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Oligomers of Parkinson's Disease-Related α-Synuclein Mutants Have Similar Structures but Distinctive Membrane Permeabilization Properties

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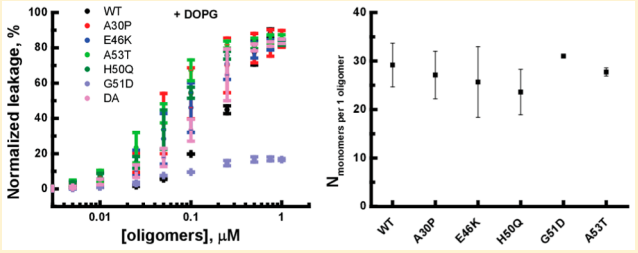
Supporting Information

ABSTRACT: Single-amino acid mutations in the human α-synuclein (αS) protein are related to early onset Parkinson's disease (PD). In addition to the well-known A30P, A53T, and E46K mutants, recently a number of new familial disease-related αS mutations have been discovered. How these mutations affect the putative physiological function of αS and the disease pathology is still unknown. Here we focus on the H50Q and G51D familial mutants and show that like wild-type αS, H50Q and G51D monomers bind to negatively charged membranes, form soluble partially folded oligomers with an aggregation number of ∼30 monomers under specific conditions, and can aggregate into amyloid fibrils. We systematically studied the ability of these isolated oligomers to permeabilize membranes composed of anionic phospholipids (DOPG) and membranes mimicking the mitochondrial phospholipid composition (CL:POPE:POPC) using a calcein release assay. Small-angle X-ray scattering studies of isolated oligomers show that oligomers formed from wild-type αS and the A30P, E46K, H50Q, G51D, and A53T disease-related mutants are composed of a similar number of monomers. However, although the binding affinity of the monomeric protein and the aggregation number of the oligomers formed under our specific protocol are comparable for wild-type αS and H50Q and G51D αS, G51D oligomers cannot disrupt negatively charged and physiologically relevant model membranes. Replacement of the membrane-immersed glycine with a negatively charged aspartic acid at position 51 apparently abrogates membrane destabilization, whereas a mutation in the proximal but solvent-exposed part of the membrane-bound α-helix such as that found in the H50Q mutant has little effect on the bilayer disrupting properties of oligomers.



α-Synuclein is an intrinsically disordered protein involved in PD.[1,2] Of all Parkinson's disease patients, 10−20% have a hereditary form of the disease. Disease-related mutations include gene duplications, triplications, and point mutations in the SNCA gene encoding αS. In the past 20 years, six different SNCA point mutations that result in specific amino acid substitutions in the αS sequence and are associated with PD have been identified: A30P,[3] E46K,[4] H50Q,[5,6] G51D,[7,8] A53T,[9] and A53E.[10,11] Two recently discovered αS mutations, H50Q and G51D, result in the rapid progression of the disease.[5−8] The pathology observed in patients with the G51D mutation is further characterized by a moderate response to treatment with levodopa, loss of autonomy, and frequent psychiatric symptoms.[8] The clinical pathology of the H50Q mutation is similar to the disease pathology of the E46K and A53T mutations.[5]

All disease-related amino acid mutations are located in the N-terminal membrane binding part of αS. These mutations may therefore directly affect αS conformation and membrane binding. As observed for wild-type (WT) αS and other disease mutants, G51D and H50Q adopt an α-helical conformation upon binding negatively charged membranes or SDS micelles.[6,8] The affinity for membranes differs between the WT protein and the known αS mutants; E46K, A53T, and A30P exhibit binding affinities for negatively charged vesicles higher than, similar to, and lower than that of WT αS, respectively.[12]

Not only membrane binding but also aggregation into amyloid fibrils is affected by the amino acid substitutions. It was

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reported that A53T and E46K aggregate faster than WT, while A30P has a slower aggregation rate.[12,13] In comparison to WT, the two newly discovered mutations, H50Q and G51D, have been reported to show faster and slower kinetics of fibril formation, respectively.[6,8,14−16]

Studies conducted in animals, cell systems, and model membranes have identified soluble αS oligomers as the potentially toxic species in PD.[17−22] To be able to relate possible toxicity to oligomer structure, αS oligomers have been characterized using various biophysical and biochemical techniques, including circular dichroism (CD),[23,24] single-molecule photobleaching,[25] small-angle X-ray scattering (SAXS),[1,26−28] atomic force microscopy (AFM),[29] nuclear magnetic resonance (NMR) spectroscopy,[30] confocal microscopy,[31] and electron microscopy.[32] These techniques have shown a variety of shapes and sizes, determined the molecular weight of the oligomers,[33,34] and established the aggregation number of the oligomers, that is, the number of monomers per oligomer.[25,26,34]

Here we quantify the membrane binding affinity of the G51D and H50Q mutants, study their aggregation into amyloid fibrils, and characterize the aggregation number (that is, the number of monomers per oligomer) and the membrane disrupting properties of oligomers formed from these αS disease mutants. SAXS studies show that oligomers formed from WT and these disease-related mutants are composed of a similar number of monomers. In contrast to the other disease mutants, no differences in G51D membrane binding, fibril formation, and oligomer structure compared to those of WT αS were observed. However, G51D oligomers were not able to permeabilize membranes. We relate the inability of the G51D oligomers to permeabilize negatively charged DOPG and mitochondrial membrane mimics to the position of the amino acid mutation and its role in membrane binding.

MATERIALS AND METHODS

Preparation of Oligomeric α-Synuclein. The expression and purification of human WT and disease-related αS mutants were performed as previously described.[35] Oligomers from WT and disease-related αS mutants were prepared as described in the literature.[36] The protein concentration was determined by measuring the absorbance using a Shimadzu spectrophotometer at 276 nm, assuming a molar extinction coefficient of 5600 M^{-1} cm^{-1}.[37] Oligomers were purified and separated from monomers using size-exclusion chromatography on a SuperdexTM 200 10/300 GL column (GE Healthcare Bio-Sciences AB, Uppsala, Sweden) using 10 mM Tris, 150 mM NaCl, and 0.01% NaN_3 as an elution buffer. Separation of oligomers from monomers is based on size, where larger particles (oligomers) elute first. On the basis of size-exclusion elution profiles, oligomer fractions were collected and concentrated up to an equivalent monomer concentration of ∼100 μM for SAXS measurements and an equivalent monomer concentration of up to ∼50 μM for membrane leakage experiments.

Preparation of SUVs and Binding of αS Monomers to SUVs. Small unilamellar vesicles (SUVs) for studying membrane binding of monomers using CD were prepared using the procedure previously described by Stefanovic et al.[36] Although the defects that are typically present in artificial SUVs may affect αS binding, the CD experiments were performed with SUVs instead of LUVs. We made this choice because the scattering of light by LUVs renders the CD spectra very noisy in the information-rich lower-wavelength regions. In the literature, it has even been suggested that in experiments with LUVs, CD signals obtained below 215 nm need to be discarded.[38] First ,we used simple negatively charged 1,2-dioleoylphosphatidylglycerol (DOPG) membranes to test the binding of the monomers. However, cell membrane lipid compositions are much more complex. αS colocalizes with mitochondrial membranes,[39] and we therefore tested if the binding of αS is comparable between DOPG and mitochondrial membrane mimics [4:3:5 18:1 cardiolipin (CL):1-palmitoyl-2-oleoylphosphatidylethanolamine (POPE):1-palmitoyl-2-oleoyl-phosphatidylcholine (POPC) ratio]. To test the binding affinities of αS for DOPG and CL:POPE:POPC membranes, monomeric WT, G51D, and H50Q αS at a concentration of 4 μM were titrated with an SUV solution. Lipid concentrations in the SUV solutions for the titration were between 0.004 and 1.2 mM. The following equation was applied to calculate the binding parameters:[40]

$$[C] = \frac{[L_{tot}] + [M_{tot}] + K_d - \sqrt{(-[L_{tot}] - [M_{tot}] - K_d)^2 - 4[M_{tot}][L_{tot}]}}{2} \quad (1)$$

where K_d is the equilibrium dissociation constant, C is the complex concentration, M_{tot} is the total monomer concentration, ($M_{tot} = M + C$) and was obtained as a function of the total lipid concentration ($L_{tot} = L + C$). To obtain the K_d values, all the data were normalized.

Preparation of LUVs and Calcein Release Assay. DOPG and CL:POPE:POPC large unilamellar vesicles (LUVs) for calcein release assays were prepared as previously described.[36] Briefly, the lipid film was hydrated with 50 mM calcein, 10 mM Hepes, and 60 mM NaCl to obtain an osmolarity of 320 mOsm kg^{-1}. After the sample had been subjected to the five freeze−thaw cycles, the solution was extruded 11 times through 100 nm pore size filters (Whatman, Maidstone, U.K.). Finally, PD-10 columns filled with Sephadex G-100 (GE Healthcare Bio-Sciences AB) were used to remove the free calcein. Calcein release kinetics of the model membranes were followed on a Varian Cary Eclipse fluorometer (Varian Inc., Palo Alto, CA), by recording the emission intensity at 515 nm for excitation at 495 nm. To completely lyse the vesicles, Triton X (0.5%) was added. All the data points were normalized using the intensity after Triton X treatment as 100% leakage.

Small-Angle X-ray Scattering. SAXS measurements on αS oligomers were performed in triplicate; i.e., three different batches of each kind of oligomer were made, except for G51D oligomers, for which we only succeeded in obtaining one batch that provided enough material. Experiments were performed on samples containing an equivalent monomer concentration of αS of ∼100 μM dissolved in 10 mM Tris, 150 mM NaCl, 0.01% NaN_3 buffer using the SAXS/WAXS setup at the BM26-DUBBLE-Dutch-Belgian Beamline (ESRF, Grenoble, France). For G51D oligomers, for two oligomer preparations we could not obtain sufficiently high concentrations to yield SAXS signals above the limit of detection. Approximately 100 μL of a sample was placed into 1.5 mm quartz capillaries, and two-dimensional images were collected using two Pilatus photon counting detectors. The sample-to-detector distance was 6.6 m. The wavelength for the incident X-ray was 0.1 nm and the beam size 2.5 mm × 4.5 mm, and the energy of the X-rays was 12 eV, resulting in a q range of ∼0.03−1.5 nm^{-1}. For data

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reduction, buffer (background) scattering values were subtracted from the protein solution scattering values and standards were used to convert the scattering values to values on an absolute scale.

First, we determined the radius of gyration, R_g, and $I(0)$, using Guinier's law: $I(q) = I(0)\exp(-q^2R_g^2/3)$. Guinier plots provide information about the average size of the particles in the solution. The oligomers studied are in the first approximation spherical structures. For spherical particles, Guinier plots, where $\ln[I(g)]$ is plotted as a function of q^2, give a linear dependency for $qR_g < 1.3$. From the slope of this curve, R_g was determined at $qR_g < 1.3$ and $I(0)$ was obtained by extrapolation to $q = 0$.

The aggregation numbers of the oligomers can be determined indirectly by an equation that describes the scattering of the particles in a solution:

$$I(q) = nV^2\Delta\rho_{\text{rel.sol}}{}^2P(q)S(q) \quad (2)$$

where n is the number of scattering particles per unit volume, V is the volume of the particle, $\Delta\rho_{\text{rel.sol}} = \rho_{\text{particle}} - \rho_{\text{solvent}}$ is the excess scattering length density or contrast, $P(q)$ is the form factor of the particle and is related to the particle's shape, and $S(q)$ is the structure factor that defines interparticle interactions in the solution.

When the SAXS intensities are extrapolated to $q = 0$, then $I(0)$ is given by[41]

$$I(0) = nV^2\Delta\rho_{\text{rel.sol}}{}^2 \quad (3)$$

In our case, $\Delta\rho_{\text{rel.sol}}$ was calculated to be 0.0002864 nm^{-2} using the contrast calculator in the SAXS utilities program (extension of Matlab),[42] using a ρ_{particle} of 1.37 g/cm^3 as the experimentally determined average protein density.[43,44] $I(0)$ and volume V can be calculated using the R_g determined via the Guinier approximation. For calculating the molecular weight, M_w, of the particles and/or proteins, the following equation was applied:[45]

$$I(0) = N(\Delta\rho V)^2 = c\Delta\rho^2v^2M_w/N_A \quad (4)$$

where N_A is Avogadro's number and c is the protein or particle concentration. The aggregation number N is extracted by dividing the M_w estimated from eq 4 by the molecular weight of the αS monomer. To visualize if disordered regions are present in the oligomer, the data obtained are also presented in a Kratky plot by plotting $I(q)q^2$ versus q.[46]

Kinetics of Aggregation. Solutions containing 100 μM αS monomers of the different disease mutants in 10 mM Tris-HCl and 100 mM NaCl (pH 7.4) were incubated at 37 °C while being constantly shaken in a Tecan SAFIRE II plate reader. Aggregation of protein into amyloid fibrils was followed in a thioflavin T (ThT) fluorescence assay. For this purpose, 5 μM ThT was used. Changes in ThT fluorescence were followed using an excitation wavelength of 446 nm and a bandwidth of 10 nm, and the emission intensity at 485 nm was recorded with an emission bandwidth of 10 nm as a function of time. Aggregation lag times were determined as previously described by Willander et al.[47]

Atomic Force Microscopy (AFM). To visualize the amyloid fibrils, samples were prepared for AFM imaging. Samples of aggregated protein (after ThT assay) were placed on the mica substrate using the procedure described by Sweers et al.[48] The dried samples were visualized by AFM using tapping mode. During these experiments, an NSC 36 tip B was used, with a force constant of 1.75 N/m (NanoAndMore GmbH, Wetzlar, Germany). All images obtained during these experiments are 4 μm × 4 μm in size with 512 pixels and a z range of 20 nm.

RESULTS

Binding of αS Monomers to SUVs. To determine the membrane binding affinities of WT, H50Q, and G51D αS for DOPG and CL:POPE:POPC SUVs, we titrated αS monomers with different concentrations of SUVs. Conformational changes of WT, G51D, and H50Q αS monomers upon binding to the membranes were followed by recording CD spectra between 190 and 260 nm. Representative CD spectra of the titration of WT monomers with DOPG and CL:POPE:POPC SUVs are given in Figure S1 of the Supporting Information. To follow α-helix formation and hence membrane binding, the mean residue ellipticities (MREs) at 222 nm are presented as a function of lipid concentration in Figure 1. As described in Materials and Methods, a simple binding model was used to calculate the equilibrium dissociation constant (K_d) from the titration of αS monomers with DOPG (Figure 1A) and CL:POPE:POPC (Figure 1B). The dissociation constants obtained by fitting the titration curves for lipid mixtures and WT, G51D, and H50Q αS are listed in Table 1. For all three αS species, the K_d values were of the same order of magnitude, but the affinity for DOPG membranes was 1 order of magnitude

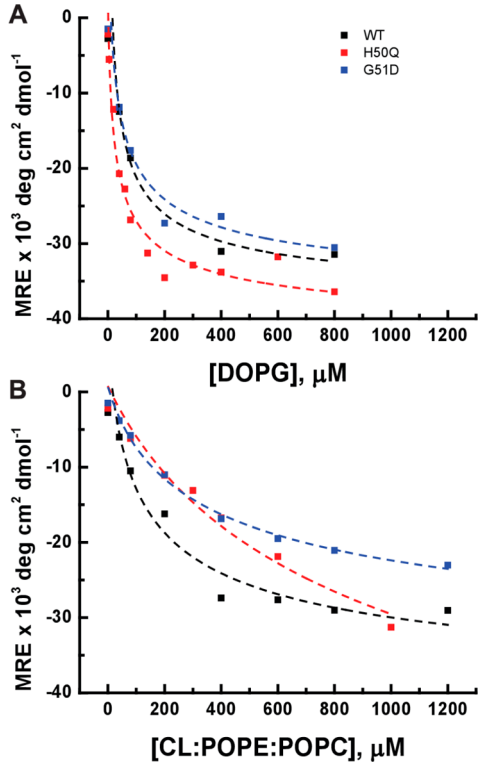

Figure 1. Titration of αS monomers by (A) DOPG and (B) CL:POPE:POPC (4:3:5) SUVs. Conformational changes in WT, H50Q, and G51D αS were followed by CD spectroscopy. The membrane-bound α-helical conformation is characterized by a negative peak at 222 nm in the CD spectrum. The binding affinity was determined from the changes in mean residue ellipticity (MRE) at 222 nm as a function of lipid concentration. For the determination of K_d, the data were normalized to 1. Experiments were performed at 25 °C for 4 μM protein in 10 mM potassium phosphate buffer (pH 7.4).

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Table 1. Binding Constants of αS Monomers with SUVs

	K_d (μM)		
lipid	WT	H50Q	G51D
DOPG	70 ± 15	32 ± 5	64 ± 14
CL:POPE:POPC	186 ± 45	1078 ± 324	307 ± 37

higher than the affinity for the CL:POPE:POPC mitochondrial membrane mimics. The observed higher affinity for DOPG membranes is in agreement with previous studies of membrane binding of WT and the A30P, A53T, and E46K amino acid mutations in αS.[24,49,50]

Aggregation Studies. To further characterize the disease-related αS mutants, the aggregation of 100 μM monomers into amyloid fibrils was studied in 10 mM Tris-HCl buffer and 100 mM NaCl (pH 7.4). Under these buffer conditions, the aggregation lag times of most disease mutants were comparable to the lag time observed for WT protein, with only A30P exhibiting significant differences (Figure 2A). The aggregation lag time of A30P was more than 2-fold longer than the lag time for the other proteins. The fibrils that were obtained with the different disease mutants were visualized using AFM. This analysis confirmed that all disease mutants are able to form amyloid fibrils. The morphologies of the amyloid fibrils formed by WT and the disease-related mutants are qualitatively comparable (Figure 2B).

Calcein Release Assay. The toxicity of αS aggregation in PD has been related to oligomeric species that bind and permeabilize membranes. We performed a calcein release assay to test the ability of oligomers of disease-related αS mutants to permeabilize membranes composed of anionic phospholipids (DOPG) and membranes mimicking the mitochondrial phospholipid composition (CL:POPE:POPC). LUVs were filled with calcein at a self-quenching concentration; upon incubation with oligomers, an increase of fluorescence emission intensity results from dye dilution due to membrane permeabilization. When oligomers were added, they induced fast calcein leakage from DOPG LUVs that was not observed for LUVs in which the mitochondrial membrane composition was mimicked. In Figure 3, the normalized leakage (percent) measured 30 min after incubation with the respective oligomeric species is represented as a function of oligomer concentration (monomer equivalent). Except for G51D, all oligomers were able to induce almost complete content loss of negatively charged DOPG membranes at the highest concentrations tested. The disease-related oligomers are slightly more efficient than WT oligomers in permeabilizing membranes; lower concentrations are required for comparable leakage (Figure 3A). Oligomer-induced calcein leakage from CL:POPE:POPC LUVs was very slow. Dye leakage reached a plateau approximately 18 h after oligomer addition.[36] G51D and E46K oligomers were not able to induce leakage from the CL:POPE:POPC LUVs (Figure 3B). Even for the A53T and A30P oligomers that were most efficient in permeabilizing LUVs with this membrane composition, the maximal leakage reached only 30%.

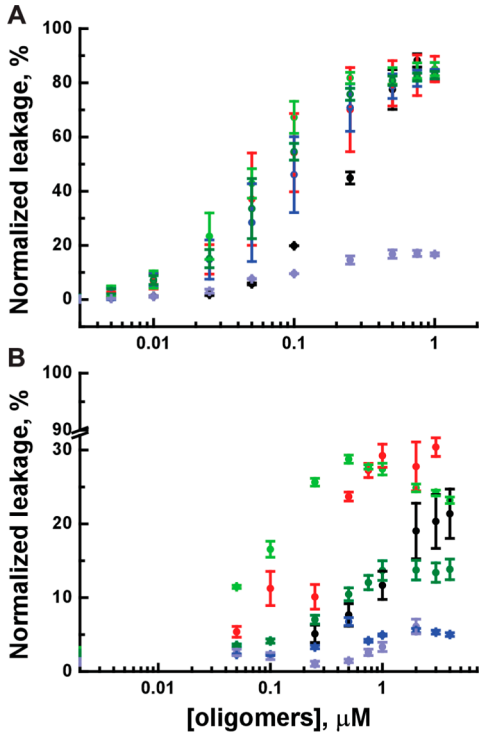

Figure 3. Release of calcein from (A) DOPG and (B) CL:POPE:POPC LUVs as a function of the concentration of oligomeric WT (black circles) and disease-related αS mutants: A30P (red), E46K (blue), A53T (light green), H50Q (dark green), and G51D (light purple). The oligomer concentration is given as the equivalent monomer concentration.

Aggregation Number. Although the membrane binding affinity and aggregation kinetics of G51D, H50Q, and WT αS

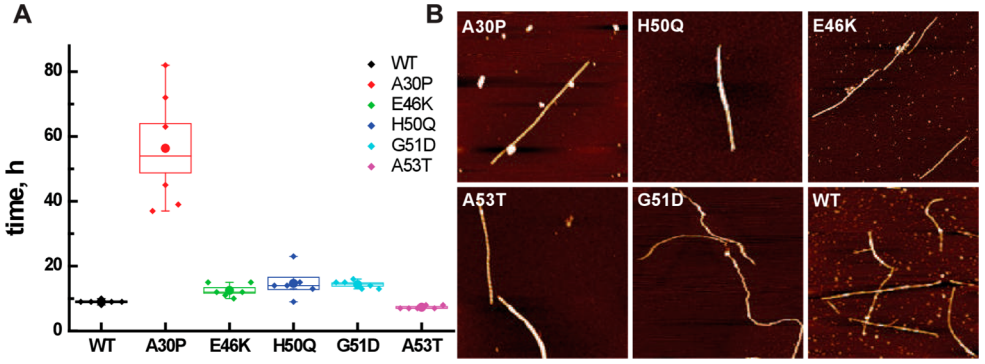

Figure 2. Aggregation of disease-related αS mutants. Fibril formation was followed using a classical ThT assay. (A) Lag times were extracted from the six individual aggregation repeats for each variant and are presented as a box plot. (B) The presence of fibrils in the plateau phase of aggregation was confirmed using AFM.

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monomers are comparable, the ability of oligomers formed from these variants to permeabilize membranes differs. The different permeabilization propensity of the oligomers may result from differences in oligomer composition and structure. The aggregation numbers of the oligomers were therefore characterized by SAXS. Figure 4A shows the scattering curves for the oligomers of WT αS and five different disease mutants. The scattering curves have very similar shapes. From these scattering curves, the aggregation numbers of the oligomers were estimated using the following procedure. R_g and $I(0)$ were determined using the Guinier approximation (Figure 4B). To calculate the aggregation number, the molecular weight (M_w) of protein oligomers has to be determined.[51,52] We used eqs 2 and 3 to determine the aggregation numbers. The values for R_g and $I(0)$ determined from the Guinier approximation and the aggregation numbers derived for the different oligomers can be found in Figure 4C and Table 2.

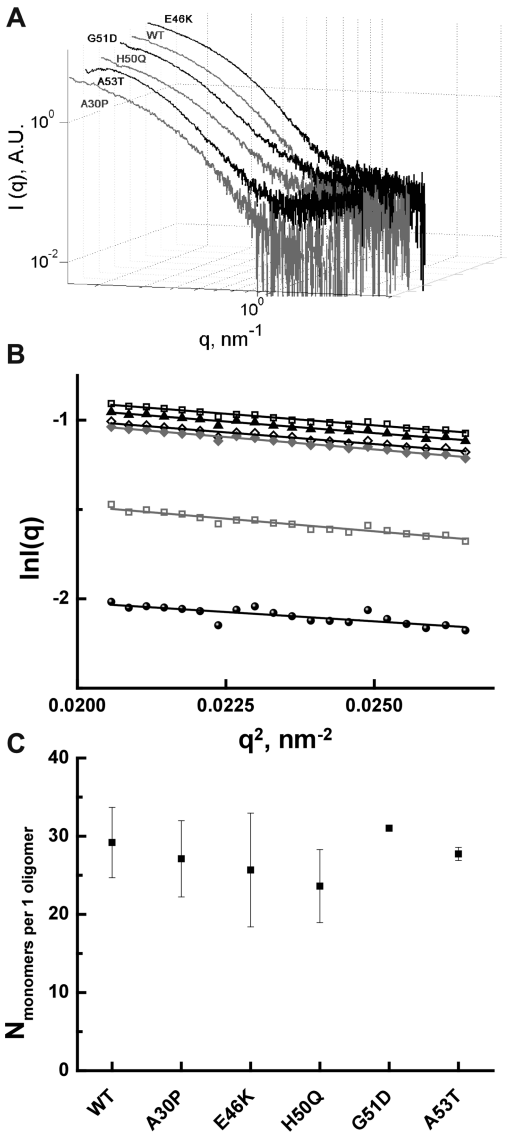

Figure 4. Small-angle X-ray scattering curves of αS oligomers. (A) Experimentally obtained SAXS curves for WT, A30P, E46K, H50Q, G51D, and A53T αS oligomers. The intensity of the buffer was subtracted from the intensity of the samples. (B) Averaged Guinier plots for all oligomers. From top to bottom: WT (black empty squares), A53T (black filled triangles), E46K (black empty diamonds), H50Q (gray filled diamonds), A30P (gray empty squares), and G51D (black filled circles), respectively. (C) From the Guinier plots, R_g and $I(0)$ are obtained and used to calculate the M_w and number of monomers in the oligomers. For each protein, three independently prepared batches of oligomers were measured, except for G51D oligomers, for which we succeeded in preparing only one batch with sufficient material.

Table 2. Summary of the Parameters Obtained from SAXS Data by Analysis of the Guinier Plot

	Guinier plot[a]		
oligomer	R_g (nm)	$I(0)$ (cm^{-1})	$N_{m/1oli}$[b]
WT	8.8 ± 0.3	0.68 ± 0.04	29 ± 4
A30P	8.3 ± 0.1	0.35 ± 0.04	27 ± 5
E46K	8.9 ± 0.4	0.66 ± 0.29	26 ± 7
H50Q	9.1 ± 0.2	0.63 ± 0.09	24 ± 4
G51D[c]	7.9	0.20	31
A53T	8.8 ± 0.4	0.66 ± 0.2	28 ± 1

[a]The errors are the standard deviations of the R_g values determined for three independently prepared batches of oligomers of the different proteins. [b]$N_{m/1oli}$ is the number of monomers per oligomer. [c]Only one batch of oligomers could be measured.

As we can observe in Figure 4C, the aggregation number (number of monomers per oligomer, $N_{m/1oli}$) estimated for the oligomers of each disease-related mutant was approximately 30, which agrees well with previously published data from our group for WT oligomers and dopamine-induced oligomers using a single-molecule photobleaching approach.[25,53] This value is also consistent with those found by Otzen and co-workers using SAXS.[34]

We further analyzed the data by plotting $q^2I(q)$ versus q in Kratky plots. These Kratky plots are used to monitor the degree of compactness of a protein to evaluate the extent of folding of proteins. Globular molecules follow Porod's law, resulting in a bell-shaped curve, whereas extended molecules, such as unfolded peptides, have a plateau or increase at higher q ranges.[54] The Kratky plots presented in Figure 5 and Figure S2

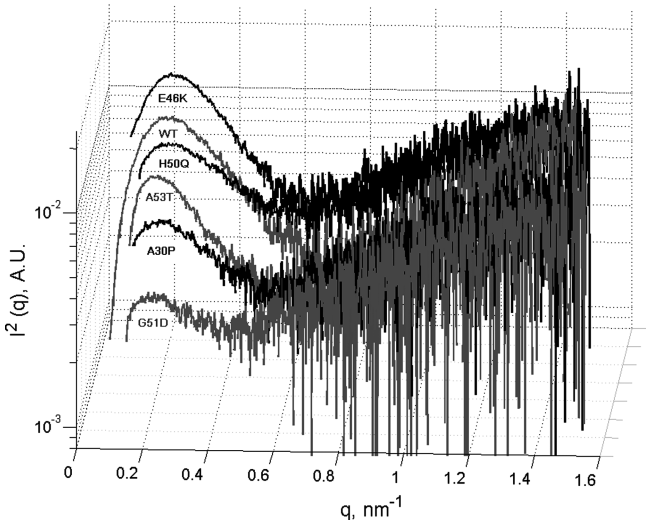

Figure 5. Composite representation of Kratky plots for oligomers composed of WT and disease-related αS mutants: E46K, H50Q, A53T, A30P and G51D. The shape of the spectra indicates that all αS oligomers are partially folded.

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of the Supporting Information show part of a bell-shaped curve and an increase in q^2I with an increasing q, which indicates that oligomers composed of WT and disease-related αS mutants are composed of partially folded proteins.

DISCUSSION

The H50Q and G51D disease mutants have been reported to adopt an α-helical conformation when binding membranes.[6,8] Here we have determined the binding affinities of monomeric WT, G51D, and H50Q for vesicles composed of negatively charged DOPG and a phospholipid composition mimicking the mitochondrial membrane. Our CD data show that WT, G51D, and H50Q have comparable binding affinities for negatively charged DOPG membranes, while small differences between the mutants were visible with CL:POPE:POPC vesicles. In the latter case, an approximately 5 times higher binding affinity was observed for WT compared to that of H50Q. The membrane composition had a large effect on the observed K_d; αS has a 1 order of magnitude higher affinity for DOPG than for CL:POPE:POPC membranes. It is known that a single amino acid substitution can cause changes in binding affinity, with A30P monomers demonstrating a binding affinity lower than those of E46K, A53T, and WT.[12] Whether and how αS amino acid mutations affect membrane binding most likely depends on the position of the amino acid substitution. However, although in G51D a membrane-immersed residue is replaced with an acidic amino acid, this change has only a small effect on K_d. The lack of a profound effect of the G51D point mutation on LUV binding may result from its proximity to the postulated break in the membrane-bound α-helix.[55] The replacement of H with Q in the H50Q mutation involves only a small change in the polarity of a solvent-exposed residue and has probably therefore little effect on membrane binding.

Under the conditions studied here, all the disease mutants form amyloid fibrils, and only for A30P was the aggregation lag time significantly increased compared to those of WT and the other disease mutants (see Figure 2A and Figure S2 of the Supporting Information). The shape of the aggregation profiles looks similar for all the mutants, suggesting that the fibril growth mechanism is comparable (Figure 2A). The morphologies of the fibrils in solution after a ThT aggregation experiment were studied by AFM, which confirmed that all mutants form qualitatively similar fibrils. Although it was previously reported that H50Q aggregated faster and G51D slower than WT,[6,8,14,15] we did not observe any significant difference in aggregation kinetics between these disease mutants and WT αS. This difference could be caused by the experimental conditions used; both the buffer and αS concentrations used in our experiments were different from those in the published reports. Whereas we studied the aggregation of 100 μM αS monomers in 10 mM Tris and 100 mM NaCl (pH 7.4), Ghosh et al.[6] used a 3-fold higher H50Q concentration in 20 mM glycine-NaOH buffer and reported a difference in aggregation kinetics between H50Q and WT. The slower aggregation kinetics of G51D compared to those of WT[8] was observed in 20 mM Tris and 150 mM KCl (pH 7.5), which is similar to our aggregation conditions.

A growing consensus suggests that oligomers are the toxic species involved in PD.[29,56] Oligomers are thought to disrupt the integrity of cellular membranes. Here we tested if oligomers of the different αS disease mutants differ in their ability to induce membrane permeabilization. Our results showed a large difference in the permeabilization of DOPG membranes by G51D oligomers compared to other oligomers. G51D oligomers could not induce more than 20% permeabilization of DOPG membranes. Oligomers of the newly discovered H50Q mutant showed permeabilization of DOPG vesicles similar to that of WT oligomers. However, oligomers of both newly discovered mutants are less prone to inducing permeabilization of mitochondrial model membranes than WT, where G51D oligomers showed almost no permeabilization (Figure 3).

WT, G51D, and H51Q monomers do not differ much in membrane affinity (Figure 1). The differences in the ability of αS oligomers to permeabilize membranes may therefore be related to differences in oligomer structure. SAXS has proven to be a very useful technique for obtaining more detailed insights into the structure and size of protein oligomers.[27,34] We therefore performed SAXS investigations of oligomers formed from WT and a range of disease-related point mutants.

However, our data show that the size and aggregation number of the six different αS oligomers studied are very similar. All oligomers studied yield aggregation numbers of ∼30 (Figure 4C), and the Kratky plots show that all oligomers are partially folded structures (Figure 5 and Figure S2 of the Supporting Information). CD measurements confirm the partially folded nature of the oligomer and show that oligomers contain β-sheets.[23,36] The partially folded nature of the oligomer is also in good agreement with results of tryptophan quenching experiments that suggest that residues 4−90 make up the core of the WT oligomer while the C-terminus remains solvent-exposed.[57] The similarities in aggregation number and structure observed by SAXS can, however, not exclude that differences exist. SAXS probes the average size of the species in solution, and it is very difficult to distinguish between two or among three different species. The mean aggregation number we derive for this WT oligomer is comparable to the values found with SAXS[34] by other investigators, and with single-molecule photobleaching experiments.[25] The difference in permeabilization cannot therefore be attributed to the oligomer aggregation number or overall structure.

Although the binding affinities of WT and G51D monomers are comparable, the exchange of a small neutral amino acid G with a polar negatively charged D resulted in a lower level of oligomer-induced permeabilization of DOPG membranes. The comparable binding affinity indicates that the G51D substitution does not result in large changes in the membrane binding α-helical structure of the monomer. Lashuel and coworkers have shown that G51D monomers show binding profiles qualitatively similar to that of WT protein but have also suggested that residues 45−55 in the G51D mutant exhibit somewhat decreased helicity, which may be attributed to N-terminal fraying of helix 2 in the presence of the glycine to aspartic acid mutation.[16] In studies with acidic vesicles, these authors have also shown that, consistent with our studies, the inherent capacity of helix formation by the G51D mutant is not abrogated, but that the mutation interferes specifically with negatively charged membranes. These changes in biochemical properties of the G51D mutant are likely also manifested in the G51D oligomer.

We note that with the G51D substitution an acidic amino acid (D) would become exposed to a lipophilic environment.[55] Although the G51D αS oligomers bind membranes, the partially folded structure of the oligomer may prevent binding of the complete α-helix. In the absence of a high-resolution structure in the membrane-bound state, we speculate that when

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fewer residues per monomer are available, the relative contribution of position 51 may become more important. Thus, the G51D oligomer may not bind membranes effectively, or the G51D substitution may make it more difficult to distort the lipid bilayer because fewer amino acid residues are immersed in the bilayer.

■ ASSOCIATED CONTENT

Supporting Information

CD spectra of the titration of WT αS with DOPG SUVS and individual Kratky plots shown in the composite graphic in Figure 5. The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/bi501369k.

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Notes

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■ ABBREVIATIONS

αS, α-synuclein; AFM, atomic force microscopy; CD, circular dichroism; CL, 18:1 cardiolipin; DOPG, 1,2-dioleoylphosphatidylglycerol; LUV, large unilamellar vesicle; MRE, mean residue ellipticity; PD, Parkinson's disease; POPC, 1-palmitoyl-2-oleoylphosphatidylcholine; POPE, 1-palmitoyl-2-oleoylphosphatidylethanolamine; SAXS, small-angle X-ray scattering; SUV, small unilamellar vesicle; WT, wild type.

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