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Doctoral dissertation summary

"Amyloid β interaction with model cell membranes – insight into the mechanism of the Alzheimer's disease etiology and a novel therapeutic approach"

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Alzheimer's disease (AD) is related to the amyloid β (Aβ) aggregation and the formation of protein deposits in the patients' brains. The amyloid cascade hypothesis suggests that Aβ over-production, aggregation, and accumulation in the human brain triggers a cascade of molecular and cellular events leading to progressive neurodegeneration. Aβ monomers (AβMs) aggregates into various kinds of Aβ oligomers (AβOs) and Aβ fibrils (AβFs). The latest research showed that both AβMs and AβFs are not toxic, while AβOs demonstrate high toxicity. AβOs permeate the cell membrane. However, the mechanism of AβO-induced membrane permeation is still under debate. Moreover, it is unclear what kind of AβOs permeate the cell membrane because AβOs are polymorphic. Therefore, this dissertation aims to unravel the AβO-induced membrane permeation mechanism and identify the membrane-permeating type of AβOs.

Aβ aggregation monitored using atomic force microscopy (AFM) to distinguish between different kinds of Aβ aggregates produced. The formed AβOs are classified into small size (SS) and large size (LS) AβOs based on their size. AFM imaging also demonstrates that LS AβOs fibrillate on the membrane surface without compromising the membrane integrity. In contrast, SS AβOs permeate the membrane via a mechanism that consists of both pore formation and lipid extraction. Interestingly, even though SS and LS AβOs interact with the membrane differently, they both reduce the membrane Young's modulus by ~45%.

Next, AβO-membrane interaction is studied by electrochemical and IR spectroscopic techniques. Moreover, the interaction of non-toxic AβMs with the lipid membrane is also studied as a control measurement. Differential capacitance measurement and electrochemical impedance spectroscopy (EIS) show that the membrane electrical properties change significantly due to membrane permeation by AβOs. Polarization-modulation infrared reflection-absorption spectroscopy (PM-IRRAS) shows that both AβMs and AβOs disorder membrane lipids, causing changes in the lipid acyl chain conformation and orientation. Interestingly, both AβMs and AβOs interact with lipid heads, but in a different manner. AβMs dehydrate lipid heads but do not affect their orientation. In contrast, AβOs do not change the lipid head hydration levels but significantly affect their orientation. Two-dimensional correlation spectroscopy (2D-COS) shows that

structural changes in lipids precede those in Aβ. Moreover, Aβ secondary structure changes in line with the Aβ aggregation mechanism.

Finally, the inhibition of AβO toxicity by a fluorene-based compound, named K162, is studied. EIS shows that K162 prevents membrane permeation by AβOs. AFM imaging shows that, in the K162 presence, no membrane pores typically formed by AβOs are present, thus confirming EIS results. AFM and molecular dynamics (MD) simulations are utilized to study the K162-Aβ interaction by monitoring Aβ aggregation in the K162 presence. MD simulations show that the aggregation of K162-bound Aβ is energetically very costly, thus unfavorable. However, AFM results show that Aβ aggregates in the K162 presence. K162 also aggregates itself, and not all K162 molecules bind to Aβ. Consequently, Aβ aggregation in the K162 presence is inhibited but not entirely prevented. K162 also modifies the Aβ aggregation pathway. In the K162 presence, only non-toxic Aβ forms, i.e., AβMs, AβDs, and AβFs, are formed, while the production of membrane-permeating AβOs is bypassed. Unlike other Aβ toxicity inhibitors, K162 preserves neurologically beneficial AβMs. The deciphered Aβ toxicity inhibition mechanism not only explains previously-reported in vivo results but also provides a novel therapeutic approach that might be explored in the future.

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