Parkinson's alpha-synuclein assembles in the disease prone stable dimers

The self - assembly of proteins into amyloid - type aggregates is a widespread phenomenon associated with many neurodegenerative diseases, including Parkinson's disease (PD). According to the current model for PD, the aggregation of alpha-Synuclein (?-Syn) and the assembly of aggregates into intracellular Lewy bodies is the hallmark of the disease. As a physiologically important protein, ?-Syn is critically involved in the trafficking of neurotransmitters at neuronal synapsis. Understanding the mechanism by which ?-Syn is converted into the disease prone species is important for the development of efficient treatments and preventative care for PD. As with other amyloid proteins, ?-Syn undergoes self-assembly kinetics, in which monomers assemble in oligomers of various sizes ending with the formation of fibrils that constitute the Lewy bodies. Current evidence suggests that the oligomeric species are more disease prone than the higher order fibrils, thereby suggesting that major efforts need to guided towards elucidating the principles by which physiologically important proteins such as ?-Syn are converted into their disease prone species. A property that can help to shed light on the ?-Syn aggregation process is the stability of the different oligomeric species; with this in mind, we began with studies of the dimers, the smallest oligomer of the protein.

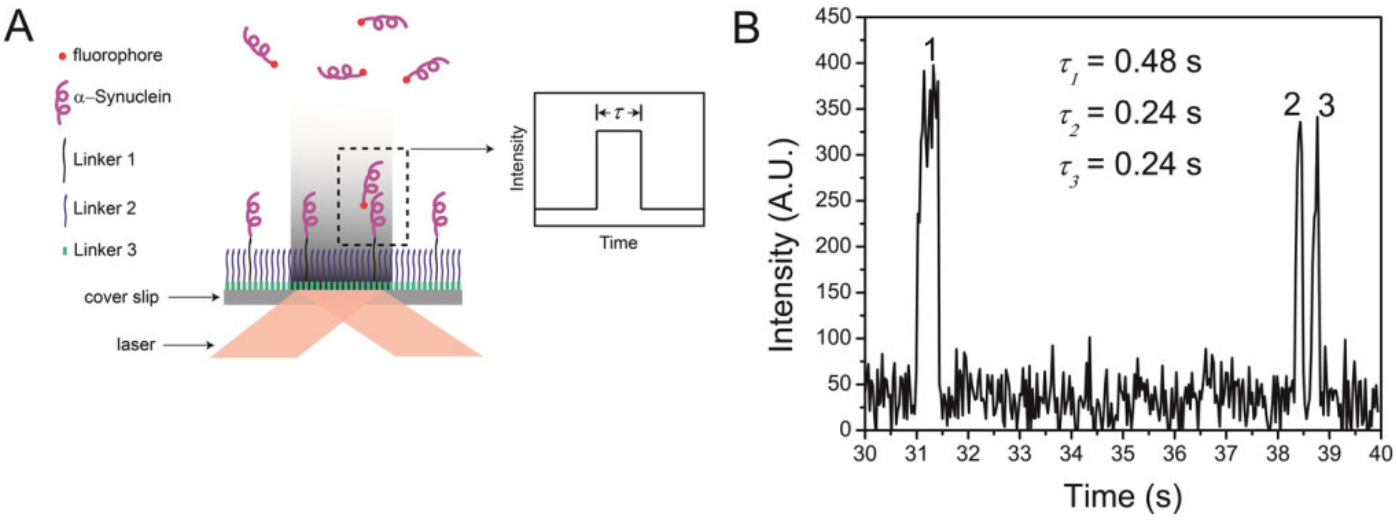

Fig. 1. (A) Schematic of the single-molecule fluorescence setup. A glass cover slip is functionalized with linkers, followed by immobilization of the unlabeled ?-Syn. The solution of the labeled ?-Syn (shown with red dots at one end) is injected into the sample chamber allowing for free ?-Syn molecules to interact with the tethered protein molecules. A laser is brought to the sample in the total internal reflection mode enabling the generation of an evanescent field and excitation of molecules in the surface proximity. The dimerization event is detected by the appearance of a fluorescence burst with duration ? defining the dimer lifetime (schematically shown in the inset). (B) Representative dimerization events observed in a time interval of less than 10 s. Event 3 appeared

in less than 2 s after event 2. The lifetimes for each event are indicated.

We developed a single-molecule imaging approach that enabled us to measure the lifetimes of ?-Syn dimers that appeared to be in the second range timescale. Through these studies, two classes of dimers with markedly different lifetimes were discovered. ?-Syn mutations involved in the development of familial PD increase dimer stability, suggesting that the onset of this disease is defined by dimer formation, which is the very first event in the aggregation process. Importantly, dimer formation was detected in solutions containing nanomolar levels of ?-Syn, suggesting that aggregation can occur at protein concentrations similar to those typically found in organisms. Also worth noting is that these low-concentration aggregation conditions were achieved in a protein tethered setup in which ?-Syn was immobilized on the surface, suggesting that the dimers were formed through an interaction of the immobilized protein with a freely diffusing ?-Syn. Moreover, aggregation on the surface occurs more rapidly than in solution regardless of the very high protein concentration in solution, which is several orders of magnitude higher than the concentrations used in the on-surface aggregation studies. Surfaces of various types are ubiquitous for intracellular and extracellular compartments, so it is highly important to understand the acceleration effect of surfaces in the protein self-assembly process. The approach described in this paper showed that the very first self-assembly step with the participation of the tethered protein occurs at the sub-nanomolar protein concentration in solution and led to the formation of stable dimers presumably in their disease-prone state.

Publication

Direct Detection of ?-Synuclein Dimerization Dynamics: Single-Molecule Fluorescence Analysis.
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Biophys J. 2015 Apr 21