Reversible Inhibition of α-Synuclein Fibrillization by Dopaminochrome-mediated Conformational Alterations*†‡

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Previous studies demonstrated that α-synuclein (α-syn) fibrillization is inhibited by dopamine, and studies to understand the molecular basis of this process were conducted (Conway, K. A., Rochet, J. C., Bieganski, R. M., and Lansbury, P. T., Jr. (2001) Science 294, 1346–1349). Dopamine inhibition of α-syn fibrillation generated exclusively spherical oligomers that depended on dopamine autoxidation but not α-syn oxidation, because mutagenesis of Met, His, and Tyr residues in α-syn did not abrogate this inhibition. However, truncation of α-syn at residue 125 restored the ability of α-syn to fibrillize in the presence of dopamine. Mutagenesis and competition studies with specific synthetic peptides identified α-syn residues 125–129 (i.e. YEMPSTM) as an important region in the dopamine-induced inhibition of α-syn fibrillation. Significantly, the dopamine oxidation product dopaminochrome was identified as a specific inhibitor of α-syn fibrillization. Dopaminochrome promotes the formation of spherical oligomers by inducing conformational changes, as these oligomers regained the ability to fibrillize by simple denaturation/renaturation. Taken together, these data indicate that dopamine inhibits α-syn fibrillation by inducing structural changes in α-syn that can occur through the interaction of dopaminochrome with the 125YEMPSTM motif of α-syn. These results suggest that the dopamine autoxidation can prevent α-syn fibrilization in dopaminergic neurons through a novel mechanism. Thus, decreased dopamine levels in substantia nigra neurons might promote α-syn aggregation in Parkinson’s disease.

Parkinson disease (PD)† is the most common neurodegenerative movement disorder, as it affects over one million people in North America and four million worldwide (1). PD is clinically diagnosed by four characteristic features, bradykinesia, postural instability, motor rigidity, and resting tremor. Pathologically, there is a progressive loss of dopaminergic neurons in the substantia nigra pars compacta, which results in a significant decrease in dopamine levels in the striatum followed by motor impairments in PD patients (1–3). In addition to neuron loss, intracellular proteinaceous lesions are found in different PD brain regions that are termed Lewy bodies (LBs) and Lewy neurites. LBs are found in the remaining dopaminergic neurons of the substantia nigra (4, 5), but they also occur in other brainstem neurons as well as in those of the thalamus, hypothalamus, cortex, olfactory bulb, and other brain regions (4, 6, 7). These inclusions are now known to be comprised of filamentous polymers of α-synuclein (α-syn) protein (5, 8–14).

α-Syn is a 140-amin acid heat-stable protein that is predominantly found in presynaptic terminals of cells of the central nervous system (15–18). Studies have shown that pathological inclusions comprised of α-syn are found in neurodegenerative disorders other than PD, including the LB variant of Alzheimer’s disease, dementia with LBs, multiple system atrophy, and related diseases collectively known as α-synucleinopathies (10, 12–14, 19, 20). Although α-syn is natively unfolded and soluble in aqueous solutions (21–25), conformational changes of this protein from random coil to β-pleated structure by unknown mechanism(s) lead to the formation of insoluble α-syn fibrils that are the building blocks of pathological inclusions (24, 26–29).

Previous studies suggest that α-syn may play a role in the regulation of synaptic dopamine levels and its secretion. For example, down-regulation of α-syn in neuronal cultures results in an alteration in the number of docked vesicles (30). Faulty synaptic transmission and altered striatal dopamine levels were observed in α-syn knock-out mice (31, 32). Furthermore, other studies have shown an interaction between the dopamine transporter and α-syn. Although some of the data are conflicting, these studies collectively suggest that dopamine transporter and α-syn form a complex that then modifies dopamine reuptake (33, 34). Dopamine/α-syn interactions also have been detected in cell culture models. For example, Xu et al. showed increased cell death in dopaminergic neurons transfected with wild type (WT) or pathologically mutant forms of α-syn (35), and Paxinou et al. reported the formation of α-syn aggregates in α-syn-overexpressing cells following simultaneous exposure to dopamine and nitric oxide (36).

Recently, Conway et al. showed that dopamine or L-dopa inhibits the fibrillization of recombinant α-syn filaments, presumably through adduct formation and stabilization of α-syn into “proteofibrillar” structures that are incompetent to...
form fibrils (37). To better understand interactions between dopamine and α-syn, we conducted in vitro studies to determine the nature of this interaction, and we also characterized the structural and conformational properties of dopamine-modified α-syn. Our results demonstrate a novel mechanism whereby dopaminochrome, an oxidized product of dopamine, inhibits α-syn fibrillization by interacting with a specific amino acid motif in the C terminus. Remarkably, the dopamine oxidation-induced formation of oligomeric spheres that are incapable of forming mature α-syn fibrils is due to conformational changes rather than covalent modifications, because these changes are reversible. Thus, our novel observations could be exploited for developing better therapies for PD-related α-synucleinopathies.

**EXPERIMENTAL PROCEDURES**

**Expression of WT, Mutant, and Truncated Recombinant Human α-Syn Proteins**—Human WT α-syn cDNA was cloned into the bacterial expression vector pKR172 at the NdeI and Hind III restriction sites. Simulated recessing point mutations or truncations of α-syn due to nonsense mutations were constructed using the QuikChange site-directed mutagenesis kit (Stratagene, La Jolla, CA) as described previously (22, 38, 39). WT, mutant, and truncated α-syn proteins were expressed in *Escherichia coli* BL21 (DE3) RIL cells and purified as described previously (22, 40).

**Synthetic α-Syn Peptides**—Peptides corresponding to human α-syn amino acid residues 116–140 (MPVDPDNEAY5MQMPEQYDVEPEA), α-syn amino acid residues 120–135 (PDNEAYMPEEGYQYDVEPEA), α-syn amino acid residues 123–133 (EAYEMPEEGY), and α-syn amino acid residues 123–133 with mutations at amino acid residues 125–129 (125EFAAFAAEYG137) were synthesized, fully deprotected, and high performance liquid chromatography-purified by the Biotechnology Resource Center at Yale University (New Haven, CT).

**Purified α-Syn Proteins**—α-Syn proteins (10 μg samples) were incubated at 37 °C with continuous shaking at 1,000 rpm at a concentration of 5 mg/ml (345 μM) in phosphate-buffered saline (PBS; 137.5 mM NaCl, 2.5 mM KCl, 10 mM Na2HPO4, and 1.75 mM KH2PO4, pH 7.5) containing 0.04% sodium azide. Dopamine was used at equimolar concentrations to α-syn (345 μM) or one-fifth of the concentration of α-syn (69 μM), and N-acetyl cysteine was used at 3-fold molar excess. Each sample (50 μl) was overlaid with ~40 μl of mineral oil to prevent evaporation. For sedimentation analysis, pelletable polymers of α-syn were collected by centrifugation at 100,000 × g for 20 min. SDS sample buffer was added to supernatants and pellets, which were heated to 100 °C for 5 min. α-Syn proteins were resolved by SDS-PAGE, stained with Coomassie Blue R-250, and quantified by densitometry. The formation of amyloid polymers of α-syn was assayed using the K14 amyloidogenic model peptide as described previously (38, 41), and α-syn fibrillation and/or oligomerization were monitored by transmission electron microscopy (EM) and atomic force microscopy (AFM) (see below).

**Purification of Dopamine-treated α-Syn Proteins**—WT or mutant α-syn proteins were exposed to equimolar concentrations of dopamine in PBS, and samples were incubated overnight at 37 °C. In some experiments, byproducts of dopamine oxidation were removed from modified α-syn by resolving the reactants using Affi-Gel blue bead chromatography. α-syn was exchanged with Microcon YM-10 centrifugal filter devices (Millipore, Bedford, MA) into 10 mM Tris, pH 7.5, and further purified through DEAE-Sepharose beads and eluted by 300 mM NaCl in 10 mM Tris, pH 7.5. α-Syn was exchanged into either 100 mM NaOAc, pH 7.5, or PBS. This purified α-syn was used for fibril assembly studies and negative staining EM, immuno-EM, AFM, circular dichroism, and Fourier transform infrared (FTIR) spectrometry.

**Visualization of α-Syn Fibril and Spherical Oligomer Formation by EM and AFM**—WT, mutant, and dopamine-modified α-syn samples were collected before and after incubation for EM, immuno-EM, and AFM analyses. Negative staining EM was used to observe fibrils of WT and mutant α-syn after fibrillation. Samples were applied to 300 mesh carbon-coated grids and negatively stained with 1% uranyl acetate. A JEOL 1010 EM was used to examine these samples at magnifications up to 100,000×, and images were captured with a Hermita camera (Birdgewater, MA) using software from AMT (Danvers, MA).

Purified dopamine-modified α-syn samples were analyzed by immuno-EM. Samples were applied onto 300 mesh carbon-coated grids, blocked with 1% bovine serum albumin in PBS, and immunostained with various primary antibodies including monoclonal antibodies that detected the N terminus (Syn505 and Syn506) and C terminus (Syn211 and Syn214) of α-syn and a polyclonal rabbit antibody raised to recombinant oligomers that were then decorated with anti-rabbit antibodies conjugated to 5-nm gold particles and negatively stained with 1% uranyl acetate. Control grids were stained with secondary antibody alone or with an anti-tau antibody (T14). EM grids were analyzed at magnifications up to 250,000×.

A multimode atomic force microscope equipped with a J scanner (Digital Instruments, Santa Barbara, CA) was used to analyze control and dopamine-modified WT and mutant α-syn samples for the presence of fibrils and/or spherical oligomers. Diluted samples were freshly prepared, 1 cm in diameter mica discs, and the specimens were air-dried overnight. Silicon nitride cantilevers with a spring constant of 0.06 newton/m were used for imaging, and the images were collected after the force applied to each specimen was minimized.

**Circular Dichroism Spectroscopy**—CD spectra were recorded using a Jasco J-810 spectropolarimeter. Spectra were collected at 25 °C in a 0.1-cm-long quartz cuvette containing the protein diluted to 0.1 mg/ml in 50 mM potassium phosphate buffer, pH 7.6.

**Protein Conformational Studies by FTIR Spectrometry**—An FTS 60A FTIR spectrometer (Bio-Rad) was used to analyze α-syn samples after the buffer was exchanged into 2 mM HEPES in D2O, pH 7.4, and then redissolved onto a germanium internal reflection element. Sample spectra were collected, rapid background scans were performed. Techniques such as smoothing, water vapor subtraction, baseline correction, and deconvolution were not performed on the raw spectra.

**Peptide Competition Experiments**—Full-length WT α-syn (345 μM) was assembled in PBS in the presence of a synthetic α-syn peptide (116–140, 120–135, 123–133, or mutant 123–133) at 5-fold molar excess with or without dopamine (345 μM). Pelletable polymers were assessed by centrifugal sedimentation analysis, and amyloid fibrils were determined by K114 fluorescence analysis as described above.

**Synthesis of Dopaminochrome**—Dopaminochrome was synthesized by dissolving dopamine at 5 mM in 10 mM sodium acetate, pH 5.8. Sodium periodate (NaIO4) was added to a final concentration of 10 mM (from a stock of 100 mM in H2O) and incubated with constant shaking for 5 min. Reverse phase high performance liquid chromatography analysis was used to confirm that the reaction was complete and resulted in dopaminochrome as the major product.

**Synthesis of Aged/Polymerized Dopamine**—Dopamine (35 mM) was incubated in PBS for 96 h. Oxidized dopamine polymers were recovered by centrifugation at 200,000 × g for 1 h and resuspended in PBS.

**Denaturation and Renaturation of Dopamine-Modified α-Syn**—α-Syn was incubated with dopamine at an equimolar concentration overnight at 37 °C without shaking. Dopamine-modified α-syn was denatured by the addition of urea to 7 M. α-Syn was subsequently re-natured by dialysis into decreasing concentrations of urea over a period of 48 h and eventually into PBS. Re-natured α-syn was then incubated with shaking at 37 °C for 96 h, and the protein was analyzed by centrifugal sedimentation and K114 fluorometry.

**RESULTS**

**Inhibition of α-Syn Protein Fibrillization by Dopamine**—Recombinant WT human α-syn was incubated under conditions that result in fibril formation in the absence or presence of dopamine with or without N-acetyl cysteine (an oxidant scavenger) or tyrosine. Sedimentation analysis (Fig. 1A) and K114 fluorescence analysis (Fig. 1B), which specifically binds amyloid fibrils (41), were employed to assess the degree of α-syn fibrillization in these samples. Both assays revealed that WT α-syn does not fibrillize in the presence of dopamine, and this inhibition is prevented by the addition of the reducing agent N-acetyl cysteine to the incubation reaction (Fig. 1, A and B). Tyrosine also was used as a control because its structure is similar to that of dopamine, but it does not self-oxidize or promote oxidation and did not inhibit the fibrillization of α-syn fibrils (Fig. 1, A and B). Most experiments in these studies were performed with an oil overlay to prevent evaporation (see “Experimental Procedures”); however, similar results were obtained without an oil overlay (see supplemental Fig. 1, available in the on-line version of this article). Furthermore, WT α-syn, which was incubated with dopamine without shaking and then purified to eliminate all dopamine oxidation byproducts,
products, also did not form pelletable polymers or amyloid fibrils upon further incubation for up to 12 days in vitro (data not shown). These results indicate that dopamine oxidation is involved in the inhibition of α-syn filament formation.

To identify α-syn structures generated by dopamine inhibition, EM and AFM analyses were conducted. Although untreated WT α-syn produced abundant fibrils after incubation for 48 h as detected by AFM (Fig. 1C) and EM (data not shown) (23 and 40), no fibrils were observed in dopamine-treated α-syn samples. Instead, there was an abundant accumulation of spherical oligomers (Fig. 1D). These spheres accumulated even within 12 h of adding dopamine and no further incubation (data not shown), indicating that dopamine promoted the conversion of α-syn from monomers to spherical oligomers. Calculations based on AFM data suggested that these spheres are ~25 nm in diameter and contain ~25 α-syn molecules. When immuno-EM was conducted using monoclonal antibodies specific for the C terminus (Syn 211 and Syn 214) (42) and N terminus (Syn 505 and Syn 506) (43) of α-syn, immunogold labeling was detected using all of the monoclonal antibodies (Fig. 1, E and F), suggesting that both the N terminus and the C terminus of α-syn within these spheres are exposed. As negative controls, anti-tau or secondary antibodies alone were used to immunostain the dopamine-modified α-syn oligomers, and no labeling was detected (data not shown).

**Fig. 1.** Dopamine inhibits α-syn fibril formation and induces structural changes in the α-syn protein. A and B, the α-syn protein was incubated under assembly conditions as described under “Experimental Procedures” for 48 h with added reagents as indicated in the keys adjacent to the graphs (Dop, dopamine; N-ac, N-acetyl cysteine). The polymerization of α-syn was analyzed by sedimentation analysis (A) and K114 fluorescence analysis (B), which measures amyloid formation. (n = 10). APUs, arbitrary fluorescence units. Error bars represent S.D. In panel A, a representative Coomassie-stained gel of supernatant (S) and pellet (P) samples after centrifugation is also shown. C, AFM images showing the filamentous state of α-syn after assembly. D, AFM analysis showing spherical oligomers that are formed after α-syn is incubated with dopamine and purified (dopaa-syn oligomers). E and F, immuno-EM images of spherical oligomers of modified α-syn. Syn 214 is a mouse monoclonal antibody that recognizes C-terminal regions of α-syn protein (E); Syn 505 is a mouse monoclonal antibody that recognizes the extreme N-terminal domain of the α-syn protein (F).
dopamine or products of dopamine oxidation. Thus, to investigate whether dopamine-mediated inhibition of α-syn fibrillation requires oxidation or other chemical modifications, α-syn proteins with the following mutations were generated: (i) single, double, triple, and quadruple Tyr → Phe mutations; (ii) single, double, and triple Met → Ala mutations; and (iii) His → Arg (H50R) mutation. The quadruple Met → Ala mutation could not be generated, because Met-1 is necessary for bacterial protein expression as the start codon.

Significantly, similar to WT α-syn, all α-syn mutants formed pelletable amyloid fibrils, and this process was inhibited by dopamine (Fig. 3, A and B). To confirm that dopamine arrested fibrillation of mutant α-syn at the spherical oligomeric stage, dopamine-treated Tyr → Phe α-syn with four types of mutations, Met → Ala α-syn with three types of mutations, and H50R α-syn were examined by AFM. Spherical oligomers with similar morphology and size to dopamine-treated WT α-syn incubated under assembly conditions as described under “Experimental Procedures.”

Inhibition of α-Syn Fibrillation by Dopamine Involves Residues 125–129 (YEMPS)—The results obtained to date suggest that amino acid residues that are generally targeted for modification by dopamine are not involved in dopamine-mediated inhibitions of α-syn fibrillation. To further investigate this mechanism, α-syn proteins with C-terminal truncations were analyzed. Fibril formation by WT α-syn 1–140 as well as by the α-syn 1–130 truncation mutants was inhibited by dopamine. However, dopamine did not inhibit the formation of fibrils assembled from α-syn 1–102, α-syn 1–110, and α-syn 1–125 truncation mutants (Fig. 3D). These results suggest that dopamine may interact or modify α-syn within residues 125–129, i.e. Tyr-125, Glu-126, Met-127, Pro-128, and Ser-129 (YEMPS).

To ensure that these results are not simply due to a faster rate of fibril formation of C-terminal truncation mutant proteins (22, 24), A53T α-syn was used as a control, because this mutant protein also fibrilizes faster than WT α-syn protein (22, 24, 40, 44, 45). However, fibril formation of A53T α-syn was also inhibited by dopamine (see Fig. 4B).

We confirmed the role of α-syn residues 125–129 in mediating the inhibitory effect of dopamine by conducting follow-up competition experiments using α-syn synthetic peptides with or without the YEMPS region. Synthetic peptides containing the YEMPS region (i.e. α-syn 116–140, α-syn 120–135, and α-syn 123–133) or peptides that contained mutations in this region (Y125F,E126A,M127A,P128F,S129A; mutant α-syn 123–133) were incubated with dopamine together with full-length α-syn at a 5-fold molar excess (Fig. 4A). As expected, full-length α-syn fibrillized into pelletable amyloids in the presence of dopamine and peptides containing the YEMPS motif, thereby indicating that these peptides competed successfully for dopamine (Fig. 4A). A YEMPS α-syn mutant peptide (mutant 123–133), where there residues YEMPS were changed to FAAFA, was unable to eliminate the inhibitory effects of dopamine (Fig. 4A).

To demonstrate directly that α-syn YEMPS residues mediate the inhibitory effect of dopamine, an α-syn mutant (designated 125–129) was generated wherein all five 125–129 amino acid residues were substituted to FAAFA, respectively. The effects of dopamine (at both equimolar and a sub-stoichiometric concentrations, i.e. 1:5 ratio of dopamine to protein) on WT α-syn,
A53T α-syn, and α-syn 125–129 fibrillation were compared. At both concentrations, dopamine inhibited fibrillation of WT α-syn (Fig. 4, B and C). Equimolar concentrations of dopamine also inhibited fibril formation of A53T α-syn, but at a ratio of 1.5 A53T α-syn was able to partially fibrillize. In contrast, the α-syn 125–129 mutant was more resilient to dopamine-mediated inhibition of fibrillation at either concentration, although some inhibition was still observed. These data were confirmed with EM analysis that showed abundant mutant α-syn 125–129 fibrils in the presence of dopamine (Fig. 4D). Taken together, these results establish that residues 125–129 in α-syn are involved in dopamine inhibition of α-syn fibrillation.

**Dopaminochrome, a Product of Dopamine Oxidation, Is Responsible for the Inhibition of α-Syn Fibril Formation**—To determine whether byproducts of dopamine autoxidation may inhibit α-syn filament formation rather than dopamine itself, the effects of dopaminochrome, a relatively stable oxidized product of dopamine, was analyzed for WT and mutant α-syn 125–129. Significantly, freshly synthesized dopaminochrome inhibited α-syn fibrillation almost as effectively as dopamine (Fig. 5, A and B). However, the mutant α-syn 125–129 protein can fibrillize in the presence of dopaminochrome. Furthermore, dopamine that has been allowed to oxidize and polymerize over time had little effect on the fibrillation of α-syn (Fig. 5C), indicating that oxidized intermediates such as dopaminochrome are responsible for the inhibition of α-syn filament formation.

**Reversibility of Dopamine Inhibition of Filament Formation**—The ability of dopaminochrome to inhibit filament formation suggests that this process may not be due to a covalent modification of α-syn. Consistent with this notion, mass spectrometry analyses of isolated dopamine-treated α-syn did not reveal significant dopamine/α-syn adducts, and [3H]dopamine incorporation analyses showed that <0.1% of the α-syn molecules were adducts of dopamine (data not shown). The lack of dopamine/α-syn adducts suggests that oxidized dopamine may induce conformational changes in α-syn, resulting in proteins that are fibrillation-incompetent. Moreover, these findings imply that this inhibition should be reversible. To test this hypothesis, dopamine-treated α-syn spherical oligomers were denatured in 7 M urea and re-natured by dialysis in PBS. Remarkably, this treatment led to the near complete recovery of the ability of dopamine-treated α-syn to assemble into fibrils (Fig. 5, D and E), which were ultrastructurally identical to those formed by untreated α-syn (Fig. 5F).

**DISCUSSION**

The studies presented here provide novel mechanistic insights into how dopamine inhibits α-syn fibrillation predominantly by inducing alterations in protein conformation that affect α-syn polymerization. Consistent with previous reports (37), our data show that dopamine prevents α-syn from forming mature amyloid fibrils and that oxidation is needed for this inhibition. However, our findings indicate that dopamine-induced covalent modification of α-syn is not required to prevent filament formation. Rather, inhibition of filament formation is due to the formation of oxidized dopamine by-products that induce conformational changes in α-syn to form species that are unable to proceed to assemble into mature fibrils.

Several key pieces of evidence support these conclusions. First, using mass spectrometry we and others (46) were unable to detect significant amounts of α-syn dopamine adducts, which is consistent with findings that dopamine can inhibit α-syn filament formation at sub-stoichiometric concentrations (37). Second, under the conditions used here that inhibit α-syn fila-
ment formation, only a minute amount of α-syn was covalently modified by dopamine (<0.1%), as demonstrated by using [3H]dopamine in radiolabeling studies (data not shown). Third, mutagenesis studies of the major amino acid residues that could be substrates for covalent modification by dopamine failed to show any effect. Although treatment with dopamine did lead to methionine oxidation as observed by the presence of species with an increased molecular mass of 64 Da using mass spectrometry (data not shown), this oxidation was not responsible for inhibition of α-syn fibrillization as demonstrated by studies of Met-to-Ala α-syn mutants and by previous studies (38). Finally, α-syn filament formation could be inhibited by a by-product of dopamine oxidation, i.e., dopaminochrome, although other intermediates may also be involved. It is noteworthy that fully oxidized/polymerized dopamine is ineffective in inhibiting α-syn fibril formation, and this failure of polymerized, oxidized dopamine to interact with α-syn might be due to chemical inactivation or steric hindrance.

The data presented here also support the notion that dopamine by-products, such as dopaminochrome, interacting with the amino acid motif YEMPS in α-syn are involved in the inhibition of filament formation. These dopamine intermediates appear to act as molecular chaperones, inducing conformational changes in α-syn as observed by FTIR spectrometry and the accumulation of structural “spheres” observed by AFM. It has been proposed that α-syn spherical oligomers generated in the absence of dopamine or dopaminochrome may be intermediates in the pathway that lead to mature fibril formation (44, 49). However, dopamine-induced α-syn spheres could be structural variants that are on an “off-filament pathway” and therefore unable to progress to form fibrils. Significantly, this altered form of α-syn can be rendered fibrillation-competent by de-/renaturation, consistent with the role of conformational alterations rather than covalent modifications in dopamine inhibition of α-syn fibrillogenesis.

These findings may have important implications for the role of dopamine oxidation in the pathogenesis of PD and future PD drug discovery efforts. It is hypothesized that dopaminergic neurons may be selectively vulnerable in PD because of their increased exposure to oxidation stress as a result of dopamine biochemistry. However, our data here suggest that intermediates in dopamine oxidation may prevent the formation of α-syn filaments, although other catecholamines might have similar effects (37, 46). Nevertheless, it remains to be determined if

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**Fig. 4.** The amino acid residues 125–129 in α-syn play a role in the inhibition of α-syn fibrillization by dopamine. A, percentage of WT α-syn pelleted during sedimentation analysis after it was incubated alone or with the indicated peptides. An identical set of samples was assayed with an equimolar concentration of dopamine. Peptides used are α-syn 116–140, α-syn 120–135, α-syn 123–133, and the mutant (Mut) α-syn 123–133 (Y125F,E126A,M127A,P128F,S129A); n = 5. B and C, analysis of the effect of equimolar or one-fifth molar concentration of dopamine (Dop) on the assembly of WT α-syn, A53T α-syn, and mutant 125–129 α-syn assessed by sedimentation analysis (B) or K114 fluorometry (C). Error bars represent S.D.; n = 4. D, negative staining electron micrographs of A53T α-syn, and mutant 125–129 α-syn challenged with one-fifth or equimolar concentrations of dopamine, as indicated in each section, and incubated under assembly conditions for 96 h.
spherical oligomers generated because of dopamine oxidation have any toxic effects. It also is difficult to assess how many oxidized dopamine intermediates exist in cells at steady state, because it is likely that these compounds readily convert to neuromelanin. It is also interesting to speculate that a reduction in dopamine levels may occur early in PD and result in an increased propensity for WT α-syn to form fibrillar inclusions, but this may partially be countered by L-dopa, which has a limited neuroprotective effect.

In summary, this study provides evidence for an alternative polymerization pathway for α-syn that does not culminate in mature α-syn fibrils. Furthermore, we show that α-syn fibrilization can be affected by physiological, molecular chaperones that may have important consequences for disease pathogenesis. These findings provide a framework for further studies to define the physiological role of dopamine on α-syn inclusion formation, and our observations could be exploited to develop novel therapies for PD and related α-synucleinopathies.

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