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Mutation E46K increases phospholipid binding and assembly into filaments of human α-synuclein

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Abstract Missense mutations (A30P and A53T) in α-synuclein and the overproduction of the wild-type protein cause familial forms of Parkinson's disease and dementia with Lewy bodies. α-Synuclein is the major component of the filamentous Lewy bodies and Lewy neurites that define these diseases at a neuropathological level. Recently, a third missense mutation (E46K) in α-synuclein was described in an inherited form of dementia with Lewy bodies. Here, we have investigated the functional effects of this novel mutation on phospholipid binding and filament assembly of α-synuclein. When compared to the wild-type protein, the E46K mutation caused a significantly increased ability of α-synuclein to bind to negatively charged liposomes, unlike the previously described mutations. The E46K mutation increased the rate of filament assembly to the same extent as the A53T mutation. Filaments formed from E46K α-synuclein often had a twisted morphology with a cross-over spacing of 43 nm. The observed effects on lipid binding and filament assembly may explain the pathogenic nature of the E46K mutation in α-synuclein.
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Keywords: α-Synuclein; Dementia with Lewy bodies; Filament assembly; Lipid binding; Parkinson's disease

1. Introduction

Parkinson's disease (PD) is the most common movement disorder. Neuropathologically, it is defined by nerve cell loss in a number of brain regions, including the substantia nigra, and the presence there of Lewy bodies and Lewy neurites [1,2]. Abundant Lewy bodies and Lewy neurites in cerebral cortex are also the defining neuropathological characteristics of dementia with Lewy bodies (DLB), a common late-life dementia that is clinically similar to Alzheimer's disease. Ultrastructurally, Lewy bodies and Lewy neurites are composed of filamentous and granular material [3]. Missense mutations (A30P and A53T) in the α-synuclein gene were identified as the cause of autosomal-dominantly inherited forms of PD [4,5] and α-synuclein has been shown to be the major component of the abnormal filamentous inclusions of Lewy bodies and Lewy neurites in idiopathic PD and DLB [6–9]. In addition, the filamentous glial and neuronal inclusions of multiple system atrophy (MSA) have also been found to contain α-synuclein as a major component [10–12]. This work has shown that PD, DLB and MSA are α-synucleinopathies.

α-Synuclein is a 140 amino acid protein of unknown function that is abundantly expressed in brain, where it is concentrated in presynaptic nerve terminals [13,14]. The amino-terminal region of α-synuclein (amino acids 7–87) consists of seven imperfect repeats, each 11 amino acids in length, with the consensus sequence KTKEGV. The repeats are continuous, except for a four amino acid stretch between repeats 4 and 5, and partially overlap with a hydrophobic region (amino acids 61–95). The carboxy-terminal region (amino acids 96–140) is negatively charged. α-Synuclein is a natively unfolded protein with little ordered secondary structure [15] that binds to lipid membranes through its amino-terminal repeats, indicating that it may be a physiological lipid-binding protein [16–21]. Upon binding to phospholipid membranes, α-synuclein adopts structures rich in α-helical character [16,18–21].

Recombinant α-synuclein readily assembles into filaments that share many of the morphological and ultrastructural characteristics of the filaments present in human brain [22–31]. Assembly is a nucleation-dependent process and occurs through sequences located in the amino-terminal 100 amino acids of α-synuclein. The carboxy-terminal region, in contrast, inhibits assembly to a certain extent. The A53T mutation in α-synuclein accelerates the rate of filament assembly. The A30P mutation has been reported to increase the total aggregation of α-synuclein, but to slow the rate of mature filament formation. It reduces the binding of α-synuclein to natural lipid membranes, suggesting that this may lead to its progressive accumulation in the cytoplasm, thus facilitating aggregation and filament formation [17,32–34]. Mutation A53T has no significant effect on the ability of α-synuclein to bind to lipid membranes [17,32–35].

Recent work has confirmed and extended the relevance of α-synuclein dysfunction for the neurodegenerative process. Triplication of a 1.6–2.0 Mb region on the long arm of chromosome 4 has been found to cause an inherited form of PD-dementia [36,37]. One of an estimated 17 genes located in this region is the α-synuclein gene, suggesting that the simple overproduction of wild-type α-synuclein may be sufficient to

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cause PD-dementia. Moreover, a novel missense mutation (E46K) in α-synuclein has been identified in a Spanish family with an autosomal-dominantly inherited form of DLB [38]. Here, we have examined the functional effects of the E46K mutation on phospholipid binding and assembly of α-synuclein into filaments.

2. Materials and methods

2.1. Expression and purification of recombinant human α-synucleins

Expression constructs of human α-synuclein, A30P α-synuclein and A53T α-synuclein in pRK172 have been described [29]. Mutation E46K in α-synuclein was introduced using site-directed mutagenesis with QuikChange (Stratagene), followed by DNA sequencing. Bacterial expression and purification of α-synuclein, as well as procedures for immunoblotting, were as described [14]. Protein concentrations were determined by quantitative amino acid analysis. For constructs encoding N-terminal glutathione *S*-transferase (GST)-fusion tags, polymerase chain reaction (PCR) was used to amplify the coding region of human α-synuclein, A30P α-synuclein, E46K α-synuclein and A53T α-synuclein in bacterial expression vector pRK172 and inserts cloned into pGEX-5X-1 (Amersham Biosciences). Constructs were verified by DNA sequencing. Bacterial expression was done as described [14]. For constructs encoding C-terminal GST-fusion tags, the Gateway Technology from Invitrogen was used. PCR primers were designed according to the manufacturer's instructions and used to amplify the coding region of human α-synuclein. Entry clones were generated in pDONR201 and expression clones in pDEST24. Constructs were verified by DNA sequencing. Expression clones were transformed into BL21(DE3) and expression induced using 0.2% L-arabinoside (Sigma–Aldrich, Poole, Dorset, UK).

Pellets from 1 L culture were resuspended in 30 ml sonication buffer [20 mM HEPES, pH 7.3, 500 mM NaCl, 1 mM EDTA, 2 mM DTT, 2% Triton X-100 and protease inhibitor cocktail (Roche Diagnostics, Ltd.)]. The bacterial suspension was sonicated (10 cycles of 20 s bursts), incubated for 20 min at 4 °C and centrifuged at 20 000 × *g* for 1 h at 4 °C. The supernatant was added to 1 ml glutathione–agarose beads. Following a 2 h rotating incubation at 4 °C, the beads were washed three times with high salt buffer (20 mM HEPES, pH 7.3, 1 M NaCl, 1 mM EDTA, and 0.1% Triton X-100) and the GST-fusion protein eluted with 3 ml elution buffer (20 mM HEPES, pH 8.5, 250 mM NaCl, 15 mM glutathione, 1 mM EDTA, and 2 mM DTT). The protein solution was dialyzed once against PBSE (phosphate-buffered saline, 1 mM EDTA) and twice against HBSE (20 mM HEPES, pH 7.3, 100 mM NaCl, and 1 mM EDTA). Protein concentrations were determined by quantitative amino acid analysis.

2.2. Preparation of fluorescence-labeled liposomes

Brain phosphatidylcholine (PC) and cholesterol were purchased from Avanti Polar Lipids (Birmingham, AL). Brain phosphatidylserine (PS) was obtained from Sigma–Aldrich. 3,3′-Dioctadecyloxacarbocyanine perchlorate (DiO) was purchased from Molecular Probes (Eugene, OR). Lipids and DiO were dissolved in chloroform–methanol (1:1, v/v) and liposomes with the following compositions prepared: PC:PS:Cholesterol 35:35:30 mol% and PC:PS:Cholesterol 52.5:17.5:30 mol%. DiO fluorescent dye was added to 0.5% (w/v). Mixed lipids were dried under a stream of nitrogen and solubilized at 3 mM with HBSE in the presence of 2% *n*-octylglucoside. Following dialysis to remove the detergent (once against PBSE and twice against HBSE), the liposome suspension was stored at 4 °C and used within 24 h. For size determination, liposomes were placed on carbon-coated 400-mesh grids and stained with 1% uranyl acetate, and micrographs recorded at a nominal magnification of 20 000× on a Philips EM208S microscope. The negatives were scanned and liposome diameters in pixels measured using Photoshop (Adobe), followed by conversion into nanometers.

Extruded liposomes were prepared as described [39,40], using the mini-extruder from Avanti Polar Lipids. Briefly, mixed lipids were dried under a stream of nitrogen and solubilized at 3 mM with HBSE. Solubilized mixed lipids were then serially extruded (21 passages for each step) at room temperature through 100, 50 and 30 nm pore diameter Nucleopore polycarbonate membranes (Whatman). The extruded liposomes were stored at 4 °C and used within 24 h.

2.3. Liposome pull-down assay

The liposome pull-down assay was done essentially as described [41,42]. Briefly, recombinant GST-fusion protein (37.5 pmol) was bound to glutathione–agarose beads (30 μl). Following three washes with HBSE, the beads were resuspended in 100 μl HBSE and mixed with 100 μl diluted liposomes (75 μM lipid), followed by a 7.5 min incubation at 37 °C with vigorous shaking. The incubation was terminated by pelleting and the beads were washed three times with HBSE at 4 °C. Bound lipids were solubilized with 0.2% Triton X-100 in HBSE and transferred to 96-well microplates. Fluorescence was measured (460 nm excitation/538 nm emission) using a microplate fluorimeter (Fluoroskan, Labsystems).

2.4. Filament assembly

Prior to setting up the assembly experiments, α-synuclein proteins were spun for 30 min at 200 000 × *g*, to remove any insoluble material. For assembly, untagged α-synuclein proteins were used at 400 μM in 30 mM. 3-[*N*-Morpholino]propanesulfonic acid (Mops), pH 7.2, containing 0.02% sodium azide and 20 μM thioflavin T (ThT, Sigma–Aldrich), and placed in a shaking incubator at 37 °C, as described [29]. For a quantitative assessment of filament formation, ThT fluorescence was used [43]. Aliquots (10 μl) were removed at various time points and brought to 400 μl with 20 μM ThT in 50 mM glycine buffer, pH 8.5. Fluorimetry was performed using a Perkin–Elmer luminescence spectrophotometer LS 50B (set at 450 nm excitation/480 nm emission, with a scan speed of 200 nm/min and with excitation and emission slit widths of 5 and 2.5 nm, respectively). Control experiments with wild-type α-synuclein had established that the continuous presence of ThT did not affect assembly (data not shown). For a semi-quantitative assessment of filament formation, electron microscopy was used, as described [29]. Briefly, aliquots of assembly mixtures were placed on carbon-coated 400-mesh grids and stained with 1% potassium phosphotungstate, and micrographs recorded at a nominal magnification of 20 000× on a Philips model EM208S microscope.

3. Results

3.1. Liposome binding of wild-type and mutant human α-synucleins

A well-established liposome pull-down assay [41,42] was used to investigate the lipid binding of wild-type, A30P, E46K and A53T α-synucleins. Incubation of GST-α-synuclein attached to glutathione–agarose (1.25 pmol/μl bead) with fluorescence-labeled PC/PS/cholesterol liposomes (75 μM lipid) resulted in the saturable binding of liposomes to GST-α-synuclein. Binding was linearly dependent on the GST-α-synuclein concentration in the range of 0.31–2.5 pmol protein/μl bead. Incubation of liposomes with GST bound to glutathione–agarose gave a figure for non-specific binding of approximately 12%, with the binding of wild-type GST-α-synuclein taken as 100%. Non-specific binding due to GST bound to beads was subtracted from total binding to give the specific binding of α-synuclein.

α-Synuclein proteins with either N- or C-terminal GST-fusion tags were used. Both types of proteins were found to bind to the same extent to liposomes (data not shown), in agreement with reports showing that N-terminal GST-fusion tags did not interfere with the lipid binding of α-synuclein [34,44]. Constructs encoding α-synuclein with the N-terminal GST-fusion tag gave a significantly higher yield of intact protein than constructs encoding C-terminally tagged protein. We, therefore, used N-terminally tagged proteins in subsequent experiments.

Liposomes of two different lipid compositions were used, PC:PS:cholesterol 52.5:17.5:30 mol% (type A, Fig. 1A) and PC:PS:cholesterol 35:35:30 mol% (type B, Fig. 1B). The former is more similar in composition to synaptic vesicles than the

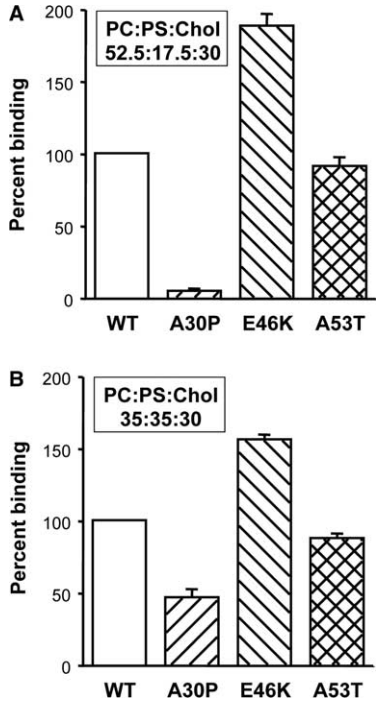

Fig. 1. Binding of wild-type (WT) and mutant (A30P, E46K, A53T) human α-synucleins to multilamellar liposomes. Liposomes of two different compositions (A and B) were used. The results are presented as percent binding of WT α-synuclein (taken as 100%) and expressed as means ± S.E.M. of the measurements obtained from three independent protein preparations, each run in duplicate.

latter [45,46]. The absolute binding of wild-type human α-synuclein to type B liposomes was approximately 4-fold higher than to type A liposomes. The two liposome types showed similar relative effects in binding of α-synuclein mutants, with the magnitude of the effects being greater for type A liposomes (Fig. 1). Mutation E46K increased the binding of α-synuclein to type A liposomes by 88% and to type B liposomes by 56%. Mutation A30P reduced the binding of α-synuclein to type A liposomes by 95% and to type B liposomes by 53%, whereas mutation A53T led to a non-significant 9–12% reduction in the binding of α-synuclein to both liposome types. Liposomes used for these measurements were heterogenous in size, with average sizes of 31 ± 15 nm (type A liposomes) and 32 ± 18 nm (type B liposomes).

Defined size (30, 50 or 100 nm) type B liposomes were produced by extrusion and the binding of wild-type and mutant (E46K) α-synucleins examined (Fig. 2). Total binding of GST α-synuclein to the extruded liposomes increased with decreasing liposome size, being 12-fold higher for 30 nm than for 100 nm liposomes. E46K α-synuclein bound significantly better than the wild-type protein to each preparation of extruded liposomes. Circular dichroism (CD) spectra from wild-type and mutant (A30P, E46K, A53T) α-synucleins in the presence of 50 nm type B liposomes confirmed that the lipid-associated conformation of wild-type α-synuclein was highly helical [16]. Of the three mutant proteins, the CD signal at 222 nm was strongest for the E46K mutation and weakest for the A30P mutation. The signal for the A53T mutation was intermediate and similar to that for wild-type α-synuclein (data not shown).

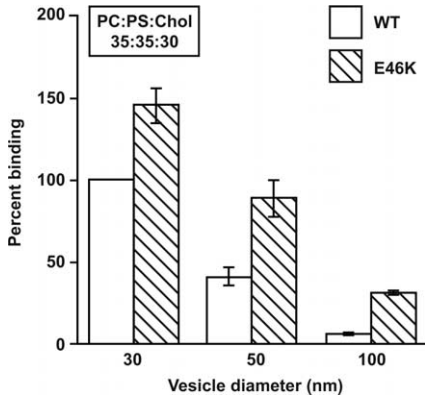

Fig. 2. Binding of wild-type (WT) and mutant (E46K) human α-synucleins to unilamellar liposomes. Type B liposomes of 30, 50 and 100 nm diameter were used. The results are presented as percent binding of WT α-synuclein to 30 nm liposomes (taken as 100%) and expressed as means ± S.E.M. of the measurements obtained from three independent protein preparations, each run in duplicate.

3.2. Filament assembly of wild-type and mutant human α-synucleins

Assembly of α-synuclein proteins was monitored quantitatively by ThT fluorescence and semi-quantitatively by electron microscopy. Both methods were used in parallel in all experiments and a close correspondence was observed between levels of ThT fluorescence and filament numbers.

The time-dependent changes in ThT fluorescence during incubation of untagged wild-type and mutant α-synucleins in a shaking incubator at 37 °C are shown in Fig. 3. E46K α-synuclein showed a faster rate of assembly and a 2.5-fold greater total assembly than the wild-type protein at 98 h (Fig. 3). The lag period was approximately 9 h for wild-type α-synuclein and 6 h for the E46K mutant. The A53T mutant behaved in a similar way to E46K α-synuclein. The rate of assembly of the A30P mutant was slower than for wild-type α-synuclein, but the amount of assembly was similar to that of the E46K and A53T mutants at 98 h (Fig. 3).

Incubation with shaking led to the bulk assembly of wild-type α-synuclein and all three mutants into filaments (Fig. 4).

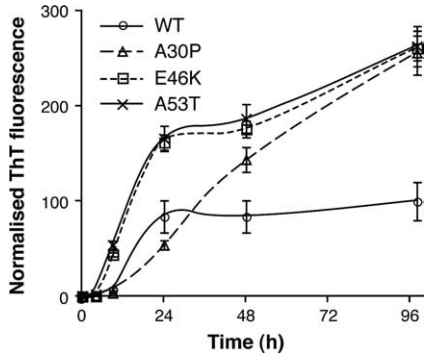

Fig. 3. Kinetics of fibrillation of wild-type (WT) and mutant (A30P, E46K, A53T) human α-synucleins, as monitored by the enhancement of thioflavin T (ThT) fluorescence intensity over time. The results are presented as normalized fluorescence (with the value for wild-type α-synuclein at 98 h taken as 100) and expressed as the means ± S.E.M. of the measurements obtained from three independent protein preparations.

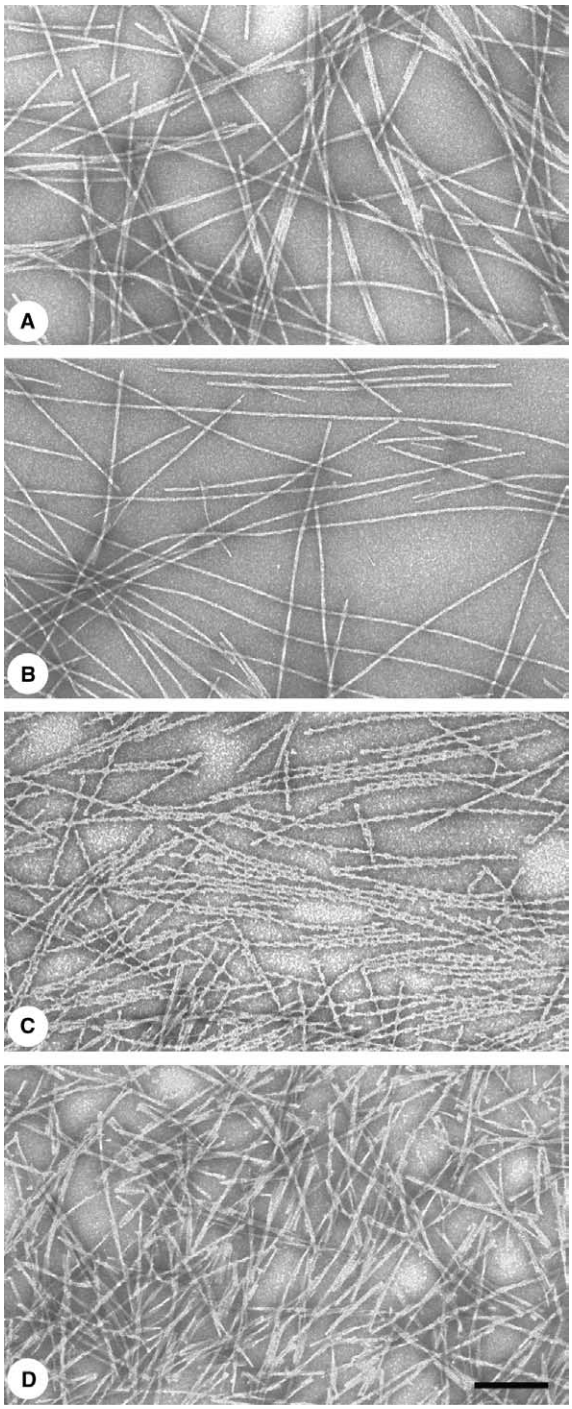

Fig. 4. Electron micrographs of wild-type and mutant human α-synuclein filaments. Filaments were assembled from recombinant wild-type α-synuclein (A), A30P α-synuclein (B), E46K α-synuclein (C) and A53T α-synuclein (D). The recombinant proteins were incubated in a shaking incubator at 37 °C for 24 h and represent samples of one of the assemblies included in Fig. 3. Scale bar, 200 nm.

The overall kinetics and amount of assembly seen by electron microscopy matched those measured with the ThT fluorescence assay. The rates of assembly of the E46K and A53T mutants were greater than those of the wild-type and A30P proteins. As we have reported previously [29], the morphologies of filaments of wild-type and A30P α-synucleins were very similar, having a filament width of 6–9 nm which varied slightly in a periodic manner along the length of the filament (Fig. 4A and B). Images of filaments made from the A53T mutant tended to show a more marked periodic variation in width between about 5 and 14 nm, giving a twisted appearance with a cross-over spacing of about 100 nm (Fig. 4D). By contrast, filaments assembled from E46K α-synuclein often showed a pronounced twisted appearance, with width varying between about 5 and 14 nm, but with a much shorter cross-over spacing of about 43 nm (Fig. 4C). The E46K filaments had a tendency to line up in register, giving arrays with a meshwork appearance.

4. Discussion

The present findings show that mutation E46K increases both the binding of α-synuclein to liposomes and its propensity to assemble into filaments. Phospholipid binding is mediated through the amino-terminal repeat region of α-synuclein, which undergoes a conformational transition from random coil to α-helical secondary structure upon lipid binding [16,18–21]. The recent analysis of the membrane interaction of α-synuclein by site-directed spin labeling has confirmed that the N-terminal repeats form extended α-helical structure, with the C-terminal region remaining unfolded [21]. It has shown moreover that equivalent positions within individual repeats are located in comparable positions with respect to membrane proximity. Each of the seven 11 amino acid repeats is believed to take up three helical turns. In membrane-bound α-synuclein, residue 46 is probably solvent-exposed, whereas lysine residues in the repeats are present at the level of the negatively charged lipid headgroups, where they contribute to binding through electrostatic interactions [21]. It remains to be determined whether substitution of the glutamic acid residue at position 46 by lysine results in increased lipid binding of α-synuclein through electrostatic interactions or whether additional mechanisms are involved. The interaction of α-synuclein with liposomes is dependent on the vesicle diameter, with small, highly curved vesicles resulting in the strongest interaction [16,47]. The same was observed here, when unilamellar vesicles of 30, 50 and 100 nm were compared. For each class of liposome, E46K α-synuclein bound significantly better than the wild-type protein, with the relative effect of the mutation increasing with liposome size.

Previously, the disease-causing mutation A53T was shown not to affect the binding of α-synuclein to artificial or natural membranes [17,32–35,44,48,49]. The present findings using type A and type B liposomes are in line with these results. In contrast to mutation A53T, mutation A30P has been shown to block the interaction of α-synuclein with rat brain membranes [17], triglyceride-rich lipid droplets in cultured cells [32] and yeast membranes [33]. However, its reported effects on the binding of α-synuclein to artificial membranes have been more variable, probably as a result of the different lipid compositions used [44,48,49]. In the present study, we detected a strong inhibitory effect of this mutation on the binding of α-synuclein to negatively charged liposomes. Unlike previous preparations [44,48,49], the liposomes used here contained cholesterol in addition to phospholipids. A recent study [34] has established that α-synuclein binds strongly to lipid rafts in nerve cells and that the A30P mutation eliminates this interaction, indicating that cholesterol may be required for both the

physiological binding of wild-type α-synuclein to lipid membranes and the strong inhibitory effect of the A30P mutation. A30 and E46 are highly conserved across many species [5,38]. A53, by contrast, is solely found in α-synuclein from humans and Old World primates, with T53 being present in rodents and several other mammalian species [50], suggesting that the A53T substitution may not influence the physiological function of α-synuclein. This is consistent with the observed lack of effect of A53T α-synuclein on lipid binding.

Human E46K α-synuclein assembled into a larger number of filaments at a significantly faster rate than the wild-type protein, as judged by ThT fluorescence and electron microscopy. The magnitude of the observed effects was similar to that of A53T α-synuclein, which is believed to cause disease through an increased tendency to form filaments [24–30]. Filaments formed from E46K α-synuclein were often strongly twisted with a cross-over spacing of approximately 43 nm. As reported previously [29], filaments formed from A53T α-synuclein were also twisted, but with a cross-over spacing of approximately 100 nm. Filaments formed from wild-type and A30P α-synuclein were straight, as described [29]. The structural relationship between straight and twisted α-synuclein filaments may be similar to that between straight and paired helical tau filaments in Alzheimer's disease [51] and various twisted ribbon morphologies in familial tauopathies [52]. It may involve an alternative strand packing, perhaps based on interfaces formed by different sequence repeats.

By ThT fluorescence, the A30P mutant showed a slower rate of filament assembly than wild-type α-synuclein, in agreement with previous studies [28,30]. However, ThT fluorescence at 98 h was approximately 2.5-fold higher for the A30P mutant than for the wild-type protein. All three mutants gave similar values of ThT fluorescence after 98 h of incubation and this was paralleled by increased numbers of filaments relative to the wild-type protein. It remains to be seen whether the E46K mutation also increases oligomerization and protofibril formation of α-synuclein, as previously shown for mutations A30P and A53T [28]. Protofibrils have been reported to permeabilize lipid vesicles upon binding [53].

Of the three disease-causing mutations, E46K is the only one to increase both lipid binding and filament assembly of α-synuclein. It is unclear whether lipid binding promotes or inhibits filament formation of α-synuclein, with some studies [32,54–57] reporting a stimulatory and others [35,58] an inhibitory effect. One study [59] has suggested that the effect of phospholipids on the assembly of α-synuclein is dependent on the ratio of protein to lipid, with high ratios accelerating filament formation and low ratios being inhibitory. In addition to a direct stimulatory effect of the E46K mutation on filament assembly, it is therefore possible that the increased binding of E46K α-synuclein to phospholipids may also contribute to disease through an indirect stimulatory effect on assembly.

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References

[1] Goedert, M. (2001) Nature Rev. Neurosci. 2, 492–501.
[2] Braak, H., Del Tredici, K., Rüb, U., de Vos, R.A.I., Jansen Steur, E.N.H. and Braak, E. (2003) Neurobiol. Aging 24, 197–211.
[3] Forno, L.S. (1996) J. Neuropathol. Exp. Neurol. 55, 259–272.
[4] Polymeropoulos, M.H., Lavedan, C., Leroy, E., Ide, S.E., Dehejia, A., Dutra, A., Pike, B., Root, H., Rubinstein, J., Boyer, R., Stenroos, E.S., Chandrasekharappa, S., Athanassiadou, A., Papapetropoulos, T., Johnson, W.G., Lazzarini, A.M., Duvoisin, R.C., Di Iorio, G., Golbe, L.I. and Nussbaum, R.L. (1997) Science 276, 2045–2047.
[5] Krüger, R., Kuhn, W., Müller, T., Woitalla, D., Graeber, M., Kösel, S., Przuntek, H., Epplen, J.T., Schöls, L. and Riess, O. (1998) Nature Genet. 18, 106–108.
[6] Spillantini, M.G., Schmidt, M.L., Lee, V.M.-Y., Trojanowski, J.Q., Jakes, R. and Goedert, M. (1997) Nature 388, 839–840.
[7] Spillantini, M.G., Crowther, R.A., Jakes, R., Hasegawa, M. and Goedert, M. (1998) Proc. Natl. Acad. Sci. USA 95, 6469–6473.
[8] Baba, M., Nakajo, S., Tu, P.S., Tomita, T., Nakaya, K., Lee, V.M.-Y., Trojanowski, J.Q. and Iwatsubo, T. (1998) Am. J. Pathol. 152, 879–884.
[9] Crowther, R.A., Daniel, S.E. and Goedert, M. (2000) Neurosci. Lett. 292, 128–130.
[10] Wakabayashi, K., Yoshimoto, M., Tsuji, S. and Takahashi, H. (1998) Neurosci. Lett. 249, 180–182.
[11] Spillantini, M.G., Crowther, R.A., Jakes, R., Cairns, N.J., Lantos, P.L. and Goedert, M. (1998) Neurosci. Lett. 251, 205–208.
[12] Tu, P.H., Galvin, J.E., Baba, M., Giasson, B., Tomita, T., Leight, S., Nakajo, S., Iwatsubo, T., Trojanowski, J.Q. and Lee, V.M.-Y. (1998) Ann. Neurol. 44, 415–422.
[13] Uéda, K., Fukushima, H., Masliah, E., Xia, Y., Iwai, A., Yoshimoto, M., Otero, D.A., Kondo, J., Ihara, Y. and Saitoh, T. (1993) Proc. Natl. Acad. Sci. USA 90, 11282–11286.
[14] Jakes, R., Spillantini, M.G. and Goedert, M. (1994) FEBS Lett. 345, 27–32.
[15] Weinreb, P.H., Zhen, W., Poon, A.W., Conway, K.A. and Lansbury, P.T. (1996) Biochemistry 35, 13709–13715.
[16] Davidson, W.S., Jonas, A., Clayton, D.F. and George, J.M. (1998) J. Biol. Chem. 273, 9443–9449.
[17] Jensen, P.H., Nielsen, M.H., Jakes, R., Dotti, C.G. and Goedert, M. (1998) J. Biol. Chem. 273, 26292–26294.
[18] Eliezer, D., Kutluay, E., Bussell, R. and Browne, G. (2001) J. Mol. Biol. 307, 1061–1073.
[19] Chandra, S., Chen, X., Rizo, J., Jahn, R. and Südhof, T.C. (2003) J. Biol. Chem. 278, 15313–15318.
[20] Bussell, R. and Eliezer, D. (2003) J. Mol. Biol. 329, 763–778.
[21] Jao, C.C., Ser-Sarkissian, A., Chen, J. and Langen, R. (2004) Proc. Natl. Acad. Sci. USA 101, 8331–8336.
[22] Crowther, R.A., Jakes, R., Spillantini, M.G. and Goedert, M. (1998) FEBS Lett. 436, 309–312.
[23] El-Agnaf, O.M.A., Jakes, R., Curran, M.D. and Wallace, A. (1998) FEBS Lett. 440, 67–70.
[24] Conway, K.A., Harper, J.D. and Lansbury, P.T. (1998) Nature Med. 4, 1318–1320.
[25] Giasson, B.I., Uryu, K., Trojanowski, J.Q. and Lee, V.M.-Y. (1999) J. Biol. Chem. 274, 7619–7622.
[26] Narhi, L., Wood, S.J., Steavenson, S., Jiang, Y., Wu, G.M., Anafi, D., Kaufman, S.A., Martin, F., Sitney, K., Denis, P., Louis, J.C., Wypych, J., Biere, A.J. and Citron, M. (1999) J. Biol. Chem. 274, 9843–9846.
[27] Wood, S.J., Wypych, J., Steavenson, S., Louis, J.C., Citron, M. and Biere, A.L. (1999) J. Biol. Chem. 274, 19509–19512.
[28] Conway, K.A., Lee, S.-J., Rochet, J.C., Ding, T.D., Williamson, R.E. and Lansbury, P.T. (2000) Proc. Natl. Acad. Sci. USA 97, 571–576.
[29] Serpell, L.C., Berriman, J., Jakes, R., Goedert, M. and Crowther, R.A. (2000) Proc. Natl. Acad. Sci. USA 97, 4897–4902.
[30] Li, J., Uversky, V.N. and Fink, A.L. (2001) Biochemistry 40, 11604–11613.
[31] Der-Sarkassian, A., Jao, C.C., Chen, J. and Langen, R. (2003) J. Biol. Chem. 278, 37530–37535.
[32] Cole, N.B., Murphy, D.D., Grider, T., Rueter, S., Brasaemle, D. and Nussbaum, R.L. (2002) J. Biol. Chem. 277, 6344–6352.
[33] Outeiro, T.F. and Lindquist, S. (2003) Science 302, 1772–1775.
[34] Fortin, D.L., Troyer, M.D., Nakamura, K., Kubo, S., Anthony, M.D. and Edwards, R.H. (2004) J. Neurosci. 24, 6715–6723.
[35] Narayanan, V. and Scarlata, S. (2001) Biochemistry 40, 9927–9934.

[36] Singleton, A.B., Farrer, M., Johnson, J., Singleton, A., Hague, S., Kachergus, J., Hulihan, M., Peuralinna, T., Dutra, A., Nussbaum, R., Lincoln, S., Crawley, A., Hanson, M., Maraganore, D., Adler, C., Cookson, M.R., Muenter, M., Baptista, M., Miller, D., Blancato, J., Hardy, J. and Gwinn-Hardy, K. (2003) Science 302, 841.
[37] Farrer, M., Kachergus, J., Forno, L., Lincoln, S., Wang, D.-S., Hulihan, M., Maraganore, D., Gwinn-Hardy, K., Wszolek, Z., Dickson, D. and Langston, W.J. (2004) Ann. Neurol. 55, 174–179.
[38] Zarranz, J.J., Alegre, J., Gomez-Estaban, J.C., Lezcano, E., Ros, R., Ampuero, I., Vidal, L., Hoenicka, J., Rodriguez, O., Atares, B., Llorens, V., Gomez Tortosa, E., del Ser, T., Munoz, D.G. and de Yebenes, J.G. (2004) Ann. Neurol. 55, 164–173.
[39] Hope, M.J., Bally, M.B., Webb, G. and Cullis, P.R. (1985) Biochim. Biophys. Acta 812, 55–65.
[40] MacDonald, R.C., MacDonald, R.I., Menco, B.P., Takeshita, K., Subbarao, N.K. and Hu, I.R. (1991) Biochim. Biophys. Acta 1061, 297–303.
[41] Davletov, B.A. and Südhof, T.C. (1993) J. Biol. Chem. 15, 26386–26390.
[42] Rickman, C., Archer, D.A., Meunier, F.A., Craxton, M., Fukuda, M., Burgoyne, R.D. and Davletov, B. (2004) J. Biol. Chem. 279, 12574–12579.
[43] Naiki, H., Higuchi, K., Hosokawa, M. and Takeda, T. (1989) Anal. Biochem. 17, 244–249.
[44] Perrin, R.J., Woods, W.S., Clayton, D.F. and George, J.M. (2000) J. Biol. Chem. 275, 34393–34398.
[45] Michaelson, D.M., Barkai, G. and Barenholz, Y. (1983) Biochem. J. 211, 155–162.
[46] Jo, E., McLaurin, J., Yip, C.M., St George-Hyslop, P. and Fraser, P.E. (2000) J. Biol. Chem. 275, 34328–34334.
[47] Nuscher, B., Kamp, F., Mehnert, T., Odoy, S., Haass, C., Kahle, P.J. and Beyer, K. (2004) J. Biol. Chem. 279, 21966–21975.
[48] Jo, E., Fuller, N., Rand, R.P., St George-Hyslop, P. and Fraser, P.E. (2002) J. Mol. Biol. 315, 799–807.
[49] Bussell, R. and Eliezer, D. (2004) Biochemistry 43, 4810–4818.
[50] Hamilton, B.A. (2004) Genomics 83, 739–742.
[51] Crowther, R.A. (1991) Proc. Natl. Acad. Sci. USA 88, 2288–2292.
[52] Crowther, R.A. and Goedert, M. (2000) J. Struct. Biol. 130, 271–279.
[53] Volles, M.J., Lee, S.-J., Rochet, J.C., Shtilerman, M.D., Ding, T.T., Kessler, J.C. and Lansbury, P.T. (2001) Biochemistry 40, 7812–7819.
[54] Perrin, R.J., Woods, W.S., Clayton, D.F. and George, J.M. (2001) J. Biol. Chem. 45, 41958–41962.
[55] Sharon, R., Goldberg, M.S., Bar-Josef, I., Betensky, R.A., Shen, J. and Selkoe, D.J. (2001) Proc. Natl. Acad. Sci. USA 98, 9110–9115.
[56] Lee, H.-J., Choi, C. and Lee, S.-J. (2002) J. Biol. Chem. 277, 671–678.
[57] Jo, E., Darabie, A.A., Han, K., Tandon, A., Fraser, P.E. and McLaurin, J. (2004) Eur. J. Biochem. 271, 3180–3189.
[58] Zhu, M. and Fink, A.L. (2003) J. Biol. Chem. 278, 16873–16877.
[59] Zhu, M., Li, J. and Fink, A.L. (2003) J. Biol. Chem. 278, 40186–40197.