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Synthetic Artificial Peptidases and Nucleases Using Macromolecular Catalytic Systems

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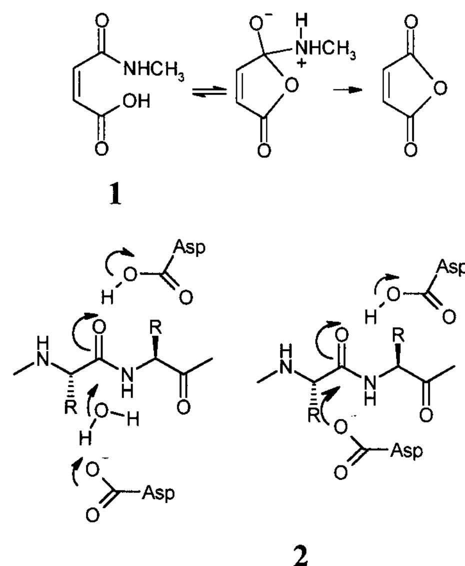
ABSTRACT
Effective artificial enzymes have been designed by adopting macromolecular systems for catalyst−substrate complexes. Artificial active sites comprising two or more organic functional groups were built on macromolecular backbones, leading to several types of organic artificial proteases. The activity of metal centers for peptide or DNA hydrolysis was greatly enhanced by attachment to polystyrene, leading to artificial metallopeptidases with substrate selectivity as well as artificial metallonucleases with high catalytic activity for double stranded DNA. A small artificial protease selective for a macromolecular target protein was synthesized. Target-specific artificial proteases can be used as protein-cleaving catalytic drugs.

Introduction

Enzymes are the most effective catalysts that exist in nature, and efforts have been made to design catalysts that mimic the catalytic principles of enzymes. Catalytic antibodies are examples of semisynthetic artificial enzymes.[1−4] Fully synthetic molecules have been also designed as enzyme mimics by using either peptidic[5,6] or nonpeptidic[7−12] molecules.

In the early stage of designing artificial enzymes, the major aims were to reproduce major characteristics of enzymatic action such as complex formation with substrates, large degrees of rate acceleration, and high selectivity. Other pursuits were to overcome limitations of enzymes such as instability to heat, incompatibility with organic solvents, inapplicability to abiotic reactions, and too narrow selectivity. As this area progresses further, research interests will focus on artificial enzymes that can solve biological problems that cannot be coped with natural enzymes. Examples are artificial proteases specifically hydrolyzing target proteins and genome restriction catalysts hydrolyzing chromosomal DNA at predetermined positions.

As a guide to designing synthetic artificial enzymes, intramolecular catalysis by organic groups may be considered. For example, hydrolysis of maleamic acid (**1**)[13] can be regarded as a model of aspartic proteases such as pepsin, renin, and HIV protease. An aspartic protease uses carboxyl groups of two Asp residues located in the active site to hydrolyze peptide bonds of substrates (**2**).[14] Maleamic acid, in which the catalytic carboxyl group is tethered to the reaction site, is hydrolyzed with a half-life of 3 h at 37 °C and optimum pH. The effective molarity (EM) of the carboxyl group in hydrolyzing the amide bond of **1** was estimated as 2×10^9 M. Even with this high EM value of the catalytic group, the reaction rate is about 10^7 times lower than that of enzymatic reactions which proceed with half-lives ($0.69/k_{cat}$) as short as 5 ms at 25 °C. This indicates that cooperation among two or more carboxyl groups and/or enhancement of intrinsic reactivity of the carboxyl groups are/is necessary to achieve high rates for peptide hydrolysis with carboxyl-based artificial proteases.



An effective enzyme-like catalyst should be able to form a complex with the substrate readily, and the complex should undergo rapid chemical transformation. In the complex, several catalytic components should take productive positions to maximize cooperativity among them and the EM of the catalytic group toward the reaction site should be sufficiently high. Moreover, the conformational freedom of the molecular framework should be controlled to maintain the productive structure. Chemical transformations in enzyme−substrate complexes often take place in hydrophobic microenvironments. It is necessary, therefore, to control the microenvironment of the complex formed between an artificial enzyme and its substrate. Construction of such a catalyst−substrate complex with a small molecular framework would not be easy.[15]

Nature has adopted polypeptides as the backbones of enzymes to tune the positions and the reactivity of catalytic elements in enzyme−substrate complexes. A better mimicry of enzymatic action would be, therefore, obtained by adopting macromolecular systems for catalyst−substrate complexes. Three combinations are possible for the macromolecular catalyst−substrate complexes: a macromolecular artificial enzyme and a macromolecular

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substrate, a macromolecular artificial enzyme and a small substrate, or a small artificial enzyme and a macromolecular substrate. Catalytic antibodies are macromolecular semisynthetic artificial enzymes mostly dealing with small substrates.[1−4] Synthetic polymers have been used as backbones of artificial enzymes catalyzing reactions of macromolecular or small substrates.[8−12,16−35] When the substrate is a macromolecule such as a protein or a nucleic acid, a small molecule may be used as the artificial enzyme.[36]

For macromolecular catalytic systems mimicking enzymatic action, it is not easy to fully characterize the structure of the catalyst−substrate complexes and to correctly determine the mechanisms of the catalytic processes. As with catalytic antibodies, therefore, strategies to design the artificial active sites and catalytic outcome of the resulting artificial enzymes are important at the current stage in the area of synthetic artificial enzymes employing macromolecular catalytic systems.

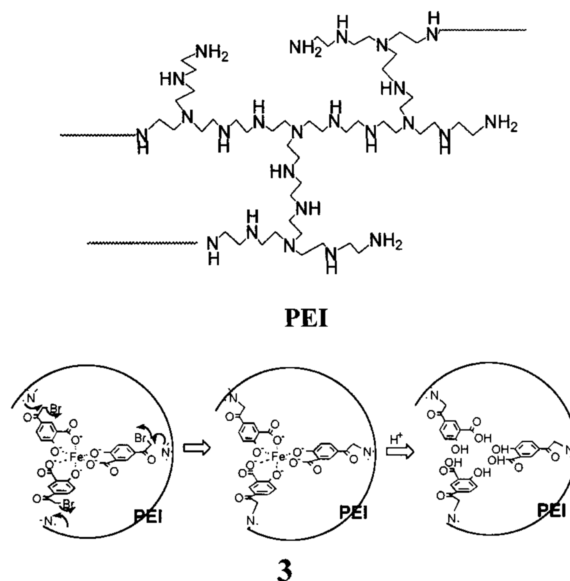
We have been involved in synthesis of enzyme-like catalysts for hydrolysis of peptide bonds of proteins and phosphodiester bonds of DNA. We chose hydrolysis as the target reaction since only one molecule is involved as the substrate except the water molecule. We selected phosphodiester and peptide bonds as the targets since DNA and protein hydrolysis is important in the era of genomics and proteomics. In this Account are summarized our strategy to design catalysts with protease or nuclease activities and the effectiveness of the artificial enzymes.

Polymeric Artificial Peptidases Exploiting Organic Catalytic Groups

As discussed above with **1**, collaboration of two or more organic functional groups is needed to achieve effective amide hydrolysis with intermolecular catalysts exploiting organic functional groups as catalytic elements. Many efforts have been made since 1960s to synthesize organic compounds that can catalyze peptide hydrolysis. They ended up mostly with hydrolyzing activated analogues of peptide bonds, leading to the criticism called “the *p*-nitrophenyl ester syndrome”.[37,38] We reported the first synthetic organic compound with proteolytic activity in 1998.[21]

The first organic artificial protease was designed by constructing an artificial active site comprising three convergent salicylate residues on the backbone of polyethylenimine (PEI).[21] PEI is a polyamine with a highly branched structure and high solubility in water. As illustrated by **3**, three molecules of 4-bromoacetylsalicylate were assembled around an Fe(III) ion and then cross-linked with PEI. Once an amino group of PEI was linked to one of the three salicylates through a covalent bond, other amino groups of PEI were located in the right positions to attack the remaining two salicylates, thus completing the cross-linkage. After removal of Fe(III) ion by treatment with acid, a water-soluble polymer with sites (Sal_3) comprising three proximal salicylates was obtained.

The Sal_3 site contained three carboxyl and three phenol groups in addition to the amino groups provided by the

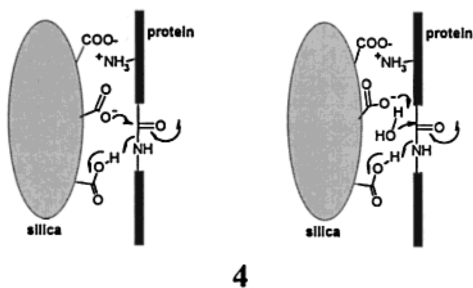


PEI backbone. Since those functional groups are catalytic groups used by enzymes, we tested whether Sal_3−PEI had catalytic properties. When Sal_3−PEI was incubated with γ-globulin (Gbn), degradation of Gbn was observed by SDS−PAGE electrophoresis. Kinetic data were collected by measuring the decrease in the intensity of the electrophoretic band. For degradation of Gbn by Sal_3−PEI, a half-life as short as 1 h at pH 7 and 50 °C was observed, which is comparable to the half-life for hydrolysis of **1**. When salicylates were attached to PEI randomly, little catalytic activity was observed, indicating that the proteolytic activity of Sal_3−PEI arose from collaboration among proximal salicylates. Optimum activity was observed at pH 6−7. Since carboxyl, phenol, and amino groups can play catalytic roles at pH 6−7, which functional groups were responsible for the proteolytic action was not clear.

We have also used cross-linked polystyrene as the backbone of our artificial enzymes. Typical examples are poly(chloromethylstyrene-*co*-divinylbenzene) (PCD) and poly(aminomethylstyrene-*co*-divinylbenzene) (PAD), which are cross-linked polystyrenes with styryl residues containing chloromethyl and aminomethyl groups, respectively. The Sal_3 site was built on polystyrene by cross-linking three molecules of 4-bromoacetylsalicylate preassembled around an Fe(III) ion with the amino groups of PAD by a procedure similar to **3**.[30] The excess amino groups on the resulting polystyrene were acetylated and then Fe(III) ion was removed with acid to obtain Sal_3−PAD−Ac. Albumin was effectively hydrolyzed by Sal_3−PAD−Ac with optimum activity manifested at pH 3 just as in the action of pepsin, the most typical aspartic protease. Protease action of Sal_3−PAD−Ac at pH 3 appears to involve collaboration of proximal carboxyl groups by analogy with **2**.

A simpler analogue of aspartic proteases was later synthesized by covering the surface of silica gel with primary amino groups and then by modifying the amines with succinic anhydride.[34] The resulting silica-based polycarboxylate hydrolyzed hemoglobin with half-life as short as 30 min at pH 8 and 50 °C. Carboxyl groups of the silica-

based catalyst can act both as binding sites recognizing ammonium or guanidinium groups of the protein and as catalytic groups playing the catalytic roles similar to those of the Asp residues of aspartic proteases (**4**).



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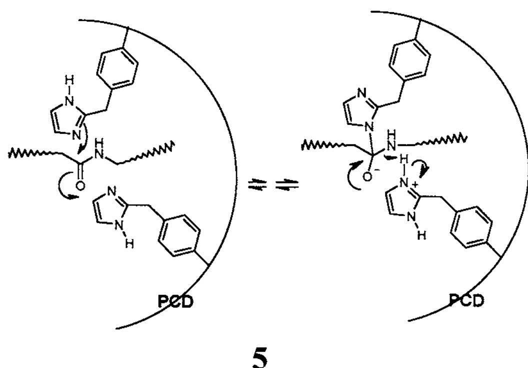
In the ribonuclease-catalyzed hydrolysis of phosphodiester bonds of RNA, two imidazole groups are involved as key catalytic groups acting as general acid/base.[39] In terms of half-life for spontaneous hydrolysis at 25 °C and pH 7, phosphodiester bonds of RNA (half-life: 100−200 y)[40] and peptide bonds of proteins (half-life: 500−1000 y)[41,42] have comparable stabilities. If RNA is hydrolyzed by the action of two proximal imidazoles, proteins may be hydrolyzed by a similar method.

We synthesized the first imidazole derivative with proteolytic activity by attaching imidazole to PCD through the carbon (C-2) atom.[28] The imidazole-containing PCD hydrolyzed albumin with a half-life as short as 20 m at pH 7 and 25 °C. The optimum activity was manifested at pH 7−9. In the PCD derivative, 24% of the styryl residues contained imidazole. When the content of the imidazole was reduced by 4.4 times, the proteolytic activity was reduced by 24 times, implicating collaboration of two or more imidazoles in the catalytic process. When imidazole was attached to PCD through the nitrogen atom instead of the C-2 atom, little catalytic activity was observed. These catalytic features are best explained by the mechanism represented by **5**, which involves cooperation between two imidazoles: one as a nucleophile and the other as a general acid.



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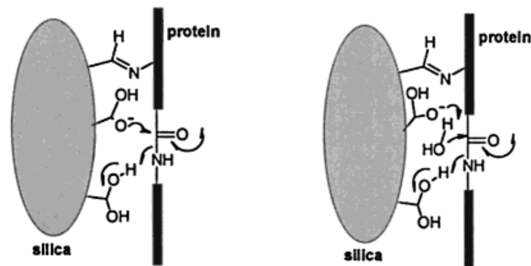
Carboxyl and imidazolyl groups are the catalytic groups present in the side chains of amino acids. It would be possible, however, to design artificial proteases by using organic functional groups unrelated to amino acids. Indeed, an artificial protease was synthesized by using aldehyde groups.[33] Silica gel covered densely with aldehyde groups manifested catalytic activity toward albumin and Gbn with half-lives as short as 1.5−3 h at pH 5−6 and 50 °C. As proposed in **6**, aldehydes can act as binding sites by forming imines with amino groups located on the protein surface. In addition, the hydrates of aldehydes can act as general acids, general bases, or nucleophiles to hydrolyze the peptide bonds of the bound protein substrate. Catalytic action of an aldehyde hydrate in a transacylation reaction has been reported.[43]

When the surface of silica gel was covered with organic functional groups such as carboxyl, hydroxyl, phenolic, imidazolyl, or mercapto groups as well as aldehyde groups, the proteolytic activity was considerably improved.[35] The best proteolytic activity was observed when silica gel was covered with histidines and aldehydes: the half-life was as short as 50 m at pH 6−9 and 25 °C, and broad substrate specificity was achieved with all of the proteins (albumin, ovalbumin, hemoglobin, and Gbn) tested in the study being hydrolyzed. Here, the aldehyde groups would act as the binding site and imidazolyl, carboxyl, and/or aldehyde hydrates as the catalytic groups by a mechanism analogous to **6**.



6

Polymeric Artificial Metallopeptidases

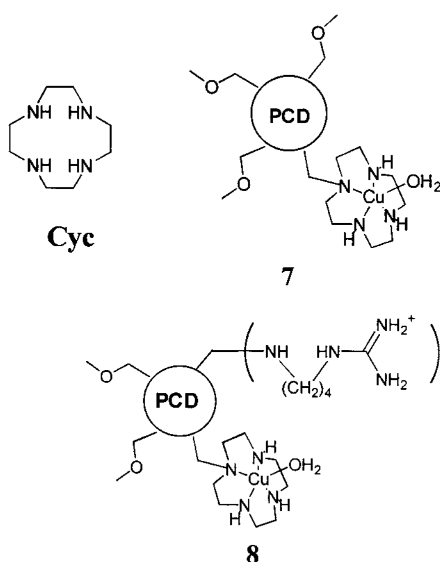
Metalloproteases use metal ions as the key catalytic groups. Metal ions can coordinate to the carbonyl oxygen of an amide group, polarizing the carbonyl group and facilitating attack by hydroxide ion.[44−47] Metal-bound hydroxide ions are potent nucleophiles for peptide cleavage.[44−47] Metal-bound water molecules can act as general acids in the rate-determining expulsion of amines from the tetrahedral intermediates.[46−48] Often, a single metal center can perform several catalytic roles.[45−47] When organic functional groups are exploited as catalytic groups for peptide hydrolysis, it is necessary to achieve collaboration among two or more catalytic groups. On the other hand, the active site of an artificial protease can be designed by using a single metal center as the catalytic group.

Metal complexes examined previously usually had low catalytic rates unless they were tethered to peptide substrates.[49,50] Proteolytic action by Pd(II) complexes untethered to proteins has been reported, but they are active at acidic pHs.[51,52] It is noteworthy that the Fe(III) complex of EDTA quickly cleaves a peptide bond when tethered to a target protein.[53] The Fe(III) complex, however, uses H_2O_2 and ascorbic acid as coreactants to form the hydrolysis products.

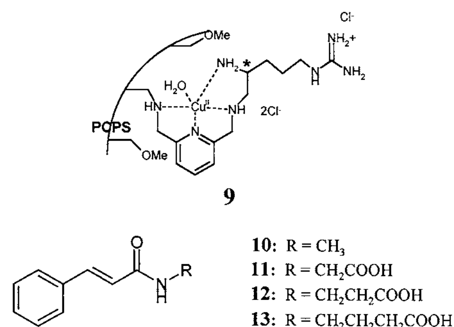
To design an effective artificial metalloprotease operating at near physiological pHs without coreactants such as H_2O_2 and ascorbic acid, it is necessary to raise the catalytic activity of metal centers substantially. We attempted to enhance the intrinsic reactivity of the metal centers by changing the microenvironment. Alternatively, we tried to increase the EM of the metal center to a sufficiently high level by forming a productive catalyst–substrate complex.

In enzyme–substrate complexes, polar interactions such as hydrogen-bonding and dipole–dipole interactions as well as electrostatic interactions between the substrate and the enzyme contribute significantly to the stabilization of the transition states.[54] Those polar interactions are enhanced in the hydrophobic microenvironments provided by the enzyme. If the microenvironment of the catalytic center of an artificial metalloprotease is modified, the proteolytic activity of metal complexes may be enhanced. The easiest way to obtain hydrophobic microenvironment in water is to attach the catalytic center to a synthetic polymer.

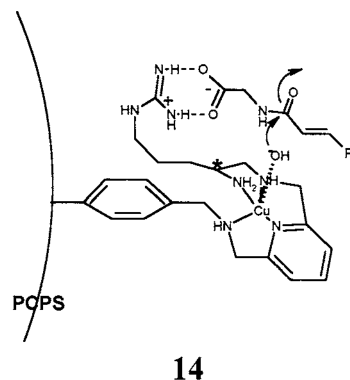
When the Cu(II) complex of cyclen (Cyc) was attached to cross-linked polystyrene, the proteolytic activity of Cu(II)Cyc was enhanced remarkably.[22] By the substitution of chloro groups of PCD with various nucleophiles, PCD derivatives **7** and **8** were prepared. Gbn was effectively hydrolyzed upon incubation with **7** and **8**. Half-life for the protein degradation was as short as 10–30 m at pH 4.5–7 and 4 °C. The rates for hydrolysis of Gbn by Cu(II)Cyc itself dissolved in water were also measured. Comparison of rate data collected at the same catalyst concentrations revealed that the proteolytic activity of Cu(II)Cyc was enhanced by up to 10^4-times upon attachment to the polystyrene. Considering that only a small fraction of Cu(II)Cyc moieties is present on the open surface on PCD and participate in hydrolysis of Gbn, the degree of activation should be substantially greater than 10^4-fold.



Since reactive metal centers were secured, it was subsequently attempted to achieve substrate selectivity in amide hydrolysis by the metal complexes.[27,29] The artificial active site of **9** was constructed on the surface of partially chloromethylated cross-linked polystyrene (PCPS) by stepwise modification of the polymer.[29] Here, the active site was chiral since L-Arg was used to introduce the guanidinium portion. Several cinnamoyl amide derivatives (**10**–**13**) were tested as substrates for **9**. Neutral amide **10** was not hydrolyzed upon incubation with **9**, but carboxyl-containing amides **11**–**13** were effectively hydrolyzed by **9** with the optimum activity observed at pH 9. Both acetyl L-Phe and acetyl D-Phe were also hydrolyzed by **9** and a small (1.5) enantioselectivity was observed as the catalyst was chiral.



In the proposed mechanism (**14**), the carboxylate anion of **11**-**13** or acetyl Phe is recognized by the guanidinium ion, and the Cu(II) center subsequently hydrolyzes the amide group. Both the electrostatic interaction between carboxylate and guanidinium ions and the Cu(II)-catalyzed amide cleavage would be facilitated by the microenvironment provided by polystyrene.



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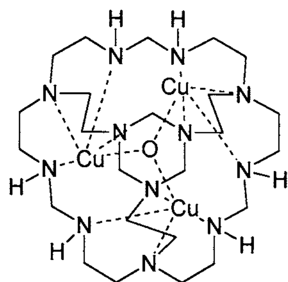
In carboxypeptidase A,[55,56] the active-site Zn(II) ion plays essential catalytic roles whereas guanidinium of Arg-145 recognizes carboxylate anion of the substrates, thus making the enzyme an exopeptidase. Important features of carboxypeptidase A reproduced by **9** include essential catalytic action of a metal ion, participation of a guanidinium group in substrate recognition, hydrolysis of unactivated amides, and substrate selectivity toward amide bonds adjacent to carboxylate groups.

Multinuclear metal centers are present in active sites of several metalloproteases. A trinuclear artificial metallopeptidase was prepared[27] by using **15**. Upon treatment of **15** with excess NaH, at least three of the six N–H protons were deprotonated. By mixing the anion of **15** with PCD, **15** was attached to PCD. In **16**, **15** is connected

to PCD by triple attachment. After the chloro groups of **16** were reacted with methoxide anion, the resulting resin was treated with acid and then with $CuCl_2$ to produce **17**. The artificial active site in **17** contains three convergent Cu(II) complexes of tris(2-aminoethyl)amine. On incubation with **17**, neutral amide **10** was not affected, whereas carboxyl-containing amides **11**−**13** were effectively hydrolyzed. Mechanism of **18** was proposed to account for the substrate selectivity manifested for the carboxyl-containing amides.



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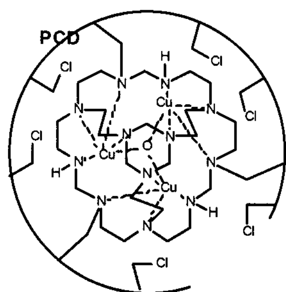

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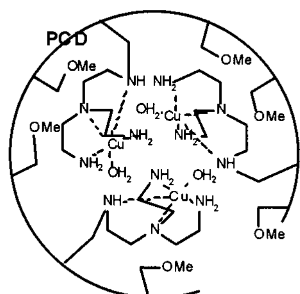

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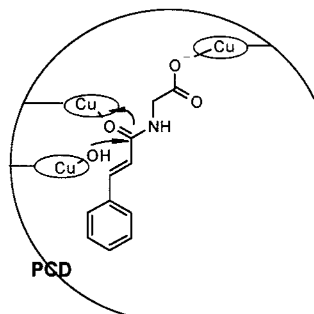

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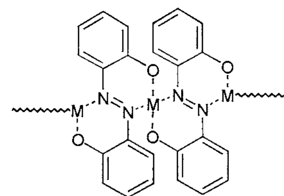
The k_{cat} value estimated for the amide hydrolysis by **9** or **17** was ≫0.1 h^{-1} at pH 8.5 and 50 °C or ≫0.2 h^{-1} at pH 8 and 50 °C, respectively. This may be compared with the k_{cat} of 0.18 h^{-1} (at the optimum pH of 9 and 25 °C) measured with a catalytic antibody[57] with peptidase activity elicited by a joint hybridoma and combinatorial antibody library approach in the hydrolysis of an amide substrate.

We have also synthesized several other multinuclear artificial metalloproteases using the metal centers of coordination polymers (**19**) formed by self-assembly of *o,o'*-dihydroxyazobenzene.[20,24] Effective hydrolysis of peptide bonds was attributed to cooperation among proximal metal centers.[24]



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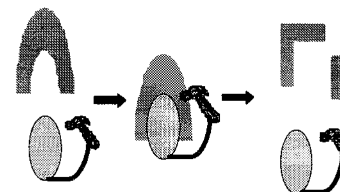
Artificial Peptidase Selective for Target Protein: Toward Design of Protein-Cleaving Catalytic Drugs

As mentioned above, we tried to increase the EM of the metal center to a sufficiently high level by forming a productive catalyst−substrate complex. When a protein is used as the substrate, such a complex may be obtained by using a catalyst that contains a recognition site specific for the substrate. If the catalyst cleaves a peptide bond after forming the complex, the catalyst can specifically cleave the target protein.

If the target protein is an enzyme, a receptor, or a toxin related to a disease, the catalyst may be used as a protein-cleaving catalytic drug. As illustrated by the cartoon of **20**, one molecule of the protein-cleaving catalytic drug can inactivate a multiple number of the target protein. On the other hand, the corresponding conventional drug can deactivate only up to an equivalent amount of the target protein. Thus, the protein-cleaving catalytic drug can reduce the drug dosage and the drug toxicity substantially.



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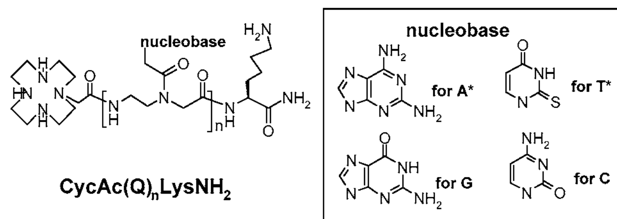
The first protein-cleaving catalyst selective for a target protein was synthesized by using myoglobin as the target.[36] From a combinatorial library of Cyc-containing peptide nucleic acid oligomers, the Cu(II) or Co(III) complex of **21** was selected as the catalyst. Here, the metal complex of Cyc acted as the catalytic site and the peptide nucleic acid oligomer as the binding site recognizing myoglobin. For cleavage of myoglobin by Co(III)**21** and

Cu(II)**21**, catalytic turnover was confirmed and the reaction rate was not affected by removal of oxygen. Higher catalytic activity was observed with Co(III)**21**, which manifested optimum k_{cat} (0.022 h^{-1} at 37 °C) at pH 7.5. MALDI-TOF mass spectrum of the reaction product obtained by incubating myoglobin with Co(III)**21** revealed that the target protein was cleaved into two protein fragments.



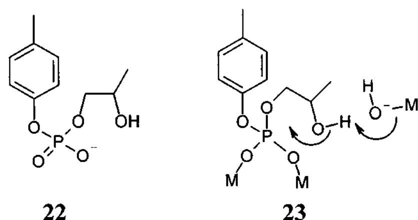
CycAc-A*-A*-T*-T*-C-G-A*-A*-C-LysNH$_2$ (**21**)

Many proteins are related to diseases and are targets of drug discovery. Numerous compounds have been discovered to have high affinity for those proteins. If such a compound is used as the binding site and connected to the catalytic group, the resulting catalyst may become an effective protein-cleaving catalytic drug specific for the target protein.

Polymeric Artificial Metallonucleases

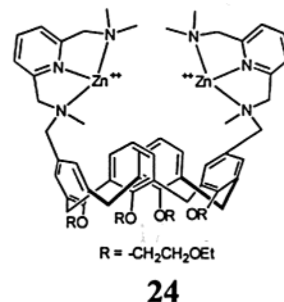
Catalysis of hydrolysis of phosphodiester bonds is related to design of artificial enzymes for RNA or DNA hydrolysis. Phosphodiester bonds of RNA and peptide bonds have comparable stabilities in terms of half-life for spontaneous hydrolysis.[40–42] On the other hand, the half-life for hydrolysis of the phosphodiester bond of DNA is longer than that of peptide bonds by more than million times.[40] Custom-made artificial restriction enzymes recognizing 17 or more[58] bases and hydrolyzing double stranded DNA can cleave the human genome at one predetermined site. To design such a genome restriction catalyst, it is necessary to secure catalytic centers that effectively hydrolyze phosphodiester bonds.

Our initial study in the area of phosphodiester bonds was performed with an activated analogue (2-hydroxypropyl-*p*-nitrophenyl phosphate, **22**) of RNA.[23] Transesterification of **22** is effectively catalyzed by two or more metal ions acting together.[59,60] The catalytic action has been explained in terms of activation of the two phosphoryl-oxygen bonds and general base assistance for the intramolecular attack of the hydroxyl group (**23**).[60]



22 **23**

As rationally designed multinuclear catalysts for transesterification of **22**, metal complexes such as **24** have been designed.[61] We synthesized[23] a macromolecular artificial metalloenzyme for the same reaction having much higher catalytic activity than **24**. The macromolecular catalyst was obtained by random attachment of lauryl groups and Ni(II) complexes of terpyridine to PEI (**25**). In water, the lauryl and the terpyridyl residues are expected to form hydrophobic clusters (**25**) on the backbone of PEI. An artificial active site comprising two or more metal ions can form in the cluster, whose structure depends on the contents of lauryl and terpyridyl residues. An effective artificial active site obtained by combinatorial attachment of the pendants catalyzed transesterification of **22** apparently by using adjacent metal centers (**26**).



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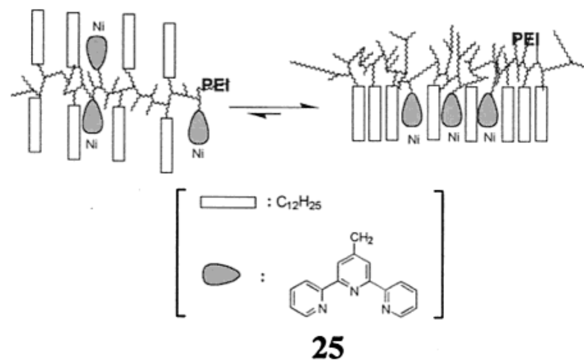

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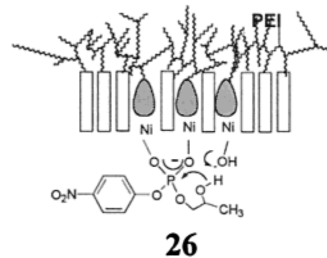

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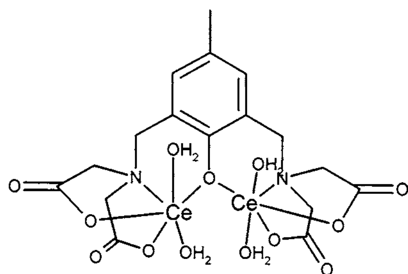
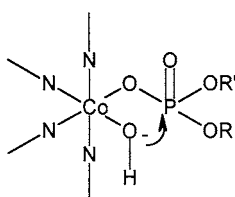
Although PEI derivative **25** demonstrated that an effective artificial active site can be obtained by self-assembly of catalytic elements, its substrate was an activated analogue of RNA. To obtain catalytic centers for DNA hydrolysis, much more effective catalytic groups are needed. Most of the known synthetic catalysts for DNA hydrolysis are metal complexes.[62–67] Although several metal complexes have been reported to promote cleavage of supercoiled and open circular DNA and single- or double-stranded oligodeoxyribonucleotides, hydrolytic cleavage of linear double-stranded polydeoxyribonucleotides by metal complexes has been seldom reported. Dicerium complex **27** was the only synthetic catalyst

reported[67] for hydrolysis of a long linear DNA duplex before publication[31,32] of our catalysts.



27

The Co(III) complex of Cyc (Co(III)Cyc) is one of the most effective synthetic catalysts discovered so far for hydrolysis of supercoiled DNAs.[65] The hydrolytic nature of the DNA cleavage by the Co(III) complexes of polyamines including Cyc has been well documented.[63,66] A mechanism (**28**) has been proposed[63] for the catalytic action of the Co(III) complexes. In view of the remarkable enhancement of the proteolytic activity of Cu(II)Cyc upon attachment to PCD,[22] we tested whether the activity of the Co(III)Cyc in phosphodiester hydrolysis is also enhanced greatly upon attachment to PCD derivatives.[31,32]



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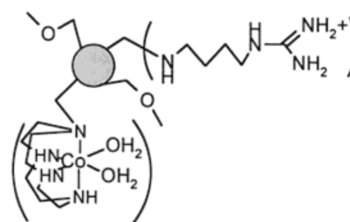
Plasmid pUC18 DNA (2,686 base pairs) was used as the supercoiled DNA substrate, whereas the linearized form of plasmid pUC18 DNA prepared by EcoR I digest was used as the linear DNA substrate. The disappearance of the supercoiled and the linear DNA substrates during incubation with several forms of PCD-supported Co(III)-Cyc such as **29** was monitored by agarose gel electrophoresis. Kinetic data were collected in the presence of excess catalyst at 4 °C for the supercoiled DNA and at 25 °C for the linear DNA. Faster rates were observed with the supercoiled DNA compared to the linear DNA for both Co(III)Cyc and PCD-supported Co(III)Cyc. Half-lives as short as 40 m at 4 °C and 30 m at 25 °C were observed for hydrolysis of the supercoiled DNA and the linear DNA, respectively, catalyzed by the PCD-supported Co(III)Cyc. Comparison of the rate constants measured for Co(III)-Cyc and for the PCD-based Co(III)Cyc at the same catalyst concentration revealed that the reactivity of Co(III)Cyc is enhanced upon attachment to PCD by more than 200 times toward the supercoiled[31] DNA and by at least 150 times toward the linear[32] DNA. Considering that only the Co(III)Cyc moieties exposed on the surface of PCD can attack the DNA, the degree of activation should be much greater than 150–200-fold. As indicated by **30**, only a minor portion of Co(III)Cyc moiety can act as the catalytic groups even among those exposed on the resin surface. Then, the degree of activation should be considerably greater than that estimated above.



29

30

With the dicerium complex (**27**), it took 24 h at 37 °C or 5 h at 55 °C to obtain hydrolysis products of a 192-base pair DNA.[67] On the other hand, the PCD-based Co-(III)Cyc derivatives degraded the linear DNA duplex into small fragments in a few hours at 25 °C.

The facile DNA hydrolysis by the PCD-based Co(III)-Cyc was shown to be due to the activation of Co(III)Cyc upon attachment to PCD rather than cooperation among two or more catalytic centers or facilitation of complex formation between DNA and the PCD-supported catalyst.[31,32]

Conclusions

Effective biomimetic catalysts with peptidase or nuclease activity have been prepared by using macromolecular catalytic systems. When macromolecular catalysts were employed, artificial active sites were built on highly branched synthetic polymers. Preassembly of the catalytic elements followed by cross-linkage with branched polymers created an artificial active site comprising those catalytic elements. Sometimes, artificial active sites comprising several catalytic elements were synthesized by random attachment of catalytic elements to the polymers or stepwise modification of the polymer surface. Effective artificial active sites were also prepared by self-assembly of catalytic elements. Synthetic polymers covered with various hydrophobic and polar groups often provided microenvironments needed for enhancement of the intrinsic reactivity of catalytic groups.

Macromolecular catalytic systems can be also obtained by using small catalysts and macromolecular substrates. For example, a small artificial protease may be designed for a target protein. The target protein is recognized by the binding site of the artificial protease. When the EM of the catalytic center in the resulting catalyst–substrate complex is sufficiently high, the artificial protease can effectively cleave the target.

Immobilized macromolecular artificial enzymes are suitable for large-scale industrial processes. In this regard,

we are trying to design immobilized artificial proteases both with broad substrate specificity and with amino acid specificities. Small artificial proteases specific for disease-related proteins can be used as drugs. We are attempting to convert known enzyme inhibitors or receptor antagonists to catalytic drugs by connecting them with the peptide-hydrolyzing catalytic centers.

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