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β-Amyloid and α-Synuclein Cooperate To Block SNARE-Dependent Vesicle Fusion

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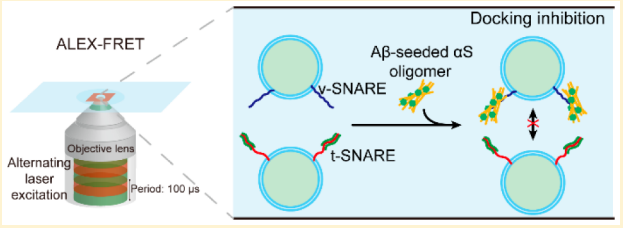
Supporting Information

ABSTRACT: Alzheimer's disease (AD) and Parkinson's disease (PD) are caused by β-amyloid (Aβ) and α-synuclein (αS), respectively. Ample evidence suggests that these two pathogenic proteins are closely linked and have a synergistic effect on eliciting neurodegenerative disorders. However, the pathophysiological consequences of Aβ and αS coexistence are still elusive. Here, we show that large-sized αS oligomers, which are normally difficult to form, are readily generated by $Aβ_{42}$-seeding and that these oligomers efficiently hamper neuronal SNARE-mediated vesicle fusion. The direct binding of the Aβ-seeded αS oligomers to the N-terminal domain of synaptobrevin-2, a vesicular SNARE protein, is responsible for the inhibition of fusion. In contrast, large-sized $Aβ_{42}$ oligomers (or aggregates) or the products of αS incubated without $Aβ_{42}$ have no effect on vesicle fusion. These results are confirmed by examining PC12 cell exocytosis. Our results suggest that Aβ and αS cooperate to escalate the production of toxic oligomers, whose main toxicity is the inhibition of vesicle fusion and consequently prompts synaptic dysfunction.



The hallmark of Alzheimer's disease (AD) is the formation of plaques and neurofibrillary tangles, mostly composed of β-amyloid (Aβ) and tau proteins.[1] In Parkinson's disease (PD) and dementia with Lewy bodies (DLB), α-synuclein (αS), a peripheral membrane-binding protein,[2] is the major component of the amyloid fibril form of Lewy bodies (LB).[3] Although the aggregations of Aβ and αS are used as the major pathological markers of AD and PD, respectively, there is ample evidence that these two pathogenic proteins are closely linked in neurodegenerative disorders.[4] For example, Aβ deposition has been found in patients with DLB,[5] and nearly half of AD patients have LB pathology.[6] Importantly, AD patients with LB pathology presented with a more rapid cognitive decline and shortened survival times compared with pure AD patients. Familial AD mutations, such as presenilin and amyloid precursor protein, also showed increased levels of LB pathology.[7−9] These observations suggested a substantial connection between AD and PD pathologies. In line with these observations, it has been demonstrated that Aβ promoted the accumulation of αS and accelerated motor and memory deficits and cognitive dysfunction in transgenic mouse models.[10,11]

Although many studies have suggested that Aβ and αS have synergistic effects on symptoms of the Lewy body variant of AD and DLB, the nature of the detailed toxicity due to the coexistence of Aβ and αS is still unknown.[12] One of the suggested models of the synergistic effects is the direct interaction between Aβ and αS,[13] which enhances the aggregation and accumulation of cross-seeded or possibly hybrid complexes.[10,14] $Aβ_{42}$, the most aggregate-prone form among the Aβ isoforms,[15] enhanced the formation of αS oligomers *in vitro* and in cell culture,[10] and the direct interaction between Aβ and αS induced a conformational change in $Aβ_{42}$.[16] Consistent with these *in vitro* studies, complex forms and coimmunoprecipitation of Aβ and αS were observed in patients' brains in the Lewy body variant of AD.[17] Thus, evidence of the synergistic effects of Aβ and αS coexistence to stimulate coaggregation and accumulation and accelerate cognitive decline is growing. However, the detailed nature of the synaptic dysfunction that the cross-seeded or hybrid complexes of Aβ and αS causes remains elusive.

While the accumulation of the fibril forms of Aβ and αS in plaques and LB are common hallmarks of AD and PD, soluble oligomeric or protofibril forms of Aβ and αS are generally regarded as the toxic species.[18−23] Because αS is abundant at presynaptic terminals, its physiological roles have been often connected to synaptic vesicle fusion and exocytosis.[24−29] Recently, Südhof and co-workers reported that αS directly binds to synaptobrevin-2, a soluble N-ethylmaleimide-sensitive factor (NSF) attachment protein receptor (SNARE) protein

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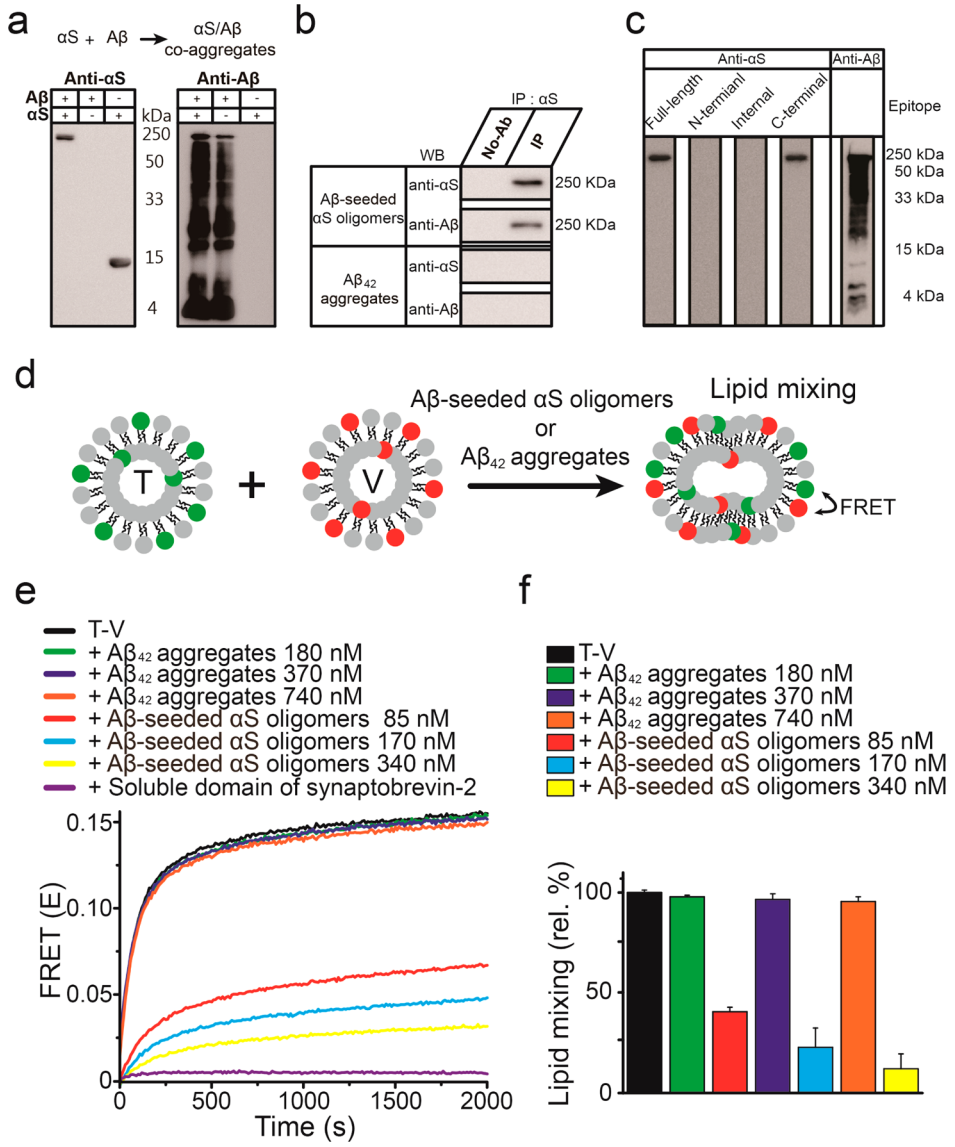

Figure 1. Generation of Aβ_{42}-seeded αS oligomers and their inhibitory effects on SNARE-mediated lipid mixing. (a) Western blot of the Aβ_{42}-seeded αS oligomers. Lane 1, purified Aβ_{42}-seeded αS oligomers obtained by incubating 10 μM αS with 20 μM Aβ_{42} monomers in 20 mM sodium phosphate buffer at 37 °C for 7 days. As a control, 20 μM Aβ_{42} or 10 μM αS was incubated under the same conditions as the Aβ-seeded αS oligomers. Lane 2, Aβ_{42}-only control. Lane 3, αS-only control. The left panel was detected using an anti-αS antibody, and the right panel used an anti-Aβ_{42} antibody. (b) Co-immunoprecipitation (Co-IP) assay of Aβ-seeded αS oligomers and Aβ_{42} aggregates. 170 nM of purified Aβ-seeded αS oligomers (by αS monomer concentration) was incubated with an αS primary antibody for 16 h at 4 °C followed by an incubation with protein A/G plus agarose beads for 2 h at 4 °C. Eluted sample were detected with anti-αS and anti-Aβ_{42} antibodies, respectively. As a control, no αS antibody was treated. (c) Western blot of Aβ-seeded αS oligomers using antibodies for various αS epitopes. Four types of αS antibodies that bind to the full-length, N-terminal, internal, or C-terminal epitopes were used to determine which motifs of αS are responsible for aggregation. Purified large Aβ-seeded αS oligomers were used for the Western blot. Aβ-seeded αS oligomers were efficiently detected when αS antibodies targeting the full-length and C-terminal epitope were used. (d–f) The effects of Aβ-seeded αS oligomers on SNARE-mediated lipid-mixing. (d) Schematics of lipid mixing assay. (e) **T-V**: **T** and **V** (20 μM in lipid concentration) were mixed together at 35 °C without any additives (black line). A negative lipid mixing control was performed by adding a soluble motif of synaptobrevin-2 (purple line). (f) Relative percentages of lipid mixing at 1800 s from d. Error bars were generated from three independent experiments.

embedded in synaptic vesicles, and promotes SNARE complex formation without alterations in neurotransmitter release.[30] This observation was explained by single-vesicle assays, which demonstrated that αS induces clustering of vesicles without affecting neurotransmitter release.[31] In line with these studies, the interactions between αS and synaptobrevin-2 were preserved in dopamine-induced large-sized αS oligomers, and the αS oligomers efficiently inhibited SNARE-mediated vesicle docking.[32] Considering the observation that Aβ induced large-sized αS oligomers and they formed complexes in brains,[10] it is highly possible that cross-seeding or αS oligomers, induced by Aβ aggregation, might interact with SNARE proteins and hamper synaptic transmission.

In this work, we showed that Aβ_{42} induced large-sized αS oligomers and that the resultant oligomers inhibited neuronal SNARE-mediated vesicle fusion. The direct binding of the Aβ-seeded αS oligomers to the N-terminal domain of synaptobrevin-2 inhibited both the lipid and content mixing of vesicle

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fusion. In contrast, αS incubated without Aβ seeding or large-sized $Aβ_{42}$ oligomers (or aggregates) generated without αS mixing had no inhibitory effects on vesicle fusion. A single-vesicle assay demonstrated that the Aβ-seeded αS oligomers blocked the docking step between vesicles. Furthermore, the inhibitory effects of the Aβ-seeded αS oligomers on exocytosis were confirmed using PC12 cells. These results suggest that Aβ and αS cooperate to accelerate the production of toxic oligomers, whose main toxicity is the inhibition of vesicle fusion and consequently the disruption of synaptic transmission, which provides a clue to the connection between AD and PD through the coexistence of Aβ and αS.

MATERIALS AND METHODS

Preparation of Aβ-Seeded αS Oligomers. Recombinant glutathione *S*-transferase (GST)-tagged αS was inserted into the pGEX-KG vector and was transformed into *Escherichia coli* Rosetta (DE3) pLysS (Novagene). The purification procedure of αS has been previously described.[32] Briefly, the cells were grown at 37 °C in LB medium and induced by adding 0.5 mM IPTG overnight at 16 °C. Pelleted cells were lysed with lysis buffer (1% sarcosine and 2 mM AEBSF in 1× PBS) and sonication. Glutathione-agarose beads were used for affinity chromatography. To prepare $Aβ_{42}$ monomers, lyophilized synthetic $Aβ_{42}$ peptides (American peptide) were dissolved in 1,1,1,3,3,3-hexafluoroisopropanol (Sigma-Aldrich) for 72 h at room temperature. Aliquoted $Aβ_{42}$ peptides were evaporated using a speed vacuum concentrator, redissolved in dimethyl sulfoxide (DMSO), and then diluted in 1× PBS (pH 7.4). To obtain Aβ-seeded αS oligomers, 10 μM αS was incubated with 20 μM $Aβ_{42}$ monomers in 20 mM sodium phosphate buffer (pH 7) for 7 days at 37 °C. After this incubation, the mixture was concentrated using an Ultracel 10k-membrane (Millipore). Aβ-seeded αS oligomers were purified by FPLC using a superdexTM200 10/300GL (GE healthcare) and concentrated again using an Ultracel 10k-membrane. For Western blotting, purified proteins were loaded onto 15% SDS-PAGE gels and transferred to PVDF membranes (Bio-Rad). An αS primary antibody (sc-52979 [Santa Cruz Bio technology], 1:750) and an Aβ primary antibody (beta amyloid 6E10 [Covance], 1:1000) were used to detect αS and Aβ proteins, respectively. An antimouse IgG peroxidase secondary antibody (Sigma; 1:2500) was used for the detection of chemiluminescence. A detailed Western blotting procedure has been described elsewhere.[32]

Preparation of Cross-Linked αS Oligomers, SNARE Proteins, and Reconstituted Proteoliposomes. The detailed procedures of preparation of cross-linked αS oligomers, SNARE proteins, and reconstituted proteoliposomes are described in detail in the Supporting Information (SI).

***In Vitro* Ensemble Vesicle Fusion Assay (Lipid Mixing and Content Mixing).** For the lipid mixing assay, two populations of proteoliposomes of t-vesicles labeled with DiI and v-vesicles labeled with DiD were mixed together to form a 20 μM lipid mixture in the presence or absence of Aβ-seeded αS oligomers or $Aβ_{42}$ aggregates. A 532 nm excitation wavelength was used for the excitation, and an emission wavelength of 690 nm was used to detect FRET. For the content mixing assay, two populations of proteoliposomes, unlabeled t-vesicles and v-vesicles (**SV**) containing sulforhodamine B (SRB), were mixed together to form a 20 μM lipid in the presence or absence of Aβ-seeded αS oligomers or $Aβ_{42}$ aggregates. A 532 nm excitation wavelength was used for the excitation, and a wavelength emission of 580 nm was used to detect SRB dequenching. Triton X-100 (0.1%) was added to reaction mixture to obtain the fluorescence signal at 100% dequenching of SRB. A temperature-controlled fluorescence spectrophotometer (Cary Eclipse, Varian) was used for both lipid mixing and content mixing assays.

Co-Immunoprecipitation of Aβ-Seeded αS Oligomers. 170 nM of purified Aβ-seeded αS oligomers (αS monomer concentration) was incubated with 2 μg αS primary antibody for 1 h at 4 °C and then incubated with 20 μL of protein A/G plus-agarose beads (Santa-cruz) for 2 h at 4 °C. Aβ-seeded αS oligomers bound to protein A/G plus-agarose beads were pelleted down by centrifugation at 2500 rpm for 5 min at 4 °C. To wash the unbound oligomers, pelleted beads were resuspended with PBS, which were centrifuged again. This washing process was repeated 4−5 times. After washing, pelleted beads were resuspended with 20 μL of 2× protein sample buffer, loaded to SDS-PAGE, and analyzed by Western blot.

RESULTS

Generation and Characterization of Aβ-Seeded αS Oligomers. Aβ-seeded αS oligomers were generated by incubating αS with $Aβ_{42}$ monomers in 20 mM sodium phosphate buffer at 37 °C. Tsigelny et al. reported that Aβ-seeded αS oligomers were effectively generated by incubating 10 μM αS with 20 μM $Aβ_{42}$ monomers.[17] We tested various concentration of $Aβ_{42}$ monomer with 10 μM αS for oligomer generation (Supplementary Figure 1, SI), which showed that the amount of Aβ-seeded αS oligomers increased as the concentration of $Aβ_{42}$ monomer increased, in line with the results of Tsigelny et al.[17] We also varied the incubation time for the oligomer formation (Supplementary Figure 2a, SI). The larger oligomers of approximately 250 kDa were not generated at 5 days of incubation but appeared at 7 days of incubation. Thus, we produced Aβ-seeded αS oligomers by incubating 10 μM αS with 20 μM $Aβ_{42}$ monomers at 37 °C for 7 days,[10,17] which were purified by size-exclusion chromatography for large-sized oligomers or protofibrils (Figure 1a). Under these incubation conditions, $Aβ_{42}$ incubated without αS generated various sizes of oligomeric and protofibril forms, typically larger than 15 kDa (Figure 1a). The incubation of αS without $Aβ_{42}$ did not produce oligomeric aggregates (Figure 1a). These results are consistent with previous studies showing that $Aβ_{42}$ promotes the aggregation of αS and functions as the intermediate for the aggregation of αS, possibly by directly interacting with αS and coaggregation.[10,16,17] Purified Aβ-seeded αS oligomers mainly have a rod-like structure (Supplementary Figure 3, SI). To assess the coexistence of two proteins in large-sized oligomeric forms, we performed a coimmunoprecipitation assay. We precipitated the oligomeric forms with an anti-αS antibody and then performed Western blot analysis using an anti-Aβ antibody (Figure 1b). The obtained results suggest that the large-sized oligomers generated by the coincubation of $Aβ_{42}$ and αS contained both $Aβ_{42}$ and αS. Alternatively, because toxic small Aβ oligomers have been formed under low temperature conditions,[33] we also attempted to produce αS oligomers in the presence of $Aβ_{42}$ at 4 °C. However, at this low temperature, no complexes or large-sized αS oligomers formed (Supplementary Figure 2b, SI).

Then, we determined which region of αS participates in the aggregation of Aβ-seeded αS oligomers. For this purpose, we

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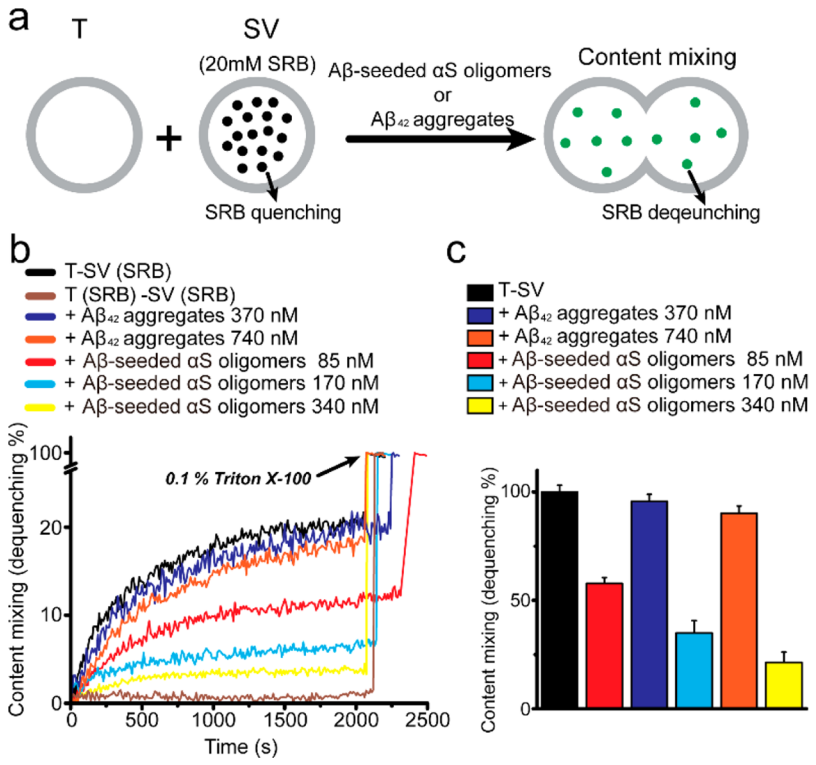

Figure 2. Inhibition of content mixing by Aβ-seeded αS oligomers. (a) Content mixing measured by the fusion reaction between unlabeled t-vesicles (**T**) (reconstituted with t-SNARE, a preformed binary complex of syntaxin-1A and SNAP-25) and v-vesicles (**SV**) (reconstituted with synaptobervin-2 and full-length synaptotagmin-1) encapsulating 20 mM SRB were detected by the dequenching of SRB. Full content release was measured by adding 0.1% Triton X-100 at the end of fusion reaction. (b) The effects of Aβ-seeded αS oligomers on the content mixing assay. **T**-**SV**: **T** and **SV** (20 μM in lipid concentration) were mixed together at 35 °C without any additives (black line). Aβ-seeded αS oligomers produced inhibitory effects on content mixing in a dose-dependent manner (85 nM, red line; 170 nM, light blue line; and 340 nM, yellow line [αS monomer concentration]). For Aβ_{42} aggregates, insignificant inhibitory effects on content mixing were observed. The leakage of SRB was tested by using t-vesicles encapsulating 20 mM SRB, **T** (SRB). The fusion of **T** (SRB) with **SV** (SRB) presented no dequenching of SRB [**T** (SRB) − **SV** (SRB)], which proved no leakage of SRB (brown line). (c) Relative percentages of SRB dequenching at 1800 s from b.

used four types of αS antibodies that bind to the full-length, N-terminal, internal, or C-terminal epitopes to identify which motifs are responsible for aggregation (Figure 1c). Aβ-seeded αS oligomers were efficiently detected when αS antibodies targeting the full-length and C-terminal epitopes were used. In contrast, the N-terminal and internal regions of αS were not targeted by any of these antibodies, indicating that the C-terminal regions of αS in Aβ-seeded αS oligomers are exposed, allowing access to the αS antibodies. Thus, the N-terminal and internal regions of αS appear to be used for the coaggregation with Aβ.

Aβ-Seeded αS Oligomers Efficiently Inhibit Neuronal SNARE-Mediated Lipid Mixing. Next, we tested if the Aβ-seeded αS oligomers inhibited neuronal SNARE-mediated vesicle fusion.[34,35] We prepared reconstituted proteoliposomes for an *in vitro* fusion assay; t-SNAREs, the heterodimer of syntaxin HT and SNAP-25, and v-SNARE synaptobrevin-2 were incorporated into t- and v-vesicles, respectively.[36] Here, t-vesicles (**T**) were doped with DiI (green dye) and v-vesicles (**V**) with DiD (red dye). When the two types of vesicles were fused together through the interactions between SNARE proteins, the mixing of the lipids from the two types of vesicles occurred (Figure 1d). This lipid mixing was monitored by the fluorescence resonance energy transfer (FRET) signal between DiI (FRET donor) and DiD (FRET acceptor) (Figure 1d). Without the addition of oligomers, the **T**-**V** mixture presented significant mixing of lipids (black line, Figure 1e). We confirmed that lipid mixing was driven by SNARE assembly because addition of the soluble synaptobrevin-2 blocked lipid mixing near completely (Figure 1e, purple line). Then, we applied Aβ_{42} oligomers generated without αS to the fusion reaction mixture, and no inhibitory effects on vesicle fusion were observed from 180 nM to 740 nM of Aβ_{42} aggregates (monomer concentration). We also assessed the products of αS incubated without Aβ_{42}, which produced no effects on vesicle fusion (Supplementary Figure 4, SI). In contrast, when we applied the Aβ-seeded αS oligomers to the fusion reaction mixture, lipid mixing was dramatically reduced (Figure 1e). The inhibition was nearly 65% with 85 nM Aβ-seeded αS oligomers (αS monomer concentration) in a 20 μM vesicle mixture (lipid concentration) (Figure 1f), and with 340 nM oligomers, nearly 80% of the fusion was blocked (Figure 1f). These results clearly demonstrate that Aβ_{42} enhanced αS oligomerization, and the resultant large-sized αS oligomers inhibited SNARE-mediated vesicle fusion.

Previous work showed that the αS monomer can inhibit vesicle fusion.[37] In this work, the authors used a high concentration of αS monomer; this concentration was greater than a few μM and is much higher than the concentration we tested (Supplementary Figure 4). At such a high concentration, the αS monomer can inhibit fusion by lipid-αS interactions rather than by direct SNARE-αS interactions. We also confirmed the fusion inhibition by a high concentration of αS monomer (Supplementary Figure 5, SI). The concentration

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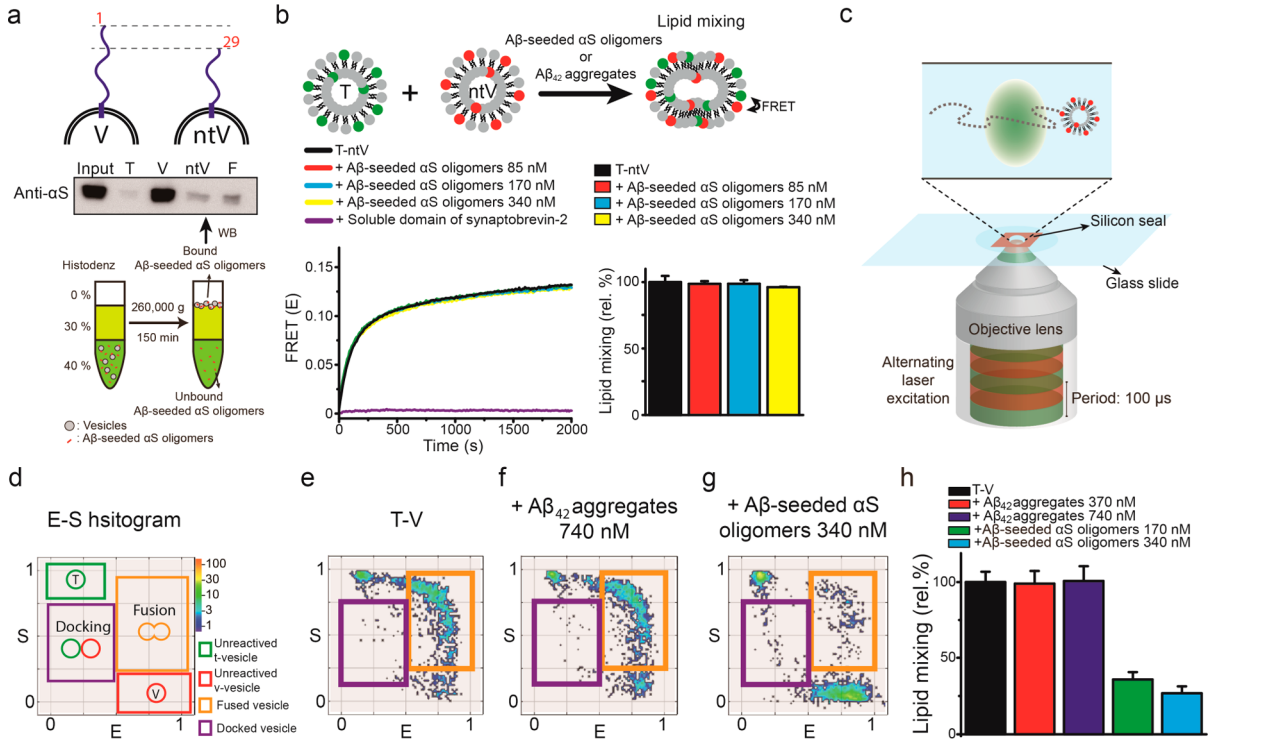

Figure 3. Aβ-seeded αS oligomers interact with the N-terminal domain of synaptobrevin-2 and block vesicle docking. (a) Co-floatation assay assessing the binding of Aβ-seeded αS oligomers to vesicles. (Bottom panel) Schematic illustration of the cofloatation assay. Each **T**, **V**, **ntV** (reconstituted with N-terminal truncated synaptobrevin-2, top cartoon) or F (protein free) was incubated with Aβ-seeded αS oligomers. Vesicle-bound Aβ-seeded αS oligomers were obtained from a gradient centrifugation in the presence of 0%, 30% (dark yellow), and 40% (green) Histodenz and quantified with Western blotting (middle panel). (b) Test of the inhibitory effect on fusion of Aβ-seeded αS oligomers on the fusion reaction with v-vesicles reconstituted with N-terminal truncated synaptobevin-2 (**ntV**). **T-ntV**: **T** and **ntV** (20 μM in lipid concentration) were mixed together at 35 °C without any additives (black line). A negative lipid mixing control was performed by adding a soluble motif of synaptobrevin-2 (purple line). No inhibitory effects of Aβ-seeded αS oligomers (85 nM, red line; 170 nM, light blue line; and 340 nM, yellow line) on **T-ntV** lipid mixing were observed. Bar graphs were obtained from three independent measurements. (c) Schematic illustration of the single-vesicle lipid-mixing assay by ALEX. Sufficient dilution to 100 pM vesicles ensures that only one vesicle passes through the excitation volume at a given time. The result of ALEX measurement is presented in ***E*** (FRET efficiency)-***S*** (sorting number) histogram. (d) Schematic representation of the ***E-S*** histogram. Three fluorescent intensities of a vesicle from the time traces in Supplementary Figure 9, SI were used to calculate ***E*** and ***S***. Unreacted, docked, and lipid-mixed vesicles localized to the different areas of the ***E*−*S*** graph (unreacted **T** [green box], unreacted **V** [red box], docked vesicles [purple box], and lipid-mixed vesicles [orange box]). (e) ***E-S*** graph of **T-V** fusion. t- and v-vesicles (20 μM final lipid concentration) were mixed and then incubated for 30 min at 35 °C prior to ALEX measurements. A significant amount of lipid-mixed vesicles was observed (orange box). (f) ***E-S*** graph of **T-V** fusion in the presence of 740 nM of Aβ_{42} aggregates. No difference in the amount of lipid-mixed vesicles was observed (orange box). (g) ***E-S*** graph of **T-V** fusion in the presence of 340 nM of Aβ-seeded αS oligomers (αS monomer concentration). (h) Relative subpopulation of lipid-mixed vesicles measured from ALEX (Supplementary Figure 8, SI). The subpopulation of lipid-mixed vesicles was significantly reduced. Error bars were obtained from three independent experiments.

required to inhibit fusion was nearly 2 orders of magnitude less for the oligomers than for the monomers.

Inhibition of Content Mixing by Aβ-Seeded αS Oligomers. In exocytosis, vesicles undergo two major reaction steps: vesicle docking, i.e., the initial complex formation reaction between t-SNARE and synaptobrevin-2 to form the vesicle complex without lipid mixing, and vesicle fusion. Vesicle fusion can be monitored by two methods. One common method is to observe lipid mixing, i.e., the mixing process between lipids of two attached vesicles (Figure 1d). However, lipid mixing does not ensure content mixing, which represents full vesicle fusion.[38] Thus, although we demonstrated that Aβ-seeded αS oligomers blocked lipid mixing (Figure 1e), we needed to test whether the oligomers also inhibited content mixing. We performed a content mixing assay (Figure 2a) using two populations of proteoliposomes, unlabeled t-vesicles and v-vesicles containing sulforhodamine B (SRB). We incorporated synaptotagmin-1 together with synaptobrevin-2 into v-vesicles (**SV**) for the content mixing assay because synaptotagmin-1 is often required for content mixing.[39] We mixed two types of vesicles with 20 μM lipid concentrations with or without aggregates. The increase in the fluorescence signal by SRB dequenching was used as an indicator of the content mixing process in this assay.[39] At appropriate time points, 0.1% Triton X-100 was added to the reaction mixture to obtain a fluorescence signal at 100% SRB dequenching. We observed that Aβ-seeded αS oligomers efficiently inhibited the neuronal SNARE-mediated content mixing, but Aβ aggregates had negligible effects on content mixing (Figure 2b). Thus, Aβ-seeded αS oligomers blocked both lipid and content mixing in vesicle fusion.

Aβ-Seeded αS Oligomers Interact with the N-Terminal Domain of Synaptobrevin-2 and Block the Vesicle Docking Step. Although various modes of interaction between αS and the reconstituted proteoliposomes are feasible, such as the binding to negatively charged membrane lipids[29,40−43] and permeabilization of vesicle membranes,[44] αS oligomer binding to synaptobrevin-2 on synaptic vesicles[30] has

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been shown to be the major factor responsible for the inhibitory effects on fusion of αS oligomers induced by dopamine.[32] The binding of αS to synaptobrevin-2 has been recently further supported by single-vesicle assays.[31] Thus, we suspected that Aβ-seeded αS oligomers also interacted with the N-terminal domain of synaptobrevin-2, which may have resulted in the inhibitory effects on fusion. To test this hypothesis, we performed a cofloatation assay.[30] In this assay, Aβ-seeded αS oligomers bound to vesicles were separated from the free Aβ-seeded αS oligomers in solution and then quantified by Western blot analysis (Figure 3a). These results show that Aβ-seeded αS oligomers bound favorably to v-vesicles (**V**), which contained synaptobrevin-2. However, when v-vesicles (**ntV**) were reconstituted with an N-terminal truncated synaptobrevin-2 (nt-synaptobrevin-2, 29−116), the binding affinity of Aβ-seeded αS oligomers to v-vesicles (**ntV**) was significantly reduced compared with the v-vesicles (**V**) reconstituted with full-length synaptobrevin-2 (Figure 3a, Western blot). These results demonstrate that αS oligomers induced by Aβ seeding also interacted with the N-terminal domain of synaptobrevin-2.

Next, we tested the effects of Aβ-seeded αS oligomers on vesicle fusion using v-vesicles (**ntV**) with nt-synaptobrevin-2 (Figure 3b). Because nt-synaptobrevin-2 did not contain the N-terminal domain to allow binding of Aβ-seeded αS oligomers, we expected that Aβ-seeded αS oligomers would not inhibit vesicle fusion. As anticipated, no inhibitory effects on vesicle fusion were observed (Figure 3b). These results are consistent with the cofloatation assays and clearly demonstrate that the interaction with the N-terminal domain of synaptobrevin-2 is a critical factor for the inhibitory effects of Aβ-seeded αS oligomers on vesicle fusion.

By adding protein-free liposomes to the fusion reaction mixture, we confirmed that Aβ-seeded αS oligomers have a negligible binding affinity to negatively charged liposomes (Supplementary Figure 6, SI). In line with this result, Aβ-seeded αS oligomers did not induce clustering of protein-free liposomes or t-vesicles, whereas the oligomers induced clustering of v-vesicles (Supplementary Figure 7, SI).

Then, we investigated how the interactions between the N-terminal domain of synaptobrevin-2 and Aβ-seeded αS oligomers inhibited vesicle fusion. We used a single-vesicle assay, termed alternating-laser excitation (ALEX), to investigate which fusion step was involved in the inhibitory effects of Aβ-seeded αS oligomers.[45] ALEX observes one vesicle or vesicle-pair at a time freely diffusing in solution (Figure 3c). By using two lasers in alternating modes, ALEX has the capability of discriminating the status of vesicle, such as unreacted, docked, and fused, and the sorted results are presented in a two-dimensional graph of ***E*** (FRET efficiency) and ***S*** (sorting number) (Figure 3d). Figure 3d depicts the expected location of each type of vesicles schematically in the ***E-S*** graph; green and red squares indicate the locations of the unreacted t- and v-vesicles, respectively, the purple square indicates docked vesicles, and the orange square indicates lipid-mixed vesicles. We incubated a mixture of **T** and **V** with or without aggregates in 20 μM of lipids concentration for 30 min at 35 °C and then performed ALEX measurements to analyze the subpopulations of the mixture (Figure 3e−g and Supplementary Figures 8 and 9). Figure 3e shows the results of **T-V** mixture without aggregates, where a substantial amount of the lipid-mixed population (orange square) was observed. When we added $A\beta_{42}$ aggregates to this mixture, no substantial differences in the subpopulations and the amount of lipid-mixed vesicles were observed (Figure 3f). However, the subpopulation of lipid-mixed vesicles was significantly reduced when we added Aβ-seeded αS oligomers (Figure 3g,h). These results are consistent with the bulk measurements, where only Aβ-seeded αS oligomers produced inhibitory effects on fusion (Figure 1e). Importantly, the subpopulation of docked vesicles (purple square) did not increase, but the subpopulation of lipid-mixed vesicles was markedly reduced by the Aβ-seeded αS oligomers (Figure 3g,h). This result directly indicates that Aβ-seeded αS oligomers inhibited vesicle fusion at the docking step; if the lipid-mixing step was inhibited, then the subpopulation of docked vesicles would have been increased. The inhibition of the docking step by Aβ-seeded αS oligomers resembles the inhibitory effects of dopamine-induced αS oligomers.[32] Thus, it is highly possible that both oligomers share the same molecular mechanism on fusion inhibition, i.e., the binding of oligomers to synaptobrevin-2 prohibiting complex formation between synaptobrevin-2 and t-SNARE in vesicle docking.

Aβ-Seeded αS Oligomers Transduced into PC12 Cells Inhibit Exocytosis. To test whether Aβ-seeded αS oligomers inhibited SNARE-mediated exocytosis at the cellular level, we used a protein transfection method to directly deliver Aβ and αS oligomers into PC12 cells (Figure 4).[32] The delivery of

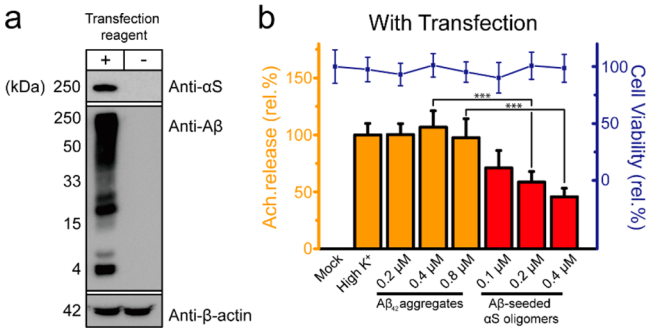

Figure 4. Aβ-seeded αS oligomers transduced into PC12 cells reduce exocytosis. (a) The transfection of Aβ-seeded αS oligomers into PC12 cells was confirmed by Western blot. Cell lysates were used for Western blot analysis and probed with an anti-αS antibody (top panel) and an anti-Aβ antibody (middle panel). The amount of protein loaded was confirmed by visualizing β-actin levels (bottom panel). When the transfection reagent was absent, no Aβ-seeded αS oligomers were detected. (b) The effects of Aβ-seeded αS oligomers delivered into PC12 cells on exocytosis. After delivering the Aβ-seeded αS oligomers or $A\beta_{42}$ aggregates generated without αS into PC12 cells with transfection reagents, the amount of released [^{14}C]-acetylcholine by a high-K^+ depolarization was measured (***$P < 0.005$). An MTT assay was performed to ensure cell viability after transfection.

oligomers into PC12 cells by this transfection method was confirmed using Western blot analysis (Figure 4a). Then, we used a high K^+ solution to depolarize and induce exocytosis and measured the level of exocytosis by quantifying the amounts of released [^{14}C]-acetylcholine (Figure 4b). When Aβ-seeded αS oligomers were transfected, the level of exocytosis was significantly reduced (Figure 4b, red bar). In contrast, the Aβ aggregates had no effect on exocytosis, which was consistent with the *in vitro* assay (Figure 4b, orange bar). An MTT assay was performed to test the cytotoxicity of both Aβ aggregates and Aβ-seeded αS oligomers, which revealed negligible toxicity (Figure 4b, blue dot line). As a control, we assessed exocytosis without treating with the transfection reagents, and no change

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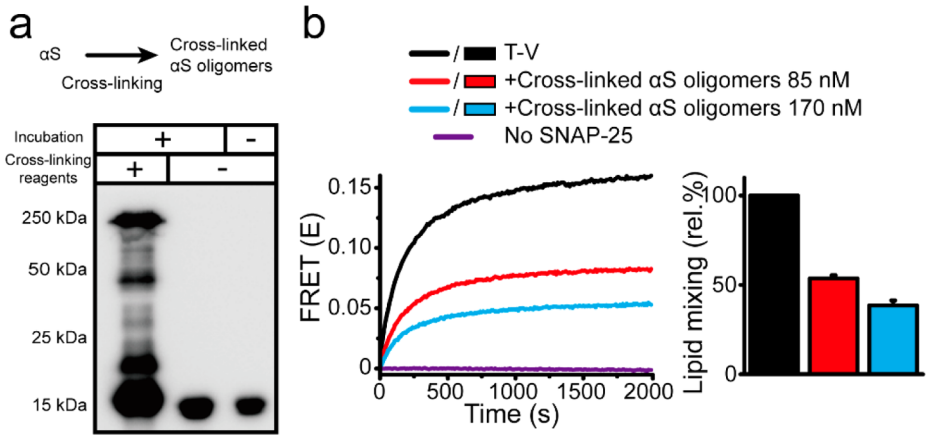

Figure 5. Cross-linked large-sized αS oligomers inhibit SNARE-mediated vesicle fusion. (a) Western blot of cross-linked αS oligomers. First lane, cross-linked αS oligomers generated by incubating 25 μM αS with 500 μM of the BS^3 (bis[sulfosuccinimidyl] suberate) cross-linker at room temperature for 1 h, followed by 25 mM Tris-HCl (pH 7.4) at room temperature for 30 min. Second lane, no BS^3 control, i.e., 25 μM αS was incubated without BS^3 at room temperature for 1 h. Third lane, αS monomers without incubation. (b) The effects of cross-linked αS oligomers on lipid mixing. A vesicle fusion reaction was performed under identical conditions as described in Figure 1d. With the addition of cross-linked αS oligomers to the **T-V** reaction mixture, a significant reduction in fusion was observed (85 nM, red line; 170 nM, light blue line; [αS monomer concentration]). The no SNAP-25 condition is a control for vesicle fusion (purple line).

in the amount of exocytosis through depolarization was observed with or without the aggregates of Aβ and Aβ-seeded αS oligomers (Supplementary Figure 10, SI). Thus, Aβ-seeded αS oligomers delivered into PC12 cells inhibited exocytosis, which is fully consistent with our observations in the *in vitro* fusion assays.

Large-Sized αS Oligomers Induced by Chemical Cross-Linking Inhibit SNARE-Mediated Vesicle Fusion. In this work, we demonstrated that Aβ-seeded αS oligomers inhibited vesicle fusion by interacting with synaptobrevin-2. The inhibitory mechanism of Aβ-seeded αS closely overlaps with the mechanism of dopamine-mediated αS oligomers.[32] These findings lead us to postulate that large-sized αS oligomers generated by any pathway would interact with synaptobrevin-2 and ultimately inhibit SNARE-mediated vesicle fusion. To test this hypothesis, we prepared large-sized αS oligomers by chemical cross-linking. We used a bis-[sulfosuccinimidyl] suberate cross-linker to generate αS oligomers (Figure 5a). After purifying the αS oligomers, we applied them to an *in vitro* vesicle fusion assay. Interestingly, the cross-linked αS oligomers also significantly reduced the amount of vesicle fusion (Figure 5b), which was similar to the inhibitory effects of Aβ-seeded αS oligomers. The inhibitory effects of the cross-linked αS oligomers on exocytosis were also confirmed using PC12 cells (Supplementary Figure 11, SI). Then, we tested whether the cross-linked αS oligomers interacted with the N-terminal domain of synpatobrevin-2 to produce an inhibitory effect using v-vesicles reconstituted with nt-synaptobrevin-2. As expected, the inhibitory effects on fusion of the cross-linked αS oligomers were absent with nt-synaptobrevin-2 (Supplementary Figure 12, SI). These results suggest that the inhibitory effect on fusion is a common feature of large-sized αS oligomers, and thus seeding molecules, such as Aβ and dopamine, play an important role in the inhibition of neurotransmission by generating harmful large-sized αS oligomers.

DISCUSSION

In this work, we studied the effects of cross-seeding between $A\beta_{42}$ and αS, which generated large-sized αS oligomers that inhibited neuronal SNARE-mediated vesicle fusion. Aβ-seeded αS oligomers binding to the N-terminal domain of synapto-brevin-2 inhibited vesicle fusion (Figure 6). In contrast, the

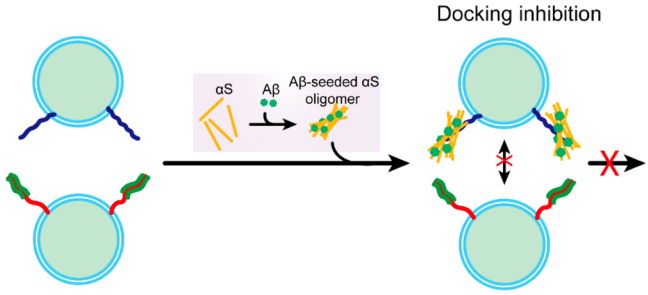

Figure 6. The proposed model for inhibitory effects of Aβ-seeded αS oligomers on vesicle fusion. Aβ escalates the production of large-sized αS oligomers, and these oligomers efficiently block vesicle docking by selectively binding to synaptobrevin-2, a vesicular SNARE protein, which would prompts synaptic dysfunction in neuron.

products of $A\beta_{42}$ and αS incubated separately without mixing had no effect on vesicle fusion. These results suggest that the cooperative effect between Aβ and αS produces toxic oligomers that inhibit vesicle fusion and consequently disrupt synaptic transmission.

Aβ is typically produced by endoproteolysis of the amyloid precursor protein in the extracellular medium. Although the plaques, mostly composed of Aβ, are located in the extracellular region, it is now generally accepted that Aβ also accumulates intracellularly.[18,46] Evidence suggests that the accumulation of intracellular $A\beta_{42}$ may be associated with an early stage of the AD pathology.[47,48] However, the role of intracellular $A\beta_{42}$ still remains a key unanswered question. Interestingly, the Aβ peptide found intracellularly mostly comprises $A\beta_{42}$ among its isoforms,[46,47] and they can be produced in the ER in neurons, with the associated synaptic pathology.[49] In addition to the generation of intracellular $A\beta_{42}$, reuptake of extracellular $A\beta_{42}$ through interactions with various receptors is a well-known process of Aβ internalization.[18,50] Evidence suggests that the oligomerization of Aβ occurs in an intracellular region prior to

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the extracellular space.[18] Because αS is one of the most abundant cytosolic proteins in the presynaptic terminal, it is possible that intracellular $A\beta_{42}$ may trigger the oligomerization of αS or co-oligomerization with αS. Our results suggest that the consequence of cross-seeding oligomers is the inhibition of SNARE functions. Recently, Sharma et al. have reported that the amount of SNARE-complex assembly is reduced in post-mortem brain tissues of AD patients.[51] This observation indicates the connection between AD and the SNARE-complex. Although multiple pathways can contribute to reduced SNARE-complex assembly, our observation that Aβ-seeded αS oligomers inhibited vesicle docking, i.e., the initial binding between SNARE-complex components, provides a potential mechanism for the reduction of SNARE-complex assembly in the brain tissues of AD patients. Because Aβ oligomer generation at the presynaptic terminal is associated with the early stages of AD,[18] the inhibition by Aβ-seeded αS oligomers of neuronal SNARE-mediated fusion may occur during the early symptoms.

Because one of the hallmarks of PD is the preferential destruction of dopaminergic neurons, dopamine has been anticipated to play a key role in PD.[52,53] Indeed, Lansbury and co-workers have demonstrated that dopamine induces the formation of αS oligomers while reducing fibril formation,[54] and the detailed mechanism of αS oligomerization by dopamine has been extensively studied.[55,56] In our previous work, we reported that dopamine-induced αS oligomers have a toxic function by inhibiting vesicle fusion.[32] In the present study, we further demonstrated that the inhibitory effects on fusion were maintained by αS oligomers generated through Aβ seeding effect and chemical cross-linking. Although αS can form oligomers by itself, many cofactors or seeding molecules, such as metal ions,[57,58] Aβ, and dopamine, accelerate αS oligomerization. Our results strongly suggest that seeding molecules, such as Aβ, dopamine, and other reactive molecules that can cross-link αS, initiate αS oligomerization, and eventually cause the inhibitory effects on fusion by generating large-sized αS oligomers (Figure 6). It may be general that large-sized αS oligomers, when produced in neurons, produce neurotoxicity by blocking SNARE-mediated neurotransmission.

■ ASSOCIATED CONTENT

Supporting Information

Supplementary Figures 1−12; methods for preparation of cross-linked αS oligomers, preparation of SNARE proteins and reconstituted proteoliposomes, *in vitro* single vesicle fusion assay by ALEX, measuring [^{14}C]-acetylcholine release from PC12 cells and MTT cytotoxicity assay, cofloatation assay used to detect the binding of Aβ-seeded αS oligomers to proteoliposomes. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) Hardy, J., and Selkoe, D. J. (2002) The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics. *Science 297*, 353−356.
(2) Iwai, A., Masliah, E., Yoshimoto, M., Ge, N., Flanagan, L., de Silva, H. A., Kittel, A., and Saitoh, T. (1995) The precursor protein of non-A beta component of Alzheimer's disease amyloid is a presynaptic protein of the central nervous system. *Neuron 14*, 467−475.
(3) Spillantini, M. G., Schmidt, M. L., Lee, V. M., Trojanowski, J. Q., Jakes, R., and Goedert, M. (1997) Alpha-synuclein in Lewy bodies. *Nature 388*, 839−840.
(4) Irwin, D. J., Lee, V. M., and Trojanowski, J. Q. (2013) Parkinson's disease dementia: convergence of alpha-synuclein, tau and amyloid-beta pathologies. *Nat. Rev. Neurosci. 14*, 626−636.
(5) Armstrong, R. A., Cairns, N. J., and Lantos, P. L. (1997) beta-Amyloid (A beta) deposition in the medial temporal lobe of patients with dementia with Lewy bodies. *Neurosci. Lett. 227*, 193−196.
(6) Hamilton, R. L. (2000) Lewy bodies in Alzheimer's disease: a neuropathological review of 145 cases using alpha-synuclein immunohistochemistry. *Brain Pathol. 10*, 378−384.
(7) Leverenz, J. B., Fishel, M. A., Peskind, E. R., Montine, T. J., Nochlin, D., Steinbart, E., Raskind, M. A., Schellenberg, G. D., Bird, T. D., and Tsuang, D. (2006) Lewy body pathology in familial Alzheimer disease: evidence for disease- and mutation-specific pathologic phenotype. *Arch. Neurol. 63*, 370−376.
(8) Tsuang, D. W., Riekse, R. G., Purganan, K. M., David, A. C., Montine, T. J., Schellenberg, G. D., Steinbart, E. J., Petrie, E. C., Bird, T. D., and Leverenz, J. B. (2006) Lewy body pathology in late-onset familial Alzheimer's disease: a clinicopathological case series. *J. Alzheimers Dis. 9*, 235−242.
(9) Rosenberg, C. K., Pericak-Vance, M. A., Saunders, A. M., Gilbert, J. R., Gaskell, P. C., and Hulette, C. M. (2000) Lewy body and Alzheimer pathology in a family with the amyloid-beta precursor protein APP717 gene mutation. *Acta Neuropathol. 100*, 145−152.
(10) Masliah, E., Rockenstein, E., Veinbergs, I., Sagara, Y., Mallory, M., Hashimoto, M., and Mucke, L. (2001) beta-amyloid peptides enhance alpha-synuclein accumulation and neuronal deficits in a transgenic mouse model linking Alzheimer's disease and Parkinson's disease. *Proc. Natl. Acad. Sci. U.S.A. 98*, 12245−12250.
(11) Clinton, L. K., Blurton-Jones, M., Myczek, K., Trojanowski, J. Q., and LaFerla, F. M. (2010) Synergistic Interactions between Abeta, tau, and alpha-synuclein: acceleration of neuropathology and cognitive decline. *J. Neurosci. 30*, 7281−7289.
(12) Marsh, S. E., and Blurton-Jones, M. (2012) Examining the mechanisms that link beta-amyloid and alpha-synuclein pathologies. *Alzheimer Res. Ther. 4*, 11.
(13) Morales, R., Moreno-Gonzalez, I., and Soto, C. (2013) Cross-seeding of misfolded proteins: implications for etiology and pathogenesis of protein misfolding diseases. *PLoS Pathog. 9*, e1003537.
(14) Ono, K., Takahashi, R., Ikeda, T., and Yamada, M. (2012) Cross-seeding effects of amyloid beta-protein and alpha-synuclein. *J. Neurochem. 122*, 883−890.
(15) Suzuki, N., Cheung, T. T., Cai, X. D., Odaka, A., Otvos, L., Jr., Eckman, C., Golde, T. E., and Younkin, S. G. (1994) An increased percentage of long amyloid beta protein secreted by familial amyloid beta protein precursor (beta APP717) mutants. *Science 264*, 1336−1340.
(16) Mandal, P. K., Pettegrew, J. W., Masliah, E., Hamilton, R. L., and Mandal, R. (2006) Interaction between Abeta peptide and alpha synuclein: molecular mechanisms in overlapping pathology of Alzheimer's and Parkinson's in dementia with Lewy body disease. *Neurochem. Res. 31*, 1153−1162.
(17) Tsigelny, I. F., Crews, L., Desplats, P., Shaked, G. M., Sharikov, Y., Mizuno, H., Spencer, B., Rockenstein, E., Trejo, M., Platoshyn, O.,

DOI: 10.1021/acs.biochem.5b00087
Biochemistry 2015, 54, 1831−1840

Yuan, J. X., and Masliah, E. (2008) Mechanisms of hybrid oligomer formation in the pathogenesis of combined Alzheimer's and Parkinson's diseases. *PloS One 3*, e3135.
(18) LaFerla, F. M., Green, K. N., and Oddo, S. (2007) Intracellular amyloid-beta in Alzheimer's disease. *Nat. Rev. Neurosci. 8*, 499−509.
(19) Bucciantini, M., Giannoni, E., Chiti, F., Baroni, F., Formigli, L., Zurdo, J., Taddei, N., Ramponi, G., Dobson, C. M., and Stefani, M. (2002) Inherent toxicity of aggregates implies a common mechanism for protein misfolding diseases. *Nature 416*, 507−511.
(20) Lotharius, J., and Brundin, P. (2002) Pathogenesis of Parkinson's disease: dopamine, vesicles and alpha-synuclein. *Nat. Rev. Neurosci. 3*, 932−942.
(21) Chiti, F., and Dobson, C. M. (2006) Protein misfolding, functional amyloid, and human disease. *Annu. Rev. Biochem. 75*, 333−366.
(22) Kim, H. Y., Cho, M. K., Kumar, A., Maier, E., Siebenhaar, C., Becker, S., Fernandez, C. O., Lashuel, H. A., Benz, R., Lange, A., and Zweckstetter, M. (2009) Structural properties of pore-forming oligomers of alpha-synuclein. *J. Am. Chem. Soc. 131*, 17482−17489.
(23) Lorenzen, N., Nielsen, S. B., Buell, A. K., Kaspersen, J. D., Arosio, P., Vad, B. S., Paslawski, W., Christiansen, G., Valnickova-Hansen, Z., Andreasen, M., Enghild, J. J., Pedersen, J. S., Dobson, C. M., Knowles, T. P., and Otzen, D. E. (2014) The role of stable alpha-synuclein oligomers in the molecular events underlying amyloid formation. *J. Am. Chem. Soc. 136*, 3859−3868.
(24) Larsen, K. E., Schmitz, Y., Troyer, M. D., Mosharov, E., Dietrich, P., Quazi, A. Z., Savalle, M., Nemani, V., Chaudhry, F. A., Edwards, R. H., Stefanis, L., and Sulzer, D. (2006) Alpha-synuclein overexpression in PC12 and chromaffin cells impairs catecholamine release by interfering with a late step in exocytosis. *J. Neurosci. 26*, 11915−11922.
(25) Gitler, A. D., Bevis, B. J., Shorter, J., Strathearn, K. E., Hamamichi, S., Su, L. J., Caldwell, K. A., Caldwell, G. A., Rochet, J. C., McCaffery, J. M., Barlowe, C., and Lindquist, S. (2008) The Parkinson's disease protein alpha-synuclein disrupts cellular Rab homeostasis. *Proc. Natl. Acad. Sci. U.S.A. 105*, 145−150.
(26) Nemani, V. M., Lu, W., Berge, V., Nakamura, K., Onoa, B., Lee, M. K., Chaudhry, F. A., Nicoll, R. A., and Edwards, R. H. (2010) Increased expression of alpha-synuclein reduces neurotransmitter release by inhibiting synaptic vesicle reclustering after endocytosis. *Neuron 65*, 66−79.
(27) Murphy, D. D., Rueter, S. M., Trojanowski, J. Q., and Lee, V. M. (2000) Synucleins are developmentally expressed, and alpha-synuclein regulates the size of the presynaptic vesicular pool in primary hippocampal neurons. *J. Neurosci. 20*, 3214−3220.
(28) Yavich, L., Tanila, H., Vepsalainen, S., and Jakala, P. (2004) Role of alpha-synuclein in presynaptic dopamine recruitment. *J. Neurosci. 24*, 11165−11170.
(29) DeWitt, D. C., and Rhoades, E. (2013) alpha-Synuclein can inhibit SNARE-mediated vesicle fusion through direct interactions with lipid bilayers. *Biochemistry 52*, 2385−2387.
(30) Burre, J., Sharma, M., Tsetsenis, T., Buchman, V., Etherton, M. R., and Sudhof, T. C. (2010) Alpha-synuclein promotes SNARE-complex assembly in vivo and in vitro. *Science 329*, 1663−1667.
(31) Diao, J., Burre, J., Vivona, S., Cipriano, D. J., Sharma, M., Kyoung, M., Sudhof, T. C., and Brunger, A. T. (2013) Native alpha-synuclein induces clustering of synaptic-vesicle mimics via binding to phospholipids and synaptobrevin-2/VAMP2. *eLife 2*, e00592.
(32) Choi, B. K., Choi, M. G., Kim, J. Y., Yang, Y., Lai, Y., Kweon, D. H., Lee, N. K., and Shin, Y. K. (2013) Large alpha-synuclein oligomers inhibit neuronal SNARE-mediated vesicle docking. *Proc. Natl. Acad. Sci. U.S.A. 110*, 4087−4092.
(33) Ahmed, M., Davis, J., Aucoin, D., Sato, T., Ahuja, S., Aimoto, S., Elliott, J. I., Van Nostrand, W. E., and Smith, S. O. (2010) Structural conversion of neurotoxic amyloid-beta(1-42) oligomers to fibrils. *Nat. Struct. Mol. Biol. 17*, 561−567.
(34) Sudhof, T. C., and Rothman, J. E. (2009) Membrane Fusion: Grappling with SNARE and SM Proteins. *Science 323*, 474−477.
(35) Jahn, R., and Scheller, R. H. (2006) SNAREs–engines for membrane fusion. *Nat. Rev. Mol. Cell. Biol. 7*, 631−643.
(36) Weber, T., Zemelman, B. V., McNew, J. A., Westermann, B., Gmachl, M., Parlati, F., Sollner, T. H., and Rothman, J. E. (1998) SNAREpins: Minimal machinery for membrane fusion. *Cell 92*, 759−772.
(37) Lai, Y., Kim, S., Varkey, J., Lou, X., Song, J. K., Diao, J., Langen, R., and Shin, Y. K. (2014) Nonaggregated alpha-synuclein influences SNARE-dependent vesicle docking via membrane binding. *Biochemistry 53*, 3889−3896.
(38) Sudhof, T. C. (2004) The synaptic vesicle cycle. *Annu. Rev. Neurosci. 27*, 509−547.
(39) Kyoung, M., Srivastava, A., Zhang, Y., Diao, J., Vrljic, M., Grob, P., Nogales, E., Chu, S., and Brunger, A. T. (2011) In vitro system capable of differentiating fast Ca^{2+}-triggered content mixing from lipid exchange for mechanistic studies of neurotransmitter release. *Proc. Natl. Acad. Sci. U.S.A. 108*, E304−313.
(40) Davidson, W. S., Jonas, A., Clayton, D. F., and George, J. M. (1998) Stabilization of alpha-synuclein secondary structure upon binding to synthetic membranes. *J. Biol. Chem. 273*, 9443−9449.
(41) Jo, E., McLaurin, J., Yip, C. M., St George-Hyslop, P., and Fraser, P. E. (2000) alpha-Synuclein membrane interactions and lipid specificity. *J. Biol. Chem. 275*, 34328−34334.
(42) Eliezer, D., Kutluay, E., Bussell, R., Jr., and Browne, G. (2001) Conformational properties of alpha-synuclein in its free and lipid-associated states. *J. Mol. Biol. 307*, 1061−1073.
(43) Comellas, G., Lemkau, L. R., Zhou, D. H., George, J. M., and Rienstra, C. M. (2012) Structural intermediates during alpha-synuclein fibrillogenesis on phospholipid vesicles. *J. Am. Chem. Soc. 134*, 5090−5099.
(44) Volles, M. J., Lee, S. J., Rochet, J. C., Shtilerman, M. D., Ding, T. T., Kessler, J. C., and Lansbury, P. T., Jr. (2001) Vesicle permeabilization by protofibrillar alpha-synuclein: implications for the pathogenesis and treatment of Parkinson's disease. *Biochemistry 40*, 7812−7819.
(45) Kim, J. Y., Choi, B. K., Choi, M. G., Kim, S. A., Lai, Y., Shin, Y. K., and Lee, N. K. (2012) Solution single-vesicle assay reveals PIP(2)-mediated sequential actions of synaptotagmin-1 on SNAREs. *EMBO J. 31*, 2144−2155.
(46) Sakono, M., and Zako, T. (2010) Amyloid oligomers: formation and toxicity of Abeta oligomers. *FEBS J. 277*, 1348−1358.
(47) Gouras, G. K., Tsai, J., Naslund, J., Vincent, B., Edgar, M., Checler, F., Greenfield, J. P., Haroutunian, V., Buxbaum, J. D., Xu, H., Greengard, P., and Relkin, N. R. (2000) Intraneuronal Abeta42 accumulation in human brain. *J. Pathol. 156*, 15−20.
(48) Chui, D. H., Tanahashi, H., Ozawa, K., Ikeda, S., Checler, F., Ueda, O., Suzuki, H., Araki, W., Inoue, H., Shirotani, K., Takahashi, K., Gallyas, F., and Tabira, T. (1999) Transgenic mice with Alzheimer presenilin 1 mutations show accelerated neurodegeneration without amyloid plaque formation. *Nat. Med. 5*, 560−564.
(49) Takahashi, R. H., Milner, T. A., Li, F., Nam, E. E., Edgar, M. A., Yamaguchi, H., Beal, M. F., Xu, H., Greengard, P., and Gouras, G. K. (2002) Intraneuronal Alzheimer abeta42 accumulates in multivesicular bodies and is associated with synaptic pathology. *J. Pathol. 161*, 1869−1879.
(50) Clifford, P. M., Zarrabi, S., Siu, G., Kinsler, K. J., Kosciuk, M. C., Venkataraman, V., D'Andrea, M. R., Dinsmore, S., and Nagele, R. G. (2007) Abeta peptides can enter the brain through a defective blood-brain barrier and bind selectively to neurons. *Brain Res. 1142*, 223−236.
(51) Sharma, M., Burre, J., and Sudhof, T. C. (2012) Proteasome inhibition alleviates SNARE-dependent neurodegeneration. *Sci. Transl. Med. 4*, 147ra113.
(52) Hirsch, E., Graybiel, A. M., and Agid, Y. A. (1988) Melanized dopaminergic neurons are differentially susceptible to degeneration in Parkinson's disease. *Nature 334*, 345−348.
(53) Halliday, G. M., McRitchie, D. A., Cartwright, H., Pamphlett, R., Hely, M. A., and Morris, J. G. (1996) Midbrain neuropathology in idiopathic Parkinson's disease and diffuse Lewy body disease. *J. Clin. Neurosci. 3*, 52−60.

DOI: 10.1021/acs.biochem.5b00087
Biochemistry 2015, 54, 1831−1840

(54) Conway, K. A., Rochet, J. C., Bieganski, R. M., and Lansbury, P. T., Jr. (2001) Kinetic stabilization of the alpha-synuclein protofibril by a dopamine-alpha-synuclein adduct. *Science 294*, 1346−1349.
(55) Cappai, R., Leck, S. L., Tew, D. J., Williamson, N. A., Smith, D. P., Galatis, D., Sharples, R. A., Curtain, C. C., Ali, F. E., Cherny, R. A., Culvenor, J. G., Bottomley, S. P., Masters, C. L., Barnham, K. J., and Hill, A. F. (2005) Dopamine promotes alpha-synuclein aggregation into SDS-resistant soluble oligomers via a distinct folding pathway. *FASEB J. 19*, 1377−1379.
(56) Rekas, A., Knott, R. B., Sokolova, A., Barnham, K. J., Perez, K. A., Masters, C. L., Drew, S. C., Cappai, R., Curtain, C. C., and Pham, C. L. (2010) The structure of dopamine induced alpha-synuclein oligomers. *Eur. Biophys. J. 39*, 1407−1419.
(57) Uversky, V. N., Li, J., and Fink, A. L. (2001) Metal-triggered structural transformations, aggregation, and fibrillation of human alpha-synuclein. A possible molecular NK between Parkinson's disease and heavy metal exposure. *J. Biol. Chem. 276*, 44284−44296.
(58) Binolfi, A., Rasia, R. M., Bertoncini, C. W., Ceolin, M., Zweckstetter, M., Griesinger, C., Jovin, T. M., and Fernandez, C. O. (2006) Interaction of alpha-synuclein with divalent metal ions reveals key differences: a link between structure, binding specificity and fibrillation enhancement. *J. Am. Chem. Soc. 128*, 9893−9901.

DOI: 10.1021/acs.biochem.5b00087
Biochemistry 2015, 54, 1831−1840