Notes

Kinetic Studies on Proteolysis by Co(III) Complex of Cyclen

Boonjae Jang and Junghun Suh*

*Department of Chemistry, Seoul National University, Seoul 151-747, Korea. *E-mail: jhsuh@snu.ac.kr*
Received September 28, 2007

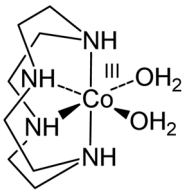
Key Words : Co(III)cyclen, Proteolysis

As a new paradigm for drug design, we have proposed catalytic drugs based on target-selective peptide-cleaving catalysts.[1-9] In the conventional approaches to design drugs that regulate the activities of disease-related proteins such as enzymes, receptors, or ion channels, attempts are made to discover small molecules that bind to the active sites of the disease-related proteins with high selectivity and high affinity. One molecule of the conventional drug can control the activity of at most one molecule of the target protein. When the substrate of a target-selective artificial protease is a protein related to a disease, the artificial protease can cleave and inactivate the target protein acting as a catalytic drug.

Peptide-cleaving catalytic drugs have several advantages in comparison with conventional drugs. First, the catalytic nature of peptide cleavage allows decreases in the amounts of the drug dose and the reduction of the side effects. Second, strong binding to the target is not required if the peptide cleavage is fast enough for the peptide-cleaving catalysts. Third, the peptide-cleaving catalysts can be obtained even for proteins or peptides lacking active sites.

We have discovered the first target-selective artificial protease by cleaving the backbone of myoglobin by hydrolysis.[3,4] In addition, we were able to design an artificial protease that selectively hydrolyzed the polypeptide backbone of peptide deformylase,[5] a bacterial enzyme regarded as an important target[10] for designing antibiotics. We also synthesized artificial proteases selective for oligomers of amyloid β-42 peptide,[9] the pathogenic species[11] for Alzheimer's disease. Moreover, we have reported catalysts for oxidative decarboxylation of *N*-terminal aspartate residues of oligopeptides such as angiotensin II[6] and melanin-concentration hormone,[7,8] which are targets for designing drugs for hypertension[12] and obesity,[13] respectively.

As the catalytic center for the peptide-cleaving catalysts, we have exploited the Co(III) complex of cyclen (Co(III)-cyclen). Co(III)cyclen itself has some activity for hydrolytic cleavage of peptide bonds. The catalytic activity of Co(III)-cyclen is enhanced to a high level by connecting it to a binding site which recognizes the target protein or peptide. If the effective concentration of Co(III)cyclen toward a reaction site on the target is sufficiently high in the target-catalyst complex, peptide cleavage occurs at a reasonably high rate.



Co(III)cyclen

If the Co(III)cyclen moiety of a peptide-cleaving catalyst cleaves non-target proteins *in vivo*, the catalyst can cause serious side effects. To utilize peptide-cleaving catalysts as catalytic drugs, therefore, it is necessary to demonstrate that random attack of the catalysts at non-target proteins is negligible.

The kinetic data for the hydrolysis of an amide (formylmorpholine) by Co(III)cyclen have been reported.[14] The second-order rate constant for the reaction was estimated as 0.26 $M^{-1}h^{-1}$ at 60 °C and pD 5.9. Unlike the amide molecule, proteins contain a large number of peptide bonds. Cleavage of any one of the peptide bonds present in a protein may alter the activity of the protein. To make sure that the attack of Co(III)cyclen moiety of peptide-cleaving catalysts at a non-target protein is negligible, we have collected kinetic data for proteolytic activity of Co(III)cyclen towards common proteins such as horse heart myoglobin, bovine serum γ-globulin, bovine serum albumin, and chicken egg white lysozyme.

Co(III)cyclen was synthesized and characterized as described in the literature.[15,16] Kinetic data for protein cleavage was collected by electrophoresis as reported for the previous works carried out in this laboratory.[17] The concentrations (S_o) of the protein substrates used in the kinetic studies were 10 μM for myoglobin, 4.0 μM for γ-globulin, 5.0 μM for albumin, and 10 μM for lysozyme. The kinetic measurements were carried out at 50 °C.

By using 5.0 mM Co(III)cyclen, the pseudo-first-order rate constant (k_o) for the disappearance of the substrate protein was measured at various pH values at 0.5 pH intervals. The highest activity was observed at pH 9.0 for myoglobin, 8.0 for γ-globulin, and 9.0 for albumin. No appreciable protein cleavage was observed for lysozyme for up to 96 hours under the same conditions.

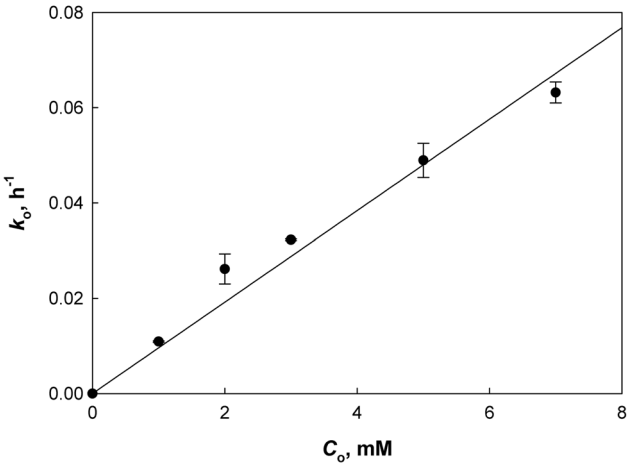


Figure 1. Plot of k_o against C_o (S_o = 10 μM) for the hydrolysis of myoglobin by Co(III)cyclen at pH 9.0 and 50 °C.

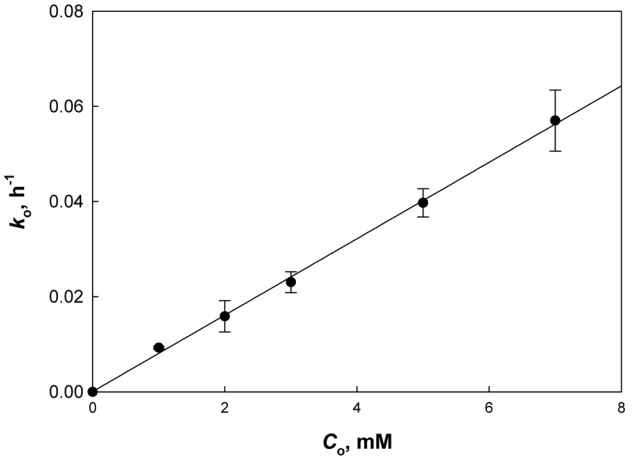


Figure 2. Plot of k_o against C_o (S_o = 4.0 μM) for the hydrolysis of the heavy chain of γ-globulin by Co(III)cyclen at pH 8.0 and 50 °C.

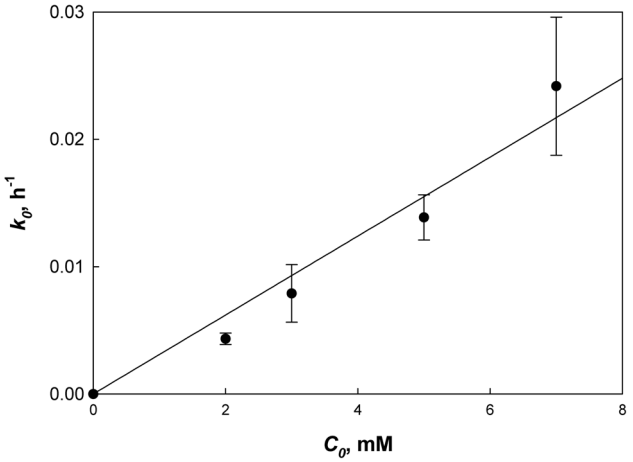


Figure 3. Plot of k_o against C_o (S_o = 5.0 μM) for the hydrolysis of albumin by Co(III)cyclen at pH 9.0 and 50 °C.

At the optimum pH, dependence of k_o on the concentration (C_o) of Co(III)cyclen was measured and the results are summarized in Figures 1-3. For γ-globulin which contains two heavy chains and two light chains, rate data were calculated for the cleavage of the heavy chain. The linearity constants calculated from the dependence of k_o on C_o are 9.6 ± 0.4 $M^{-1}h^{-1}$ for myoglobin, 8.1 ± 0.1 $M^{-1}h^{-1}$ for γ-globulin, and 3.1 ± 0.2 $M^{-1}h^{-1}$ for albumin.

The peptide-cleaving catalysts discovered so far for myoglobin, peptide deformylase, or oligomers of amyloid β-42 peptides show significant activity at or lower than 5 μM concentrations at 37 °C. It is appropriate, therefore, to evaluate the proteolytic activity of Co(III)cyclen at 5 μM concentration. The kinetic data obtained in the present study indicate that the half-lives for the cleavage by 5 μM Co(III)cyclen at 50 °C are 600 days for myoglobin, 710 days for γ-globulin, and 1900 days for albumin. The half-life is even much greater for lysozyme. The half-lives would increase by 3-4 fold when the temperature is lowered from 50 °C to 37 °C. Myoglobin, the heavy chain of γ-globulin, albumin, and lysozyme contain 153, *ca*. 460, 583, and 129 amino acid residues, respectively. Proteins containing more peptide bonds have higher chance for peptide cleavage by Co(III)cyclen. Proteins containing even more peptide bonds would resist hydrolysis by 5 μM Co(III)cyclen at least for several weeks at 37 °C.

The kinetic data collected in the present study indicate that the attack of Co(III)cyclen-based peptide cleaving catalysts at non-target proteins is negligible when the catalysts are added at or below micromolar concentrations. This provides a piece of evidence supporting the safety of catalytic drugs based on Co(III)cyclen-containing artificial proteases.

Experimental Section

Horse heart myoglobin, bovine serum γ-globulin, bovine serum albumin, and chicken egg white lysozyme were purchased from Sigma and used without further purification. All buffer solutions were filtered with 0.45 μm Millipore microfilter and autoclaved before use in the kinetic measurements. Buffers (0.05 M) used in this study were acetate (pH 5.5), 2-(*N*-morpholine)ethanesulfonate (pH 6.0-6.5), *N*-2-hydroxyethylpiperazine-*N*'-ethansulfonate (pH 7.0-8.0), *N*'-[tris(hydroxymethyl)methyl]-3-aminopropanesulfonate (pH 8.5) and borate (pH 9.0-9.5). pH measurements were carried out with a Dongwoo Medical DP-880 pH/Ion meter. The degree of cleavage of proteins was measured by SDS-PAGE with a Mighty Small II SE 250 model.[17] Densities of the electrophoretic bands were analyzed with a AlphaImager 2200 model and a AlphaEase model.

Acknowledgment. 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 and Notes

1. Suh, J. *Acc. Chem. Res.* **2003**, *36*, 562-570.
2. Chei, W. S.; Suh, J. *Prog. Inorg. Chem.* **2007**, *55*, 79-142.
3. Jeon, J. W.; Son, S. J.; Yoo, C. E.; Hong, I. S.; Song, J. B.; Suh, J.

Org. Lett. **2002**, *4*, 4155-4158.
4. Jeon, J. W.; Son, S. J.; Yoo, C. E.; Hong, I. S.; Suh, J. *Bioorg. Med. Chem.* **2003**, *11*, 2901-2910.
5. Chae, P. S.; Kim, M.-s.; Jeung, C.-S.; Lee, S. D.; Park, H.; Lee, S. Y.; Suh, J. *J. Am. Chem. Soc.* **2005**, *127*, 2396-2397.
6. Kim, M.-s.; Jeon, J. W.; Suh, J. *J. Biol. Inorg. Chem.* **2005**, *10*, 364-372.
7. Kim, M. G.; Kim, M.-s.; Lee, S. D.; Suh, J. *J. Biol. Inorg. Chem.* **2006**, *11*, 867-875.
8. Kim, M. G.; Kim, M.-s.; Park, H.; Lee, S.; Suh, J. *Bull. Korean Chem. Soc.* **2007**, *28*, 1151-1155.
9. 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. Intl. Ed. Engl.* **2007**, *46*, 7064-7067.
10. Ravi Rajagopalan, P. T.; Grimme, S.; Pei, D. *Biochemistry* **2000**, *39*, 779-790.
11. Hardy, J.; Selkoe, J. *Science* **2002**, *297*, 353-356.
12. Touyz, R. M.; Schifferin, E. L. *Pharmacol. Rev.* **2000**, *52*, 639-672.
13. Gibson, W. T.; Pissios, P.; Trombly, D. J.; Luan, J.; Keogh, J.; Wareham, N. J.; Maratos-Flier, E.; O'Rahilly, S.; Farooqu, I. S. *Obes. Res.* **2004**, *12*, 743-749.
14. Takasaki, B. K.; Kim, J. H.; Rubin, E.; Chin, J. *J. Am. Chem. Soc.* **1993**, *115*, 1157-1159.
15. Kim, J. H.; Britten, J.; Chin, J. *J. Am. Chem. Soc.* **1993**, *115*, 3618-3622.
16. Gómez, K.; González, G.; Martínez, M.; Mendoza, C.; Sienra, B. *Polyhedron* **2006**, *25*, 3509-3518.
17. Yoo, S. H.; Lee, B. J.; Kim, H.; Suh, J. *J. Am. Chem. Soc.* **2005**, *127*, 9593-9602.