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Cu(II) promotes amyloid pore formation

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ABSTRACT

The aggregation of α-synuclein is associated with dopamine neuron death in Parkinson's disease. There is controversy in the field over the question of which species of the aggregates, fibrils or protofibrils, are toxic. Moreover, compelling evidence suggested the exposure to heavy metals to be a risk of PD. Nevertheless, the mechanism of metal ions in promoting PD remains unclear. In this research, we investigated the structural basis of Cu(II) induced aggregation of α-synuclein. Using transmission electron microscopy experiments, Cu(II) was found to promote in vitro aggregation of α-synuclein by facilitating annular protofibril formation rather than fibril formation. Furthermore, neuroprotective baicalein disaggregated annular protofibrils accompanied by considerable decrease of β-sheet content. These results strongly support the hypothesis that annular protofibrils are the toxic species, rather than fibrils, thereby inspiring us to search novel therapeutic strategies for the suppression of the toxic annular protofibril formation.

1. Introduction

The aggregation of α-synuclein is thought to play a role in the onset of Parkinson's disease (PD) [1]. This small natively disordered protein can convert to mature fibrils with β-sheet conformation through an aggregation pathway relevant to intermediate species referred to as 'protofibrils' (or 'oligomers') [1,2]. Among various protofibril structures, annular protofibrils have generated great interest due to their toxin-like morphology and pore-forming potential on cell membranes [3–7]. Two PD linked mutants, A53T and A30P, were reported to have a high propensity to form annular protofibrils that have a strong affinity for membranes, implying a pore-like mechanism related to the toxicity of annular structures that are rich in β-sheet [8–10]. The pore-like mechanism was also supported by the electrophysiological analysis of bilayer conductance with the protofibrils [6,7,11]. In addition, the pore-like morphology and activity on cell membranes of annular protofibrils have also been observed in other protein misfolding diseases, which may imply a common molecular mechanism for the toxicity of protein aggregates [12,13]. On the other hand, the structural flexibility and instability of protofibrils considerably hindered progresses in annular protofibril study. Using cryo-electron microscopy (cryo-EM), our group reported, for the first time, on α-synuclein protofibril structural characteristics, which was also direct proof of the existence of annular protofibrils in solution, and illustrated the aggregation pathway visually from annular protofibrils to fibrils [14]. However, there is still controversy in the field over the question of which species are toxic. Are annular protofibrils the toxic species? More evidence is desired before drawing a definite conclusion.

Compelling epidemiological evidence indicated a risk of PD upon the occupational exposure to heavy metals [15]. In brains of patients with PD, copper, iron, and zinc in high concentration were detected in the cerebrospinal fluid or the substantia nigra [16–18]. That oxidative stress was considered to be a pathogenic factor for PD led to the hypothesis that redox-active metal ions concentrated in substantia nigra could result in more oxidative conditions favorable for radical species production [19,20]. It was also proposed that metal ions might promote the fibrillization of α-synuclein during aggregation, possibly through the structural changes of α-synuclein caused by direct interactions between the protein and metal ions [21–24]. Nevertheless, the mechanism of metal ions in

Abbreviations: ATR, attenuated total reflectance; cryo-EM, cryo-electron microscopy; DMSO, dimethylsulfoxide; EGCG, epigallocatechin gallate; FTIR, Fourier transform infrared; PBS, phosphate buffered saline; PD, Parkinson's disease; TEM, transmission electron microscopy; ThT, Thioflavin T; WT, wild type.

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promoting α-synuclein neurotoxicity remains unclear. Given that increasing evidence suggested fibrils were not the toxic species, it is reasonable to reconsider the roles of metal ions in α-synuclein aggregation.

Polyphenols are a large structural constellation of aromatic compounds characterized by multiple phenol groups. Recent research showed that a number of these natural compounds, such as baicalein and epigallocatechin gallate (EGCG), suppressed toxic aggregation of amyloid proteins [25]. One report in particular showed that EGCG led α-synuclein monomers to form off-pathway, nontoxic oligomers [26]. Baicalein exhibited neuroprotective effect as the treatment of experimental parkinsonism in both in vivo and in vitro research [27,28]. Moreover, it was found to inhibit the fibril formation of α-synuclein and disaggregate existing fibrils, resulting in the production of a stabilized soluble oligomer [29,30]. However, other studies revealed that baicalein inhibited the oligomer formation of α-synuclein in living cells [31], and thus the disaggregation of existing fibrils by baicalein might not be the key to its neuroprotective effect.

Cu(II) and baicalein were both known to bind α-synuclein and modulate its fibrillization, but their influences on α-synuclein protofibrils were less well understood. Here, we report on investigations of the effects of Cu(II) and baicalein on α-synuclein aggregation in vitro, while also testing the hypothesis that annular protofibrils are toxic species.

2. Material and methods

2.1. In vitro incubation of α-synuclein

Wild type (WT) α-synuclein (rPeptide LLC, purity>95%) was reconstituted in 1 × phosphate buffered saline (PBS), 0.02% (w/v) NaN_3. 400 μM WT α-synuclein was then incubated at 37 °C in presence or absence of 40 μM copper sulfate on a rolling rack for the investigation of the effect of copper(II) on α-synuclein aggregation. Aliquots from protein samples were collected at different incubation periods and stored at −80 °C.

To study the impact of baicalein on annular protofibrils, the solution of 400 μM α-synuclein with 40 μM Cu collected after 60 h incubation as a good source of annular protofibrils was mixed with equal molar baicalein in 2% dimethylsulfoxide (DMSO) to get a final concentration of 200 μM α-synuclein and baicalein in 1% DMSO. The protein solution treated by DMSO without baicalein was prepared as the control. Both solutions were incubated for 8 h at 37 °C before transmission electron microscopy (TEM) and Fourier transform infrared (FTIR) tests.

2.2. Thioflavin T (ThT) fluorescence

ThT fluorescence test was conducted following the procedures described in our previous report [14]. Briefly, 100 μL aqueous solution of 400 μM WT α-synuclein and 100 μM ThT in presence or absence of copper sulfate (40 μM) in 1 × PBS, pH7.4, 0.02% NaN_3 were tested in a 96-well plate. The in vitro aggregation was monitored by a Gemini EM Fluorescence Microplate Reader (Molecular Devices, Sunnyvale, CA) with an excitation wavelength of 440 nm and an emission wavelength of 482 nm at 37 °C. The fluorescence signal was collected every 15 min after the 30-s shaking.

2.3. TEM image collection

Negative stain specimens were placed onto carbon-coated copper grids after glow-discharge. Grids were then rinsed by three drops of PBS buffer and stained by uranyl acetate (1%). TEM images were recorded on Gatan 1 k CCD with a FEI CM200 TEM operating at 200 kV. Cryo-EM samples were prepared with C-flat 2/2 holey carbon grids (Protochips, Raleigh, NC) by a Cp3 cryoplunger (Gatan, Pleasanton, CA). Cryo-EM images were collected with SO-163 films (Kodak, Rochester, NY).

2.4. FTIR

FTIR data collection and analysis were performed using the procedures reported previously [14]. Attenuated total reflectance (ATR)-FTIR data was measured on a Spectrum 100 FTIR spectrometer with universal ATR sampling accessory (Perkin Elmer, Waltham, MA). Protein solutions treated with baicalein and DMSO were spread out on a Diamond/ZnSe crystal and dried to form a thin film [32]. Spectra data was further analyzed using the OriginPro software from OriginLab Co.. Curve-fitting was applied for the estimation of secondary structure components and the percentages of each components in terms of the peak positions identified using second derivatives and Fourier self-deconvolution [32,33].

3. Results and discussion

3.1. Cu(II) promoted annular protofibril formation

In our previous research, rat primary midbrain cultures were transduced with adenovirus encoding the human WT α-synuclein in the absence or presence of Cu(II) gene to determine the effects of Cu(II) on the toxicity of α-synuclein to post-mitotic dopaminergic neurons [34]. The results showed that WT aSyn neurotoxicity was potentiated in the presence of 10 μM Cu(II), apparently via a mechanism involving oxidative stress, whereas the metal ion alone displayed no significant toxic effects at this concentration. Similar results were also reported by treating neurons with exogenous α-synuclein and copper ion [35]. Cu(II) modulates α-synuclein aggregation either through binding [21–24] or through increased oxidative stress [36]. However, the effects of Cu(II) on different α-synuclein assemblies formed during the protein's aggregation are poorly understood. To answer this question, 400 μM α-synuclein was incubated in presence or absence of 40 μM copper ion at 37 °C in vitro and the aggregation was monitored with ThT fluorescence and TEM assays. In the ThT fluorescence assay, the β-sheet content of α-synuclein, which is indicative of the aggregation process, increased during the incubation following a sigmoidal behavior, and the elongation phase of the aggregation process was accelerated by ionic copper (Fig. 1A). This observation is in good agreement with previous reports [21,22,37].

However, from the ThT fluorescence data it is difficult to determine what kinds of species of α-synuclein aggregates were prevalent upon the addition of copper ion. Thus, negative stain TEM was applied to better illustrate the Cu^{2+} promoted aggregation. The typical structures of α-synuclein aggregates in the absence or in the presence of ionic copper after different incubation periods are displayed in Fig. 2. No fibrils or discernible protofibrils were found at the beginning of the incubation (Fig. 2 and 0 h) regardless of the presence of ionic copper. This result implied that only small species, such as monomers, dimers, or small oligomers existed in the system at the early aggregation stage, showed by the lag phase of the aggregation in the fluorescence curves (Fig. 1A), and these small species could not be identified using negative stain TEM. Annular protofibrils were initially found after 48 h incubation for both groups (in absence and presence of copper ion), however, comparatively fewer were found in the absence of copper (Fig. 2 and 48 h). The existence of annular protofibrils in solution was proved by cryo-EM (Fig. 1B). Fibrils were first found at t = 48 h in the protein sample without ionic copper, earlier than that in

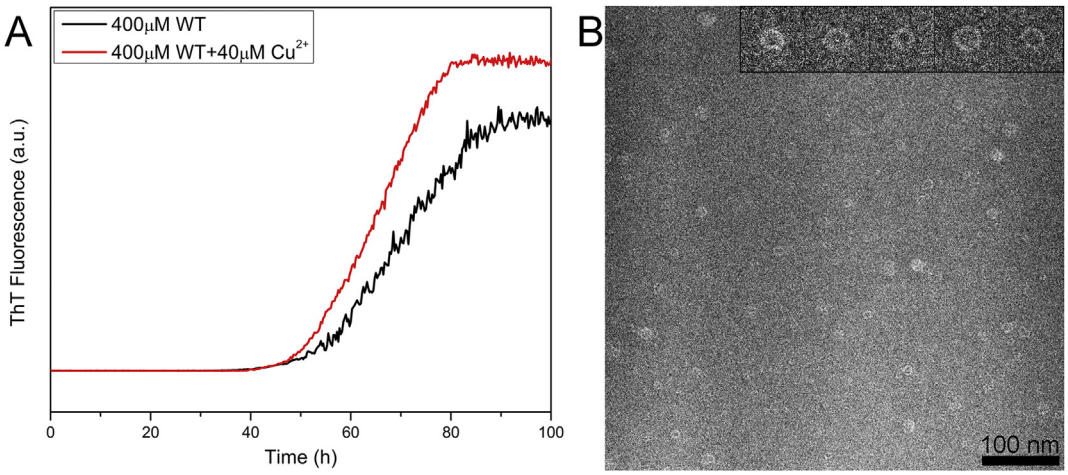

Fig. 1. (A) The aggregation kinetics of 400 μM α-synuclein in absence and presence of 40 μM Cu^{2+} monitored with ThT fluorescence. (B) A typical cryo-EM image showing annular protofibrils of 400 μM α-synuclein in presence of 40 μM Cu^{2+}. The inset shows typical annular protofibrils. The size of each square box is 30.5 nm.

presence of ionic copper (t = 56 h) (data not shown). In TEM images, the major species in the protein alone sample after 48 h were fibrils and large spherical aggregates, whereas annular protofibrils dominated the copper containing sample before 68 h, and were replaced by fibrils after 68 h (Fig. 2). Therefore, it appears that the addition of Cu^{2+} altered the distribution of α-synuclein among various aggregate structures.

Ionic copper was found to promote α-synuclein aggregation by facilitating annular protofibril formation rather than fibril formation. One TEM image at each time point was hardly enough to represent the complex composition and distribution of difference structures. Considering the highly heterogeneous distribution of the aggregates, 100 TEM images were randomly collected from the protein samples in presence and absence of Cu^{2+} at each incubation time point for the quantitative analysis of fibrils and annular protofibrils. The random collection, however, resulted in the inclusion of fibril bundles, which could not be used for fibril counting. It is simple to selectively image dispersed fibrils, which are countable regardless the problems caused by the significant variance of the fibril length. Nevertheless, the selective counting of dispersed fibrils fails to yield data that properly reflect the fibril distribution without artifacts. Accordingly, the number of images that showed fibrils (set as N_f) out of a total of 100 images in each group was determined. The N_f values of the protein samples in absence and presence of Cu^{2+} were plotted against the incubation time in Fig. 3A. Both curves roughly followed sigmoidal behavior similar to the aggregation kinetics curves in Fig. 1A. However, the protein sample with copper ion displayed a longer lag phase for fibrillization than the protein alone sample in opposition to the ThT fluorescence results, in which β-sheet contents were measured. The N_f value, though imperfect, is an appropriate parameter for this analysis given the sampling constraints outlined above and likely provides a reasonable estimate for the amount of fibrils and. It is worth noting that fibril bundles were observed only in the protein alone sample. Therefore, the addition of copper ion might actually postpone fibril formation.

The question to be addressed next is why ThT fluorescence results seem to indicate that, contrary to the TEM results above, Cu^{2+} promoted α-synuclein aggregation. Annular protofibrils were counted for each TEM image and the total amount of annular protofibrils in each group of 100 images was set as N_p. The N_p values of the protein samples in absence and presence of Cu^{2+} were plotted against the incubation time in Fig. 3B. The rapid accumulation of annular protofibrils in the sample with Cu^{2+} started in parallel with the ThT fluorescence signal, suggesting that annular protofibrils contributed to the initial β-sheet accumulation of the protein sample with copper ion in Fig. 1. Moreover, the decrease in the number of annular protofibrils coincided with the increase in the number of fibrils, which strongly supported our previous report that annular protofibrils could convert to fibrils [14]. Importantly, there were considerably more annular protofibrils in the protein sample in presence of Cu^{2+} than that in absence of Cu^{2+} during the incubation period. The C-terminal region enriched in acidic amino acid residues was found to play a role in copper ion binding that could induce oligomerization of α-synuclein [22,23,38]. Specifically, the truncation of the C-terminal region of α-synuclein with endoproteinase Asp-N could suppress the copper-induced oligomerization [39]. Therefore, the interaction between the negatively charged C-terminal region and the positively charged copper ion might induce and stabilize annular structures. We conclude that rather than accelerating mature fibril formation, Cu^{2+} in fact accelerated annular protofibril formation and postponed fibril formation, thereby impacting the aggregation pathway.

3.2. Annular protofibrils are the toxic species

In our previous research, we found that Cu^{2+} significantly aggravated the toxicity of α-synuclein aggregates [34]. Here the quantitative analysis of the aggregates elucidated that copper ion actually promoted annular protofibril formation rather than fibril formation. Therefore, annular protofibrils might be the toxic species and copper ion could aggravate the toxicity by facilitating the formation of annular protofibrils. PD linked mutants, A53T and A30P, were reported to accelerate oligomerization rather than fibrillization, suggesting that protofibrils or oligomers were the toxic species, not fibrils [40]. In an impressive research, a series of techniques were applied to study the morphology as well as the size distribution of α-synulcein protofibrils [41]. Both A30P and A53T were found to facilitate annular protofibril formation compared with WT α-synulcein. Considering the high affinity of annular protofibrils to membranes and the conductance increase of lipid bilayers caused by α-synuclein protofibrils [6,9,10,13], we concluded that annular protofibrils were toxic to dopaminergic neurons and could be an important species involved in the pathogenesis of PD.

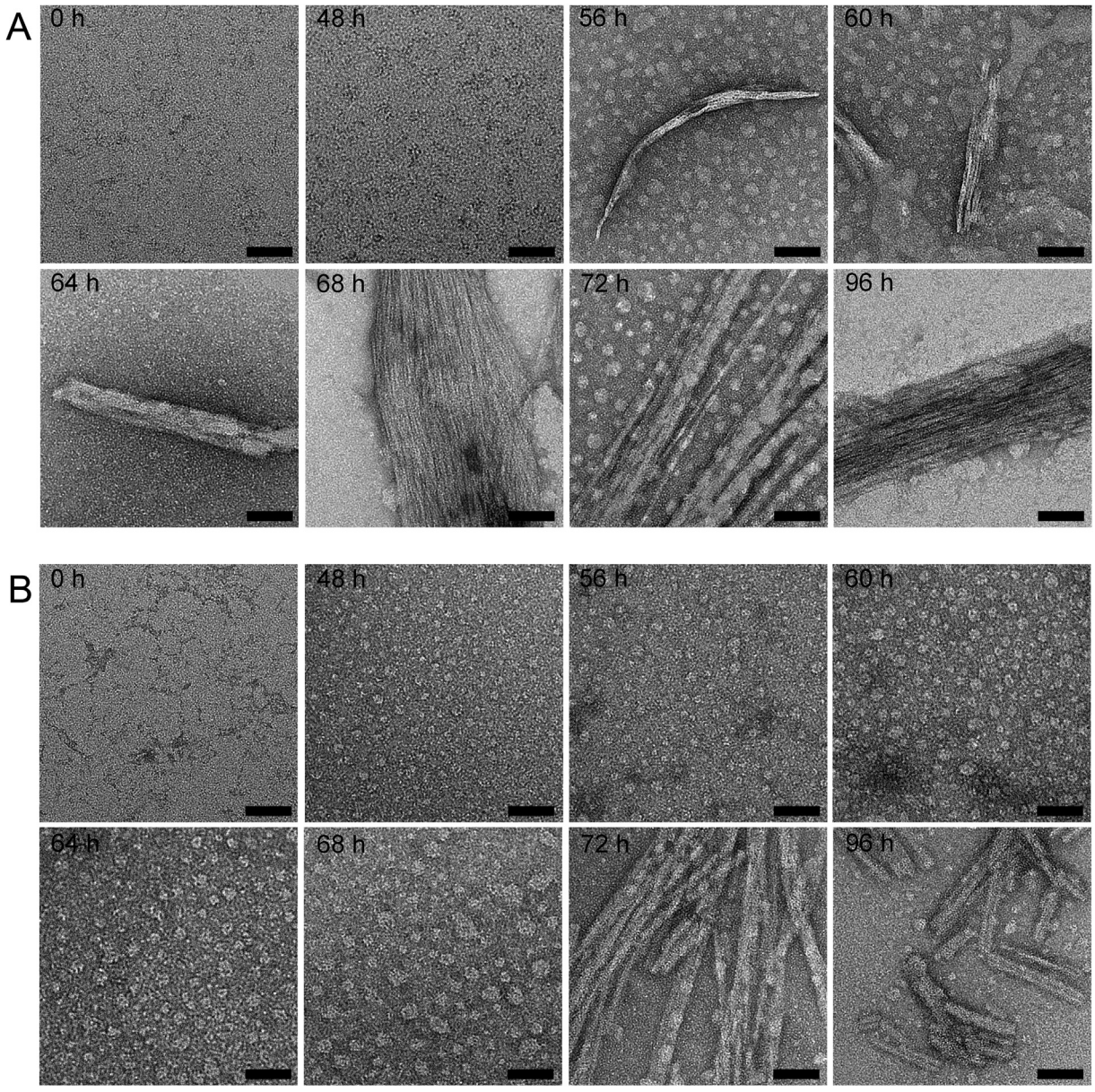

Fig. 2. Negative stain TEM images exhibiting the structures of the aggregates of 400 μM WT α-synuclein in absence (A) and in presence of 40 μM Cu^{2+} (B) at a series of incubation times. (Scale bars: 50 nm).

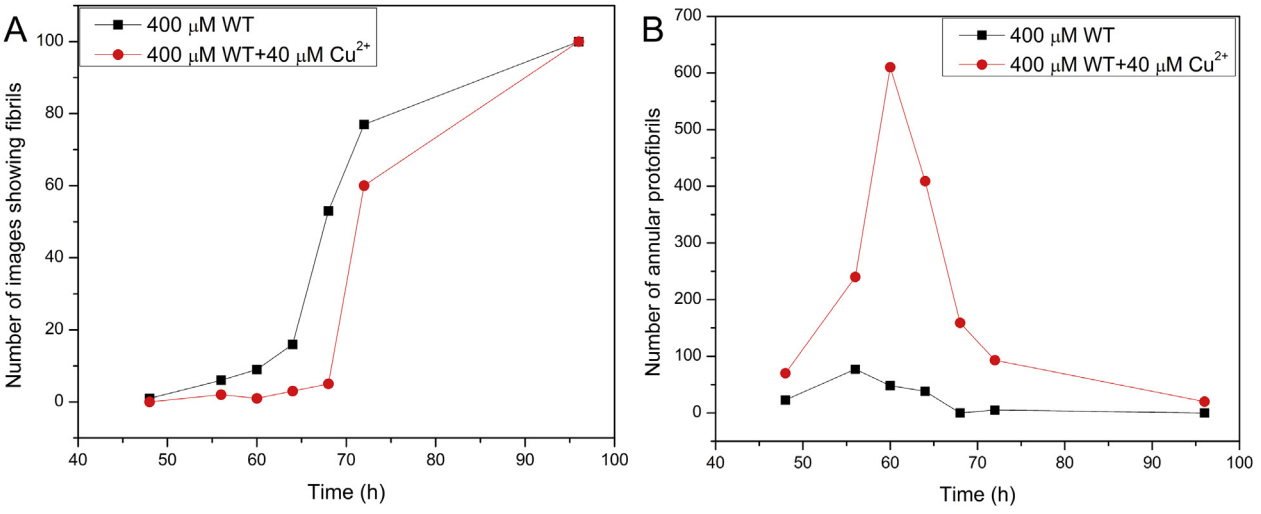

Fig. 3. Quantitative analysis of fibrils (A) and annular protofibrils (B) of 400 μM WT α-synuclein in absence and presence of 40 μM Cu^{2+} during the incubation.

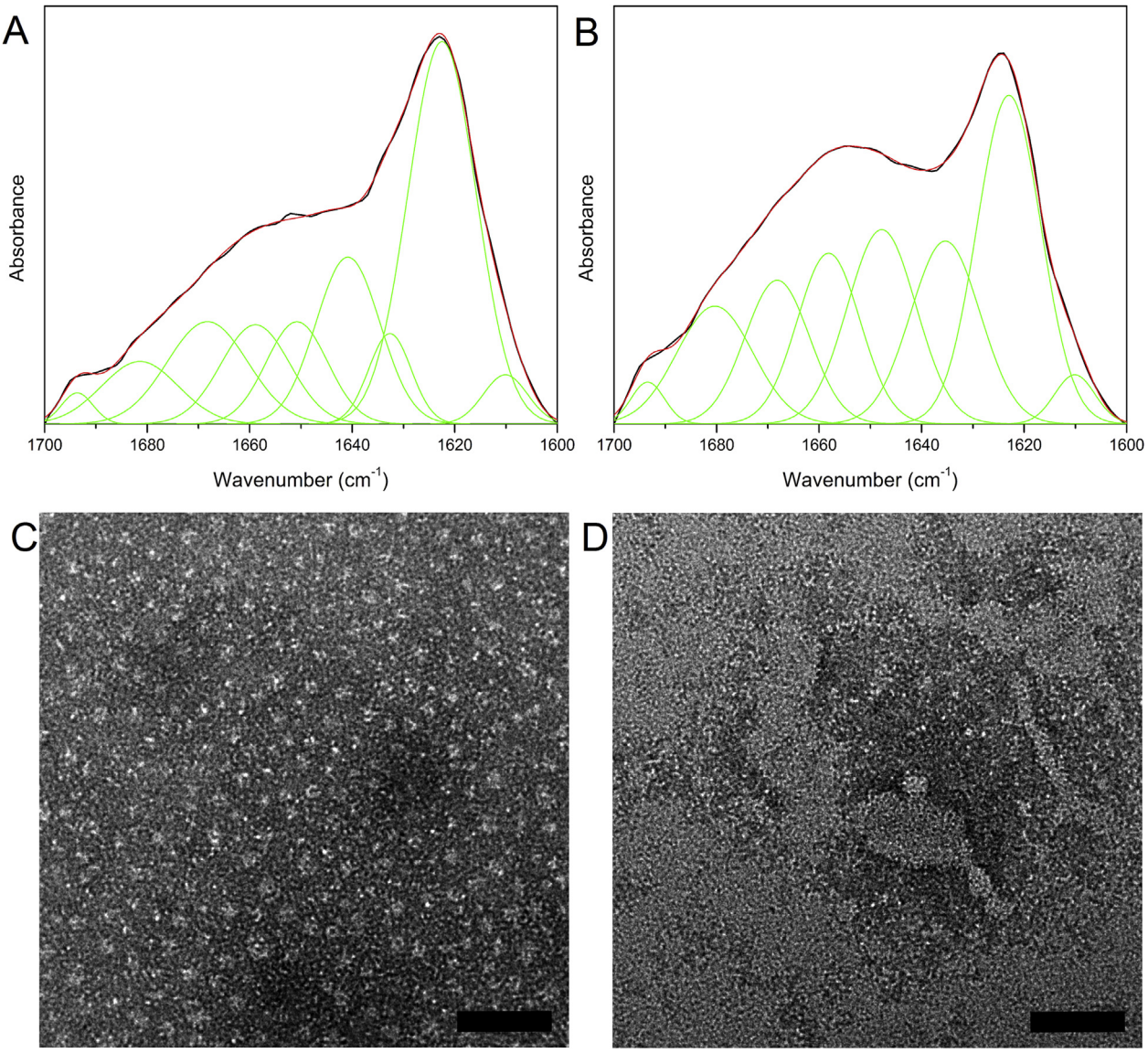

Fig. 4. The analysis of control (A and C) and baicalein-treated (B and D) α-synuclein aggregates by FTIR (A and B) and negative stain TEM (C and D). The mixtures were incubated for 8 h. The FTIR spectra (black) were disintegrated upon fitting into Gaussian curves (green). The cumulative fit curve was displayed in red. Scale bars in TEM images: 50 nm. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

3.3. *Baicalein decomposed annular protofibrils*

Baicalein disaggregated existing protofibrils and destroyed β-sheet structures, as revealed by TEM and ATR-FTIR spectroscopy. The solution of 400 μM α-synuclein with 40 μM Cu^{2+} collected after 60 h incubation was selected as a good source of annular protofibrils. The protein solution treated by DMSO without baicalein was prepared as the control. Both solutions were incubated for 8 h at 37 °C before TEM and FTIR tests.

FTIR analysis was used to investigate the β-sheet content in control and baicalein-treated α-synuclein aggregates (Fig. 4A and B). The spectra were disintegrated by fitting into Gaussian curves in light of peak positions determined by second derivatives and Fourier self-deconvolution. The control sample without baicalein exhibited high β-sheet content at 59.5% attributed by the peaks at about 1622 cm^{-1}, 1633 cm^{-1}, 1641 cm^{-1} and 1694 cm^{-1} in the FTIR spectrum (Fig. 4A). On the contrary, baicalein-treated sample showed much lower β-sheet contribution (44.2%) with the peaks at around 1623 cm^{-1}, 1635 cm^{-1} and 1693 cm^{-1} (Fig. 4B). The loss of the β-sheet structure indicated that baicalein damaged β-sheet conformation, which is the structural basis of annular protofibrils and fibrils.

Furthermore, annular protofibrils were widely distributed in the control (Fig. 4C), while they were hardly observed by TEM in the baicalein-treated protein sample (Fig. 4D). Given that the annular protofibrils dominated the untreated protein sample and were not decomposed by after the incubation in presence of 1% DMSO, baicalein disaggregated these toxic species and disrupted β-sheet structure through the interaction with hydrophobic groups in α-synuclein molecules [30]. During this process, fibrils would also be disaggregated along with the β-sheet rich annular protofibrils, due to the destruction of their own β-sheet structures [30].

To summarize, Cu(II) was found to promote the formation of annular protofibrils rather than fibrils in vitro using TEM. Moreover, baicalein, a known compound with neuroprotective effect [27,28,42], disaggregated the α-synuclein annular protofibrils, as

revealed by the significant decrease of β-sheet content. Rapid annular protofibril formation in the presence of Cu^{2+}, correlated with our recently published cell culture studies showing enhanced toxicity of WT α-synuclein in the presence of Cu(II) [34]. In addition, the fact that baicalein reverse β-sheet formation in protofibril-rich solutions supports the conclusion that annular protofibrils could be responsible for toxicity. In addition to having direct effects on α-synuclein self-assembly, Cu(II) and baicalein likely alleviate α-synuclein neurotoxicity via other mechanisms involving modulation of cellular pathways such as antioxidant responses and the ubiquitin-proteasome pathways [25,28,34]. These findings may inspire us to move forward towards setting a goal in the discovery of novel therapeutic strategies aimed at suppressing the formation of the toxic α-synuclein annular protofibrils.

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References

[1] A.L. Fink, The aggregation and fibrillation of alpha-synuclein, Acc. Chem. Res. 39 (2006) 628–634.
[2] D.R. Brown, Oligomeric alpha-synuclein and its role in neuronal death, IUBMB Life 62 (2010) 334–339.
[3] C.A. Lasagna-Reeves, C.G. Glabe, R. Kayed, Amyloid-β Annular Protofibrils Evade Fibrillar Fate in Alzheimer Disease Brain, J. Biol. Chem. 286 (2011) 22122–22130.
[4] H. Jang, F.T. Arce, S. Ramachandran, R. Capone, R. Azimova, B.L. Kagan, R. Nussinov, R. Lal, Truncated β-amyloid peptide channels provide an alternative mechanism for Alzheimer's disease and down syndrome, Proc. Natl. Acad. Sci. 107 (2010) 6538–6543.
[5] R. Kayed, A. Pensalfini, L. Margol, Y. Sokolov, F. Sarsoza, E. Head, J. Hall, C. Glabe, Annular protofibrils are a structurally and functionally distinct type of amyloid oligomer, J. Biol. Chem. 284 (2009) 4230–4237.
[6] F. Schmidt, J. Levin, F. Kamp, H. Kretzschmar, A. Giese, K. Bötzel, Single-channel electrophysiology reveals a distinct and uniform pore complex formed by α-Synuclein oligomers in lipid membranes, PLoS One 7 (2012) e42545.
[7] H.-Y. Kim, M.-K. Cho, A. Kumar, E. Maier, C. Siebenhaar, S. Becker, C.O. Fernandez, H.A. Lashuel, R. Benz, A. Lange, M. Zweckstetter, Structural properties of pore-forming oligomers of α-Synuclein, J. Am. Chem. Soc. 131 (2009) 17482–17489.
[8] H.A. Lashuel, D. Hartley, B.M. Petre, T. Walz, P.T. Lansbury, Neurodegenerative disease – amyloid pores from pathogenic mutations, Nature 418 (2002), 291–291.
[9] T.T. Ding, S.J. Lee, J.C. Rochet, P.T. Lansbury, Annular alpha-synuclein protofibrils are produced when spherical protofibrils are incubated in solution or bound to brain-derived membranes, Biochemistry 41 (2002) 10209–10217.
[10] M.J. Volles, S.J. Lee, J.C. Rochet, M.D. Shtilerman, T.T. Ding, J.C. Kessler, P.T. Lansbury, Vesicle permeabilization by protofibrillar alpha-synuclein: implications for the pathogenesis and treatment of Parkinson's disease, Biochemistry 40 (2001) 7812–7819.
[11] B.D. van Rooijen, M.M.A.E. Claessens, V. Subramaniam, Membrane permeabilization by oligomeric alpha-Synuclein: in search of the mechanism, PLoS One 5 (2010).
[12] H.A. Lashuel, P.T. Lansbury, Are amyloid diseases caused by protein aggregates that mimic bacterial pore-forming toxins? Q. Rev. Biophys. 39 (2006) 167–201.
[13] A. Quist, L. Doudevski, H. Lin, R. Azimova, D. Ng, B. Frangione, B. Kagan, J. Ghiso, R. Lal, Amyloid ion channels: a common structural link for protein-misfolding disease, Proc. Natl. Acad. Sci. U. S. A. 102 (2005) 10427–10432.
[14] H. Zhang, A. Griggs, J.-C. Rochet, Lia A. Stanciu, In vitro study of α-Synuclein protofibrils by Cryo-EM suggests a Cu2+-dependent aggregation pathway, Biophys. J. 104 (2013) 2706–2713.
[15] A.L. Feldman, A.L.V. Johansson, G. Nise, M. Gatz, N.L. Pedersen, K. Wirdefeldt, Occupational exposure in Parkinsonian disorders: A 43-year prospective cohort study in men, Park. Relat. Disord. 17 (2011) 677–682.
[16] K.J. Barnham, A.I. Bush, Metals in Alzheimer's and Parkinson's diseases, Curr. Opin. Chem. Biol. 12 (2008) 222–228.
[17] K. Jomova, D. Vondrakova, M. Lawson, M. Valko, Metals, oxidative stress and neurodegenerative disorders, Mol. Cell. Biochem. 345 (2010) 91–104.
[18] K. Jomova, M. Valko, Advances in metal-induced oxidative stress and human disease, Toxicology 283 (2011) 65–87.
[19] S.J. Chinta, J.K. Andersen, Redox imbalance in Parkinson's disease, Biochimica Biophysica Acta (BBA) – General Subj. 1780 (2008) 1362–1367.
[20] M. Bisaglia, I. Tessari, S. Mammi, L. Bubacco, Interaction between α-Synuclein and metal ions, still looking for a role in the pathogenesis of Parkinson's disease, NeuroMolecular Med. 11 (2009) 239–251.
[21] V.N. Uversky, J. Li, A.L. Fink, Metal-triggered Structural Transformations, Aggregation, and Fibrillation of Human α-Synuclein: a possible molecular link between Parkinson's disease and heavy metal exposure, J. Biol. Chem. 276 (2001) 44284–44296.
[22] R.M. Rasia, C.W. Bertoncini, D. Marsh, W. Hoyer, D. Cherny, M. Zweckstetter, C. Griesinger, T.M. Jovin, C.O. Fernández, Structural characterization of copper(II) binding to α-synuclein: Insights into the bioinorganic chemistry of Parkinson's disease, Proc. Natl. Acad. Sci. U. S. A. 102 (2005) 4294–4299.
[23] C.G. Dudzik, E.D. Walter, G.L. Millhauser, Coordination features and affinity of the Cu2+ site in the α-Synuclein protein of Parkinson's disease, Biochemistry 50 (2011) 1771–1777.
[24] A. Binolfi, A.A. Valiente-Gabioud, R. Duran, M. Zweckstetter, C. Griesinger, C.O. Fernandez, Exploring the structural details of Cu(I) binding to α-Synuclein by NMR spectroscopy, J. Am. Chem. Soc. 133 (2010) 194–196.
[25] B. Cheng, H. Gong, H.W. Xiao, R.B. Petersen, L. Zheng, K. Huang, Inhibiting toxic aggregation of amyloidogenic proteins: A therapeutic strategy for protein misfolding diseases, Biochim. Biophys. Acta-General Subj. 1830 (2013) 4860–4871.
[26] D.E. Ehrnhoefer, J. Bieschke, A. Boeddrich, M. Herbst, L. Masino, R. Lurz, S. Engemann, A. Pastore, E.E. Wanker, EGCG redirects amyloidogenic polypeptides into unstructured, off-pathway oligomers, Nat. Struct. Mol. Biol. 15 (2008) 558–566.
[27] S. Tian, G.R. He, J.K. Song, S.B. Wang, W.Y. Xin, D. Zhang, G.H. Du, Pharmacokinetic study of baicalein after oral administration in monkeys, Fitoterapia 83 (2012) 532–540.
[28] M.L. Jiang, Y. Porat-Shliom, Z. Pei, Y. Cheng, L. Xiang, K. Sommers, Q. Li, F. Gillardon, B. Hengerer, C. Berlinicke, W.W. Smith, D.J. Zack, M.A. Poirier, C.A. Ross, W.Z. Duan, Baicalein reduces E46K alpha-synuclein aggregation in vitro and protects cells against E46K alpha-synuclein toxicity in cell models of familiar Parkinsonism, J. Neurochem. 114 (2010) 419–429.
[29] D.P. Hong, A.L. Fink, V.N. Uversky, Structural characteristics of alpha-Synuclein oligomers stabilized by the flavonoid baicalein, J. Mol. Biol. 383 (2008) 214–223.
[30] M. Zhu, S. Rajamani, J. Kaylor, S. Han, F. Zhou, A.L. Fink, The flavonoid baicalein inhibits fibrillation of α-Synuclein and disaggregates existing fibrils, J. Biol. Chem. 279 (2004) 26846–26857.
[31] J.H. Lu, M.T. Ardah, S.S.K. Durairajan, L.F. Liu, L.X. Xie, W.F.D. Fong, M.Y. Hasan, J.D. Huang, O.M.A. El-Agnaf, M. Li, Baicalein inhibits formation of alpha-synuclein oligomers within living cells and prevents A beta peptide fibrillation and oligomerisation, Chembiochem 12 (2011) 615–624.
[32] D.-P. Hong, A.L. Fink, V.N. Uversky, Structural characteristics of α-Synuclein oligomers stabilized by the flavonoid baicalein, J. Mol. Biol. 383 (2008) 214–223.
[33] J. Kong, S. Yu, Fourier transform infrared spectroscopic analysis of protein secondary structures, Acta Biochim. Biophys. Sinica 39 (2007) 549–559.
[34] A. Anandhan, H. Rodriguez-Rocha, I. Bohovych, A.M. Griggs, L. Zavala-Flores, E.M. Reyes-Reyes, J. Seravalli, L.A. Stanciu, J. Lee, J.-C. Rochet, O. Khalimonchuk, R. Franco, Overexpression of alpha-synuclein at non-toxic levels increases dopaminergic cell death induced by copper exposure via modulation of protein degradation pathways, Neurobiol. Dis. (2014), http://dx.doi.org/10.1016/j.nbd.2014.11.018 (in press).
[35] J.A. Wright, X.Y. Wang, D.R. Brown, Unique copper-induced oligomers mediate alpha-synuclein toxicity, FASEB J. 23 (2009) 2384–2393.
[36] N.B. Cole, D.D. Murphy, J. Lebowitz, L. Di Noto, R.L. Levine, R.L. Nussbaum, Metal-catalyzed oxidation of α-Synuclein, J. Biol. Chem. 280 (2005) 9678–9690.
[37] J.T. Pedersen, J. Ostergaard, N. Rozlosnik, B. Gammelgaard, N.H.H. Heegaard, Cu(II) mediates kinetically distinct, Non-amyloidogenic aggregation of amyloid-beta peptides, J. Biol. Chem. 286 (2011) 26952–26963.
[38] D.R. Brown, Metal binding to alpha-synuclein peptides and its contribution to toxicity, Biochem. Biophys. Res. Commun. 380 (2009) 377–381.
[39] S.R. Paik, H.J. Shin, J.H. Lee, C.S. Chang, J. Kim, Copper(II)-induced self-oligomerization of alpha-synuclein, Biochem. J. 340 (1999) 821–828.
[40] K.A. Conway, S.J. Lee, J.C. Rochet, T.T. Ding, R.E. Williamson, P.T. Lansbury, Acceleration of oligomerization, not fibrillization, is a shared property of both alpha-synuclein mutations linked to early-onset Parkinson's disease: implications for pathogenesis and therapy, Proc. Natl. Acad. Sci. U. S. A. 97 (2000) 571–576.
[41] H.A. Lashuel, B.M. Petre, J. Wall, M. Simon, R.J. Nowak, T. Walz, P.T. Lansbury, alpha-synuclein, especially the Parkinson's disease-associated mutants, forms pore-like annular and tubular protofibrils, J. Mol. Biol. 322 (2002) 1089–1102.
[42] X. Mu, G. He, Y. Cheng, X. Li, B. Xu, G. Du, Baicalein exerts neuroprotective effects in 6-hydroxydopamine-induced experimental parkinsonism in vivo and in vitro, Pharmacol. Biochem. Behav. 92 (2009) 642–648.