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DOI
10.1016/j.bpj.2014.11.377

Publication date
2015

Document Version
Final published version

Published in
Biophysical Journal

Link to publication

Citation for published version (APA):
Nowosielski, M. A., & Bolhuis, P. G. (2015). Exploring the Phase Space of Alpha-Synuclein with Replica Exchange Simulations. *Biophysical Journal*, *108*(2, suppl. 1), 63A. https://doi.org/10.1016/j.bpj.2014.11.377

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oligomerization behavior of tau, it is feasible that loss of foldedness and increased rigidity of the secondary structure content of soluble protein may contribute to a loss-of-function and eventually causes cytotoxicity, independent of intramolecular association.

314-Pos Board B94

Disease Related Point Mutations and Solution Conditions Determine Fibrillization Behavior of α-Synuclein

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In vitro fibrillization 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. α-Synuclein (αSyn), 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 fibrillization process. By selecting specific solution conditions we were able to produce morphologically homogeneous fibrils of wt and disease mutant αSyn 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. Specifically, the morphology of wt αSyn fibrils appears to be dictated by two distinct mechanisms that is competitive growth of different polymorphs during the fibrillization phase followed by structural rearrangements during the process of aging. In contrast, the disease mutant αSyn variants aggregate with faster kinetics and result in fibrils with well defined and stable morphology over time. Additional cross seeding experiments of wt αSyn 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 fibrillization kinetics in ThT assays. Moreover, our experiments indicate differential interaction of ThT with morphologically different αSyn amyloid fibrils.

315-Pos Board B95

Fibril Breaking Accelerates α-Synuclein Fibrillization

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The formation of amyloid fibrils of α-synuclein is a pathological hallmark of the Parkinson's disease. The fibrillization is an autocatalytic process that is seeded by mature α-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 α-synuclein aggregation that includes monomer binding to fibril ends and formation of new growing centers by fibril breaking. Such an autocatalytic fibrillization mechanism accounts for distinctive features of the experimentally observed fibrillization 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 exponential 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 reversible process with equilibrium dissociation constant (Kd) less than 3 μM. The proposed model provides a quantitative means to compare α-synuclein aggregation rates and affinity to fibril ends under different conditions, and could be useful in characterizing and designing aggregation inhibitors.

316-Pos Board B96

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

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α-Synuclein (AS) is a intrinsically disordered protein involved in the pathogenesis of Parkinson's disease, which is known to form toxic aggregates as well as fibrils. From a computational point of view, it represents a challenging system, as the protein can be found in multiple conformers, separated by many high free energy barriers. To overcome these barriers, we conducted replica exchange molecular dynamics. Using the Amber force field in implicit solvent for the AS monomer, we find transitions between states with both alpha helix, beta sheet and disordered structure. We also conducted molecular dynamics simulations for AS fragments to study the propensity for fibril formation. The results will be discussed in the context of existing and recent experimental findings.

317-Pos Board B97

Single Molecule Fluorescence Assay of Alpha Synuclein Dimerization

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The aggregation of α-Synuclein (α-Syn) is linked to Parkinson's disease. The mechanism of early aggregation steps and the effect of pathogenic single-point mutations remain elusive. We report here a single molecule fluorescence study of α-Syn dimerization and the effect of pathological single point mutations. Specific interactions between covalently immobilized fluorophore-free α-Syn monomers on a substrate and fluorophore-labeled monomers diffusing freely in solution were observed using total internal reflection fluorescence microscopy. The results showed that WT α-Syn dimers adopt two types of dimers, type 1 and type 2. The lifetimes of type 1 and type 2 dimers were determined to be 197 ± 3 ms and 3334 ± 145 ms, respectively. All three single point mutations, A30P, E46K and A53T, increased the lifetime of type 1 dimer as well as enhanced the relative contribution of type 2 dimer in the overall population with type 1 dimer being the major fraction. The kinetic stability of type 1 dimers (expressed in terms of lifetimes) had the following order A30P (693 ± 14 ms) > E46K (292 ± 5 ms) > A53T (226 ± 6 ms) > WT (197 ± 3 ms). More stable, type 2 dimers had lifetimes in the range of several seconds. The strongest effect, observed for the A30P mutant, resulted in a lifetime 3.5 times higher than WT for type 1 dimer. Moreover, the stability of WT dimers at pH 5 (938 ± 15 ms) was substantially enhanced compared with that at neutral pH. Our data suggests that α-Syn dimers are heterogeneous, and single-point mutations and acidic pH promote dimerization as evidenced by longer lifetimes. It also suggests that heterogeneity of the α-Syn dimerization could give rise to different aggregation pathways.

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Alpha Synuclein's Anomalous Structural Fluctuations in X-ray Single Molecule Observations

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The structures of alpha-synuclein (α-syn) in solutions resemble that of a random coil because α-syn is one of the intrinsically disordered protein (IDP). Alpha-syn is of great interest to Parkinson's researchers because it is a major constituent of Lewy bodies. Here, we observed characteristic of structural fluctuation of wild type (WT), phosphorylated mimic (S129E) and familial mutants (A53T, E46K) by using Diffracted X-ray Tracking (DXT) as x-ray single molecule detection systems. DXT is a method of measuring internal motions of the proteins by using trajectories of labeled gold nanocrystal. In order to measure movements of the specific binding sites of proteins, DXT monitors of X-ray diffraction spots from gold nanocrystal that were labeled in the active sites of the proteins. We measured structural fluctuations of individualα-syn molecules with both high time-resolution (36ms/frame and 0.1ms/frame) and high precision (0.1nm scale). DXT experiments used the energy of quasi-white x-rays (energy peak-width of 2%, 10-20 keV, BL40XU, SPring-8). As a result, we discovered that WT's fluctuation is lower than those of other mutants (S129E, A53T and E46K) from observed dynamical motion's histograms. Furthermore, we found that modes of motions in E46K and A53T have closer to that of S129E than that of WT. From motion's histograms in rotatory direction for the chain axis of α-syn's amino acid, we confirmed that motion's histograms in WT has simple single Gaussian distribution. However, those in other mutants have complex ones. From our DXT results, it is very clear that there is specific different motions between WT of α-syn and other mutants. In addition to, we found that there is different number of X-ray diffraction spots from gold nanocrystal between WT of α-syn and other mutants.