

Amyloid cascade hypothesis for Alzheimer's disease. Does it work under physiological conditions?

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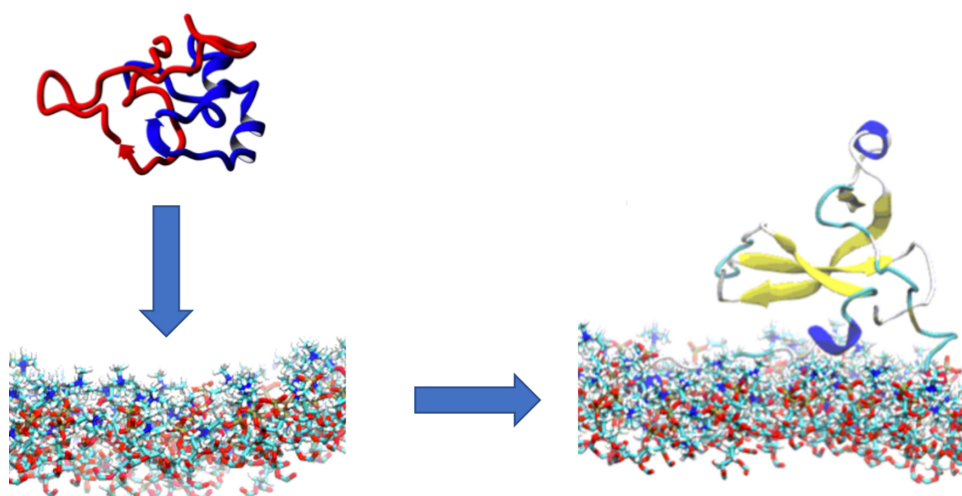
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Submitted: May 19, 2023

Accepted: June 5, 2023

Published: June 7, 2023

Graphical abstract:



Abstract

Plaques in the brain consisting of proteins are a hallmark of diseases like Alzheimer's disease (AD) and Parkinson's disease (PD). Such aggregates can be assembled spontaneously by specialized proteins such as amyloid beta ($A\beta$) proteins in the case of AD. Numerous in vitro studies made a foundation for the Amyloid Cascade Hypothesis (ACH), according to which the misfolding of proteins leads to their self-assembly into toxic oligomers along with the formation of amyloid fibrils assembled as plaques in the brain. Notably physiological concentration of $A\beta$ proteins in the brain is in the low nanomolar concentration, so no spontaneous aggregation of $A\beta$ protein occurs at such conditions, questioning the validity of the ACH model. However, recent studies revealed that surfaces could play a role as a catalyst of the aggregation process, so self-assembly of $A\beta$ can be observed at physiologically low concentrations of $A\beta$ proteins, although no spontaneous aggregation occurs in the bulk solution. The catalytic property of membrane surfaces towards $A\beta$ aggregation depends on the membrane composition. This finding suggests a number of novel ideas on molecular mechanisms of amyloid self-assembly, which lay a foundation for the development of treatments and preventions for AD, as discussed in this article.

Keywords:

Amyloid aggregation, Alzheimer's disease, Neurodegenerative diseases, Cellular membrane, Lipid bilayer, AFM imaging

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Rationale and Purpose

The formation of amyloid protein aggregates is considered the central model explaining the development of various human disorders, including fatal neurodegenerative diseases. At the same time, some inconsistencies with the test tube studies and dynamics of amyloid proteins *in vivo*, along with the lack of treatment of the disease based on the use of anti-aggregation small molecules, put the amyloid cascade hypothesis in question. However, recent successes with treating Alzheimer's disease (AD) with anti-amyloid antibodies supported the amyloid cascade hypothesis as a model for developing the disease. Moreover, recent studies revealed a critical role of membranes in the transition of functionally important amyloid proteins to their disease-prone state by the self-assembly in aggregates.

This novel model, the major point of this article, is a paradigm shift and opens novel avenues in developing treatments and prevention for Alzheimer's disease and other neurovegetative disorders.

Introduction

The formation of amyloid protein aggregates is the molecular mechanism behind a variety of human disorders, including fatal neurodegenerative diseases like Alzheimer's disease (AD), Huntington's disease, and Parkinson's disease (PD) (1-3). Such aggregates are the major components of amyloid plaques in the AD patient's brain, considered pathogenic signatures of the disease (4). Numerous test-tube studies demonstrated that monomeric amyloid protein spontaneously assembled in aggregates size of those increases over time, so the long filamentous aggregates are primary morphologies at the latest stage of the kinetics process (reviewed in (5, 6). According to these models, the nucleation-dependent mechanism of amyloid formation explains a typical sigmoidal shape curve as a function of time. The nucleation step of the aggregation process is complex. The assembly of nuclei from monomers is the primary process of aggregation kinetics. The polymerization elongation corresponds to the fast part of the kinetic curve followed by the stationary or saturation phase (7-14). The self-assembly process depends on various factors, including

the protein monomer concentration, presence of metal ions, pH of the medium, and interaction with small molecules (15, 16). These studies resulted in the amyloid cascade hypothesis (ACH), which has been extended from AD to other neurodegenerative diseases (17-21). According to this model, assembling A β into aggregates is the first step of AD development in which oligomers rather than fibrils are the neurotoxic species (22). Fibrils are formed at the later stages of disease development; these species constitute plaques in the brain. The similarity in structures of amyloids in plaques and assembled *in vitro* (the recent paper (23) and references therein) justify the implementation of the knowledge of the *in vitro* studies to the self-assembly process *in vivo*.

Several problems have been identified that complicate the translation of the *in vitro* results of amyloid aggregation to control the aggregation process *in vivo*. The decrease in the concentration of monomers decelerates the kinetics of the self-assembly of amyloids. However, drug development approaches based on decreasing A β concentration and disaggregating the plaques were unsuccessful (reviewed in (17, 24, 25). This failure is explained by the fact that in the monomeric state, all amyloidogenic proteins are functionally important, and the findings in (26-28) point to neuroprotective features of monomeric A β . Therefore, approaches focused on decreasing the concentration of amyloids can impair the positive functional roles of amyloid proteins. However, the most serious problem is the concentration range of amyloids *in vivo* and experiments *in vitro* (24). Specifically, *in vitro* aggregation experiments use A β concentrations in the micromolar range, whereas A β levels *in vivo* are several orders of magnitude lower. According to publications (29-31), the range of A β in the soluble fraction from the brain is in the low nanomolar range. No A β and other amyloidogenic protein aggregation occur *in vitro* at such low concentrations (e.g., (32, 33)). At the same time, plaques are formed *in vivo*, suggesting that A β aggregation, regardless of the low concentration, does occur, but the mechanism allowing for the amyloid protein to aggregate is unknown. Numerous experiments in bulk solution performed at various conditions did not provide the answer to potential self-

assembly mechanisms utilized *in vivo*, suggesting that traditional test tube experiments miss some critical features of the *in vivo* environment. Novel approaches are needed to understand the aggregation process *in vivo*.

Results

Surfaces as catalysts for amyloid aggregation at physiologically relevant low concentrations

The authors in (34) studied the aggregation of A β at physiologically low nanomolar concentrations and found that the assembly of A β in aggregates does occur regardless of the low concentration range of the peptide. Interestingly, these studies found that oligomeric species assembled in such experiments remain stable in the presence of sodium dodecyl sulfate (SDS). This contrasts with the instability of oligomeric species and their dissociation in the presence of SDS.

Another approach allowing for direct visualization of the aggregation process with AFM for studies of aggregation at low concentrations was described in (32). The mica sheets were immersed in a solution of the protein in nanomolar concentration. After a set incubation time, a mica sheet was removed from the solution, rinsed with water, dried, and imaged using AFM. Experiments with A β protein demonstrated that globular aggregates appear on the mica surface, and the number and sizes of aggregates increase with the time of incubation. The control aggregation experiments of protein aggregation without a mica surface did not reveal protein aggregation.

Additionally, time-lapse studies, in which the selected area was imaged continuously, revealed that oligomers could dissociate from the surface, creating a pool of aggregates in bulk solution. This important property was confirmed by directly measuring the concentration of aggregates in the bulk solution above the surface. These studies suggest that the on-surface aggregation is the mechanism by which amyloid oligomers in the solution can be produced, regardless of the low concentrations of monomers.

This conclusion aligns with experiments on the aggregation of α -synuclein (α -syn) using a highly sensitive fluorescence approach (35). The authors detected the aggregation of α -syn

at nanomolar concentration and revealed the self-assembly of α -syn aggregates, including fibrils at glass surfaces and supported lipid bilayers. Additional support for the on-surface catalysis of amyloid aggregation came from the single-molecule fluorescence approach (36, 37). In this approach, the monomeric amyloid protein was immobilized on the surface. The fluorescently labeled protein can make a dimer with the tethered monomer, resulting in a fluorescence burst, with the burst's duration corresponding to the dimer's lifetime. Of note, the concentration of the fluorescently labeled monomer was in the nanomolar range, so the dimers were formed regardless of a low concentration of amyloids in the bulk solution.

The on-surface aggregation experiments laid the foundation for the model explaining the on-surface aggregation catalysis (38). According to that model, aggregation starts with protein monomers transiently attaching to the surface due to non-specific interactions. This process increases the local concentration of proteins, increasing the probability of oligomerization reactions occurring on the surface. The process starts with immobilizing a monomer, and the aggregation proceeds by assembling oligomers on these transiently bound monomers. The key factor defining the on-surface aggregation process is the transient binding of monomers to the surface that plays the role of nuclei in the assembly of aggregates.

Interaction of A β with lipid membrane

The findings on the catalytic effect of surfaces in the amyloid point suggest that a similar result can be observed for membranes. Lipid vesicles have also been used to probe the aggregation kinetics of A β , and the acceleration of the aggregation has been observed (39). It was shown in (39) that the enhanced aggregation rate is mainly initiated by vesicle-fibril interaction. Zwitterionic 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC) vesicles do not alter the primary nucleation step; instead, monomer-dependent secondary nucleation on the fibril surface helps to enhance the overall rate of aggregation. Importantly, the catalytic effect of the surface in amyloid aggregation explains the aggregation experiments with A β 40 in cell culture, in which the protein concentration was in the low nanomolar range (34).

AFM imaging was applied to directly visualize the aggregation of A β 42 protein with phospholipid bilayers containing 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) and 1-palmitoyl-2-oleoyl-sn-glycero-3-phospho-L-serine (POPS) physiologically low concentrations (33). A solution of 10 nM A β 42 monomers was placed on the top of the bilayer, and AFM images were taken at various times. Aggregates appeared on the surface, gradually increasing their numbers and sizes over time (40). Like studies described above for aggregation on mica, time-lapse imaging over the same surface area revealed that A β 42 aggregates, assembled on phospholipid membranes, can dissociate from the surface into the bulk solution. Such a process could lead to the appearance of oligomers in the bulk solution, and it was confirmed by measuring the concentration of A β 42 aggregates in the solution above the membrane. Notably, the surface after the oligomer dissociation remained smooth, indicating no damage to the surface bilayer after the aggregates were dissociated. Note that similar observations were obtained with α -syn, suggesting the assembly of the disease-prone nano-aggregates on the membrane surface can be a general phenomenon for protein aggregation diseases. Damage to membranes caused by A β 42 (including pore formation) is considered a major mechanism of neurotoxicity of A β 42 oligomers (41, 42). However, no changes to the surface morphology were identified after the oligomers dissociated from the surface in the AFM experiments (40). These findings align with the results in paper (41), in which no pores were observed for A β 42 monomers or when monomeric A β 42 solution was used.

The effect of lipid composition on the interaction of A β with the bilayer

Numerous reports indicate that the composition of the membrane plays a significant role in the development of the disease state by facilitating the formation of A β oligomers (43). Lipid rafts are a membrane microdomain rich in cholesterol and sphingolipids and serve a critical role in neuronal functions. Cholesterol was known to modulate the A β interaction with lipid bilayers (44). In addition, cholesterol depletion in hippocampal neurons reduced the production of

insoluble A β (45). Recent studies show that cholesterol in 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC) vesicles play a catalytic role in enhancing the aggregation of A β by increasing the primary nucleation rate 20-fold (46). However, the role of cholesterol in the A β self-assembly has been controversial, and conflicting results have been reported. An increase in the cholesterol level in neuroblastoma cells has been found to inhibit the binding of A β with gangliosides (47). Such results demonstrate that not a single component of the lipid bilayer modulates A β aggregation; instead, multiple components in the bilayer are involved. This led to the investigation of the role of different lipids in the development of AD. It has been found that the level of gangliosides (48), which are glycosphingolipids predominantly found in the central nervous system, directly relates to AD pathology. A recent study shows that A β binds with the GM1 cluster, and the A) monomer is mainly involved in the binding, not the oligomers (49). GM1 enriched membrane induced the fibril formation and oligomer deposition (50). Ganglioside bound A β has been found to possess a strong seeding ability toward the amyloid formation (51). This evidence indicates that different membrane components have a crucial and decisive role in forming A β aggregates.

The AFM time-lapse studies were performed to clarify the role of lipids at the physiologically relevant conditions, which included the concentrations of A β 42 and the lipid compositions. It was shown that the latter is more catalytically active among two phospholipids, POPC and POPS (33, 40, 52). A stronger effect was found in the time-lapse AFM experiments for the lipid bilayers POPC-POPS (1:1 mol) with the presence of cholesterol (20 mol%) (PC-PS-Chol) (53). The incorporation of cholesterol was verified by the increase of the bilayer stiffness measured by AFM force spectroscopy. The data analysis revealed a 6-fold increase in aggregates in the presence of cholesterol. The accelerating effect of lipids in the membrane was reported for GM1 ganglioside in the membrane (54).

Notably, the time-lapse AFM imaging experiments of the aggregation on the PC-PS-Chol membrane in the presence of free

cholesterol revealed a robust synergetic effect of cholesterol (55). The experiments demonstrate that free cholesterol and its presence inside the membrane further accelerate the aggregation process by producing aggregates more rapidly and of larger sizes. These aggregates, formed on the lipid bilayer, can dissociate from the surface and accumulate in the bulk solution, and the presence of free cholesterol also accelerates this dissociation.

Computational modeling of the interaction of amyloid monomers with lipid bilayer surfaces

All-atom molecular dynamics simulations (MD) were applied to obtain insight into the underlying molecular mechanism of A β 42 aggregation on the POPC and POPS bilayer surfaces (53). The simulations show that in solution, A β 42 monomer is unstructured, but at the surface, they generally adopt conformations with extended β -sheet segments. The β -sheets are characteristic features for A β 42 fibrils, suggesting that the membrane-bound A β 42 monomer adopts the aggregation-prone conformation.

The MD simulations suggest that interaction with the membrane surface rapidly changes the conformation of A β 42 monomer by forming extended β -structure motifs, considered major building blocks within A β proteins, facilitating their assembly into amyloid aggregates (e.g., reviewed in (56)). This model was directly confirmed by simulations of A β 42 dimer assembly by the interaction of unstructured

A β 42 monomer with the surface-bound one (53). Additionally, MD simulations for the dimer assembly demonstrate that a misfolded A β 42 monomer rapidly dimerizes when another monomer appears near the misfolded one. The unstructured dimer undergoes conformation changes after interaction with the bilayer. Importantly, neither monomers nor dimers stay firmly bound to the bilayer surface; instead, they are dynamic and mobile, able to dissociate, associate, and tumble over the surface, exposing different segments of the molecules to the surface.

The MD simulations explain the effect of cholesterol on the amyloid aggregation observed in the experiments. These simulations showed that cholesterol binds A β 42 monomers and significantly changes the conformational sampling of the A β 42 monomer (55). These changes double the fraction of low-energy conformations compared to those in the absence of cholesterol, and this effect can contribute to the aggregation process.

Discussion

Amyloid self-assembly at membranes and model for the disease development.

The overall conclusion of these recent studies is that the phospholipid bilayer works as a catalyst enabling amyloid aggregates to assemble on the surface at physiologically relevant concentrations of A β , which leads to the model for the formation of the disease-prone amyloid aggregates schematically shown in Figure 1 (40, 53, 57, 58).

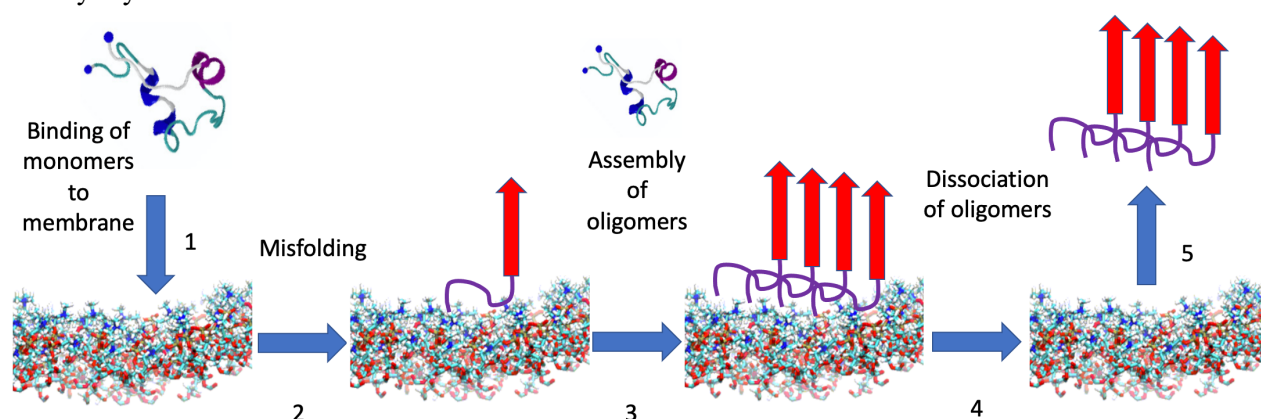


Figure 1. Model for the self-assembly of amyloid aggregates on the membrane surface. (40, 53, 57, 58)

According to this model, the interaction of A β protein with the membrane (step 1) leads to a conformational change of the peptide leading to the formation of the aggregation-prone conformation of the peptide (step 2, misfolding). Interaction of this monomer with another monomer from the solution leads to the formation of the dimer, followed by the growing aggregates (step 3). Assembled aggregates can dissociate from the surface, accumulating neurotoxic aggregates inside the cell. Overall, membrane catalysis is a pathway for the spontaneous assembly of amyloid oligomers at physiologically relevant concentrations. This mechanism has several properties explaining AD development.

The membrane composition is one of the factors defining the catalytic aggregation properties of the membrane, which can explain the role of lipid homeostasis on the development of AD (reviewed in (59-63)). Note reference (69) in which the link between the membrane composition and properties of receptors associated with A β synthesis and oligomers degradation, along with other pathways influencing A β production and metabolism, is reviewed. The role of cholesterol in AD pathogenesis is discussed in

(60), and the elevated level of low-density lipoprotein cholesterol with early onset of AD has been revealed in (64). Hypercholesterolemia is a risk factor in AD, accelerating the A β aggregation and memory impairment (65, 66). In addition to cholesterol, membrane components, such as sphingomyelin (Sph) and monosialoganglioside GM1, have a substantial effect on the amyloid fibril's formation on membranes, and the variability of their concentration in the membrane is associated with the AD development (reviewed in (42, 67, 68)). These can form rafts in the membrane (69), which can be responsible for the elevated amyloid aggregation process. The properties of these lipids align with the on-surface aggregation model, which includes the effects of free lipids and those within membranes. Moreover, the composition of the membrane can be a factor modulating the membrane catalysis of amyloids assembly; therefore, changes in membrane composition towards the elevated affinity of amyloid proteins to membranes can shift the equilibrium between monomeric and aggregated states of A β towards aggregated ones which define the disease state.

Conclusion

The proposed model is a paradigm shift for developing efficient treatments and diagnostics for AD, PD, and other protein aggregation diseases. According to this model, treatment efforts should focus on decreasing the affinity of amyloid proteins to membranes by developing small molecules that interfere with membrane-amyloid interactions and the change in the membrane composition via regulation of lipid metabolism. Control of the membrane composition is expected to be the major focus for preventative means that can be proven by corresponding experimental studies. The feasibility of such an approach is supported by the findings on the role of lipids in AD development (reviewed in (61, 62)). Diet as one of the preventive factors is widely discussed (e.g., (59, 60)), and detailed characterization of the membrane composition with the identification of the one that corresponds to the healthy state would provide a basis for the development of necessary diet approaches. The second direction is associated with the use of small molecules disrupting the association of A β with membranes. In both cases, potential treatments are related to the interaction of A β with membranes without changing their concentration as other treatments do. Given that in the monomeric state, all amyloidogenic proteins are functionally important, and the findings in (26-28) point to neuroprotective features of monomeric A β , the approach to controlling A β aggregation without changing the concentration of monomeric A β is entirely novel. It allows us to avoid impairing the positive functional roles of amyloid proteins, which are concentration dependent. Corresponding drug design requires detailed characterization of amyloid-membrane interaction as a function of the membrane composition and the intracellular level of lipids, and the current progress in these studies provides a basis for future studies. Computational modeling can provide the necessary critical structural information (70).

Acknowledgments

The work was supported by grants to YLL from NSF (MCB 1515346) and NIH (GM096039, GM100156). The author thanks former and current lab members for stimulating comments, Shaun Filliaux for proofreading the manuscript, and his helpful comments.

Conflict of Interest

The author is an associate editor of the journal and declares no other conflicts of interest. For a signed statement, please contact the journal office at editor@precisionnanomedicine.com.

Quote this article as Lyubchenko Y, Amyloid cascade hypothesis for Alzheimer's disease. Does it work under physiological conditions? *Precis. Nanomed.* 2023, 6(2):1013-1022, <https://doi.org/10.33218/001c.77826>

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