DOI 10.5012/bkcs.2011.32.5.1751

Cu(II) Cyclen Cleavage Agent with BTA-derived Binding Group for h-IAPP

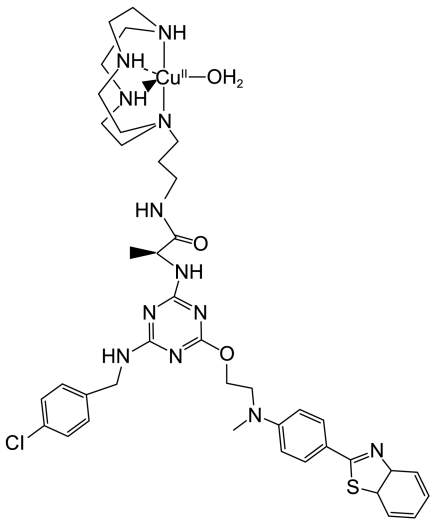
Keunhong Jeong,* Woo Young Chung, Young-Sik Kye, Dongwook Kim, and Si-uk Song

*Department of Chemistry, Korea Military Academy, Seoul 139-799, Korea. *E-mail: doas1mind@kma.ac.kr*
Received May 18, 2010, Accepted March 19, 2011

Key Words : Type 2 diabetes mellitus, Human islet amyloid peptide, Cu(II) cyclen cleavage agent, BTA-derived binding group

Type 2 diabetes mellitus (T2DM) is a fatal disorder disease that is characterized by substantial β-cell mass reduction in the context of insulin deficiency.[1-4] Previous researches have demonstrated that human islet amyloid polypeptide (h-IAPP), an oligopeptide monomer with 37 amino acid residues, induces apoptotic cell-death in β-cells and causes a development of T2DM.[5,6] Previous studies have also shown that fibrils, which are the final form of aggregated h-IAPP, are not necessarily the most toxic form of amyloid protein in general.[7,8] Oligomers may induce β-cell apoptosis.[9-12] Therefore, tremendous efforts have been conducted to develop drugs which prevent h-IAPP from forming oligomers. For instance, the use of oligomer-specific antibodies has been investigated,[13] as well as the use of Cu(II) ion, which prevents h-IAPP from forming the β-sheet conformers, and cleavage agents with Co(III) cyclen and Cu(II) cyclen.[14-16]

Cleavage agent with Cu(II) cyclen has been proposed as novel catalytic drug. Candidates for catalytic drug are mainly composed of two parts. One part is the catalytic group that hydrolyzes the peptide bond of h-IAPP. This part is composed of Cu(II) cyclen complex, which allows for secreted Cu(II) ions to act as oligomer inhibitors. The other part is the binding group that binds to h-IAPP through hydrophobic interactions. In regards to the hydrolysis reaction by Cu(II) cyclen complex, reaction time is critical and closely connected with the binding group's capacity since the cleavage agent cleaves only the oligomer form and has limited reaction time. Therefore, it is necessary to synthesize the catalytic drug candidate with the other binding group. Proposed binding group is I-Box-derived one. Benzothiazole-anilines (BTAs) functional group has higher affinity for the hydrophobic amyloid than I-Box(2-(4'-dimethylaminophenyl)-6-iodobenzoxazole) and BTAs also have a ability to cross the blood-brain barrier (BBB) well.[17,18] Figure 1 shows the proposed structure of Cu(II) cyclen cleavage agent with BTA-derived binding group (Fig. 1).



Cu(II) cleavage agent with BTA-derived binding group

Figure 1. Synthesized Cu(II) cleavage agent with BTA-derived binding group.

New Cu(II) cleavage agent with BTA-derived binding group was synthesized and the efficiency of this noble agent for h-IAPP cleavage was tested. Figure 2 shows that the MALDI-TOF TOF MS spectrum of fragmentation pattern when h-IAPP was incubated with new Cu(II) cleavage agent with BTA-derived binding group.

According to the previous literatures, residues 20-29 and 30-37 of h-IAPP (37mer) are considered as amyloidogenic region.[19-21] Peaks shown in Figure 2 indicate the fragments of h-IAPP. This spectrum demonstrates that highly amyloidogenic regions of h-IAPP can exist as dissolved form in solution. This indicates that self aggregation of h-IAPP can be inhibited by this noble agent. In our previous study, we

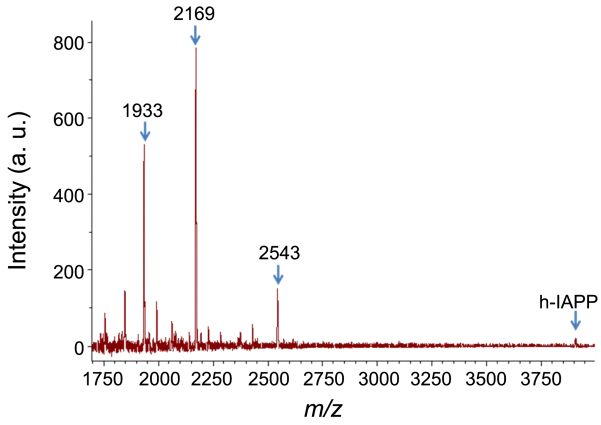


Figure 2. MALDI-TOF mass spectrum of the product obtained by incubation of h-IAPP (8 μM) with BTA-derivative cleavage agent (1 μM) for 36 h at pH 7.50 (50 mM HEPES) in 37 °C. The peaks with *m/z* of 1933, 2169, and 2543 correspond to h-IAPP$_{19-37}$, h-IAPP$_{16-37}$, and h-IAPP$_{14-37}$, respectively.

synthesized Cu(II) cyclen cleavage agent with I-Box-derived binding group.

To compare the cleavage efficiency of two cleavage agents, h-IAPP was incubated with each agents and cleavage yield was quantified by using fluorescamine assay. The resulting cleavage yield by BTA-derived binding group was 11.8 mol %, which was higher than that of the cleavage agent with I-Box-derived binding group (8.3 mol %).[22]

Batch experiment was designed to verify whether this agent can cleave the fibril form of h-IAPP. The h-IAPP was incubated for 36 h before the addition of cleavage agents. Then, cleavage agent with BTA-derived binding group was added and the mixture was incubated for an additional 36 h. There was no significant peak of h-IAPP fragments. In our previous study, full aggregation of h-IAPP was observed after 20 hours of incubation at the same condition (pH 7.5, 37 °C, 50 mM HEPES). Thus, no peak observation implies that this noble cleavage cannot break down the fully aggregated form of h-IAPP because monomer or oligomers of h-IAPP cannot exist in 36 hours of incubation.

Peaks shown in Figure 2 except parent peak of h-IAPP indicate fragments of h-IAPP containing hydrophobic region that plays critical role in amyloid formation. These fragments do not form amyloid fibril while parent compound h-IAPP does. It is uncertain whether the cleavage agent is active on monomenr or oligomers of h-IAPP. However, it is clear in our study that new Cu(II) cyclen agent has a capability to break down the h-IAPP monomer or oligomer before it fully aggregates to form fibrils.

Therefore, Cu(II) cyclen cleavage agent with BTA-derived binding group must be considered as a candidate for catalytic drugs.

Experimental

The cleavage agent moiety, without metal ion, was synthesized as described previously.[23] Complex with Cu(II), in order to obtain the Cu(II) cyclen aqua complex on the catalyst, was carried out as described in the literature.[24] The structure of Cu(II) aqua complex of 1,4,7,10-tetraazacyclododecane (Cu(II) cyclen) was previously identified by crystallographic methods.[25]

The synthesized cleavage agent moieties were dissolved in methanol. Then 0.95 equiv. of $CuCl_2$ (in methanol) was added to the solution of the cleavage agent moiety to avoid excess amount of Cu^{2+} ion being present. If free Cu^{2+} ion exists, they may be directly combined with h-IAPP. After solvent evaporation, the Cu(II) cyclen cleavage agent stock solution was obtained by dissolving in purified water. The stock solution was incubated at 37 °C for 4 h before use (Fig. 1).

h-IAPP was synthesized and purchased from Anaspec Inc. 1mg of h-IAPP was dissolved in 1 mL of hexafluoroisopropanol (HFIP) and separated into 32 samples. The solution was sonicated for 5 min, lyophilized, and stored frozen (−70 °C). The lyophilized sample was re-dissolved in 1 mL HEPES buffer (50 mM, pH 7.5, Sigma, USA) to make 8 μM of h-IAPP solution. Incubation of the cleavage agent with h-IAPP and subsequent identification of the cleaved fragments was performed as described previously.[22] Briefly, after the cleavage agent was activated at 37 °C for 4 h, it was added to 8 μM h-IAPP solution and adjusted to final concentrations of 1 μM for the cleavage agent and 8 μM for h-IAPP. Cleavage agent incubation was carried out at 37 °C for 36 h. After incubation, the molecular mass of the peptides was measured using MALDI-TOF MS (Bruker Daltonics Autoflex II MALDI-TOF/TOF mass spectrometer).

Cleavage yield was evaluated by fluorescamine assay.[16,26] After incubation, the mixture was filtered through a 10,000 Da MW-cutoff filter (Millipore Microcon centrifugal filter device YM-10) at 13,000 × g for 30 min at room temperature with a microcentrifuge (Hanil Model Micro 12). Filtration process cuts off aggregated fibril weighing over 10,000 Da and leaves only h-IAPP monomer, dimer, and cleaved fragments. The 100 μL of filtrate was transferred to a 500 μL microfuge tube. After addition of 10 μL of 13.5 M NaOH, the sample was placed in an autoclave at 120 °C for 2 h in order to hydrolyze amino acids. After cooling down to room temperature, the mixture was neutralized with methanesulfonic acid (15.4 M, 7.5 μL), then boric acid solution (0.7 M, 48 μL) was added to adjust the pH to 9.0. After brief vortexing and centrifugation, sample was loaded onto a 96 well plate (F96 Cert. Maxisorp, Nunc-Immuno Plate). Fluorescamine in acetonitrile (3.0 mg/mL, 10 μL) was also loaded onto the 96 well plate and subsequently mixed. The relative fluorescence of the samples was measured with alpha-imager (Model 1220 INT). As a control, after the h-IAPP buffer solution without cleavage agent was incubated, the same molar cleavage agent (1 μM) was added to the solution. The mean fluorescence intensity of 3 experiments was used to determine the peptide quantity.

Acknowledgments. This study was funded by the Korea Military Academy Hwarang-dae Research Institute research program. We acknowledge Prof. Suh, Junghun of Seoul National University for allowing us to use their research facilities during the course of this study.

References

1. Clark, A.; Wells, C.; Buley, I.; Cruickshank, J.; Vanhegan, R.; Matthews, D.; Cooper, G.; Holman, R.; Turner, R. *Diabetes Research (Edinburgh, Scotland)* **1988**, *9*, 151.
2. Butler, A.; Janson, J.; Bonner-Weir, S.; Ritzel, R.; Rizza, R.; Butler, P. *Diabetes* **2003**, *52*, 102.
3. Westermark, P.; Wernstedt, C.; O'Brien, T.; Hayden, D.; Johnson, K. *Am. J. Pathol.* **1987**, *127*, 414.
4. Cooper, G.; Willis, A.; Clark, A.; Turner, R.; Sim, R.; Reid, K. *Proc. Natl. Acad. Sci.* **1987**, *84*, 8628.
5. Hayden, M. *JOP. J Pancreas (Online)* **2002**, *3*, 126.
6. Roberts, A.; Leighton, B.; Todd, J.; Cockburn, D.; Schofield, P.; Sutton, R.; Holt, S.; Boyd, Y.; Day, A.; Foot, E. *Proc. Natl. Acad. Sci.* **1989**, *86*, 9662.
7. Brender, J.; Lee, E.; Cavitt, M.; Gafni, A.; Steel, D.; Ramamoorthy, A. *J. Am. Chem. Soc.* **2008**, *130*, 6424.
8. Brender, J.; Hartman, K.; Reid, K.; Kennedy, R.; Ramamoorthy, A. *Biochemistry* **2008**, *47*, 12680.

9. Janson, J.; Ashley, R.; Harrison, D.; McIntyre, S.; Butler, P. *Diabetes* **1999**, *48*, 491.
10. Kayed, R.; Sokolov, Y.; Edmonds, B.; McIntire, T.; Milton, S.; Hall, J.; Glabe, C. *J. Biol. Chem.* **2004**, *279*, 46363.
11. Ritzel, R.; Meier, J.; Lin, C.; Veldhuis, J.; Butler, P. *Diabetes* **2007**, *56*, 65.
12. Kayed, R.; Head, E.; Thompson, J.; McIntire, T.; Milton, S.; Cotman, C.; Glabe, C. *Science* **2003**, *300*, 486.
13. Lin, C.; Gurlo, T.; Kayed, R.; Butler, A.; Haataja, L.; Glabe, C.; Butler, P. *Diabetes* **2007**, *56*, 1324.
14. Masad, A.; Hayes, L.; Tabner, B.; Turnbull, S.; Cooper, L.; Fullwood, N.; German, M.; Kametani, F.; El-Agnaf, O.; Allsop, D. *FEBS Lett.* **2007**, *581*, 3489.
15. Ward, B.; Walker, K.; Exley, C. *J. Inorg. Biochem.* **2008**, *102*, 371.
16. Suh, J.; Chei, W.; Lee, T.; Kim, M.; Yoo, S.; Jeong, K.; Ahn, J. *J. Biol. Inorg. Chem.* **2008**, *13*, 693.
17. Klunk, W. E.; Wang, Y.; Huang, G.-F.; Debnath, M. L.; Holt, D. P.; Mathis, C. A. *Life Sci.* **2001**, *69*, 1471.
18. Nordberg, A. *Neuropsychologia* **2008**, *46*, 1636.
19. Glenner, G.; David Eanes, E.; Wiley, C. *Biochem. Biophys. Res. Commun.* **1988**, *155*, 608.
20. Westermark, P.; Engstrom, U.; Johnson, K.; Westermark, G.; Betsholtz, C. *Proc. Natl. Acad. Sci.* **1990**, *87*, 5036.
21. Scrocchi, L.; Chen, Y.; Waschuk, S.; Wang, F.; Cheung, S.; Darabie, A.; McLaurin, J.; Fraser, P. *J. Mol. Biol.* **2002**, *318*, 697.
22. Jeong, K.; Chung, W. Y.; Kye, Y.-S.; Kim, D. *Bioorg. Med. Chem.* **2010**, *18*, 2598.
23. Jeong, K.; Kim, M.; Chung, W.; Kye, Y.; Kim, J.; Suh, J. *J. Ind. Eng. Chem.* **2009**, *15*, 342.
24. Wu, W.; Lei, P.; Liu, Q.; Hu, J.; Gunn, A.; Chen, M.; Rui, Y.; Su, X.; Xie, Z.; Zhao, Y. *J. Biol. Chem.* **2008**, *283*, 31657.
25. Felix, V.; Delgado, R.; Amorim, M.; Chaves, S.; Galvao, A.; Duarte, M.; Carrondo, M.; Moura, I.; Silva, J. *J. Chem. Soc., Dalton Trans.* **1994**, *1994*, 3099.
26. Jeong, K.; Suh, J.; Chung, W.; Kye, Y.; Kim, D.; Lee, T. *Bull. Korean Chem. Soc.* **2010**, *31*, 1029.