ORGANIC LETTERS

2008
Vol. 10, No. 3
481−484

Proteolytic Activity of Cu(II) Complex of 1-Oxa-4,7,10-triazacyclododecane

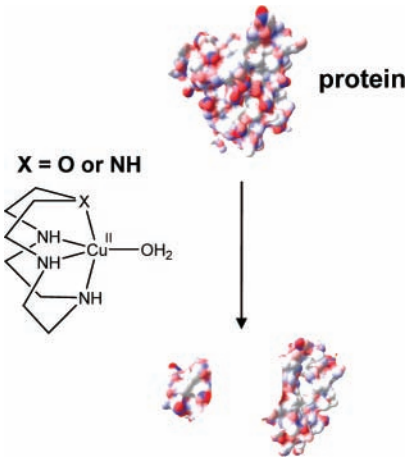
Seung Woo Jang and Junghun Suh*

Department of Chemistry, Seoul National University, Seoul 151-747, Korea

jhsuh@snu.ac.kr

Received November 27, 2007

ABSTRACT



Proteolytic activity of the Cu(II) complex of 1-oxa-4,7,10-triazacyclododecane (oxacyclen) was compared with that of the Cu(II) complex of 1,4,7,10-tetraazacyclododecane by using albumin, γ-globulin, and myoglobin as substrates. Values of k_{cat}/K_m were greater for Cu(II)oxacyclen by 40–80 times. The enhanced activity is attributed to the increased Lewis acidity of Cu(II) due to substitution of one nitrogen donor atom with oxygen.

A variety of organic reactions are catalyzed by metalloenzymes by using the metal ions as Lewis acid catalysts.[1] Metalloproteases are the most typical among them, and various efforts have been made to mimic metalloproteases with synthetic compounds.[2,3]

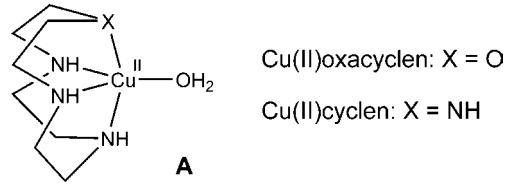
When a metal ion acts as a catalyst in the hydrolysis of a peptide bond, the metal-bound hydroxide ion and the metal-bound water molecule as well as the metal ion itself play several catalytic roles.[4] Various metal complexes promote hydrolysis of peptide bonds.[1,5−17] Some of them end up with complexes formed with the product fragments and/or manifest activity only at acidic pH values. To exploit the catalytic center of artificial metalloproteases, the metal complexes

(1) Chei, W. S.; Suh, J. *Prog. Inorg. Chem.* **2007**, *55*, 79−142.
(2) Suh, J. *Acc. Chem. Res.* **2003**, *36*, 562−570.
(3) Suh, J. *Bull. Korean Chem. Soc.* **2004**, *25*, 1917−1923.
(4) Suh, J. *Acc. Chem. Res.* **1992**, *25*, 273−279.
(5) Boreham, C. J.; Buckingham, D. A.; Keene, F. R. *J. Am. Chem. Soc.* **1979**, *101*, 1409−1421.
(6) Chin, J.; Jubian, V.; Mreje, K. *J. Chem. Soc., Chem. Commun.* **1990**, 1326−1328.
(7) Burgeson, I. E.; Kostić, N. M. *Inorg. Chem.* **1991**, *30*, 4299−4305.
(8) Takasaki, B. K.; Kim, J. H.; Rubin, E.; Chin, J. *J. Am. Chem. Soc.* **1993**, *115*, 1157−1159.
(9) Zhu, L.; Kostić, N. M. *J. Am. Chem. Soc.* **1993**, *115*, 4566−4570.
(10) Murthy, N. N.; Mahroof-Tahir, M.; Karlin, K. D. *J. Am. Chem. Soc.* **1993**, *115*, 10404−10405.
(11) Hegg, E. L.; Burstyn, J. N. *J. Am. Chem. Soc.* **1995**, *117*, 7015−7016.
(12) Takarada, T.; Yashiro, M.; Komiyama, M. *Chem. Eur. J.* **2000**, *6*, 3906−3913.
(13) Zhang, L.; Mei, Y.; Zhang, Y.; Li, S.; Sun, X.; Zhu, L. *Inorg. Chem.* **2003**, *42*, 492−498.
(14) Milović, N. M.; Badjić, J. D.; Kostić, N. M. *J. Am. Chem. Soc.* **2004**, *126*, 696−697.
(15) Kassai, M.; Ravi, R. G.; Shealy, S. J.; Grant, K. B. *Inorg. Chem.* **2004**, *43*, 6130−6132.
(16) Erxleben, A. *Inorg. Chem.* **2005**, *44*, 1082−1094.
(17) de Oliveira, M. C. B.; Scarpellini, M.; Neves, A.; Terenzi, H.; Bortoluzzi, A. J.; Szpoganics, B.; Greatti, A.; Mangrich, A. S.; de Souza, E. M.; Fernandez, P. M.; Soares, M. R. *Inorg. Chem.* **2005**, *44*, 921−929.

10.1021/ol702860h CCC: $40.75
Published on Web 01/09/2008

should be regenerated after peptide hydrolysis and should manifest considerable activity at neutral pH values.

The Cu(II) complex (Cu(II)cyclen) of 1,4,7,10-tetraazacyclododecane (cyclen) has been employed as the proteolytic center of artificial metalloproteases with potential applicability in protein industry.[18,19] The Cu(II)cyclen immobilized on a cross-linked polystyrene manifested broad substrate selectivity, cleaving all of the protein molecules examined.[19] The half-life for the protein cleavage by the polystyrene-linked Cu(II)cyclen was as short as 5 min at 50 °C. The hydrolytic nature of the peptide cleavage by Cu(II) complexes containing various ligands has been well established.[18−21]



A

Enhancement of catalytic activity of the metal centers is essential to improvement of artificial metalloenzymes. One way to tune the reactivity of the metal center is to modify the chelating ligand of the metal complex. In an attempt to improve the catalytic activity of Cu(II) complexes in peptide hydrolysis, one of the nitrogen atoms of Cu(II)cyclen was substituted with oxygen to obtain the Cu(II) complex (Cu(II)-oxacyclen) of 1-oxa-4,7,10-triazacyclododecane[22] (oxacyclen) in the present study. It was hoped that the Cu(II) ion of Cu(II)oxacyclen possesses greater proteolytic activity owing to its stronger Lewis acidity compared with that of Cu(II)cyclen. Approximate shapes of both Cu(II)oxacyclen and Cu(II)cyclen containing aqua ligands are illustrated by **A** on the basis of the crystallographic structures reported[23,24] for $[Cu(cyclen)H_2O]^{2+}$ and $[Cu(oxacyclen)Br]^{2+}$.

Spectral titration of the HBr salt[22] of oxacyclen with $CuCl_2$ in water at 741 nm revealed stoichiometric formation of a 1:1-type complex. By adding 0.95 equiv of $CuCl_2$ to oxacyclen to avoid possible kinetic complications arising from extra Cu^{2+} ion added to the reaction mixture, the aqueous stock solution of Cu(II)oxacyclen was prepared. Protein substrates employed in the kinetic measurements were bovine serum albumin (Alb), bovine serum γ-globulin (Gbn), and horse heart myoglobin (Mb). Cleavage of a protein substrate was followed by sodium dodecyl sulfate polyacrylamide gel electrophoresis[25,26] (SDS-PAGE). Typical data obtained for the SDS-PAGE experiments are illustrated in Figure 1. From the logarithmic plots of intensities of the

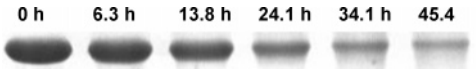


Figure 1. Typical data of SDS-PAGE carried out during cleavage of a protein substrate: The data were collected by incubating Alb (12 μM) with Cu(II)oxacyclen (0.30 mM) at pH 9.0 and 50 °C.

bands measured at various time intervals, the pseudo-first-order rate constant (k_o) was calculated as reported previously.[18,19,27,28] For Gbn, rate data were collected for cleavage of the heavy chain.

For Alb, Gbn, and Mb (4−12 μM), the pH dependence of k_o was measured with 5.0 mM Cu(II)oxacyclen at pH 5.5−9.5. At higher pH values, slow degradation of the protein was observed even in the absence of Cu(II)-oxacyclen. At the pH values where Cu(II)oxacyclen manifested the highest k_o values without showing any appreciable background protein cleavage, k_o values were measured at various initial concentrations (C_o) of Cu(II)-oxacyclen. At the same pH, the rate data were also obtained for Cu(II)cyclen.

The kinetic data collected for degradation of Mb, Gbn, and Alb by Cu(II)oxacyclen or Cu(II)cyclen are summarized in Figures 2−4. Saturation kinetic behavior was observed

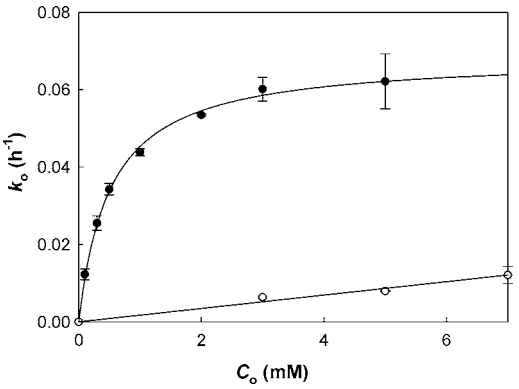


Figure 2. Dependence of k_o on C_o for cleavage of Alb at pH 9.0 and 50 °C by Cu(II)oxacyclen (●) or Cu(II)cyclen (○).

for the dependence of k_o on C_o for Cu(II)oxacyclen. Much slower rates were observed for Cu(II)cyclen, displaying linear dependence of k_o on C_o.

(18) Yoo, C. E.; Chae, P. S.; Kim, J. E.; Jeong, E. J.; Suh, J. *J. Am. Chem. Soc.* **2003**, *125*, 14580−14589.
(19) Yoo, S. H.; Lee, B. J.; Kim, H.; Suh, J. *J. Am. Chem. Soc.* **2005**, *127*, 9593−9602.
(20) Moon, S.-J.; Jeon, J. W.; Kim, H.; Suh, M. P.; Suh, J. *J. Am. Chem. Soc.* **2000**, *122*, 7742−7749.
(21) Suh, J.; Moon, S.-J. *Inorg. Chem.* **2001**, *40*, 4890−4895.
(22) Rossiter, C. S.; Mathews, R. A.; Morrow, J. R. *Inorg. Chem.* **2005**, *44*, 9397−9404.
(23) Félix, V.; Delgado, R.; Amorim, M. T. S.; Chaves, S.; Galvão, A. M.; Duarte, M. T.; Carrondo, M. A. A. F. C. T.; Moura, I.; Fraústo da Silva, J. J. R. *J. Chem. Soc., Dalton Trans.* **1994**, 3099−3106.
(24) Ren, Y. W.; Li, J.; Zhao, S. M.; Zhang, F. X. *Struct. Chem.* **2005**, *16*, 439−444.
(25) Lameli, U. K. *Nature* **1970**, *227*, 680−685.
(26) Hames, B. D. In *Gel Electrophoresis of Proteins*; Hames, B. D., Rickwood, D., Eds.; IRL Press: New York, 1990; Chapter 1.
(27) Suh, J.; Hah, S. S. *J. Am. Chem. Soc.* **1998**, *120*, 10088−10093.
(28) Jang, B. B.; Lee, K. P.; Min, D. H.; Suh, J. *J. Am. Chem. Soc.* **1998**, *120*, 12008−12016.

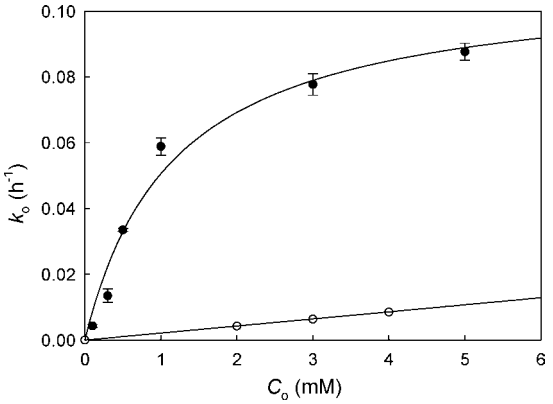


Figure 3. Dependence of k_o on C_o for cleavage of the heavy chain of Gbn at pH 8.0 and 50 °C by Cu(II)oxacyclen (●) or Cu(II)-cyclen (○). Data for Cu(II)cyclen were taken from ref 28.

The kinetic data of Figures 2−4 can be analyzed in terms of a Michaelis−Menten scheme (eq 1)

$$C + S \underset{K_m}{\rightleftharpoons} CS \xrightarrow{k_{cat}} C + P \quad (1)$$

as was done for the kinetic data obtained with macromolecular artificial metalloproteases.[18,19] Under the conditions of $C_o \gg S_o$, pseudo-first-order kinetic behavior is expected with k_o being derived as eq 2.

$$k_o = k_{cat}C_o/(K_m + C_o) \quad (2)$$

Values of kinetic parameters obtained by nonlinear regression of the kinetic data measured with Cu(II)oxacyclen according to eq 2 are summarized in Table 1.

For the proteolytic action of Cu(II)cyclen, k_o was proportional to C_o which can be also analyzed in terms of eq 2. When $K_m \gg C_o$, the proportionality constant for the plot of

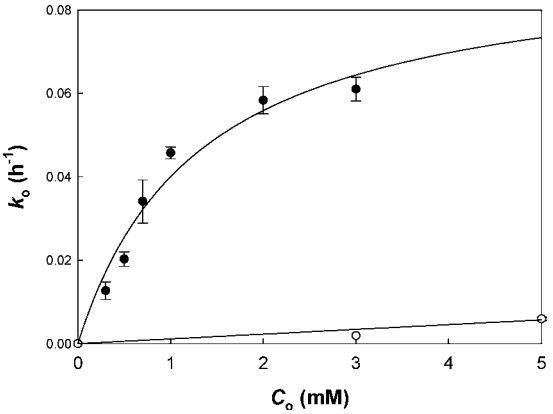


Figure 4. Dependence of k_o on C_o for cleavage of Mb at pH 9.5 and 50 °C by Cu(II)oxacyclen (●) or Cu(II)cyclen (○).

Table 1. Values of Kinetic Parameters Measured for Cleavage of Mb, Gbn, or Alb with Cu(II)oxacyclen or Cu(II)cyclen at 50 °C[a]

protein	ligand	k_{cat} (10^{-2} h^{-1})	K_m (mM)	k_{cat}/K_m (h^{-1} M)
Alb	oxacyclen	6.9 ± 0.1	0.51 ± 0.03	130 ± 10
Alb	cyclen	-	-	1.7 ± 0.1
Gbn	oxacyclen	11 ± 1	1.2 ± 0.3	94 ± 2
Gbn	cyclen[b]	-	-	2.1 ± 0.1
Mb	oxacyclen	9.3 ± 1.2	1.3 ± 0.4	70 ± 11
Mb	cyclen	-	-	1.0 ± 0.2

[a] Measured at pH 9.0 (0.05 M sodium borate) for Alb, pH 8.0 (0.05 M *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonate) for Gbn, and pH 9.5 (0.05 M sodium borate) for Mb. [b] Data taken from ref 28.

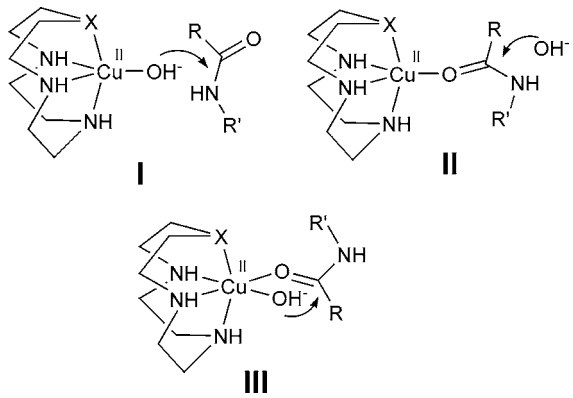
k_o against C_o is k_{cat}/K_m. The values of k_{cat}/K_m measured with Cu(II)cyclen are summarized in Table 1.

The proteolytic activities of Cu(II)oxacyclen and Cu(II)-cyclen can be best compared in terms of k_{cat}/K_m. The value of k_{cat}/K_m for Cu(II)oxacyclen is greater than that for Cu(II)-cyclen by 76 times for Alb, 45 times for Gbn, and 70 times for Mb.

The much greater proteolytic activity of Cu(II)oxacyclen may be explained in terms of the greater Lewis acidity of Cu(II) center. Substitution of one of the nitrogen atoms of cyclen with oxygen will lower the Lewis basicity of the chelating ligand, raising the Lewis acidity of the Cu(II) center. This is evidenced by the pK_a value of 8.7 for the aqua ligand coordinated to the Cu(II) atom of Cu(II)-oxacyclen.[29] The corresponding pK_a is 12.9 for Cu(II)-cyclen.[30]

In accordance with mechanisms generally proposed for ester or peptide hydrolysis by metal complexes,[1,4] three mechanisms summarized in Scheme 1 can be proposed for

Scheme 1. Possible Mechanisms for Proteolytic Action of the Cu(II) Complexes



the proteolytic action of Cu(II)oxacyclen and Cu(II)cyclen. These mechanisms have the same rate expression. In agreement with these mechanisms, the proteolytic rate by Cu(II)oxacyclen decreased as pH was lowered from the pH values employed in the kinetic study of Figures 2−4.

In mechanisms II and III, the enhanced Lewis acidity of the Cu(II) center would facilitate coordination of carbonyl oxygen of the amide group and polarization of the coordinated carbonyl group, leading to enhanced catalytic activity. In mechanisms I and III, the enhanced Lewis acidity of Cu(II) center would promote ionization of the Cu(II)-bound water molecule and raise the fraction of the Cu(II)-bound hydroxo nucleophile at pH values lower than the pK_a values. The higher Lewis acidity of Cu(II) would, however, reduce the nucleophilicity of the Cu(II)-bound hydroxo ion. The net enhancement in proteolytic activity would be, therefore, attained when the effect of enhanced ionization of the Cu(II)-bound water outweighs the effect of reduced nucleophilicity of the Cu(II)-bound hydroxo ion.

In addition to the difference in the Lewis acidity of the Cu(II) center, slight structural differences in transition states for the proteolytic actions of Cu(II)oxacyclen and Cu(II)-cyclen may affect the activity. Moreover, affinity toward certain parts of the protein substrates may be improved by the change in the ligand structure.

Detailed mechanistic information is not available at this stage for the proteolysis by Cu(II)oxacyclen and Cu(II)-cyclen. Nevertheless, the present study demonstrates that the proteolytic activity of metal ions can be considerably affected by a slight change in the ligand structure.

Artificial metalloproteases selectively cleaving target proteins or oligopeptides can be used as catalytic drugs.[2,31−34] The peptide-cleaving catalysts can inactivate even the proteins lacking active sites, for which conventional drugs targeting active sites of disease-related proteins cannot be obtained. Thus, artificial metalloprotease has provided a new therapeutic option for amyloid diseases such as Alzheimer's disease.[34] Improvement of the proteolytic activity of the metal centers of the target-selective artificial proteases is important in designing peptide-cleaving catalytic drugs. In this regard, the results of the present study will provide a guideline on improvement of proteolytic activity of the peptide-cleaving catalysts.

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.

OL702860H

(29) Amorim, M. T. S.; Chaves, S.; Delgado, R.; Fraústo da Silva, J. J. R. *J. Chem. Soc., Dalton Trans*. **1991**, 3065−3072.

(30) Kruppa, M.; Frank, D.; Leffler-Schuster, H.; König, B. *Inorg. Chim. Acta* **2006**, *359*, 1159−1168.

(31) Jeon, J. W.; Son, S. J.; Yoo, C. E.; Hong, I. S.; Song, J. B.; Suh, J. *Org. Lett.* **2002**, *4*, 4155−4158.

(32) Jeon, J. W.; Son, S. J.; Yoo, C. E.; Hong, I. S.; Suh, J. *Bioorg. Med. Chem.* **2003**, *11*, 2901−2910.

(33) Chae, P. S.; Kim, M.; Jeung, C.; Lee, S. D.; Park, H.; Lee, S.; Suh, J. *J. Am. Chem. Soc.* **2005**, *127*, 2396−2397.

(34) Suh, J.; Yoo, S. H.; Kim, M. K.; Jeong, K.; Ahn, J. Y.; Kim, M.; Chae, P. S.; Lee, T. Y.; Lee, J.; Lee, J.; Jang, Y. A.; Ko, E. H. *Angew. Chem., Int. Ed.* **2007**, *46*, 7064−7067.