Cleavage Agents for α-Synuclein

Jaehwa Lee, Sang Ho Yoo, Keunhong Jeong, Tae Yeon Lee, Jae Young Ahn, and Junghun Suh*

*Department of Chemistry, Seoul National University, Seoul 151-747 Korea. *E-mail: jhsuh@snu.ac.kr*
Received February 13, 2008

Key Words : Peptide-cleaving catalyst, α-Synuclein, Parkinson's disease

Previously, we reported artificial proteases for soluble oligomers of amyloidogenic peptides such as amyloid β-42 (Aβ_{42}) or human islet amyloid polypeptide (h-IAPP).[1,2] Several reports suggest that soluble oligomers of Aβ_{42} and h-IAPP are the pathogenic intermediates in Alzheimer's disease and type 2 diabetes mellitus, respectively. Soluble oligomers of other amyloidogenic peptides or proteins (AmPs) such α-synuclein, prion, and polyglutamine are also implicated as the primary toxic species in amyloidoses such as Parkinson's disease, spongiform encepahlopathies, and Huntington's disease, respectively.[3]

The association process of an AmP involves formation of several oligomers, protofibrils, and fibrils as summarized in Figure 1.[4-6] Here, the species placed in the rectangle stand for the soluble oligomers. Conversion of large assemblies such as protofibrils and fibrils into smaller ones is slow, and formation of the large assemblies can be considered as irreversible or partially irreversible.[4]

As a new therapeutic option for amyloidoses, we have proposed reduction of the level of the pathogenic oligomers of AmPs through peptide cleavage.[1,2,7] As shown in Figure 1 where the cleavage agent is indicated as (R)-(LCo^{III}), cleavage of an AmP included in a target oligomer reduces the concentration of the target oligomer, leading to decreases in the concentrations of other oligomers which readily transform into the target oligomer.

The successful discovery of cleavage agents for oligomers of Aβ_{42} and h-IAPP suggested that new therapeutic methods for amyloidoses may be obtained by using artificial proteases. In the present study, we have tested whether artificial proteases can be also designed for the oligomers of α-synuclein which is implicated as the pathogenic species of Parkinson's disease.

A slightly modified form (MW 15500) of α-synuclein was obtained in the present study as described in the Experimental Section. To examine the degree of aggregation of α-synuclein, a filtration experiment was performed: A solution (70 μM) of α-synuclein was shaken (600 rpm) at 37 °C and pH 7.50 (50 mM HEPES). At various time intervals, an aliquot (20 μL) of the mixture was passed through a membrane with the pore size of 0.22 μm (Millipore Millex-GV 4MM). The amount of α-synuclein that passed through the filter was measured according to the procedure described previously.[1,2] Results of the filtration experiment are summarized in Figure 2. Because the pore of the filter was fairly large, the fraction blocked by the filter represents large protofilbrils and fibrils as well as aggregates adsorbed onto the reaction vessel (Eppendorf tube). The results reveal that a major portion of α-synuclein remains as aggregates that can pass through the filter with a pore size of 0.22 μm within several days.

The four cleavage agents (**A**-**D**) reported[1,2] to cleave soluble oligomers of Aβ_{42} and h-IAPP were tested with α-synuclein. In **A**-**D**, the Co(III) complex of cyclen (Co(III)-cyclen) is exploited as the catalytic site for peptide cleavage and the organic moieties are employed as binding site to recognize the target oligomers of AmPs. A solution of α-synuclein (70 μM) was incubated with one of **A**-**D** at 37 °C for various periods and cleavage of α-synuclein was examined by MALDI-TOF MS. Cleavage agents **A** and **B** exhibited cleavage activity whereas **C** and **D** manifested no activity.

The product solution obtained by the reaction of α-

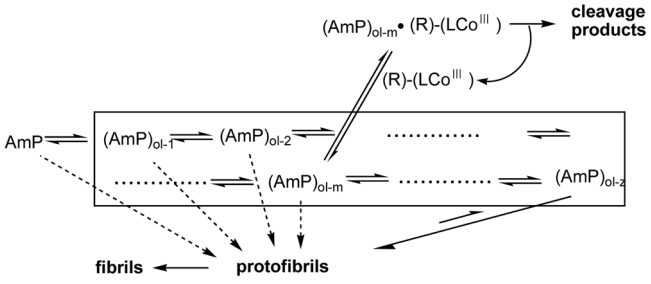


Figure 1. Formation of various assemblies of an AmP and reduction of the assemblies by the action of cleavage agents.

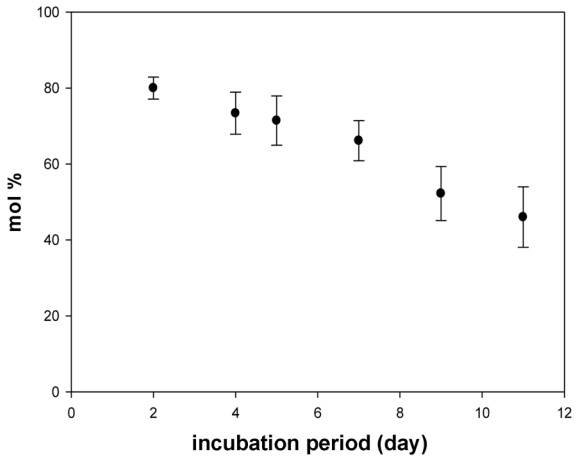
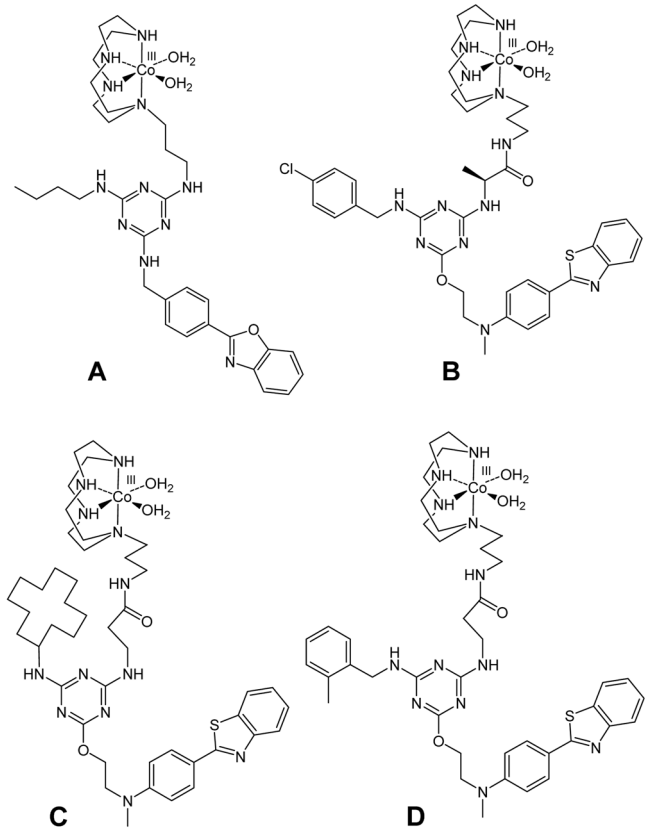


Figure 2. Fraction of α-synuclein (70 μM) passing through the membrane with pore size of 0.22 μm after incubation for various periods at 37 °C and pH 7.50.



synuclein with **A** or **B** was filtered through a membrane with the cutoff MW of 10000 to remove aggregates of α-synuclein. The resulting solution was subjected to HPLC separation to isolate the oligopeptide fragments and to ensure exclusion of uncleaved α-synuclein. Since α-synuclein has molecular weight of about 15000, some large cleavage fragments may have not passed the membrane. The oligopeptide fragments were hydrolyzed under alkaline conditions, and the resulting amino acids were quantified with fluorescamine as described previously.[1,2] The amounts of peptide fragments obtained by the action of **A** and **B** were estimated as mol % of the initially added amount of α-synuclein and are defined as the cleavage yields. The cleavage yields measured after reaction with various initial concentrations (C_o) of α-synuclein for 3 days at 37 °C and pH 7.50 are plotted against log C_o/M in Figures 3 and 4. Plateau values of the cleavage yields are about 30% and significant yields were observed at 1-10 nM C_o.

Effective cleavage of α-synuclein and catalytic turnover were achieved with **A** and **B** at submicromolar concentrations of the cleavage agents. Cleavage agents **A** and **B** were also active in cleaving soluble oligomers of amyloid β-40 (Aβ_{40}), Aβ_{42}, and h-IAPP. Since **A** and **B** do not cleave various common proteins,[1,2] it is also likely that **A** and **B** cleave the oligomer, instead of monomer, of α-synuclein.

Cleavage of a soluble oligomer of an AmP by a cleavage agent is schematically described in Figure 5.[2] The interaction among aromatic side chains of an AmP has a central role in the self-assembly process leading to formation of oligomers.[8] The aromatic interaction may produce an

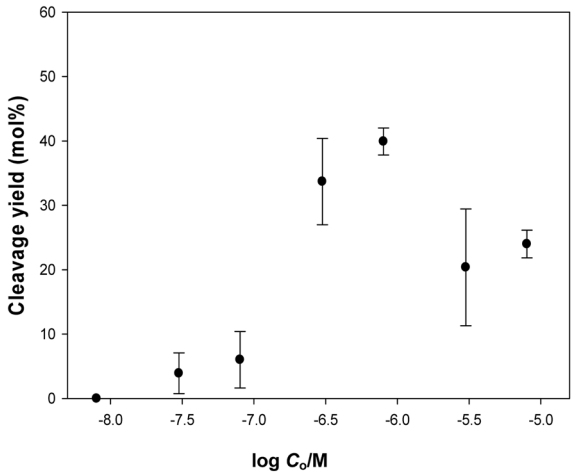


Figure 3. Plot of the cleavage yield against log C_o/M for cleavage of α-synuclein (70 μM) by **A** measured after reaction for 3 days at 37 °C and pH 7.50.

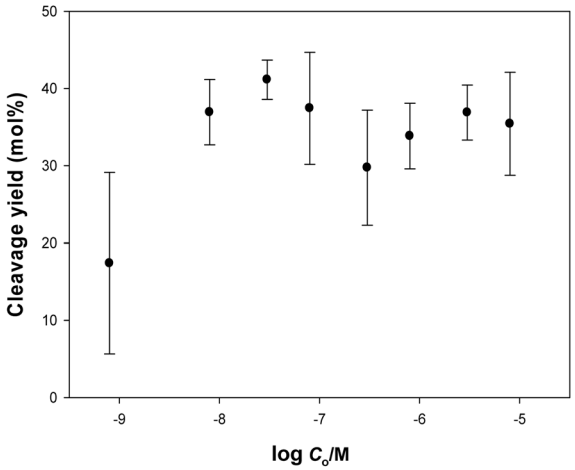


Figure 4. Plot of the cleavage yield against log C_o/M for cleavage of α-synuclein (70 μM) by **B** measured after reaction for 3 days at 37 °C and pH 7.50.

aromatic domain in $(AmP)_{ol\text{-}m}$. Thus, that cleavage agents such as **A** and **B** may form complexes with $(AmP)_{ol\text{-}m}$ by exploiting their aromatic moieties to recognize the aromatic domain of $(AmP)_{ol\text{-}m}$.

In the complexes formed between α-synuclein and the cleavage agents, the Co(III)cyclen moieties of **A** and **B** must have high effective concentrations towards peptide bonds of the target AmP. The lack of activity for **C** and **D** to cleave the oligomer of α-synuclein may be ascribed either to failure to form complexes with the target oligomer or to low effective concentrations of Co(III)cyclen moiety towards the peptide bonds in the complexes.

The oligomers of α-synuclein, Aβ_{40}, Aβ_{42}, and h-IAPP should have distinctly different structures. In this regard, it is interesting that **A** and **B** cleave soluble oligomers of α-synuclein, Aβ_{40}, Aβ_{42}, and h-IAPP.[1,2] Cleavage of the soluble oligomers of two or more AmPs by the same cleavage agent suggests that the target oligomers of h-IAPP, Aβ_{40}, Aβ_{42}, and α-synuclein contain microdomains of similar structures and that those microdomains facilitate complex

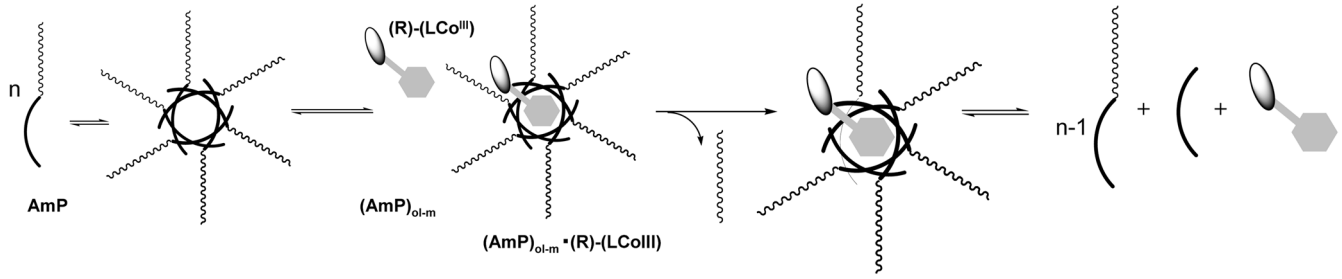


Figure 5. Schematic description of pathway for cleavage of a soluble oligomer of an AmP by a cleavage agent.

formation with the same cleavage agent such as **A** and **B**.

The antibody raised against Aβ_{42} oligomer recognized not only the Aβ_{42} oligomer but also the oligomers of other AmPs such as Aβ_{40}, α-synuclein, h-IAPP, polyglutamine, lysozyme, insulin, prion peptide 106-126.[9] This was taken to indicate that soluble oligomers of a variety of AmPs have a common conformation-dependent structure. In addition, this led to speculation[10] that a single drug might be able to cure a variety of amyloidoses. Both the recognintion of soluble oligomers of α-synuclein, Aβ_{40}, Aβ_{42}, or h-IAPP by **A** or **B** and recognition of soluble oligomers of various AmPs by the antibody can be attributed to the presence of the common microdomains of the oligomers.

We have proposed that target-selective artificial proteases may be used as catalytic drugs and may become a new paradigm in drug design.[1,2,7,11-19] The present study indicates that the target-selective artificial proteases may be especially effective for amyloidoses such as Alzheimer's disease, diabetes, Parkinson's disease, Huntington's disease, and mad cow's disease for which the conventional drugs targeting active sites of proteins cannot be designed.

Experimental Section

The recombinant plasmid containing the α-synuclein gene was provided by Prof. Seung R. Peik. *E. coli* C41 (DE3) containing the plasmid was cultured at 37 °C. From the cell pellet obtained therefrom, α-synuclein was purified by Ni-NTA affinity chromatography. The eluted fractions were examined by SDS-PAGE analysis. The product was further purified by ultrafiltration using a membrane with cut-off MW of 30000 for 30 minutes to remove proteins with large MW and concentrated by passing through an membrane with cut-off MW of 10000 for 2 hours. The concentration of the protein was determined by measuring the absorbance value at 280 nm according to the literature.[20] The solution was lyophilized in small aliquots (1 mg α-synuclein) and the lyophilized protein was stored at −70 ºC. Right before use, the α-synuclein powder was dissolved in 2 mM NaOH.[21] The resultant α-synuclein contained the *C*-terminal tag of LEHHHHHH to facilitate purification by Ni-NTA affinity chromatography. In addition, Met in the 5th position was replaced by Leu to prevent error in the transcription and translation processes. The α-synuclein derivative is composed of 148 amino acid residues and has a molecular weight of 15507 due to the presence of the eight-residue tag of the *C*-terminus. Structure of the α-synuclein derivative obtained in the present study was confirmed by trypsin digestion followed by analysis with MALDI-TOF MS. Synthesis of **A** and **B**, filtration experiment, measurement of cleavage yields, and data analysis were carried out as described in the previous reports.

Acknowledgments. This work was supported by the Korea Science and Engineering Foundation through the National Research Laboratory Program (No. M10500000001-06J0000-00110) funded by the Ministry of Science and Technology.

References

1. Suh, J.; Yoo, S. H.; Kim, M. G.; Jeong, K.; Ahn, J. Y.; Kim, M. S.; Chae, P. S.; Lee, T. Y.; Lee, J.; Lee, J.; Jang, Y. A.; Ko, E. H. *Angew. Chem. Int. Ed.* **2007**, *46*, 7064-7067.
2. Suh, J.; Chei, W. S.; Lee, T. T.; Kim, M. G.; Yoo, S. H.; Jeong, K.; Ahn, J. Y. *J. Biol. Inorg. Chem.* in press.
3. Demuro, A.; Mina, E.; Kayed, R.; Milton, S. C.; Parker, I.; Glabe, C. G. *J. Biol. Chem.* **2005**, *280*, 17294-17300.
4. Lesné, S.; Koh, M. T.; Kotilinek, L.; Kayed, R.; Glabe, C. G.; Yang, A.; Gallagher, M.; Ashe, K. H. *Nature* **2006**, *440*, 352-357.
5. Bitan, G.; Kirkitadze, N. D.; Lomakin, A.; Vollers, S. S.; Benedek, G. B.; Teplow, D. B. *Proc. Natl. Acad. Sci. USA* **2003**, *100*, 330-335.
6. Hull, R. L.; Westermark, G. T.; Westermark, P.; Kahn, S. E. *J. Clin. Endocrinol. Metab.* **2004**, *89*, 3629-3643.
7. Suh, J.; Chei, W. S. *Curr. Op. Chem. Biol.* in press.
8. Cohen, T.; Frydman-Marom, A.; Rechter, M.; Gazit, E. *Biochemistry* **2006**, *45*, 4727-4735.
9. Kayed, R.; Head, E.; Thompson, J. L.; McIntire, T. M.; Milton, S. C.; Cotman, C. W.; Glabe, C. G. *Science* **2003**, 300, 486-489.
10. Dobson, C. M. *Science* **2004**, *304*, 1259-1262.
11. Jeon, J. W.; Son, S. J.; Yoo, C. E.; Hong, I. S.; Song, J. B.; Suh, J. *Org. Lett.* **2002**, *4*, 4155-4158.
12. Suh, J. *Acc. Chem. Res.* **2003**, *36*, 562-570.
13. Jeon, J. W.; Son, S. J.; Yoo, C. E.; Hong, I. S.; Suh, J. *Bioorg. Med. Chem.* **2003**, *11*, 2901-2910.
14. Chae, P. S.; Kim, M.; Jeung, C.; Lee, S. D.; Park, H.; Lee, S.; Suh, J. *J. Am. Chem. Soc.* **2005**, *127*, 2396-2397.
15. Jeon, J. W.; Suh, J. *J. Biol. Inorg. Chem.* **2005**, *10*, 364-372.
16. Kim, M. G.; Kim, M.-s.; Lee, S. D.; Suh, J. *J. Biol. Inorg. Chem.* **2006**, *11*, 867-875.
17. Kim, M. G.; Kim, M.-s.; Park, P.; Lee, S.; Suh, J. *Bull. Korean Chem. Soc.* **2007**, *28*, 1151-1155.
18. Chei, W. S.; Suh, J. *Prog. Inorg. Chem.* **2007**, *55*, 79-142.
19. Jang, B.; Suh, J. *Bull. Korean Chem. Soc.* **2008**, *29*, 202-204.
20. Antony, T.; Hoyer, W.; Cherny, D.; Heim, G.; Jovin, T. M.; Subramaniam, V. *J. Biol. Chem.* **2003**, *278*, 3235-3240.
21. Munishkina, L. A.; Phelan, C.; Uversky, V. N.; Fink, A. L. *Biochemistry* **2003**, *42*, 2720-2730.