Exploring the Phase Space of Alpha-Synuclein with Replica Exchange Simulations

Nowosielski, M.A.; Bolhuis, P.G.

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In vitro fibrilization of proteins into amyloid fibrils provides critical insights into the factors influencing protein aggregation and has a key role in understanding the molecular basis of several neurodegenerative diseases caused by amyloids. \(\alpha\)-Synuclein (\(\alpha\)-Syn), an intrinsically disordered protein implicated in Parkinson’s disease, aggregates readily in vitro into fibrils that exhibit appreciable structural polymorphism. This inherent polymorphism is a major obstacle in elucidating structural features and understanding the fibrillation process. By selecting specific solution conditions we were able to produce morphologically homogeneous fibrils of wt and disease mutant \(\alpha\)-Syn at the plateau phase of Thioflavin-T (ThT) assays, as evident from atomic force microscopy (AFM) imaging and analyses. Our results indicate that the in vitro aggregation conditions as well as the disease related point mutations of the protein determine the dominant morphology and the maturation behavior of the fibrils produced. The morphology of wt \(\alpha\)-Syn fibrils appear to be dictated by two distinct mechanisms that is competitive growth of different polymorphic species during the fibrilization phase followed by structural rearrangements during the process of aging. In contrast, the disease mutant \(\alpha\)-Syn variants aggregate with faster kinetics and result in fibrils with well defined and stable morphology over time. Additional cross seeding experiments of wt \(\alpha\)-Syn with disease mutant proteins have shown faithful transmission of the mutant fibril morphologies across two generations. The aggregation into homogeneous fibril populations with mutant-specific morphology is characterized by distinct fibrilization kinetics in ThT assays. Moreover, our experiments indicate differential interaction of ThT with morphologically different \(\alpha\)-Syn amyloid fibrils.

Fibril Breaking Accelerates \(\alpha\)-Synuclein Fibrilization

Volodymyr V. Shvadchak,1 Mireille M.A.E. Claessens,2 Vinod Subramaniam1,2

1Nanoscale Biophysics, AmOLF, Amsterdam, Netherlands, 2Nanobiophysics, MESA+-& MIRA, University of Twente, Enschede, Netherlands.

The formation of amyloid fibrils of \(\alpha\)-synuclein is a pathological hallmark of the Parkinson’s disease. The fibrilization is an autocatalytic process that is seeded by mature \(\alpha\)-Synuclein fibrils. We studied dependence of the fibril growth rate on the concentrations of monomers and seeds and on mechanical shaking intensity and proposed a mechanism of \(\alpha\)-synuclein aggregation that includes monomer binding to fibril ends and formation of new growing centers by fibril breaking. Such an autocatalytic fibrilization mechanism accounts for distinctive features of the experimentally observed fibrilization process: exponential growth of the fibril concentration at the beginning of (seeded) aggregation – the linear dependence of the observed aggregation rate constant on the square root of monomer concentration - strong acceleration of aggregation by shaking. Based on the experimental distribution of fibril lengths we expect that fibril breaking is random and that the probability of breaking is proportional to the fibril length. The relatively low efficiency of the formation of primary fibrils explains the highly stochastic nature of the observed lag time compared to the aggregation rate. The rate constant of monomer binding to fibril end could was calculated based on the aggregation rate and the average length of formed fibrils and corresponds to attachment of monomer to particular fibril end approximately every 10s. Aggregation rates at low concentrations show that binding of monomer to the fibril ends is a irreversible process with equilibrium dissociation constant (Kd) less than 3 mM. The proposed model provides a quantitative means to compare \(\alpha\)-synuclein aggregation rates and affinity to fibril ends under different conditions, and could be useful in characterizing and designing aggregation inhibitors.

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