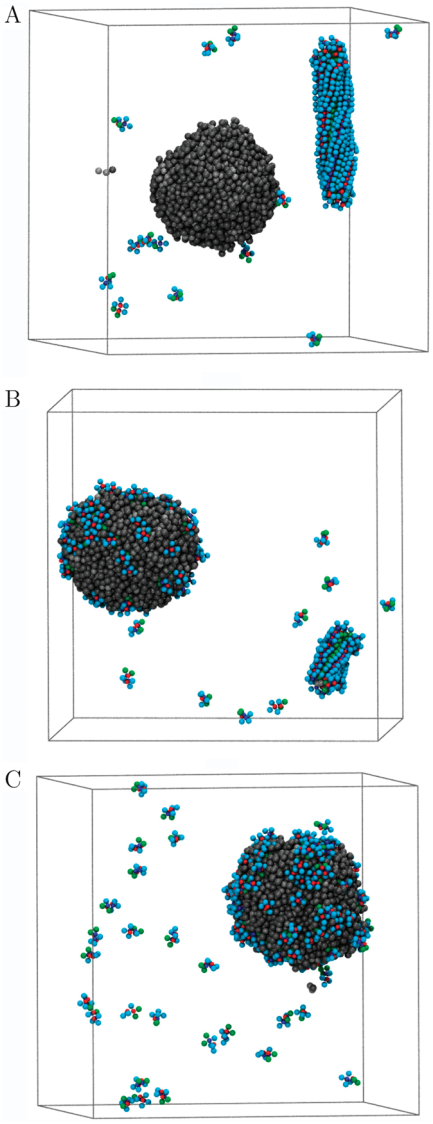


Figure 1. Simulation of amyloid fibril disaggregation in the presence of a lipid vesicle. (A) At the start of the simulation, the fibril consists of around 100 peptides arranged in four protofilaments. The vesicle consists of 1000 lipids. (B) After 2 μs of simulation time, roughly 50% of the peptides are bound to the vesicle. The fibril has degraded into a diffusible protofibrillar oligomer of about 40 peptides, which is metastable on a 1−10 μs time scale. (C) After 3.5 μs, the protofibril has fully disintegrated. The peptides are either bound to the vesicle or dispersed in the bulk.

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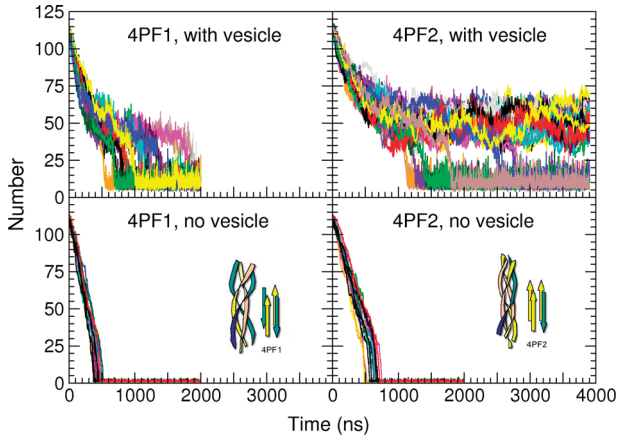

Figure 2. Fibril disaggregation kinetics in individual simulations. In 4PF1 fibrils, there are two up and two down protofilaments, while in 4PF2, there are three up and one down (insets in bottom plots). Moreover, fibrils of type 4PF1 are thicker and shorter than 4PF2 fibrils. The size of the largest peptide aggregate as a function of simulation time is shown for 20 (4PF1, with vesicle), 38 (4PF2, with vesicle), 20 (4PF1, no vesicle, dilute conditions), and 19 (4PF2, no vesicle, dilute conditions) runs. In the presence of a vesicle, fibrils with the 4PF1 morphology disaggregate within $< 2\ \mu$s, while full fibril disaggregation is observed in only 10 of 38 simulations with the 4PF2 morphology. A plateau region, where the size of the largest aggregate is almost constant, is observed in the 4PF2 simulations, but only in the presence of the vesicle.

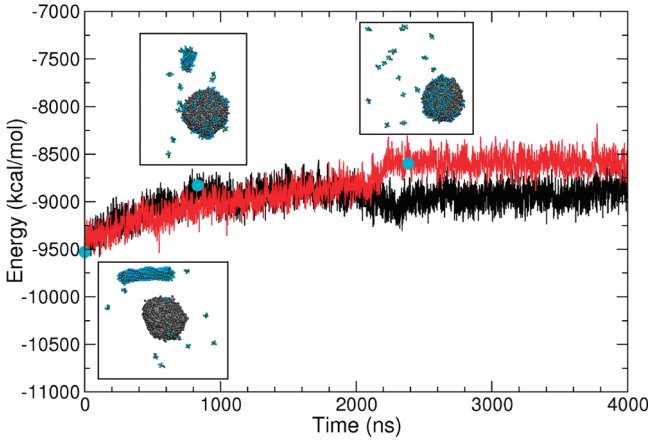

Figure 3. Potential energy of the whole system (i.e., 125 peptides and 1000 lipids) during the simulations. The potential energy is shown for two runs, one in which the fibril fully disaggregated into monomers (red, see the structures in the insets) and one in which the end state was a metastable oligomer (black). The cyan circles correspond to the energy of the conformations shown in the insets. Note that the initial, oligomeric, and monomeric phases are characterized by distinct energy levels.

presence of a lipid vesicle is necessary for the formation of backward oligomers.

In a previous simulation study with the two-state model of aggregating peptides,[8] the fibrils observed at the end of the run consisted of four protofilaments arranged in two distinct morphologies (termed 4PF1 and 4PF2) that differed in the orientation of the protofilament and the thickness of the fibrils (Figure 2). The pathways of fibril generation were very similar for the two morphologies, and the same on-pathway oligomers were observed. However, the two morphologies are characterized by monomer dissociation constants of K_d = 1.59 and 1.27 mM for 4PF1 and 4PF2, respectively, so that fibrils with the 4PF2 morphology are more stable (note that K_d = 1.75 mM for peptide binding to the vesicle). Simulations of fibril disaggregation were initiated with fibrils of the two forms to assess if the small difference in their dissociation constants leads to any variations in their disaggregation pathways or kinetics. Remarkably, oligomers observed during the defibrillation of the less stable 4PF1 fibrils were relatively short-lived, and the end state was monomeric in all simulations (see Figure 2 and Figure S2, Supporting Information). Thus, different fibril morphologies can lead to major differences in the stability of disaggregation oligomers and determine the product of the defibrillation process.

In conclusion, simulations of amyloid-like fibrils have been carried out in the presence of a lipid vesicle that is attractive to peptide monomers. The fibrils disaggregate into soluble backward oligomers that are larger, more ordered, and more stable than the oligomers observed during amyloid aggregation. The backward oligomers are not observed in simulations of fibril disaggregation under highly dilute conditions without lipid vesicles. Finally, simulations of fibrils with two different morphologies, having a small variation in the fibrils' dissociation constants, resulted in different rates of disaggregation and stability of the oligomers. This suggests that defibrillation can be used to isolate structurally uniform fibrils from inhomogeneous mixtures in vitro for nanotechnological applications[15] or structural investigations such as solid-state NMR. As an example, a dialysis chamber that is only permeable to monomers could be employed to purify the most stable fibrillar morphology.

METHODS

Simplified Phenomenological Model. The phenomenological models for lipids and peptides, as used in the simulation, are described in detail elsewhere.[8,9] Simplified peptides have a single degree of freedom, and their conformation can be either aggregation-prone (β) or aggregation-protected (π), where the latter is 21 times more populated than the former using a value of $dE = E_\pi - E_\beta = -2.25$ kcal/mol.[8] Fibrils are stabilized by electrostatic interactions between peptide dipoles, resulting in equilibrium where, in the absence of lipids, 110−120 of the peptides are part of the fibril. van der Waals interactions between the peptides and lipids are scaled by a factor (λ). In the original setup, we used λ = 0.87 or 0.90, and approximately 50% of the peptide monomers were bound to the vesicle surface. Here, we set λ = 0.95, resulting in about 80% of the peptides bound to the vesicle upon fibril disaggregation.

Simulations of Fibrils with a Lipid Vesicle. The fibril and vesicle were extracted from simulations of 125 peptides and 1000 lipids, respectively,[8,9] in a cubic box (l = 29 nm). Two different forms of fibrils (4PF1 and 4PF2), differing in the orientation of the dipoles along the fibrillar, axis were studied separately. The concentration of the peptides corresponds to 8.5 mM. The vesicle was put in the center of the simulation box and then moved randomly until the distance between any lipid atom and any peptide atom was at least 0.8 nm. The

DOI: 10.1021/jz900318t | J. Phys. Chem. Lett. 2010, 1, 471–474

system was then energy-minimized by 150 steps of the steepest descent and 200 steps of the conjugate gradient algorithms. After minimization, the temperature of the system was gradually increased to 310 K over 1000 steps of Newtonian dynamics with a time step of 2 fs before equilibration for a further 10000 steps at constant temperature. A second equilibration phase of 10000 steps was initiated with Langevin dynamics and a longer time step of 50 fs. The friction in the Langevin simulations was determined by a coefficient of 0.01 ps^{-1}. To make sure that the internal degrees of freedom equilibrate properly, a single bead of each peptide molecule was kept fixed during this phase. Following equilibration, a production Langevin dynamics run was initiated with none of the beads fixed. All simulations were carried out with the computer program CHARMM,[16,17] version 31. The simulations were runs for 2 and 3.9 μs with the 4PF1 and 4PF2 fibrils, respectively. A 1 μs run with 1000 lipids and 125 peptides requires about 10 days on a single core of a Xeon 5410 at 2.33 GHz. Disaggregation simulations were repeated 20 and 38 times (for 4PF1 and 4PF2 fibrils, respectively), with different random seeds for the initial distribution of velocities and placement of the peptides.

Simulations of Disaggregation by Dilution. The simulation of fibril disaggregation at high dilution was carried out as above, with the exception that a vesicle was not present and the box was approximately 30 times larger in volume (l = 90 nm). The simulations were repeated 20 and 19 times for fibrils of types 4PF1 and 4PF2, respectively.

Visual Presentation and Analysis. Figures of snapshots from the simulations were prepared by the computer program VMD.[18] Analysis was carried out by home-written software and by analysis programs from the Gromacs simulation package.[19,20] The computer program Wordom[21] was used to convert the CHARMM trajectories to a format that can be handled by Gromacs. The analysis program g_clustsize, available in Gromacs, was modified to allow the study of lipid or peptide clusters. A cutoff of 6 Å was used for clustering.

SUPPORTING INFORMATION AVAILABLE

Supplementary Figures 1−3. This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author:

*To whom correspondence should be addressed. E-mail: caflisch@bioc.uzh.ch.

ACKNOWLEDGMENT The authors thank Andrea Magno for critically reading this manuscript. This work was supported by the NCCR Neural Plasticity and Repair of the Swiss National Science Foundation, the Research Program for young scientists of the University of Zürich, the Olga Mayenfisch Foundation, and the Holcim Foundation.

REFERENCES

(1) Fändrich, M. on the Structural Definition of Amyloid Fibrils and Other Polypeptide Aggregates. *Cell. Mol. Life Sci.* **2007,** *64*, 2066–2078.

(2) Tuite, M. F.; Melki, R. Protein Misfolding and Aggregation in Ageing and Disease: Molecular Processes and Therapeutic Perspectives. *Prion* **2007,** *1*, 116–120.

(3) Zhao, H.; Tuominen, E. K.; Kinnunen, P. K. Formation of Amyloid Fibers Triggered Byphosphatidylserine-Containing Membranes. *Biochemistry* **2004,** *43*, 10302–10307.

(4) Engel, M. F. M.; Khemtémourian, L.; Kleijer, C.; Meeldijk, H.; Jacobs, J.; Verkleij, A.; de Kruijff, B.; Killian, J.; Höppener, J. Membrane Damage by Human Islet Amyloid Polypeptide through Fibril Growth at the Membrane. *Proc. Natl. Acad. Sci. U.S.A.* **2008,** *105*, 6033–6038.

(5) Lorenzo, A.; Yankner, B. A. Amyloid Fibril Toxicity in Alzheimer'S Disease and Diabetes. *Ann. N.Y. Acad. Sci.* **1996,** *777*, 89–95.

(6) Martins, I. C.; Kuperstein, I.; Wilkinson, H.; Maes, E.; Vanbrabant, M.; Jonckheere, W.; Van Gelder, P.; Hartmann, D.; D'Hooge, R.; De Strooper, B.; Schymkowitz, J.; Rousseau, F. Lipids Revert Inert Aβ Amyloid Fibrils to Neurotoxic Protofibrils That Affect Learning in Mice. *EMBO J.* **2008,** *27*, 224–233.

(7) Caflisch, A. Computational Models for the Prediction of Polypeptide Aggregation Propensity. *Curr. Opin. Chem. Biol.* **2006,** *10*, 437–44.

(8) Pellarin, R.; Caflisch, A. Interpreting the Aggregation Kinetics of Amyloid Peptides. *J. Mol. Biol.* **2006,** *360*, 882–892.

(9) Friedman, R.; Pellarin, R.; Caflisch, A. Amyloid Aggregation on Lipid Bilayers and Its Impact on Membrane Permeability. *J. Mol. Biol.* **2009,** *387*, 407–415.

(10) Khemtémourian, L.; Lahoz Casarramona, G.; Suylen, D. P.; Hackeng, T. M.; Meeldijk, J. D.; de Kruijff, B.; Hoppener, J. W.; Killian, J. A. Impaired Processing of Human Pro-Islet Amyloid Polypeptide Is Not a Causative Factor for Fibril Formation or Membrane Damage in Vitro. *Biochemistry* 2009.

(11) Martinez, Z.; Zhu, M.; Han, S.; Fink, A. L. GM1 Specifically Interacts with α-Synuclein and Inhibits Fibrillation. *Biochemistry* **2007,** *46*, 1868–1877.

(12) O'Nuallain, B.; Shivaprasad, S.; Kheterpal, I.; Wetzel, R. Thermodynamics of Aβ(l-40) Amyloid Fibril Elongation. *Biochemistry* **2005,** *44*, 12709–12718.

(13) Yip, C. M.; McLaurin, J. Amyloid-β Peptide Assembly: A Critical Step in Fibrillogenesisand Membrane Disruption. *Biophys. J.* **2001,** *80*, 1359–1371.

(14) Pellarin, R.; Guarnera, E.; Caflisch, A. Pathways and Intermediates of Amyloid Fibril Formation. *J. Mol. Biol.* **2007,** *374*, 917–924.

(15) Cherny, I.; Gazit, E. Amyloids: Not Only Pathological Agents but Also Ordered Nanomaterials. *Angew. Chem., Int. Ed.* **2008,** *47*, 4062–4069.

(16) Brooks, B. R.; Bruccoleri, R. E.; Olafson, B. D.; States, D. J.; Swaminathan, S.; Karplus, M. CHARMM: A Program for Macromolecular Energy Minimization and Dynamics Calculations. *J. Comput. Chem.* **1983,** *4*, 187–217.

(17) Brooks, B. R.; et al. CHARMM: The Biomolecular Simulation Program. *J. Comput. Chem.* **2009,** *30*, 1545–614.

(18) Humphrey, W.; Dalke, A.; Schulten, K. VMD: Visual Molecular Dynamics. *J Mol. Graphics* **1996,** *74*, 33–38.

(19) Berendsen, H. J. C.; van der Spoel, D.; Vandrunen, R. Gromacs — A Message-Passing Parallel Molecular-Dynamics Implementation. *Comput. Phys. Commun.* **1995,** *91*, 43–56.

(20) Lindahl, E.; Hess, B.; van der Spoel, D. Gromacs 3.0: A Package for Molecular Simulationand Trajectory Analysis. *J Mol. Model.* **2001,** *7*, 306–17.

(21) Seeber, M.; Cecchini, M.; Rao, R; Settanni, G.; Caflisch, A. Wordom: A Program for Efficientanalysis of Molecular Dynamics Simulations. *Bioinformatics* **2007,** *23*, 2625–2627.

DOI: 10.1021/jz900318t | *J. Phys. Chem. Lett.* **2010,** *1*, 471–474