Functional Molecules Based on Polyazometals. (2) Insoluble Artificial Metalloproteinase Obtained with a Cu(II)-Polyazometal

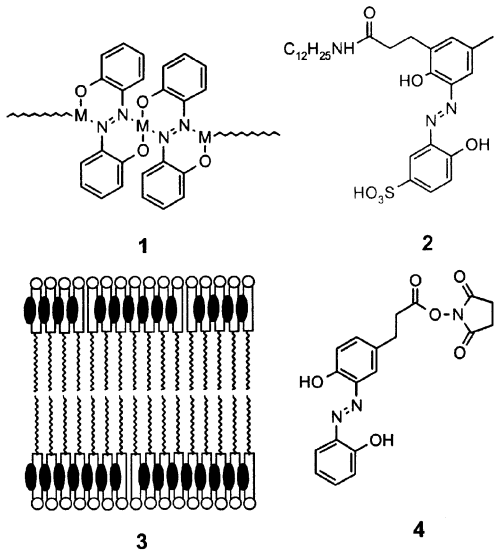
Junghun Suh,* Youn-Gu Lee, and Chul-Seong Jeung

School of Chemistry and Molecular Engineering and Center for Molecular Catalysis, Seoul National University, Seoul 151-742, Korea

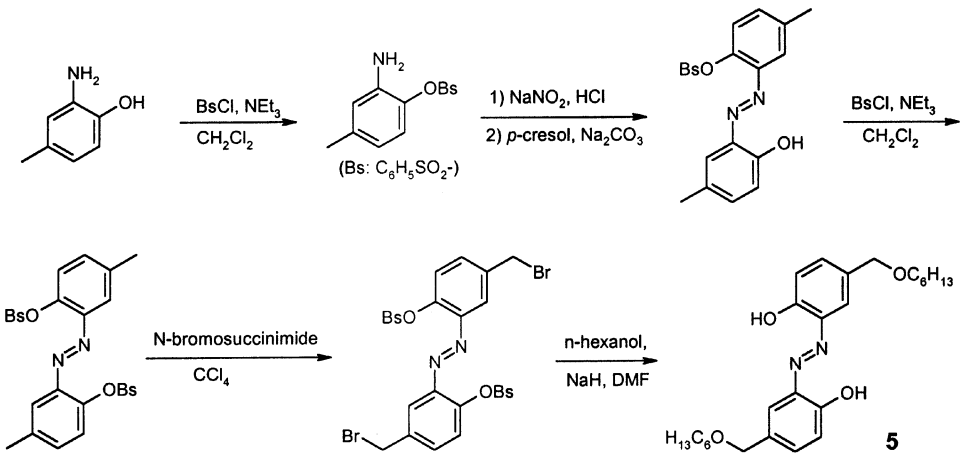
Received March 7, 2000

As a novel methodology for designing the active sites of artificial enzymes, we have developed self-assembly of active sites from catalytic elements.[1-3] For example, an artificial active site was formed by self-assembly from the Ni(II)-complex of a terpyridine derivative and the lauryl group attached to PEI, manifesting high catalytic power in transesterification of an RNA model.[1] In addition, we have designed active sites by self-assembly from metal ions and 2,2'-dihydroxyazobenzene (DHAB), a ligand equipped with two chelating sites. The assembly of a metal ion and a DHAB derivative forms a polyazometal (PAM), the coordination polymer or molecular cluster obtained with the metal ion bound to the azo ligand. Effective artificial proteinases have been designed with PAMs.[2,3]

In previous studies,[4] DHAB mixed with Fe(II) and Fe(III) ions was found to form solid materials with intrinsic conductivity. To explain the intrinsic conductivity, formation of coordination polymers such as **1** was proposed. In addition, sonication of amphiphile **2** and its analogue in the presence of transition metal ions produced coordinatively polymerized bilayer membranes (CPBMs) as schematically illustrated by **3**.[5] Upon complexation with the transition metal ions, stability of the bilayer membranes was remarkably improved. Amphiphile **2** forms complexes with transition metal ions with 1 : 1 molar ratio. The CPBMs of **2** obtained in the presence of Co(III) or Fe(III) ion were found to cleave chymotrypsin and carboxypeptidase A.[2] Inactivation of chymotrypsin by multiple cleavage was complete within a few minutes at 4 °C and pH 7.5 when the amphiphile concentration was 5.12 mM.[2] This can be compared with the half-life of 500-1000 yr for the spontaneous hydrolysis of peptide bonds at 25 °C and pH 7.[6]



Another type of PAM derivative has been obtained by conjugation of poly(allylamine) with a PAM prepared by mixing **4** with Fe(III) or Co(III) ion in DMSO.[3] Hydrolytic cleavage of bovine serum albumin (BSA) (M.W. 66000) was effectively catalyzed by the PAM-PAA conjugates. Pseudo-first-order rate constant (k_o) for disappearance of BSA was



Scheme 1

proportional to C_o (the initially added concentration of the catalyst expressed as the concentration of metal center). The k_o values measured with the PAM-PAA conjugate (C_o=2.4 × 10^{-5} M) corresponded to the half-life of 40 min at 50 °C and pH 8.5.

In the present study, 2,2'-dihydroxy-5,5'-di(hexyloxymethyl)-azobenzene (**5**: mp 80-81 °C, Anal. C, H, N) was synthesized according to Scheme 1 in an attempt to design an insoluble artificial proteinase based on PAM. In 40%(v/v) aqueous ethanol, Cu(II) complex of **5** precipitated when the molar amount of added Cu(II) exceeded that of added **5**. After a hot ethanol solution of **5** was added to an aqueous solution of equimolar $CuCl_2$, the resulting solution in 40% (v/v) aqueous ethanol was evaporated under a reduced pressure to obtain the powders of PAM of Cu(II) **5**.

The PAM of Cu(II) **5** was insoluble in water. In water containing 0.05 M buffers, the insoluble PAM of Cu(II) **5** manifested catalytic activity in hydrolysis of BSA as checked by SDS-PAGE electrophoresis[7] of the reaction mixture. At pH 9, accumulation of intermediate proteins was not observed during the cleavage of BSA, whereas formation and breakdown of several intermediate proteins were observed at pH 10-11. The progress of the cleavage of BSA was followed by measuring the density of the electrophoretic bands corresponding to BSA as described previously.[3,8] Plot of log [BSA] against time manifested pseudo-first-order kinetic behavior at least up to 4 half-lives. Pseudo-first-order rate constant (k_o) thus obtained was proportional to C_o. Here, C_o was expressed as the total concentration of Cu(II) ion obtainable when the insoluble PAM was assumed to be dissolved. From the plot of k_o against C_o, proportionality constant k_{bi} was estimated. The pH profile of k_{bi} is illustrated in Figure 1, which reveals that the insoluble PAM catalyst manifests optimum activity at pH ≥ 9. At pH 9 and 37 °C, half-life of 140 min was achieved with the PAM (C_o=2.3 × 10^{-3} M) for hydrolysis of BSA (3.7 × 10^{-7} M). This catalytic activity is comparable to or better than several other known artificial proteinases[3,8a,9] including catalytic antibodies, except for CPBMs[2] of **2** or a polystyrene derivative[8b] containing Cu(II) complex of cyclen. It is noteworthy that the reaction rates were not affected appreciably by changing the speed (0-1200 rpm) of stirring the reaction mixture. This suggests that diffusion of BSA onto the insoluble PAM is a fast process.

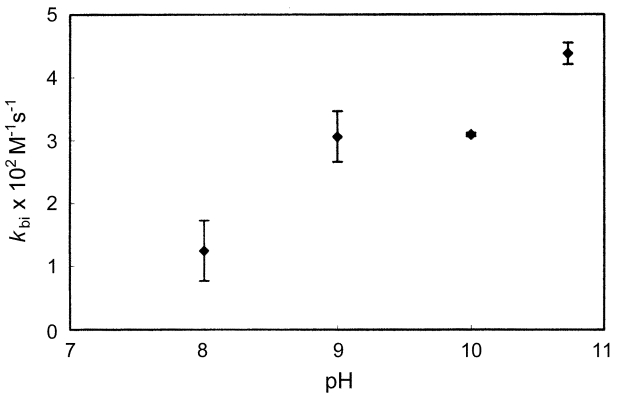


Figure 1. pH dependence of k_{bi} for hydrolysis of BSA by PAM of Cu(II) **5** at 37 °C.

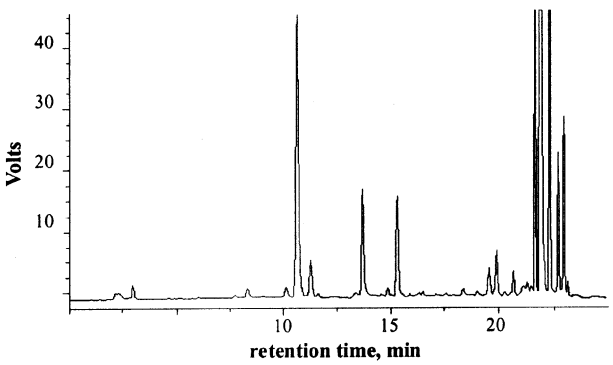
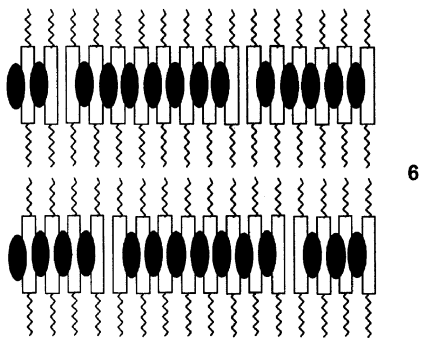


Figure 2. HPLC chromatogram of peptide products obtained after hydrolysis of BSA by PAM of Cu(II) **5**.

After BSA (3.3 × 10^{-5} M) was incubated with the insoluble PAM of Cu(II) **5** (C_o=9.0 mM) for 3 hr at pH 10 and 37 °C, the product was separated by filtration. Analysis by MALDI-TOF MS of the product mixture, indicated the presence of peptides with M.W. of 59600, 55900, 33900, 22900, 16400, 13900, 11800, 11100, 8600, and 7500 as well as other smaller fragments, whereas that of BSA indicated M.W. of 67100. After treatment of the amino groups of product peptides with phenyl isocyanate, the N-labeled peptides were subjected to HPLC analysis. Results of the HPLC analysis illustrated in Figure 2 indicate that a large number of peptides are obtained by the catalytic action. Labeling of the products by phenyl isocyanate indicates that amino groups are generated by cleavage of peptide bonds, providing evidence for hydrolytic nature of the cleavage.

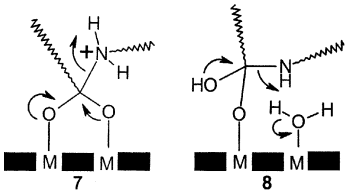
A coordination polymer (**6**) may be formed when Cu(II) ion and **5** are mixed together. In **6**, the rectangles, dark ellipses, and tails stand for DHAB moieties, Cu(II) ions, and hexyl tails, respectively. The actual structure formed by an equimolar mixture of Cu(II) and **5** may be more complicated than this. It is possible than each Cu(II) **5** complex may exist as an insoluble molecular cluster in water. Since PAM is defined above as the coordination polymer or molecular cluster obtained with a metal ion bound to a azo ligand, the insoluble material obtained by dispersing Cu(II) ion and **5** in water can be described as a PAM. It is noteworthy that the insoluble powders obtained by mixing Cu(II) and **5** in a 1 : 2 molar ratio did not manifest any catalytic activity in the hydrolysis of BSA as checked by a separate experiment. This implicates



6

that the proteolytic activity of the PAM of Cu(II) **5** is related to the unique structure of the PAM.

In the insoluble PAM of Cu(II) **5**, many proximal metal pairs would exist. Thus, collaboration between the two proximal metal centers can lead to effective catalysis in protein hydrolysis. Although the exact mechanism for the proteolytic action of the PAM cannot be determined, the metal centers of the PAM can perform several catalytic roles[10] discovered from various previous studies on metal-catalyzed hydrolysis of amides. Possible mechanisms for the collaboration between two metal centers are indicated by **7** and **8**. Since the rate-determining step in the peptide hydrolysis is most likely the expulsion of amine from the tetrahedral intermediate, the mechanistic analysis is focused on this step. In **7**, the intermediate is presumed to form from attack of metal-bound hydroxide ion at the carbonyl carbon of the scissile amide bond coordinated to another metal ion. In **8**, one metal ion stabilizes the tetrahedral intermediate whereas the other metal ion provides a metal-bound water molecule, which can act as a general acid catalyst for the expulsion of the amine.



7 8

Acknowledgment. This work was supported by a grant from Non-Directed Research Fund (1997) of Korea Research Foundation.

References

1. Suh, J.; Hong, S. H. *J. Am. Chem. Soc.* **1998**, *120*, 12545.
2. Suh, J.; Oh, S. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 1067.
3. Suh, J.; Moon, S. J. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 2751.
4. (a) Suh, J.; Oh, E. *Synth. Met.* **1990**, *39*, 177. (b) Suh, J.; Oh, E.; Kim, H. C. *Synth. Met.* **1992**, *48*, 325.
5. (a) Suh, J.; Lee, K. J.; Bae, G.; Kwon, O.-B.; Oh, S. *Langmuir* **1995**, *11*, 2626. (b) Suh, J.; Shim, H.; Shin, S. *Langmuir* **1996**, *12*, 2323. (c) Suh, J.; Shin, S.; Shim, H. *Bull. Korean Chem. Soc.* **1997**, *18*, 190.
6. (a) Bryant, R. A. R.; Hansen, D. A. *J. Am. Chem. Soc.* **1996**, *118*, 5498. (b) Radzicka, A.; Wolfenden, R. *J. Am. Chem. Soc.* **1996**, *118*, 6105. (c) Smith, R. M.; Hansen, D. A. *J. Am. Chem. Soc.* **1998**, *120*, 8910.
7. (a) Lameli, U. K. *Nature* **1970**, *227*, 680. (b) Hames, B. D. In *Gel Electrophoresis of Proteins*; Hames, B. D., Rickwood, D., Eds.; IRL Press: New York, 1990; Chap. 1.
8. (a) Suh, J.; Hah, S. S. *J. Am. Chem. Soc.* **1998**, *120*, 10088. (b) Jang, B.-B.; Lee, K. P.; Min, D. H.; Suh, J. *J. Am. Chem. Soc.* **1998**, *120*, 12008.
9. (a) Zhu, L.; Qin, L.; Parac, T. N.; Kostic, N. M. *J. Am. Chem. Soc.* **1994**, *116*, 5218. (b) Martin, M. T.; Angeles, T. S.; Sugasawara, R.; Aman, N. I.; Napper, A. D.; Darsley, M. J.; Sanchez, R. I.; Booth, P.; Titmas, R. C. *J. Am. Chem. Soc.* **1994**, *116*, 6508. (c) Hegg, E. L.; Burstyn, J. N. *J. Am. Chem. Soc.* **1995**, *117*, 7015. (d) Gao, C.; Lavey, B. J.; Lo, C.-H. L.; Datta, A.; Wentworth, P. Jr.; Janda, K. D. *J. Am. Chem. Soc.* **1998**, *120*, 2211.
10. (a) Suh, J. *Acc. Chem. Res.* **1992**, *25*, 273. (b) Suh, J. In *Perspectives in Bioinorganic Chemistry*; Hay, R. W., Dilworth, J. R., Nolan, K. B., Eds.; JAI Press: London, 1996; Vol. 3, pp 115-149.