BIOCHEMISTRY
including biophysical chemistry & molecular biology

Rapid Report

pubs.acs.org/biochemistry

β-Cyclodextrin and Curcumin, a Potent Cocktail for Disaggregating and/or Inhibiting Amyloids: A Case Study with α-Synuclein

Saurabh Gautam, Sandip Karmakar, Abhik Bose, and Pramit K. Chowdhury*

Department of Chemistry, Indian Institute of Technology Delhi, Hauz Khas, New Delhi 110016, India

Supporting Information

ABSTRACT: Aggregation of α-synuclein has been implicated in Parkinson's disease (PD). While many compounds are known to inhibit α-synuclein aggregation, dissolution of aggregates into their constituent monomers cannot be readily achieved. In this study, using a range of techniques, we have shown that an optimized cocktail of curcumin and β-cyclodextrin, at appreciably low concentrations, not only inhibited aggregation but also broke up the preformed aggregates almost completely. We propose that these compounds exhibit synergy in their action and thus provide us with the exciting prospect of working toward the development of a suitable drug candidate for prevention and treatment of PD.

Misfolding or unfolding often results in protein aggregation, which is a known causative agent of a number of neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease (PD), Huntington disease, and prion disease, to name a few. In humans, aggregation of α-synuclein, a 140-amino acid presynaptic and intrinsically disordered protein, has been implicated in PD.[1] Many small molecule inhibitors have been reported to inhibit the aggregation of α-synuclein,[2−4] curcumin being one of them.[5] However, use of these compounds *in vivo* and disaggregation of preformed aggregates by the same still remain challenging tasks.

Curcumin is a polyphenolic compound (Figure S1a, Supporting Information) and is extracted from the spice plant *Curcuma longa* (turmeric),[6] which is extensively used as a food ingredient in the Indian subcontinent. Curcumin has been reported to inhibit the aggregation of α-synuclein by binding to its monomers[5] and binding to its oligomers.[7] However, its insolubility in water and high instability in the gastrointestinal tract have so far been the limiting factors in its use for the treatment or prevention of PD and aggregation-related diseases in general.[6]

Cyclodextrins (CDs) are oligosaccharides with six (α-CD), seven (β-CD), or eight (γ-CD) glucose molecules linked through α-1−4-glycosidic linkages forming a hydrophobic internal cavity (Figure S1b,c, Supporting Information), which has been frequently used for the encapsulation of compounds that are sparingly soluble in water. Cyclodextrins (CDs) are known to interact with curcumin and increase its water solubility, stability, and bioavailability.[8] Here we have investigated the influence of the combination of β-CD and curcumin on the aggregation of α-synuclein. Our data reveal that this combination not only can block α-synuclein aggregation but also can break up preformed protein aggregates substantially. We also investigated the effect of curcumin or β-CD individually on the inhibition of aggregation and dissolution of preformed aggregates. On the basis of our observations, we found that the presence of any of these two compounds increased the effectiveness of the other considerably by bringing about a concomitant decrease in the dosage required.

Aggregation of α-synuclein in this study was conducted in the presence of 20% (v/v) ethanol and 0.1 mM $FeCl_3$ with stirring at 37 °C and was monitored by the widely used methods of thioflavin T (ThT) fluorescence and congo red (CR) absorbance. Figure S2a (Supporting Information) shows the progression of the aggregation and structural transition of native α-synuclein as followed using the enhancement of ThT fluorescence and circular dichroism spectral evolution, respectively, the latter monitored at 218 nm. Also, as is evident from the changes in secondary structure, native α-synuclein has a disordered conformation in its monomeric state that changes over with time to one having a high β-sheet content, this being one of the defining characteristics of amyloid aggregates (Figure S2b, Supporting Information). Subsequently, the aggregation of α-synuclein was studied in the presence of different concentrations of curcumin. For all such samples, the data reveal a decrease in the extent of aggregation (Figure 1a), with 0.1 μM curcumin (the lowest concentration of curcumin used) being the least effective and while 20 μM curcumin being able to completely inhibit the aggregation *in vitro* (Figure 1a). The observed results were further validated by circular dichroism spectroscopy and CR absorbance (Figures S3a and S4a, Supporting Information) and are in good agreement with previous studies that have shown curcumin to be effective in blocking the aggregation of α-synuclein.[5]

Having probed the influence of curcumin on α-synuclein aggregation, we next investigated whether β-CD alone could modulate the aggregation profile. CDs have been shown earlier to inhibit the neurotoxicity and aggregation of amyloid-β (Aβ) peptides[9] at higher concentrations, but a recent study has proposed that at a concentration of 10 or 100 mM, these may even enhance the aggregation of these peptides.[10] Indeed, our study shows that β-CD by itself suppressed the formation of aggregates of α-synuclein also in a concentration-dependent manner, with 10 and 60 μM being the least and most effective concentrations of β-CD, respectively, in blocking aggregation (Figure 1b). Subsequently, we decided to investigate both curcumin and β-CD together, to determine their effect on the

Received: May 27, 2014
Revised: June 16, 2014
Published: June 16, 2014

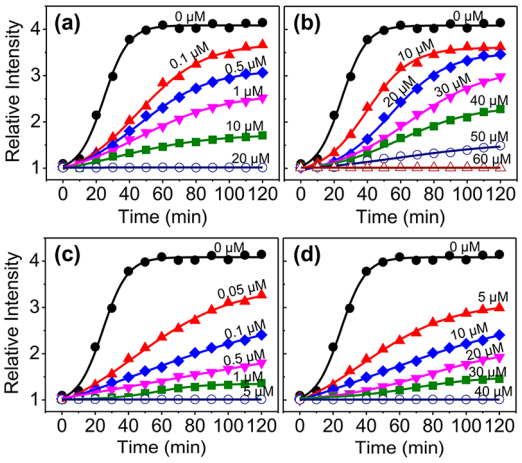

Figure 1. Effects of curcumin and β-CD on the aggregation of α-synuclein using ThT fluorescence in the presence of (a) varying concentrations of curcumin, (b) varying concentrations of β-CD, (c) β-CD (10 μM) with varying concentrations of curcumin, and (d) curcumin (0.1 μM) with varying concentrations of β-CD.

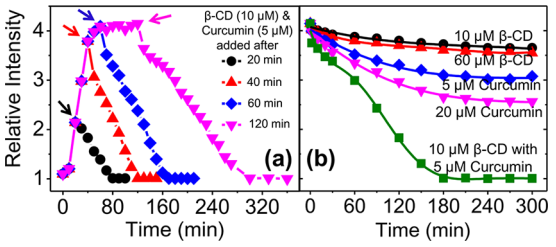

Figure 2. Disaggregation of preformed aggregates of α-synuclein using ThT fluorescence. (a) Disaggregation monitored in the presence of β-CD (10 μM) and curcumin (5 μM) after initial aggregation for different time intervals as mentioned (arrows show the time points at which curcumin and β-CD were added). (b) Disaggregation profile of preformed aggregates (obtained after 120 min) of α-synuclein after addition of curcumin and/or β-CD.

aggregation, with the caveat that the presence of β-CD might help better solubilize curcumin and hence enhance its inhibition ability. β-CD at a concentration of 10 μM (least effective when used alone) along with 5 μM curcumin completely abolished the aggregation (Figure 1c). It should be noted that both concentrations for these two compounds were much lower than the concentrations of any of those used alone (i.e., not in combination) for complete blocking.

Moreover, it was also apparent that the presence of one of the components significantly lowered the dosage requirements of the other for blocking to be effective. For example, in the presence of 0.1 μM curcumin, the β-CD concentration required for complete inhibition of aggregation decreased from 60 to 40 μM (Figure 1d), while when 10 μM β-CD was used, the curcumin concentration was reduced to 5 μM (from 20 μM) (Figure 1c). These results, also confirmed using circular dichroism spectroscopy, CR absorbance, and native polyacrylamide gel electrophoresis (PAGE) (Figures S3, S4, and S5a, Supporting Information), therefore strongly hint at the potency of the combination of curcumin and β-CD for inhibiting α-synuclein aggregation.

Even more desirable and challenging than inhibition is the dissolution of preformed aggregates. While curcumin has been used for the inhibition of aggregation of α-synuclein, only limited information is available with regard to its effect on the disaggregation of preformed aggregates.[5,7] On the other hand, β-CD has rarely been used for the breaking up of such preformed aggregates. Therefore, in this work, we also analyzed the effect of the aforementioned optimized combination (10 μM β-CD and 5 μM curcumin) on the aggregates of α-synuclein (Figure 2a). Aggregates formed at different time points were incubated with β-CD (10 μM) and curcumin (5 μM) at 37 °C. Aggregate formation was reversed in all the cases as shown by the decrease in ThT fluorescence (Figure 2a) and CR absorbance (Figure S6, Supporting Information). For aggregates formed after incubation for 20 min, the curcumin−β-CD combination reversed the aggregation almost completely in ∼60 min as shown. Similarly, the aggregates formed after 40, 60, and 120 min were broken up within incubation times of ∼80, ∼110, and ∼180 min, respectively, after the addition of the curcumin−β-CD cocktail (Figure 2a and Figure S6a, Supporting Information). The aggregates obtained after incubation for 12 h had a reversal trend similar to those obtained after incubation for 120 min, with the subsequent disaggregation process requiring ∼180 min (Figure S7, Supporting Information). Secondary structure analyses also showed that α-synuclein aggregates reverted back to the native unstructured conformation of the protein after incubation with the curcumin−β-CD mixture (Figure S8, Supporting Information). The findings mentioned above were further confirmed using native PAGE analyses (Figure S5a,b, Supporting Information). However, it should be noted again that either of these two compounds used individually and also at higher concentrations was far less successful in breaking up the aggregates of α-synuclein. β-CD used alone at two different concentrations (10 and 60 μM) was almost completely ineffective, while curcumin showed a small decrease in the level of aggregation at 5 μM, with 20 μM curcumin being relatively more efficient (Figure 2b and Figure S6b, Supporting Information).

To further confirm our observations due to concerns over interpretation of ThT data,[11] we have undertaken a host of independent assays (CR absorbance, circular dichroism, and native gel studies have already been mentioned), including transmission electron microscopy (TEM) measurements and size exclusion chromatography (SEC) analyses. TEM also supported the findings described above showing the inhibition of aggregation (Figure S9a,b, Supporting Information) and clear disaggregation (Figure 3) of preformed synuclein aggregates in the presence of the optimized combination (10 μM β-CD and 5 μM curcumin), with the aggregates reverting to monomeric α-synuclein. On the other hand, β-CD and curcumin used alone showed little change in the density or morphology of amyloid aggregates (Figure S10, Supporting Information). SEC data also confirmed the disaggregation of aggregates by the optimized cocktail (Figure S11, Supporting Information). Moreover, to ascertain the fact that our observations are not biased due to the solvent composition, the aforementioned experiments (inhibition and disaggregation) were also performed individually in buffer, 20% (v/v) ethanol and $FeCl_3$ (ThT and CR data in Figures S12 and S13, Supporting Information, native PAGE and TEM images in Figures S5c, S9c,d, and S14, Supporting Information). Except for the differences in time incurred for formation of the α-synuclein aggregates {∼60, ∼7, and ∼30 h for buffer only, ethanol only [20% (v/v)], and $FeCl_3$ only (0.1 mM), respectively} and dissolution of the same, our studies clearly reveal the solvent composition independence of our results,

dx.doi.org/10.1021/bi500642f | *Biochemistry* 2014, 53, 4081−4083

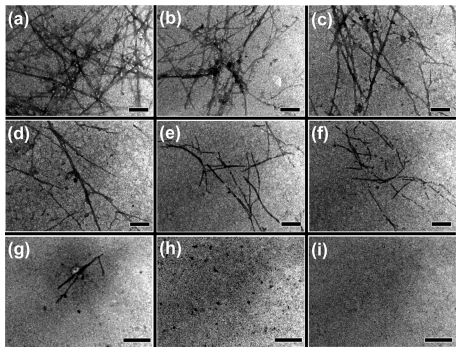

Figure 3. TEM images of aggregated α-synuclein before and after incorporation of the combination of β-CD (10 μM) and curcumin (5 μM). (a) α-Synuclein aggregated for 120 min in the presence of ethanol [20% (v/v)] and $FeCl_3$ (0.1 M). (b–i) Fate of aggregated α-synuclein after addition of β-CD (10 μM) and curcumin (5 μM) at (b) 0, (c) 10, (d) 30, (e) 60, (f) 90, (g) 120, (h) 150, and (i) 180 min. The scale bar is 200 nm.

thereby further lending credence to the potency of the curcumin–β-CD cocktail.

Our data taken together therefore suggest that β-CD not only can be used as a carrier of curcumin for increased bioavailability and utilization by the cells but also can itself prevent the aggregation of α-synuclein. More importantly, it works through a synergistic combination with curcumin and disaggregates the amyloids. The effective concentrations of both compounds required for these processes were significantly lower than those of similar low-molecular weight compounds of different chemical classes used earlier for inhibition of aggregation or disaggregation of α-synuclein.[3,4] Moreover, these low concentrations (<10 μM for each) are also suitable for use *in vivo* and crossing the blood–brain barrier.[12]

The mechanism of inhibition of α-synuclein aggregation by curcumin has recently been proposed wherein the inhibitor effected an increase in the level of intramolecular diffusion of protein chain(s), thereby enhancing the reconfiguration rate of the same.[5] However, in the aforementioned report and other related studies, curcumin had to be present in at least stoichiometrically equivalent proportions to that of α-synuclein for the small molecule inhibitor to be effective.[5] Here, our optimal combination in which the curcumin concentration is 7-fold lower than that of the protein not only is a significant improvement but also points toward the important role played by β-CD in increasing its potency. It should be kept in mind that though the starting concentration of curcumin (and of β-CD) is lower than that of the monomeric protein, the scenario changes after aggregation wherein depending on the size of the oligomers or fibrils formed, both curcumin and β-CD are available in excess (per aggregated unit). Also, on the basis of the fact that curcumin itself could bring about better disassembly of aggregates than β-CD (Figure 2b and Figure S6b, Supporting Information), we hypothesize that curcumin initiates the process by interacting with and starting to disentangle the higher-order oligomers and/or fibrils of α-synuclein. Indeed, it has been shown that polyphenols having multiple phenyl rings and -OH groups exhibit efficient association with the β-sheet-rich aggregates, with the former aiding in interaction with the hydrophobic groups, and the -OH groups facilitate the weakening of the intrastrand H-bonds in the aggregates, thereby encouraging disassembly into monomeric subunits.[13,14] Subsequently, β-CD sequesters the disentangled monomer units so exposed after the action of curcumin on the aggregate, in its hydrophobic cavity, thereby preventing their further association. In this regard, CDs have been shown to function as pseudochaperones and thus used as proper folding aids for a variety of proteins.[15] Moreover, the hydrophobic interior of these bucket-shaped molecules (CDs) can interact with nonpolar amino acid side chains, the latter often being the nucleation sites for the initiation of protein aggregation. Incidentally, the NAC (non-amyloid-β component) stretch of α-synuclein is predominantly hydrophobic,[2,4] being rich in glycine, alanine, and valine residues along with a phenylalanine residue, with the phenyl ring of the latter known to interact directly with the nonpolar interior of β-CD.[9] Thus, if curcumin's ability to hinder and even break up aggregates is kept in mind, this along with the chaperone-like action of β-CD provides us with an exciting prospect for developing a realistic drug candidate in the form of a curcumin–β-CD cocktail for the prevention and treatment of PD.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Supporting details and Figures S1–S14. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: pramitc@chemistry.iitd.ac.in. Phone: +91-11-26591521.

Funding

This work was financially supported by the Fast Track Scheme for Young Scientists (SR/FT/CS-007/2010) from the Department of Science and Technology (DST) to P.K.C. and start-up funds from IIT Delhi. S.G. and S.K. are recipients of fellowships from CSIR and IIT Delhi, respectively.

Notes

The authors declare no competing financial interests.

■ REFERENCES

(1) Surguchov, A. (2013) *J. Neurosci. Res. 91*, 161–166.
(2) Fink, A. L. (2006) *Acc. Chem. Res. 39*, 628–634.
(3) Masuda, M., Suzuki, N., Taniguchi, S., Oikawa, T., Nonaka, T., Iwatsubo, T., Hisanaga, S., Goedert, M., and Hasegawa, M. (2006) *Biochemistry 45*, 6085–6094.
(4) Meng, X., Munishkina, L. A., Fink, A. L., and Uversky, V. N. (2009) *Biochemistry 48*, 8206–8224.
(5) Ahmad, B., and Lapidus, L. J. (2012) *J. Biol. Chem. 287*, 9193–9199.
(6) Anand, P., Kunnumakkara, A. B., Newman, R. A., and Aggarwal, B. B. (2007) *Mol. Pharmaceutics 4*, 807–818.
(7) Singh, P. K., Kotia, V., Ghosh, D., Mohite, G. M., Kumar, A., and Maji, S. K. (2013) *ACS Chem. Neurosci. 4*, 393–407.
(8) Yallapu, M. M., Jaggi, M., and Chauhan, S. C. (2012) *Drug Discovery Today 17*, 71–80.
(9) Qin, X. R., Abe, H., and Nakanishi, H. (2002) *Biochem. Biophys. Res. Commun. 297*, 1011–1015.
(10) Wang, M. S., Boddapati, S., and Sierks, M. R. (2009) *Biochem. Biophys. Res. Commun. 386*, 526–531.
(11) Hudson, S. A., Ecroyd, H., Kee, T. W., and Carver, J. A. (2009) *FEBS J. 276*, 5960–5972.
(12) Baum, L., and Ng, A. (2004) *J. Alzheimer's Dis. 6*, 367–377.
(13) Porat, Y., Abramowitz, A., and Gazit, E. (2006) *Chem. Biol. Drug Des. 67*, 27–37.
(14) Berhanu, W. M., and Masunov, A. E. (2010) *Biophys. Chem. 149*, 12–21.
(15) Machida, S., Ogawa, S., Xiaohua, S., Takaha, T., Fujii, K., and Hayashi, K. (2000) *FEBS Lett. 486*, 131–135.