139b Role of Aggregation Conditions in Structure, Stability and Toxicity of Intermediates in the Beta Amyloid Fibril Formation Pathway

Sungmun Lee and Theresa A. Good

β-amyloid peptide (Aβ) is among the main protein components of senile plaques that are one of the histopathological hallmarks in Alzheimer's disease (AD). Aβ readily forms fibrils that have high β-sheet content, and it is this fibril or aggregated structure of Aβ that is associated with senile plaques in AD. From recent studies, it is known that Aβ is toxic when aggregated. In particular, soluble oligomers have comparable neurotoxicity. However, the relationship between neurotoxicity and the size of Aβ*f*naggregates or oligomers is still under investigation. Here, we show that different Aβ incubation conditions in vitro can affect both the rate of Aβ fibril formation and the conformation of intermediates in the aggregation pathway. When mixed, Aβ aggregates faster than non-mixed Aβ as determined by Congo red binding, however, the morphology of fibrils formed at the end of aggregation with or without mixing, as observed in electron micrographs, are comparable. Interestingly, intermediates formed during Aβ aggregation differ greatly under mixing and non-mixing conditions. CD data suggest that without mixing, Aβ undergoes a significant conformational change upon aggregation to a fibril form that is not seen when Aβ is mixed during aggregation. Unfolding studies in guanidine hydrochloride indicate that fibrils formed without mixing are more stable to unfolding in detergent than aggregation intermediates or Aβ fibrils formed with mixing. In addition, Aβ fibrils formed without mixing were less toxic to differentiated SH-SY5Y cells than the Aβ aggregation intermediates of fibrils formed with mixing. These results suggest that it is not the tertiary structure alone of Aβ that correlates with its toxicity, but possibly other characteristics such as stability to unfolding in detergent that influence the toxic properties of Aβ. Given that aggregation of Aβ in vivo may differ significantly from in vitro aggregation conditions, understanding the other factors that influence toxicity outside of tertiary structure may help guide development of agents to prevent Aβ toxicity associated with Alzheimer's disease.