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SDS-Induced Fibrillation of α-Synuclein: An Alternative Fibrillation Pathway

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A structural investigation of the sodium dodecyl sulfate (SDS)-induced fibrillation of α-synuclein (αSN), a 140-amino-acid protein implicated in Parkinson's disease, has been performed. Spectroscopic analysis has been combined with isothermal titration calorimetry, small-angle X-ray scattering, and transmission electron microscopy to elucidate a fibrillation pathway that is remarkably different from the fibrillation pathway in the absence of SDS. Fibrillation occurs most extensively and most rapidly (starting within 45 min) under conditions where 12 SDS molecules are bound per αSN molecule, which is also the range where SDS binding is associated with the highest enthalpy. Fibrillation is only reduced in proportion to the fraction of SDS below 25 mol% SDS in mixed surfactant mixtures with nonionic surfactants and is inhibited by formation of bulk micelles and induction of α-helical structure. In this fibrillogenic complex, 4 αSN molecules initially associate with 40–50 SDS molecules to form a shared micelle that gradually grows in size. The complex initially exhibits a mixture of random coil and α-helix, but incubation results in a structural conversion into β-sheet structure and concomitant formation of thioflavin-T-binding fibrils over a period of several hours. Based on small-angle X-ray scattering, the aggregates elongate as a beads-on-a-string structure in which individual units of ellipsoidal SDS–αSN are bridged by strings of the protein, so that aggregates nucleate around the surface of protein-stabilized micelles. Thus, fibrillation in this case occurs by a process of continuous accretion rather than by the rate-limiting accumulation of a distinct nucleus. The morphology of the SDS-induced fibrils does not exhibit the classical rod-like structures formed by αSN when aggregated by agitation in the absence of SDS. The SDS-induced fibrils have a flexible worm-like appearance, which can be converted into classical straight fibrils by continuous agitation. SDS-induced fibrillation represents an alternative and highly reproducible mechanism for fibrillation where protein association is driven by the formation of shared micelles, which subsequently allows the formation of β-sheet structures that presumably link individual micelles. This illustrates that protein fibrillation may occur by remarkably different mechanisms, testifying to the versatility of this process.

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Abbreviations used: αSN, α-synuclein; PD, Parkinson's disease; LB, Lewy bodies; LN, Lewy neurites; CMC, critical micelle concentration; DDM, dodecyl maltoside; DecM, decyl maltoside; ITC, isothermal titration calorimetry; TEM, transmission electron microscopy; SAXS, small-angle X-ray scattering; PBS, phosphate-buffered saline; ThT, thioflavin T; ACBP, acyl-coenzyme-A-binding protein; IFT, indirect Fourier transformation.

Introduction

Four million individuals worldwide are affected by Parkinson's disease (PD).[1] Two major hallmarks of PD are proteinaceous intraneuronal inclusions known as Lewy bodies (LB) and thread-like proteinaceous inclusions within neurons called Lewy neurites (LN). The main protein component of LB and LN is the 140-amino-acid protein α-synuclein (αSN),[2,3] which is natively unfolded.[4] Recombinant human αSN has been shown to form filaments or fibrils under physiologically relevant pH and buffer conditions, which have characteristic staining and morphology similar to those of filaments extracted from the LB and LN of PD-affected brains.[5–8] Consequently, αSN is believed to play a critical, but not fully understood, role in the onset and progress of PD.

αSN is predominantly expressed in the human brain and concentrated in presynaptic nerve terminals.[9–13] Although the precise physiological function of αSN is unclear, several physiological roles have been proposed. These include involvement in neurotransmitter release and vesicle recycling,[14] maintenance of SNARE protein complexes,[15] modulation of neural plasticity,[13] dopamine neurotransmission,[16] and endoplasmic reticulum–Golgi trafficking.[17] Thus, a lipid-mediated function of αSN is plausible, and it is generally accepted that membrane interactions play an important role in the physiological function of αSN.[18,19] This is also supported by the presence of seven imperfect repeats of KTKEGV mainly located in the N-terminal region known to interact with lipid vesicles. Indeed, αSN is known to bind acidic lipid vesicles, and an acidic lipid-mediated interaction induces an α-helical structure in αSN,[20–22] which in turn prevents αSN from forming filaments and fibrils.[23] This putative function makes it interesting to explore αSN's interaction with lipids and amphiphiles in general. Several groups have investigated the interaction of αSN with lipid membranes and surfactant micelles.[24–27] However, the influence of lipid interaction on fibrillation is complex, and whether membranes favor fibrillation or inhibit fibril formation is still widely debated.

The anionic surfactant sodium dodecyl sulfate (SDS) is widely used in biophysical studies to mimic membrane environments for proteins, since SDS has at least some of the features of a lipid membrane.[28] Thus, the negative charge that renders SDS a powerful protein denaturant is also critical for attracting αSN to lipids. The natively unfolded nature of αSN gives rise to a multistate folding when titrated with SDS, going from random coil to different α-helical folded states around the critical micelle concentration (CMC) of SDS.[29–32] It can take days to months for fibrillation to occur.[7,33,34] However, addition of negatively charged polymers or colloids such as heparin, heparin sulfate, dextran sulfate, and anionic surfactants accelerates the kinetics of αSN fibrillation.[35–37] Knowledge of the anionically stimulated fibrillation of αSN is important to understanding whether intracellular anionic surfaces could be potential αSN-nucleating surfaces.[37] Although the precise action of the SDS-induced fibrillation of αSN is not yet fully understood, different models have emerged. The ability of SDS to stimulate aggregation has been attributed both to the induction of α-helical structure[29–31] and the ability of SDS micelles to form a scaffold for αSN nucleation.[37] Both hypotheses combine in the work performed by Ahmad *et al.*, who investigated the effect of SDS on seed-induced fibrillation of αSN. They showed two types of αSN ensembles: namely, (1) a fibrillogenic ensemble characterized by enhanced hydrophobic exposure and partially helical conformations (0.5–0.75 mM SDS and 1 mg/mL αSN), and (2) an ensemble with a less accessible hydrophobic surface and a high helical content formed at high SDS concentrations (2 mM SDS and 1 mg/mL αSN) with reduced fibrillogenicity.[38] Thus, increased α-helix content decreases the ability of αSN to fibrillate.

In this study, we investigate the fibrillogenic intermediate that SDS induces in αSN. A major difference between SDS and vesicle-forming lipids is that SDS has a high CMC (7 mM) in water and thus only forms micelles at millimolar concentrations. This means that there will be, in general, a significant concentration of monomeric SDS present, which can interact with αSN in ways that are different from the micelle. The key issue is "Is the SDS-induced fibrillation of αSN dependent on the bulk monomer concentration of SDS, the formation of micellar species, or the density of negative charge in micelles?" To address this, we use mixed micelles of SDS and two nonionic surfactants, dodecyl maltoside (DDM; *n*-dodecyl-β-D-maltoside) and decyl maltoside (DecM; *n*-decyl-β-D-maltoside), which have CMC values of 0.18 and 1.8 mM,[39] respectively. Consequently, we can manipulate both the CMC and the mole fraction of SDS, allowing us to test these key issues. We identify an optimal concentration of submicellar SDS for the induction of αSN fibrillation and demonstrate that this process is mediated by the formation of micelle-like clusters at the surface of αSN, but inhibited by bulk micelles and reduced in parallel with the reduction of negative charge by the inclusion of nonionic surfactants. Using isothermal titration calorimetry (ITC), we determine that ~12 SDS molecules bind to αSN in the fibrillogenic state. Transmission electron microscopy (TEM) shows that the SDS-induced fibrils have a more flexible morphology than agitation-induced SDS-free αSN fibrils, but can be induced to form straight fibrils by subsequent agitation. Based on our small-angle X-ray scattering (SAXS) measurements, we propose a model for the stepwise growth of SDS-decorated αSN fibrils, which indicates that fibrillation in this case occurs by a process of continuous accretion rather than by the rate-limiting accumulation of a distinct nucleus.