β-Synuclein Inhibits Formation of α-Synuclein Protofibrils: A Possible Therapeutic Strategy against Parkinson's Disease†

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ABSTRACT: Parkinson's disease (PD) is an age-associated and progressive movement disorder that is characterized by dopaminergic neuronal loss in the substantia nigra and, at autopsy, by fibrillar α-synuclein inclusions, or Lewy bodies. Despite the qualitative correlation between α-synuclein fibrils and disease, in vitro biophysical studies strongly suggest that prefibrillar α-synuclein oligomers, or protofibrils, are pathogenic. Consistent with this proposal, transgenic mice that express human α-synuclein develop a Parkinsonian movement disorder concurrent with nonfibrillar α-synuclein inclusions and the loss of dopaminergic terminii. Double-transgenic progeny of these mice that also express human β-synuclein, a homologue of α-synuclein, show significant amelioration of all three phenotypes. We demonstrate here that β- and γ-synuclein (a third homologue that is expressed primarily in peripheral neurons) are natively unfolded in monomeric form, but structured in protofibrillar form. β-Synuclein protofibrils do not bind to or permeabilize synthetic vesicles, unlike protofibrils comprising α-synuclein or γ-synuclein. Significantly, β-synuclein inhibits the generation of A53T α-synuclein protofibrils and fibrils. This finding provides a rationale for the phenotype of the double-transgenic mice and suggests a therapeutic strategy for PD.

Parkinson's disease (PD)[1] is a neurodegenerative movement disorder that results from the loss of dopaminergic neurons projecting from the substantia nigra to the dorsal striatum (*1*). A small fraction of the remaining neurons of the postmortem PD substantia nigra are characterized by fibrillar inclusions known as Lewy bodies. The major fibrillar protein component of Lewy bodies is α-synuclein. A causative role for α-synuclein in PD pathogenesis is strongly supported by (a) genetics: two different α-synuclein missense mutations (A53T and A30P) cause autosomal-dominant PD (*2*, *3*); (b) biophysics: the familial PD mutations accelerate α-synuclein fibril (A53T) and oligomer (A30P) formation (*4*, *5*); and (c) animal modeling: expression of α-synuclein in *Drosophila* or mice induces PD-like behavioral and pathological phenotypes. In transgenic *Drosophila*, development of fibrillar α-synuclein-containing inclusions is associated with selective loss of dopaminergic neurons and locomotor impairment (*6*). However, in several lines of transgenic mice, locomotor impairment, and loss of dopaminergic terminii are observed in the presence of *nonfibrillar* α-synuclein inclusions (*7*). In other lines, fibrillar α-synuclein inclusions and neurodegeneration are observed outside the substantia nigra (*8*, *9*).

The fact that several lines of transgenic mice develop a Parkinsonian phenotype without ever developing fibrillar inclusions (*7*) suggests that the α-synuclein protofibril, an intermediate in the fibrillization process, rather than the fibril itself, may be pathogenic (*10*). In support of this hypothesis, biophysical studies demonstrate that factors that exacerbate PD in vivo also promote protofibril formation in vitro. First, both mutant forms of α-synuclein (designated A53T and A30P) undergo more rapid protofibril formation than wild type α-synuclein (WT), whereas A30P fibril formation is relatively slow (*4*, *5*). Second, mouse α-synuclein (Mo) inhibits WT fibril formation, leading to the accumulation of protofibrils (*11*). This observation is consistent with the lack of fibrillar inclusions in the "symptomatic" transgenic mice. Third, the oxidative products of several catecholamines, including dopamine and L-DOPA, inhibit the conversion of protofibrils to fibrils, causing accumulation of the α-synuclein protofibrils (*12*). This observation is consistent with the known hypersensitivity of dopaminergic neurons, especially those expressing high levels of cytoplasmic dopamine, to cell death in PD. Finally, the unusual pore-like properties of α-synuclein protofibrils suggest a mechanism linking protofibril formation to neuronal death (the "amyloid pore") (*13*−*18*).

If α-synuclein protofibrils are indeed the pathogenic species in PD, then blocking their formation would be a useful therapeutic strategy. This could be accomplished with an exogenous drug-like molecule or by activation of an endogenous protein inhibitor. The latter strategy has been utilized against sickle cell anemia, where inducible hemoglobin homologues inhibit the in vitro polymerization of the mutant hemoglobin and ameliorate disease in mouse models (*19*). The strategy has recently been demonstrated to be effective in the Parkinsonian α-synuclein transgenic mouse, by crossing that mouse with a mouse that expresses

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[1] Abbreviations: A30P, human A30P α-synuclein; A53T, human A53T α-synuclein; CD, circular dichroism spectroscopy; Mo, mouse α-synuclein; PD, Parkinson's disease; PG, phosphatidylglycerol; WT, human wild type α-synuclein.

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Alpha    MDVFMKGLSK AKEGVVAAAE KTKQGVAEAA GKTKEGVLYV     40
Beta     MDVFMKGLSM AKEGVVAAAE KTKQGVTEAA EKTKEGVLYV     40
Gamma    MDVFKKGFSI AKEGVVGAVE KTKQGVTEAA EKTKEGVMYV     40

Alpha    GSKTKEGVVH GVATVAEKTK EQVTNVGGAV VTGVTAVAQK     80
Beta     GSKTREGVVQ GVASVAEKTK EQASHLGGAV FSG------      74
Gamma    GAKTKENVVQ SVTSVAEKTK EQANAVSEAV VSSVNTVATK     80

Alpha    TVEGAGSIAA ATGFVKKDQL GKN--EE GAPQEGILED        115
Beta     --AGNIAA ATGLVKREEF PTDLKPEEVA QEAAEEPLIE 109
Gamma    TVEEAENIAV TSGVVRKEDL RPSAPQQEGV ASKEKEEVAE     120

Alpha    MPVDPDNEAY EMPSEEGYQD YEPEA            140
Beta     PLMEPEGESY EDPPQEEYQE YEPEA            134
Gamma    EAQSGGD                 127
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FIGURE 1: The sequences of the three human synuclein variants. The red amino acids indicate six repeats of the hexameric motif KTK-(E/Q)GV. The region marked with the blue line (residues 61−95 of α-synuclein) shows the NAC (**n**on-**a**myloid **c**omponent of amyloid plaque) region.

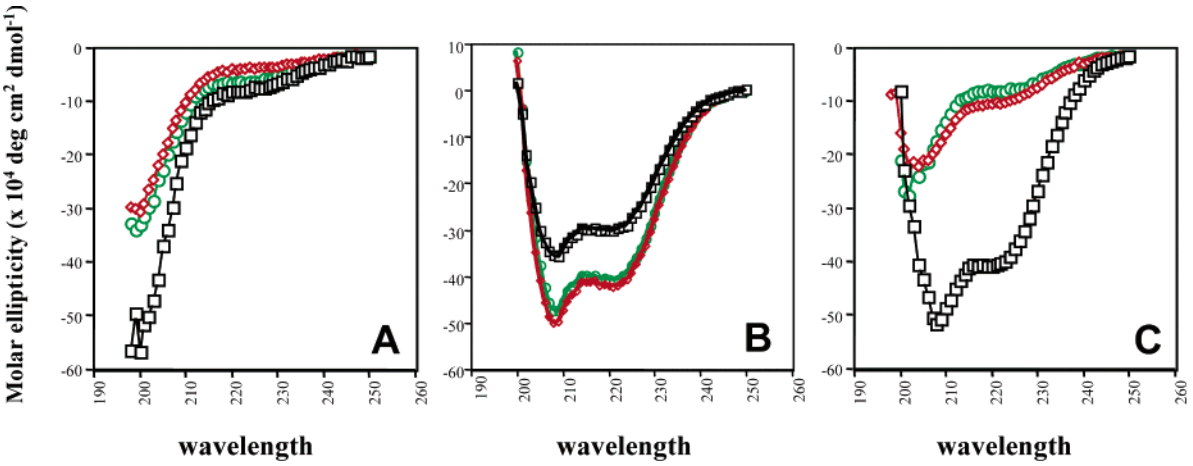

FIGURE 2: The secondary structures of the monomeric α- (black squares), β- (red diamonds), and γ- (green circles) synucleins are different, whether in (A) phosphate buffer solution (20 mM potassium phosphate, pH 7.4), (B) SDS (20% SDS in 20 mM potassium phosphate, pH 7.4), or (C) the presence of PG vesicles (1 mg/mL PG vesicles in 1 mM HEPES, 14.5 mM KCl, 1 mM EDTA, pH 7.4).

β-synuclein (*20*), a homologue of α-synuclein. In the resultant double-trangenic mice, all three of the Parkinsonian phenotypes, movement disorder, inclusions, and dopaminergic terminal loss, are ameliorated (*21*). In vitro studies of α/β-synuclein mixtures indicate that the two proteins interact (*21*) and that β-synuclein inhibits fibril formation by α-synuclein (*22*). However, there is no report of the effect of β-synuclein on the critical first step of the α-synuclein fibrillization process, that is, protofibril formation. The "toxic protofibril hypothesis" (*10*) would predict, given the phenotype of the double-trangenic mice, that β-synuclein should inhibit protofibril formation by α-synuclein. The studies reported here were therefore undertaken as a test of the "toxic protofibril hypothesis".

We report here that β-synuclein (but not a third congener, γ-synuclein) inhibits A53T α-synuclein protofibril formation, consistent with the inclusion phenotype of the double-trangenic mice and with the "toxic protofibril hypothesis". We also report that β-synuclein protofibrils are unable to bind or permeabilize synthetic vesicles; they do not have pore-like properties. The ramifications of these findings relevant to a therapeutic strategy against PD are discussed.

MATERIALS AND METHODS

Purification of Recombinant α-, β-, and γ-Synucleins. Recombinant α-, β-, and γ-synucleins were cloned from human cDNA (Clontech) by PCR. The expression vector in *Escherichia coli* system pET-31a was used for β- and γ-synuclein proteins. The sequences of all DNA constructs were confirmed by ABI377 fluorescent DNA sequencing. Both proteins were purified using the same protocols as used for α-synuclein (*4*). Recombinant α-synuclein was expressed and purified at the center for Biocatalysis and Bioprocessing at the University of Iowa (Iowa City, IA). All protein concentrations in this report were determined by Quantitative Amino Acid Composition Analysis.

Preparation of Oligomeric (Protofibrillar) Synucleins. Purified, lyophilized synucleins were dissolved in phosphate-buffered saline [PBS: 0.01 M phosphate buffer, 2.7 mM KCl, 137 mM NaCl, pH 7.4; or, in the case of membrane permeability assays (see below), in 10 mM HEPES, pH 7.4, 145 mM KCl] with 0.02% NaN_3 to a final concentration of 1 mM. Without additional incubation, the solution was filtered using Microcon 0.22 μm filter (Millipore, Pittsburgh, PA). The filtrate was loaded onto a Superdex-200 gel filtration column (HR 10/30, Pharmacia) and eluted in PBS at a flow rate of 0.5 mL/min as described previously (*11*). Oligomeric (protofibrillar) species were detected in the eluted volume 8∼9.5 mL (void fractions) and monomeric α-synuclein was eluted in 13∼15 mL.

Oligomerization and Fibrillization of Synucleins. Purified, lyophilized synucleins were dissolved in PBS with 0.02%

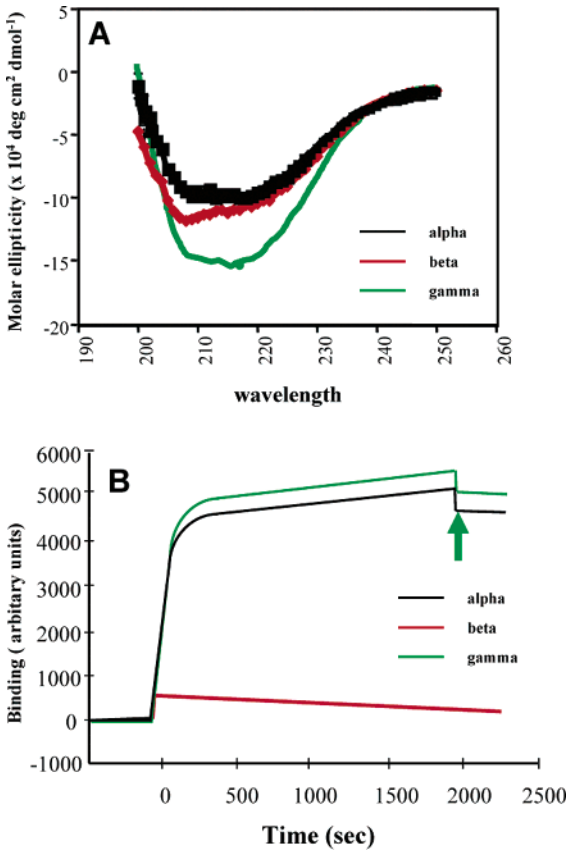

FIGURE 3: The α-, *β*-, and *γ*-protofibrils have similar structure, but different vesicle-binding properties. (A) CD spectra of void fractions of synucleins (10 *μ*M) were measured at 22 °C. The final spectrum was the mean of three individual scans, after background subtraction. (B) The binding of void fractions of α- and *γ*-synucleins to the immobilized PG vesicles. Only the void fractions of α- and *γ*-synucleins bind to the immobilized vesicles. Monomeric α-, *β*-, and *γ*-synuclein did not bind (data not shown).

NaN_3 and were filtered in using Microcon 0.22 *μ*m filter and Microcon 100K MWCO filter (Millipore, Pittsburgh, PA). For the measurement of oligomerization (protofibril formation), samples were prepared at 300 *μ*M and incubated at 37 °C under rotating conditions (*23*, *24*). After sedimentation of fibrils from aliquots, the supernatant was subjected to gel-filtration chromatography to identify and quantitate protofibrillar synucleins. For fibrillization, samples at 70 *μ*M were incubated under the same conditions as oligomerization experiments; the fibril was measured by Thioflavin T (*4*, *11*). A53T α-synuclein was used in the mixtures with *β*- or *γ*-synuclein (Figure 5) owing to its rapid oligomerization and fibrillization. We have demonstrated that the fibrillization of wild-type α-synuclein is also inhibited by *β*-synuclein (not shown).

Far-UV Circular Dichroism (CD) Spectroscopy. Far-UV CD spectra of monomeric synucleins were collected at 22 °C using an Aviv 62A DS spectropolarimeter and a 0.1-cm cuvette. The data were acquired at 1-nm intervals with a response time of 4 s per measurement. The final spectrum was obtained by calculating the mean of three individual scans and subtracting the background. A sample of monomeric or oligomeric synucleins was prepared for CD measurements by elution from a Superdex-200 gel filtration column in 20 mM potassium phosphate (pH 7.4).

Vesicle Binding and Permeabilzation. Phosphatidylglycerol (PG) was purchased from Avanti Polar Lipids, Inc. (Alabaster, AL) and small unilamellar vesicles (SUV) were prepared as described previously (*13*). The binding of each synuclein variant to PG vesicles was measured using BIAcore (BIA, Sweden) as described in Volles et al. (*13*).

The membrane permeability assay was accomplished according to the method of Blau and Weissmann (*13*, *25*). A fluorescence detector based on the common stopped-flow device was assembled (M. Volles, unpublished): the flow cell of an HPLC fluorescence detector [Kratos (Chestnut Ridge, NY) FS 950 Fluoromat with F4T5BL lamp; excitation filter: 334 nm with 10 nm band-pass; emission filter: long-pass with 495 nm cutoff] was connected to the central port of a T-type mixing chamber, the remaining two ports of which were connected to 100 mL syringe. A solution of synuclein was mixed with a solution of PG vesicles (50 *μ*L of a solution containing 10 mM $CaCl_2$) in a plastic tube and aliquots were injected the fluorescence detector using an HPLC pump (Waters 600E). The carrier solvent was 10 mM HEPES, 145 mM KCl, 5 mM $CaCl_2$, pH 7.4 at 0.5 mL/min. Effects are reported as percentages of the effect of 25 *μ*M ionomycin.

RESULTS

Monomeric β- and γ-Synucleins Have Similar Conformational Properties to Each Other and Differ Slightly from α-Synuclein. Human *β*-synuclein is 78% identical to α-sy-

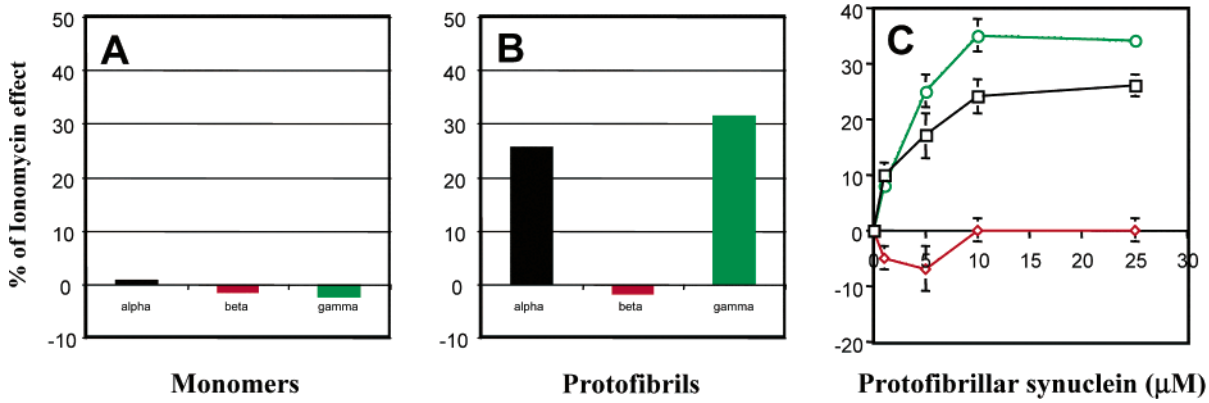

FIGURE 4: Protofibrils comprising α- and *γ*-synucleins permeabilize synthetic vesicles. All three monomers at 100 *μ*M had no activity (A), whereas the protofibrillar α- and *γ*-synuclein were active at 30 *μ*M (B). The permeabilization activity of the protofibrils was dose-dependent under a certain threshold (ca. 10 *μ*M) (C). Effects are reported as percentages of the effect of 25 *μ*M ionomycin. Each value represents the average of three different experiments.

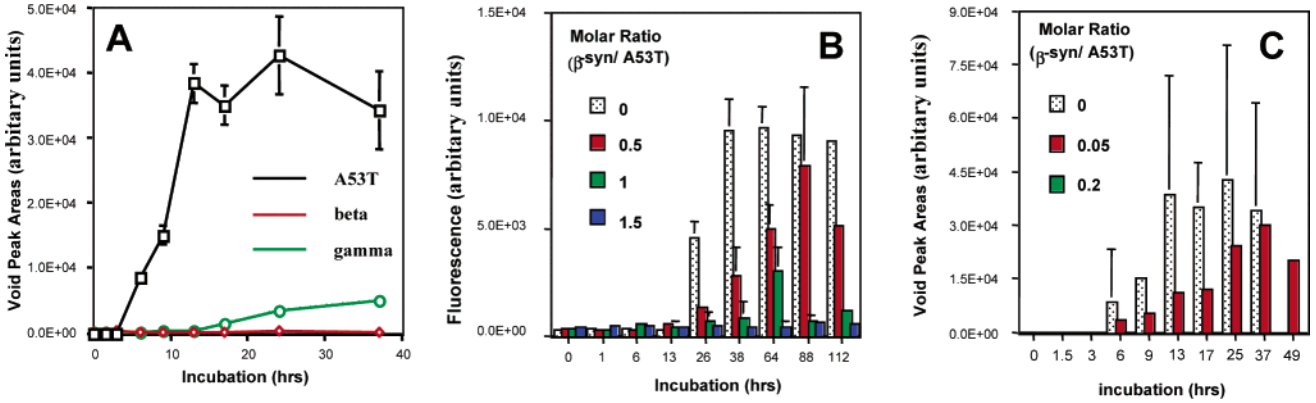

FIGURE 5: β-Synuclein inhibits fibril and protofibril formation by α-synuclein. (A) Oligomerization of α-, β-, and γ-synucleins, followed according to Rochet et al. (*11*). Inhibition by β-synuclein of (B) fibril formation by α-synuclein and of (C) oligomerization of α-synuclein. γ-Synuclein had no effect on fibril formation or protofibril formation (data not shown).

nuclein, while γ-synuclein is 60% identical (Figure 1). All three synucleins have very similar N-terminal hexamer repeat regions (Figure 1). β-Synuclein lacks a portion of the NAC (nonamyloid component) region (blue bar in Figure 1), while γ-synuclein lacks the tyrosine-rich carboxy-terminal domain. Both β- and γ-synuclein share with α-synuclein a natively unfolded structure under "physiological" conditions (Figure 2A) (*26*). Both β- (red spectra, Figure 2) and γ- (green spectra) have a slighter greater intrinsic propensity to adopt helical structure [in 20% SDS, Figure 2B or 5 mM HFIP (1,1,1,3,3,3-hexafluoro-2-propanol), not shown] than α-synuclein (black spectra), but α-synuclein takes on more helical structure than β- or γ- when bound to PG vesicles (Figure 2C). However, the weak interactions between monomeric synucleins and PG vesicles were not detected by surface plasmon resonance (SPR, see below, data not shown).

β-Synuclein and γ-Synuclein Both Generate Structured Oligomers, But only the γ-Derived Oligomers Behave like α-Synuclein Protofibrils; Tightly Binding and Permeabilizing Synthetic Vesicles. Protofibrils comprising β- and γ-synuclein were purified by gel filtration chromatography (*13*). CD analysis of the protofibrillar fraction showed that γ-synuclein (green spectrum, Figure 3A) and β-synuclein (red) protofibrils resemble α-synuclein (black) protofibrils with respect to secondary structure. However, β-synuclein protofibrils differed in that they did not tightly bind to immobilized PG vesicles (Figure 3B), as did α- and γ-synuclein protofibrils (no binding of α, β, or γ monomers could be detected by this method, data not shown). This difference seemed to be related to the structure/properties of the β-synuclein protofibril, since β-protofibrils also did not bind to rat brain-derived vesicles (data not shown) (*15*).

β-Synuclein Protofibrils, Unlike α- and γ-Synuclein Protofibrils, Do Not Form Amyloid Pores. None of the monomeric synucleins had any significant effect on the integrity of synthetic PG vesicles (Figure 4A). However, protofibrillar γ-synuclein, like protofibrillar α-synuclein, produced a dose-dependent pore-like permeabilization effect (Figure 4B,C). Protofibrillar β-synuclein had no effect on membrane integrity, consistent with its inability to bind vesicles (see above). This result suggests that the sequence between residues 71 and 84 of α-synuclein (Figure 1), previously implicated in fibril formation (*24*), is important for amyloid pore formation as well. The fact that the same sequence is important in both fibril and pore formation suggests that it may be difficult to selectively inhibit or promote either event with a small molecule or a point mutation.

β- and γ-Synuclein Are Slow to Form Protofibrils. Having demonstrated that γ-synuclein protofibrils have amyloid pore properties, whereas β-synuclein protofibrils do not, we sought to compare the kinetics of protofibril formation by the three variants. Previous studies by others had shown that only α-synuclein is found in Lewy bodies (*27*) and that α-synuclein fibrillizes much more rapidly in vitro than either β- or γ-synuclein (*28*, *29*). For our kinetic studies, we utilized A53T rather than WT, because of the fact that it rapidly formed the quantities of protofibrils required to allow observation of inhibition (below). Under conditions where a thioflavin T signal from A53T was first detected after 16 h (WT produced a thioT signal at ca. 28 h, not shown), no significant thioflavin T signal was detected from β- or γ-synuclein until the 14th day of incubation (*15*) (data not shown). From A53T, the amount of oligomeric material increased substantially after 5 h at 37 °C (Figure 5A). No β-synuclein oligomers were observed for the entire time course of over 30 days. γ-Synuclein oligomers were produced very slowly and did not accumulate or convert to fibrils. Thus β- and γ-synucleins, relative to α-synuclein, are not prone to generate protofibrils or fibrils.

Only β-Synuclein Inhibits Fibril and Protofibril Formation by A53T α-Synuclein. The fact that β-synuclein can suppress α-synuclein inclusion formation in vivo suggests that this homologue can influence the aggregation and/or fibrillization of α-synuclein. Indeed, these two proteins interact (*21*) and β-synuclein can inhibit fibril formation by α-synuclein (*22*). To investigate whether the nonfibrillogenic synucleins can affect the oligomerization and fibrillization of α-synuclein, mixtures containing A53T and either β- or γ-synuclein were incubated.

The lag time of A53T fibrillization was significantly delayed by substoichiometric amounts of β-synuclein (Figure 5B) (*22*). Fibril formation by WT was also inhibited under these conditions (data not shown). However, comparable concentrations of γ-synuclein had no significant effect (data not shown). The lag time for A53T oligomerization/protofibril formation was elongated in A53T/β mixtures and oligomerization was effectively eliminated by 0.2 molar equivalents of β-synuclein (Figure 5C). Once again, γ-synuclein showed no effect on the oligomerization of α-synuclein, even when added in 4-fold excess (data not shown).

Due to the slowness of WT protofibril formation, we have not measured the effect of β-synuclein or γ-synuclein on that process. However, given the analogy at the level of fibril formation, it is likely that the observed effects will hold for WT as well as A53T.

DISCUSSION

In addition to α-synuclein, which is linked to PD, the synuclein family includes β-synuclein (phosphoneuroprotein-14; ref *20*), and γ-synuclein (breast carcinoma-specific factor; ref *30*) (Figure 1). There is no clear picture as to the biological activity of any members of this family, although α-synuclein has been implicated in fatty acid binding (*31*) and phospholipase D regulation (*32*, *33*). Whether β- and γ-synuclein have pathological roles in addition to their undetermined biological roles is unclear. However, only α-synuclein is found in Lewy bodies (*27*) and only α-synuclein efficiently fibrillizes in vitro (*28*, *29*). Although there is no direct information linking β-synuclein to PD, a change in the relative amounts of the α- and β- messages is characteristic of the PD substantia nigra (α-synuclein is up and β-synuclein is down) (*34*). This PD-specific increase in the α/β ratio is consistent with the observation that β-synuclein expression can suppress the Parkinsonian phenotype induced by α-synuclein overexpression in mice (*21*). The suggestion that one could produce a therapeutic benefit against PD by effecting a decrease in the α/β ratio led us to carry out the series of biophysical studies described here, designed to elucidate the interaction between α- and β-synuclein.

We demonstrate here that β-synuclein is unique among the three synucleins in that it does not form "amyloid pores", which are proposed to be the neurotoxic oligomeric species (*15*−*18*). In addition, a small amount of β-synuclein is able to completely suppress the oligomerization of α-synuclein. This finding is consistent with the phenotype of the α/β double-trangenic mice, in which inclusions are not observed, and with the toxic protofibril hypothesis (*10*), since abolishing nonfibrillar inclusions and amelioration of symptoms and neurodegeneration are linked (*21*).

The inhibitory activity of β-synuclein contrasts with that of other proteins whose sequence is closely related to that of α-synuclein: mouse α-synuclein and a dopamine-modified form of α-synuclein both inhibit fibril formation, but not protofibril formation, leading to accumulation of protofibrils (*11*, *12*). Neither of these congeners was observed to inhibit protofibril formation. Thus, these two congeners may actually increase the pathogenicity of α-synuclein.

The findings reported here are not unprecedented: sickle-cell hemoglobin fibrillization is inhibited by closely related hemoglobin variants, including a fetal form that is not normally expressed after birth. Induction of fetal hemoglobin expression can be accomplished with a drug (hydroxyurea) that has beneficial effects in vivo (*19*). A similar strategy could be effective against PD, that is, upregulation of β-synuclein expression using a drug-like small molecule.

REFERENCES

1. Lansbury, P. T., and Brice, A. (2002) *Curr. Opin. Genet. Dev. 12*, 299−306.
2. Kruger, R., Kuhn, W., Muller, T., Woitalla, D., Graeber, M., Kosel, S., Przuntek, H., Epplen, J. T., Schols, L., and Riess, O. (1998) *Nat. Genet. 18*, 106−8.
3. Polymeropoulos, M. H., Lavedan, C., Leroy, E., Ide, S. E., Dehejia, A., Dutra, A., Pike, B., Root, H., Rubenstein, 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*, 2045−7.
4. Conway, K. A., Harper, J. D., and Lansbury, P. T. (1998) *Nat. Med. 4*, 1318−20.
5. Conway, K. A., Lee, S. J., Rochet, J. C., Ding, T. T., Williamson, R. E., and Lansbury, P. T., Jr. (2000) *Proc. Natl. Acad. Sci. U.S.A. 97*, 571−6.
6. Feany, M. B., and Bender, W. W. (2000) *Nature 404*, 394−8.
7. Masliah, E., Rockenstein, E., Veinbergs, I., Mallory, M., Hashimoto, M., Takeda, A., Sagara, Y., Sisk, A., and Mucke, L. (2000) *Science 287*, 1265−9.
8. Giasson, B. I., Ischiropoulos, H., Lee, V. M., and Trojanowski, J. Q. (2002) *Free Radic. Biol. Med. 32*, 1264−75.
9. Lee, M. K., Stirling, W., Xu, Y., Xu, X., Qui, D., Mandir, A. S., Dawson, T. M., Copeland, N. G., Jenkins, N. A., and Price, D. L. (2002) *Proc. Natl. Acad. Sci. U.S.A. 99*, 8968−73.
10. Goldberg, M. S., and Lansbury, P. T., Jr. (2000) *Nat. Cell Biol. 2*, E115−9.
11. Rochet, J. C., Conway, K. A., and Lansbury, P. T., Jr. (2000) *Biochemistry 39*, 10619−26.
12. Conway, K. A., Rochet, J. C., Bieganski, R. M., and Lansbury, P. T., Jr. (2001) *Science 294*, 1346−9.
13. Volles, M. J., Lee, S. J., Rochet, J. C., Shtilerman, M. D., Ding, T. T., Kessler, J. C., and Lansbury, P. T., Jr. (2001) *Biochemistry 40*, 7812−9.
14. Volles, M. J., and Lansbury, P. T., Jr. (2002) *Biochemistry 41*, 4595−602.
15. Ding, T. T., Lee, S. J., Rochet, J. C., and Lansbury, P. T., Jr. (2002) *Biochemistry 41*, 10209−17.
16. Lashuel, H. A., Hartley, D., Petre, B. M., Walz, T., and Lansbury, P. T., Jr. (2002) *Nature 418*, 291.
17. Lashuel, H. A., Petre, B. W., Simon, M., Nowak, R. J., Walz, T., and Lansbury, P. T., Jr. (2002) *J. Mol. Biol. 322*, 1089−102.
18. Anguiano, M., Nowak, R. J., and Lansbury, P. T., Jr. (2002) *Biochemistry 41*, 11338−43.
19. He, Z., and Russell, J. E. (2002) *Proc. Natl. Acad. Sci. U.S.A. 99*, 10635−40.
20. Jakes, R., Spillantini, M. G., and Goedert, M. (1994) *FEBS Lett. 345*, 27−32.
21. Hashimoto, M., Rockenstein, E., Mante, M., Mallory, M., and Masliah, E. (2001) *Neuron 32*, 213−23.
22. Uversky, V. N., Li, J., Souillac, P., Millett, I. S., Doniach, S., Jakes, R., Goedert, M., and Fink, A. L. (2002) *J. Biol. Chem. 277*, 11970−8.
23. Biere, A. L., Wood, S. J., Wypych, J., Steavenson, S., Jiang, Y., Anafi, D., Jacobsen, F. W., Jarosinski, M. A., Wu, G. M., Louis, J. C., Martin, F., Narhi, L. O., and Citron, M. (2000) *J. Biol. Chem. 275*, 34574−9.
24. Giasson, B. I., Murray, I. V., Trojanowski, J. Q., and Lee, V. M. (2001) *J. Biol. Chem. 276*, 2380−6.
25. Blau, L., and Weissmann, G. (1988) *Biochemistry 27*, 5661−6.
26. Weinreb, P. H., Zhen, W., Poon, A. W., Conway, K. A., and Lansbury, P. T., Jr. (1996) *Biochemistry 35*, 13709−15.
27. Hurtig, H. I., Trojanowski, J. Q., Galvin, J., Ewbank, D., Schmidt, M. L., Lee, V. M., Clark, C. M., Glosser, G., Stern, M. B., Gollomp, S. M., and Arnold, S. E. (2000) *Neurology 54*, 1916−21.
28. Uversky, V. N., E, M. C., Bower, K. S., Li, J., and Fink, A. L. (2002) *FEBS Lett. 515*, 99−103.
29. Goedert, M. (2001) *Nat. Rev. Neurosci. 2*, 492−501.
30. Jia, T., Liu, Y. E., Liu, J., and Shi, Y. E. (1999) *Cancer Res. 59*, 742−7.
31. Sharon, R., Goldberg, M. S., Bar-Josef, I., Betensky, R. A., Shen, J., and Selkoe, D. J. (2001) *Proc. Natl. Acad. Sci. U.S.A. 98*, 9110−5.
32. Ahn, B. H., Rhim, H., Kim, S. Y., Sung, Y. M., Lee, M. Y., Choi, J. Y., Wolozin, B., Chang, J. S., Lee, Y. H., Kwon, T. K., Chung, K. C., Yoon, S. H., Hahn, S. J., Kim, M. S., Jo, Y. H., and Min do, S. (2002) *J. Biol. Chem. 277*, 12334−42.
33. Jenco, J. M., Rawlingson, A., Daniels, B., and Morris, A. J. (1998) *Biochemistry 37*, 4901−9.
34. Rockenstein, E., Hansen, L. A., Mallory, M., Trojanowski, J. Q., Galasko, D., and Masliah, E. (2001) *Brain Res. 914*, 48−56.

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