

Mutations associated with familial Parkinson's disease alter the initiation and amplification steps of α -synuclein aggregation

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Parkinson's disease is a highly debilitating neurodegenerative condition whose pathological hallmark is the presence in nerve cells of proteinaceous deposits, known as Lewy bodies, composed primarily of amyloid fibrils of α -synuclein. Several missense mutations in the gene encoding α -synuclein have been associated with familial variants of Parkinson's disease and have been shown to affect the kinetics of the aggregation of the protein. Using a combination of experimental and theoretical approaches, we present a systematic *in vitro* study of the influence of disease-associated single-point mutations on the individual processes involved in α -synuclein aggregation into amyloid fibrils. We find that lipid-induced fibril production and surface catalyzed fibril amplification are the processes most strongly affected by these mutations and show that familial mutations can induce dramatic changes in the crucial processes thought to be associated with the initiation and spreading of the aggregation of α -synuclein.

lipid-induced aggregation | seeded aggregation | kinetic analysis | familial Parkinson's disease | neurodegenerative disease

The protein α -synuclein is expressed abundantly in the brain [representing up to 1% of all proteins in the neuronal cytosol (1)], especially in dopaminergic neurons, where it has been suggested to interact with the membranes of synaptic vesicles containing dopamine (2–7). During their life cycle in dopaminergic neurons, synaptic vesicles undergo variations in pH ranging from 7.4, during the process of fusion with the plasma membrane, to 5.5, during storage as a resting pool of vesicles and when the vesicles are being loaded with neurotransmitters (8–11). Furthermore, α -synuclein has been suggested to be degraded via lysosomal pathways which can have pH values as low as 5.5 (12). These examples indicate that the local pH environment experienced by α -synuclein can change very significantly during its functional cycle. α -Synuclein has also been identified as the major constituent of Lewy bodies, intraneuronal inclusions found in the brains of patients suffering from Parkinson's disease and a number of related neurodegenerative disorders including dementia with Lewy bodies (13). In addition, studies of families with a history of Parkinson's disease have resulted in the identification of a series of familial mutations (Fig. 1), leading to early-onset (A30P, E46K, A53T, G51D) or late-onset (H50Q) forms of the disease (14–19). Large variations in the age of disease onset have, however, been observed in each case (Fig. 1) (19).

The effects of these sequence changes on the kinetics of the overall aggregation of α -synuclein have been the subject of intense studies since the discovery of this protein as the main constituent of Lewy bodies (13, 22–31). The A53T variant is generally considered to accelerate aggregation compared with the WT protein (22), but less agreement exists concerning the effects of the A30P mutation, because this variant has been variously reported to aggregate more slowly (28), more rapidly (23–26), or with the same rate (22) as the WT protein. Moreover, for the E46K (27) and H50Q (29, 30) variants, the aggregation process has been reported to be

accelerated compared with WT α -synuclein, but the G51D variant has been reported to aggregate more slowly than the WT protein (31). These studies provide key insights into the influence of the mutations on the overall aggregation process, but a more detailed knowledge of how the individual microscopic processes of the aggregation reaction are affected by each of the disease-associated, single-point mutations would be of considerable additional value (32–34) (Fig. 1).

A distinctive feature of the process of amyloid formation by α -synuclein, as indicated by *in vitro* studies, is that homogeneous primary nucleation (i.e., the formation of growth-competent nuclei directly from monomeric species in bulk solution) is undetectably slow (20). It is well established, however, that the nucleation process is strongly enhanced in the presence of surfaces, such as those of lipid vesicles (21, 35) or hydrophobic polymers [in the form of nanoparticles (36) or macroscopic surfaces (37)]. In the case of lipid membranes, the chemical properties of the lipids composing the membrane were shown to influence very significantly the magnitude by which they can trigger the aggregation of α -synuclein, suggesting a role of the membrane composition in balancing normal and aberrant behavior of the protein (38). In addition, although aggregation is not normally observed under quiescent conditions in the absence of appropriate surfaces, the addition of preformed seed fibrils allows the growth of aggregates

Significance

Proteinaceous deposits composed primarily of amyloid fibrils of α -synuclein are a hallmark of a range of neurological disorders including Parkinson's disease. Mutations of the gene encoding α -synuclein have been associated with familial variants of Parkinson's disease and lead to early- and late-onset forms of the disease. Using a combination of experimental and theoretical approaches specifically designed for the study of α -synuclein, we report that the mutations dramatically affect the rate of lipid-induced fibril production and surface-catalyzed fibril amplification processes. Such a systematic study provides new insights into the influence that single-amino acid replacements in α -synuclein can have on the two steps of α -synuclein aggregation that are likely to be crucial for disease: the initial formation of aggregates and their proliferation.

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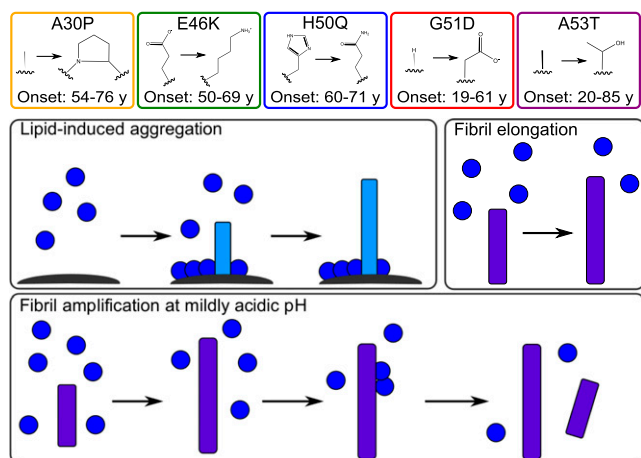


Fig. 1. Disease-associated mutations of α -synuclein and specific steps in the mechanism of aggregation. (Upper) Schematic illustration of the mutational variants of α -synuclein associated with familial Parkinson's disease. The amino acid substitution and the range of the age of onset reported in patients are indicated in each case (19). (Lower) Schematic summary of individual processes involved in the aggregation of WT α -synuclein (20, 21).

to be studied under well-defined conditions (20, 39). Indeed, if experiments are carried out at low concentrations of seed fibrils, secondary processes, by which existing fibrils amplify in number, can be directly studied. In this context, it has recently been found that the aggregation mechanism of α -synuclein is highly dependent on the solution conditions, in particular the pH of the solution (20). The quantities of added seed fibrils and the pH of the solution can, therefore, be used to adjust the relative contributions of the growth and proliferation of α -synuclein fibrils.

On the basis of this progress in our understanding of the complex mechanism of α -synuclein aggregation, we have developed and used a strategy in which individual microscopic processes in the overall aggregation reaction are probed by experiments under specifically designed conditions. To study the initial formation of fibrils from soluble monomeric species, we investigated lipid-induced aggregation by adding lipid vesicles to solutions of monomeric α -synuclein at neutral pH (21). The growth of fibrils was separately studied by addition of high (micromolar) concentrations of preformed fibrils to solutions of monomeric α -synuclein at neutral pH (20). Finally, we studied fibril amplification through secondary nucleation, by adding low (nanomolar) concentrations of preformed fibrils to solutions of monomeric α -synuclein under mildly acidic pH conditions (20, 37). Such a three-pronged strategy is particularly well suited for the study of aggregation and fibril formation by α -synuclein, because it considerably simplifies the mechanistic analysis of kinetic data, reflects the fact that the protein is exposed to varying environmental conditions during its cellular life cycle (2–7, 40, 41) and allows the study of the effect of each point mutation on each of the microscopic steps of α -synuclein aggregation.

Our results reveal that the rate of fibril elongation is only affected to a small extent by the changes in the amino acid sequence of α -synuclein associated with familial Parkinson's disease. However, we find that these mutations can alter by several orders of magnitude the rates of the initiation of fibril production induced by lipid vesicles and of their proliferation through autocatalysis at the surfaces of existing fibrils and hence dramatically change the relative importance of specific steps within the mechanism of amyloid fibril formation.

Results

The Rate of Lipid-Induced α -Synuclein Aggregation Is Dramatically Altered by the Mutations. The first set of experiments was carried out in the presence of negatively charged lipid vesicles, conditions

we have recently reported to increase the rate of primary nucleation of α -synuclein amyloid formation by several orders of magnitude (21). First, we used CD to monitor the transition from the highly disordered solution state to the α -helical membrane bound state of the mutational variants associated with the binding to vesicles prepared from 1,2-dimyristoyl-sn-glycero-3-phospho-L-serine (DMPS) (21) and to determine their binding affinity to these vesicles, as described previously for WT α -synuclein (21) (*SI Appendix*, Fig. S1 *A* and *B*). All of the lipid-binding curves are well described by a one-step binding model (21), and both the WT and mutant forms of α -synuclein bind to the vesicles in conformations with high degrees of α -helicity and with submicromolar affinity (*SI Appendix*, Fig. S1*A*). Interestingly, the number of lipid molecules associated with each molecule of α -synuclein, determined from these fits, is very similar for WT (28.2 ± 0.8), A30P (33.2 ± 3.4), E46K (30.4 ± 1.6), H50Q (35.2 ± 2.1), and A53T (26.7 ± 1.1) (within experimental error; *SI Appendix*, Fig. S1) but is notably higher for G51D (42.5 ± 4.5) (see *SI Appendix* for more details). This observation, together with the fact that the lipid-bound G51D shows a smaller degree of α -helicity (less than 70% of that of WT), is consistent with the expectation that a smaller region of each G51D molecule is associated with the membrane surface in a compact helical state and that a larger region of G51D is unfolded and therefore could generate greater steric hindrance than that of WT at the surface of the vesicles. In addition, binding of each of the α -synuclein variants to DMPS vesicles induces a reduction in the melting temperature of the constituent lipids (*SI Appendix*, Fig. S1*C*), as previously observed for WT α -synuclein (21), indicating a higher binding affinity of the WT protein and the mutational variants studied here for the membrane in its fluid phase rather than in its gel phase (38).

Having characterized the binding of the proteins to the lipid vesicles, we monitored the kinetics of amyloid formation by the mutational variants in the presence of DMPS vesicles (Fig. 2 and *SI Appendix*, Fig. S2). The aggregation rate of A30P was slightly enhanced (Fig. 2*B*) and that of A53T was markedly enhanced (Fig. 2*F*) compared with that of the WT protein (Fig. 2*A*), whereas the rates of H50Q (Fig. 2*D*), E46K (Fig. 2*C*), and G51D (Fig. 2*E*) were much slower than that of WT. The fibrils formed by A30P and A53T in the presence of the DMPS vesicles were found to have a morphology very similar to that of the fibrils formed by WT α -synuclein under these conditions; in all these cases, thin fibrillar structures characteristic of protofilaments [2.6 ± 0.4 nm (WT), 3.4 ± 0.6 nm (A30P), and 2.9 ± 0.7 nm (A53T) in diameter] attached to small spherical species (attributable to the vesicles) were the dominant species visible in the AFM images (Fig. 2*A*, *B*, and *F*, *Insets*). It is interesting in this context that the amino acid substitution in the familial Parkinson's disease-associated variants of α -synuclein are all within the region containing residues 26–97, which have been reported to modulate the interaction and affinity of the protein to membranes (42) (Fig. 2*G*). Interestingly, in the case of H50Q, a second aggregation phase was observed at longer times (~ 70 h) under quiescent conditions, which is characterized by the formation of fibrils with a morphology similar to that of the mature fibrils formed by WT α -synuclein under conditions where the nucleation reaction takes place at air–water or water–polymer interfaces (6.6 ± 0.9 nm in diameter; *SI Appendix*, Fig. S2*B*) (20). The intensity of the thioflavin-T (ThT) fluorescence of the H50Q fibrillar sample at the end of the second phase was observed to be an order of magnitude higher than that of the first phase and indeed of that of the other variants (i.e., WT, A30P, E46K, and A53T) (Fig. 2 and *SI Appendix*, Fig. S2*B*) under these conditions, indicating that ThT binds differently to the mature fibrils than to lipid-induced protofilaments.

In the light of these observations, we then analyzed the aggregation kinetics in detail using a one-step nucleation model (21), in which the nucleation reaction is assumed to occur at the

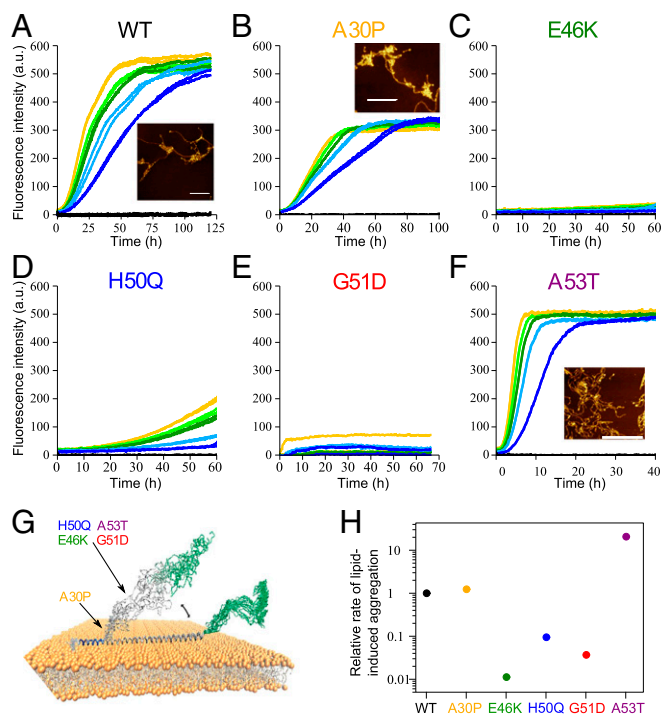


Fig. 2. The rate of lipid-induced α -synuclein aggregation is dramatically altered by the disease-related mutations. (A–F) Change in ThT fluorescence intensity when monomeric α -synuclein [WT (A), A30P (B), E46K (C), H50Q (D), G51D (E), and A53T (F)] was incubated in the absence (black) and in the presence of 100 μ M DMP5 under quiescent conditions at pH 6.5 and 30 $^{\circ}$ C. The protein concentrations used in this study were: 20 μ M (dark blue), 40 μ M (light blue), 60 μ M (green), 80 μ M (light green), and 100 μ M (yellow). The insets in A, B, and F show the AFM images of the fibrils formed under these conditions. (Scale bars: 1 μ m.) a.u., arbitrary units. (G) Schematic illustration of α -synuclein interacting with a lipid bilayer highlighting the positions of the mutations in the membrane sensor region which has been reported to modulate the membrane binding affinity. Adapted from ref. 42 with permission from Macmillan Publishers Ltd: *Nature Communications*, copyright (2014). (H) Relative rate of lipid-induced α -synuclein aggregation, determined by fitting the early times of the aggregation curves of each mutant to a one-step nucleation model (*SI Appendix*, Fig. S3).

surface of the vesicles and is then followed by the growth of fibrils from the vesicle-bound nuclei (Fig. 2H and *SI Appendix*, Fig. S3). Our global analysis shows that the A30P mutation does not notably affect the lipid-induced aggregation rate, whereas the A53T mutation increases this rate by approximately two orders of magnitude relative to that of WT α -synuclein. The mutations E46K, H50Q, and G51D all decrease the lipid-induced aggregation rate, by one (H50Q) or two (E46K, G51D) orders of magnitude, relative to that of WT α -synuclein (Fig. 2H).

α -Synuclein Fibril Elongation at Neutral pH Is Only Weakly Influenced by the Mutations. We next carried out experiments that involved measurements of the aggregation kinetics of WT α -synuclein and variants (A30P, E46K, H50Q, G51D, and A53T) in the presence of 5 μ M preformed seed fibrils (monomer equivalent concentration) at pH 6.5 under quiescent conditions, where the kinetics are dominated by elongation of the added seeds (20). We observed that the monomeric forms of all of the sequence variants of α -synuclein were able to elongate the preformed fibrils of all variants (Fig. 3A and *SI Appendix*, Fig. S4). To facilitate direct comparison of the variants, in each case, the measured elongation rates were normalized relative to that of “homogeneous” elongation (i.e., to the elongation rate of fibrils of a given variant by monomeric protein molecules of the same variant). We found

that in all cases, such normalized elongation rates varied by less than one order of magnitude, except for the value determined for monomeric G51D, which elongates the fibrils of different variants notably more slowly (Fig. 3B). Interestingly, the variants in which the amino acid substitution is situated within the region known to form the β -sheet rich core of the fibrils (i.e., E46K, H50Q, G51D, and A53T) were found to have the highest rates of elongation when the monomers elongated their own seed fibrils (i.e., for homogeneous elongation) (Fig. 3B). The morphologies of all of the fibrils formed from the cross-seeding experiments with the mutational variants were observed to be very similar (\sim 7 nm in diameter; *SI Appendix*, Fig. S5A).

The Rate of Fibril Amplification of α -Synuclein Under Mildly Acidic pH Conditions Is Strongly Influenced by the Mutations. We next performed sets of cross-seeding experiments (Fig. 4A and *SI Appendix*, Fig. S6) at mildly acidic pH and under quiescent conditions, where α -synuclein fibrils have been shown to amplify autocatalytically and to form higher order assemblies (20). Under these conditions, the observed change in the overall aggregation rate (see *SI Appendix*, Eq. 10) is therefore attributable to the simultaneous changes in the monomer concentration and the number of growing fibrils, the latter being the sum of processes that increase and decrease the number of fibril ends [i.e., secondary nucleation and higher order assembly (20), respectively].

The results show that under these conditions the A30P, E46K, and A53T variants, like the WT protein (20, 37), show sigmoidal aggregation profiles, which are indicative of the contribution of secondary nucleation to the growth kinetics, and that are independent of the specific variant from which the seed fibrils are formed (Fig. 4A and B and *SI Appendix*, Fig. S6). Detailed analysis of these data shows that the rates of fibril amplification (*SI Appendix*, Fig. S7A) for the WT, A30P, E46K, and A53T variants are within an order of magnitude of each other and that the reaction half times (*SI Appendix*, Fig. S7B) display only a weak dependence on the initial monomer concentration in the range studied in the present work; this finding suggests that in each of these cases, the autocatalytic process is independent of the monomer concentration, which could, for example, be the result of the saturation of all of the effective nucleation sites on the surfaces of the fibrils (43). The behavior of the H50Q and G51D variants is, however, very different from that of the WT protein as only very slow aggregation is observed for these variants (Fig. 4 and *SI Appendix*, Fig. S6). To be able to distinguish whether this striking difference in behavior originates from differences in the rates of elongation or secondary nucleation under these conditions, we carried out experiments at higher seed concentrations at acidic pH. Here, the depletion of monomeric protein molecules through fibril elongation is so rapid that secondary nucleation does not detectably contribute to the aggregation process, as suggested by the exponential, rather than sigmoidal kinetic curves. We found that the elongation rates, although significantly faster than at neutral pH (20), are the same within an order of magnitude for all variants (*SI Appendix*, Fig. S8). Taken together, these findings suggest that the α -synuclein variants H50Q and G51D have a very slow secondary nucleation rate. The amino acid substitutions in each of the two variants (H for Q and G for D) imply a change in the net charge from that of the WT protein under these conditions, and so we probed the pH dependence of their aggregation rates at pH values between 7.4 and 5.2, at both low and high seed concentrations. We did not detect aggregation at low seed concentrations for either H50Q or G51D under these conditions (*SI Appendix*, Fig. S9A and B), suggesting the absence of notable secondary nucleation throughout this pH range. At higher seed concentrations, we found that the elongation rate increased in magnitude, notably from pH 6.6 to pH 5.8 (*SI Appendix*, Fig. S9C–E), a behavior very similar to that reported for the WT protein (20). The very low aggregation rates for the H50Q and G51D variants at low seed concentrations and acidic pH, therefore, indicate a negligible rate of aggregate formation through secondary nucleation.

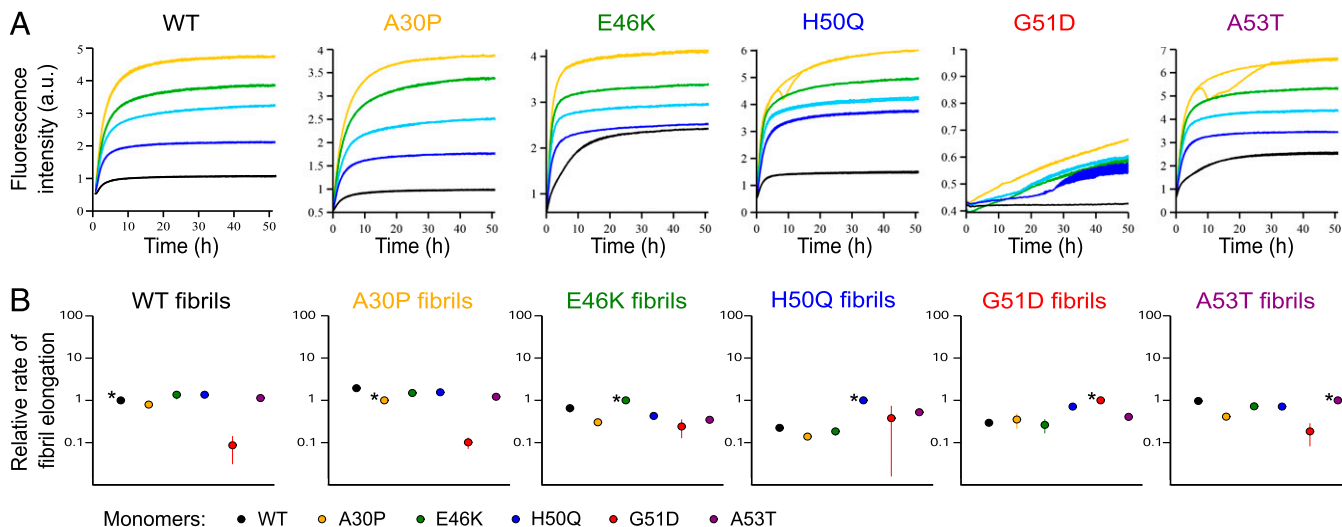


Fig. 3. The elongation rate of α -synuclein is only mildly affected by the disease-associated mutations. (A) Change in ThT fluorescence when monomeric α -synuclein at different concentrations [10 μ M (black), 30 μ M (blue), 50 μ M (light blue), 70 μ M (green), and 100 μ M (yellow)] was incubated in the presence of 5 μ M preformed fibrils of the WT protein under quiescent conditions at pH 6.5 and 37 $^{\circ}$ C. a.u., arbitrary units. (B) Normalized ratios of the elongation rates of the disease-associated variants relative to that of each variant monomer elongating its own preformed fibrils. Note that the elongation of seeds by monomers of the same type (marked by *) is always among the fastest (except for A30P).

Discussion

Although the biological function of α -synuclein has not yet been elucidated in detail, suggestions include, among others, the regulation of vesicular plasticity (40), oxidative stress, and mitochondrial function (41). Furthermore, α -synuclein has been shown to experience heterogeneous cellular environments, including differences in pH ranging from 5.5 to 7.4 (2–7, 40, 41). In the light of these findings, and of our knowledge of the high dependence of α -synuclein aggregation on the pH of its environment (20, 37) and on the presence of surfaces (21, 36), we have developed a comprehensive strategy to further our overall understanding of the way that α -synuclein converts from its normally soluble state into amyloid fibrils. This strategy involves

studying separately the different processes involved in the aggregation reaction, namely the initial formation steps, the growth of the fibrils, and their amplification (Figs. 2–4). Our approach relies on the presence of a variety of features to induce aggregation, such as negatively charged lipids or acidic solution conditions, and represents a step toward a characterization of amyloid formation by α -synuclein that is relevant to key aspects of physiological conditions.

This approach has been applied in the present study to characterize the effects of five mutational variants, namely A30P, E46K, H50Q, G51D, and A53T, in α -synuclein that are associated with familial Parkinson’s disease (14–18). Our results show that single-amino acid substitutions in the 140-residue chain of α -synuclein not only increase or decrease the overall rate of

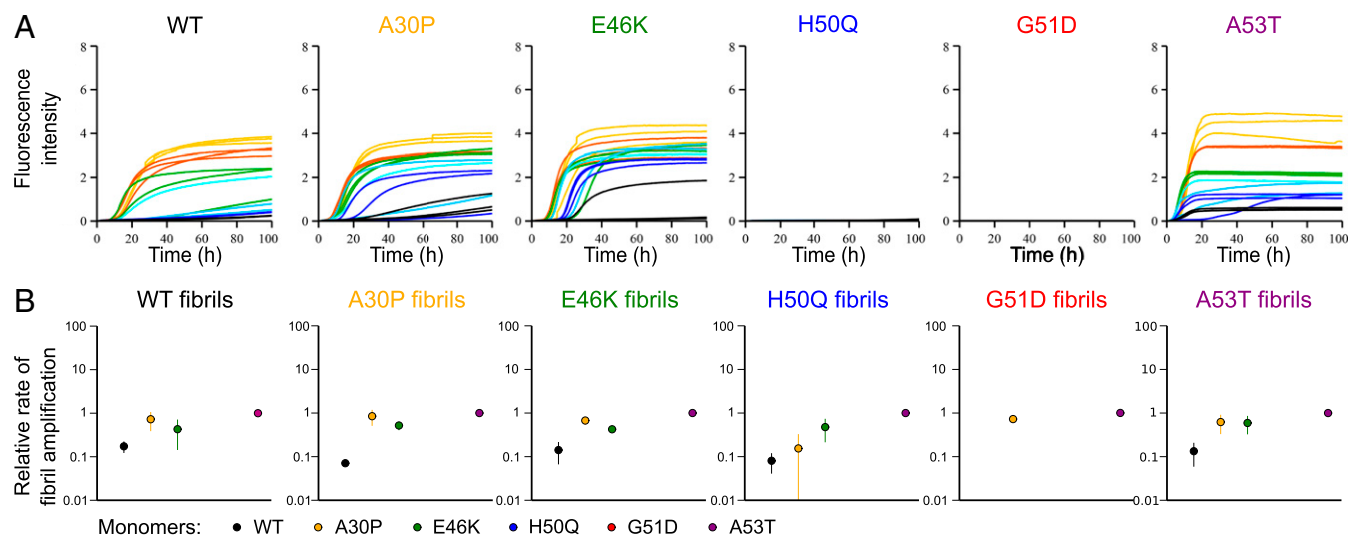


Fig. 4. The rates of fibril amplification of WT α -synuclein, A30P, E46K, and A53T, but not that of H50Q and G51D, are enhanced at mildly acidic pH. (A) Change in ThT fluorescence intensity when monomeric α -synuclein (WT, A30P, E46K, H50Q, G51D, and A53T) was incubated in the presence of 35 nM preformed fibrils of the WT protein under quiescent conditions at pH 4.8 and 37 $^{\circ}$ C. The protein concentrations used in this study were 20 μ M (black), 30 μ M (dark blue), 40 μ M (light blue), 50 μ M (green), 70 μ M (red), and 100 μ M (yellow). (B) The relative rates of fibril amplification of the α -synuclein variants were averaged over the concentrations studied here and normalized relative to the rate of the fastest aggregating monomeric variant (i.e., A53T α -synuclein).

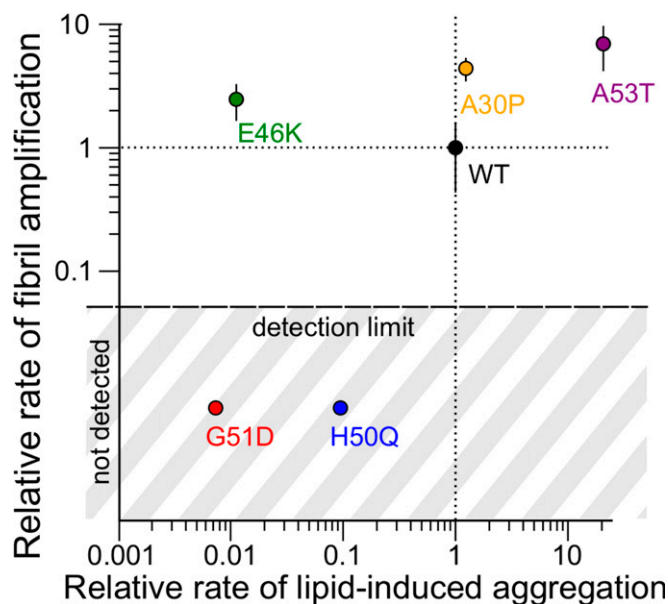


Fig. 5. Mutations associated with familial Parkinson's disease alter the lipid-induced aggregation and fibril amplification steps of α -synuclein amyloid formation. The plot summarizes the effects of the five single-amino acid mutations studied in this work on the rates of lipid-induced aggregation and fibril amplification at mildly acidic pH. The data were normalized relative to the rates of WT (black circle) α -synuclein.

aggregation, as noted in earlier studies (22–30) and as reproduced by us (*SI Appendix*, Fig. S10), but also dramatically affect the relative importance of individual microscopic steps (Fig. 5). More specifically, our findings indicate that the process of fibril elongation by addition of monomeric protein molecules to preformed fibrils is only relatively weakly affected by the mutations, with the measured rates spanning less than one order of magnitude (*SI Appendix*, Fig. S11). The data reveal, however, that the elongation rates of E46K, H50Q, G51D, and A53T are faster for monomeric proteins that add to fibrils formed by the same sequence compared with those formed from different variants (Fig. 3). All of those sequence changes are located well within the region of the α -synuclein sequence that is thought to make up the cross- β core of the amyloid fibrils [ca. residues 30–110 (44, 45)].

Although the fibril elongation step in the aggregation of α -synuclein is not very strongly influenced by the disease-associated single point mutations, we find that the rate of surface-induced fibril production processes, via interaction with lipid vesicles or fibril surfaces at mildly acidic pH, can be dramatically affected by these sequence modifications (Fig. 5). Indeed, the rate of lipid-induced aggregation was found to vary by three orders of magnitude across the five variants (Figs. 2*H* and 5) with values both higher (A53T) and lower (E46K, H50Q, and G51D) than that of the WT protein; these findings are consistent with previous descriptions of the overall relative rates of aggregation under nonquiescent reaction conditions where surface effects are also likely to be dominant (22, 31).

These observations reveal that lipid-induced generation of fibrils is highly sensitive to the specific sequence of the protein, in particular, the region encompassing the residues 46–51. Similar effects on the aggregation rate are also evident at mildly acidic pH, where secondary nucleation and higher-order assembly processes are strongly enhanced (20). In particular, the rates of fibril amplification for A53T, A30P, E46K, and WT α -synuclein were observed to be within an order of magnitude of each other, whereas the rates of secondary nucleation were found to be undetectably slow for H50Q and G51D (Fig. 4), suggesting that a decrease in charge from +1 to 0 or from

0 to –1 in this region interferes with the secondary nucleation process. Overall, we note that the rates of individual microscopic steps in the process can differ by several orders of magnitude between different variants, whereas in previous reports under shaking conditions, the observed differences appear to be much smaller. This effect is likely to stem from the fact that shaking leads not only to enhanced primary nucleation rates (37) but also to strongly enhanced rates of aggregate fragmentation that are likely to be rate determining in such cases (46). The latter effect, therefore, is likely to mask, at least in part, the intrinsic differences in the nucleation rate constants.

Because both lipid-induced fibril production and secondary nucleation of α -synuclein aggregation are heterogeneous processes [i.e., they occur on surfaces (of lipid vesicles and fibrils, respectively)], it is interesting to ask whether these two types of events are effectively the same process occurring on different surfaces or if they are fundamentally different events. We find that mutations associated with a change in the protonation state of the protein induce a change either in the rate of the lipid-induced aggregation alone [E46K (+2)] or in the rates of both surface-induced fibril production processes [H50Q (–1) and G51D (–1)] (Fig. 5), suggesting that the two types of processes are significantly different. However, given that these nucleation events are both linked to a pre-equilibrium of α -synuclein binding to the respective surfaces, such differences could be caused by differential surface binding affinity or else by differences in the nucleation event itself. In any case, our results suggest that electrostatic interactions play an important role in the various nucleation events.

Translating these findings into a medically relevant context is, however, far from straightforward, not least because of the large variations in the ages of onset reported for even the same familial forms of Parkinson's disease (19). Moreover, biological processes such as the pathways by which wild-type α -synuclein is normally degraded were shown to be affected by some of the disease-associated mutations (A30P and A53T), leading to the accumulation of the corresponding mutants and a gain of their toxicity (47). Finally, the lack of a simple correlation between the *in vitro* aggregation propensity and the age of disease onset reinforces the view that cellular factors are able to modulate the intrinsic aggregation propensities that are defined by the amino acid sequence.

In summary, the present work represents a detailed analysis of the influence of mutations associated with familial Parkinson's disease on the different microscopic steps in the process of α -synuclein aggregation. In particular, our results show that the ability of a given sequence to elongate preformed seed fibrils is affected only to a relatively small degree by the identities of the monomeric and fibrillar variants. By contrast, the rates of the processes responsible for the *de novo* production of fibrils (lipid-induced aggregation and secondary nucleation) have been found to differ by multiple orders of magnitude depending on which mutational variant is involved in monomeric form. In conclusion, the results reveal that single-point mutations in α -synuclein can lead to dramatic changes in the balance of the individual molecular steps of α -synuclein aggregation and hence in the overall mechanism of amyloid formation.

Materials and Methods

Detailed information on experimental measurements and on methods of kinetic analysis can be found in *SI Appendix*. WT α -synuclein and the mutational variants were expressed and purified as previously described (20). Lipid vesicles were prepared by freeze-thaw cycles and sonication (21). Differential scanning calorimetry thermograms were acquired using a Microcal VP-DSC calorimeter. Far-UV circular dichroism spectra were recorded on a JASCO J-810 spectrophotometer. Aggregation experiments were performed in low-binding, clear-bottomed half-area 96-well plates under quiescent conditions and amyloid formation was monitored by the increase in ThT fluorescence. Atomic force microscopy images were obtained with a Nanowizard II atomic force microscope using tapping mode in air.

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