Communication

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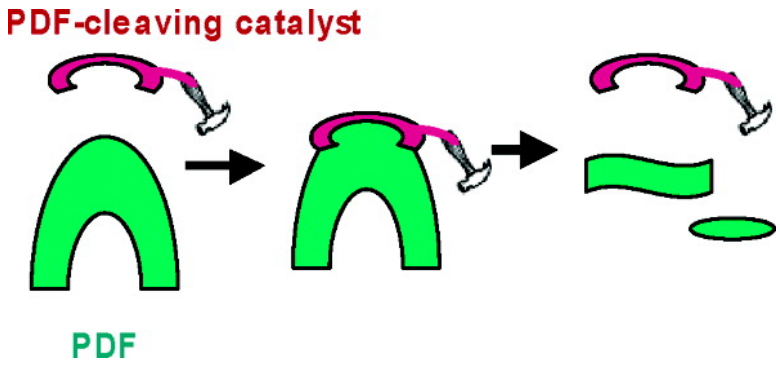

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Peptide-Cleaving Catalyst Selective for Peptide Deformylase

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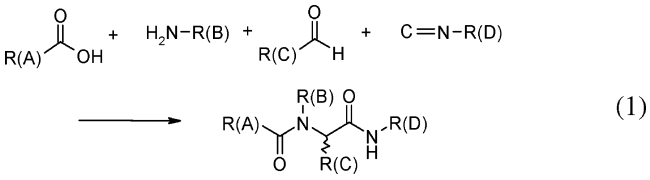
Previously, we reported[1] the first artificial protease selective for a target protein by designing synthetic homogeneous catalysts that recognized myoglobin (Mb) and cleaved the polypeptide backbone of Mb by hydrolysis using metal complexes[2−4] as the catalytic center. If the target protein for a peptide-cleaving catalyst is an enzyme related to cancers or is a viral or bacterial enzyme, destruction of the enzyme by cleavage of the polypeptide backbone may cure the disease. Since a catalytic amount may be sufficient for a peptide-cleaving catalytic drug, the dosage and the side effects of the drug may be reduced substantially. In addition, harmful proteins lacking active sites, such as prion related to mad-cow diseases or β-amyloid related to Alzheimer's disease, can be destroyed by the peptide-cleaving catalysts. Although the Mb-cleaving catalysts were the first target-selective artificial proteases, their molecular weights (ca. 3000) were too large to use the catalysts as drugs and to analyze the mechanism of the catalytic action. In addition, Mb is not related to a disease. To establish drug discovery exploiting peptide-cleaving catalysts, it is desirable to design artificial proteases possessing considerably smaller molecular weights as well as high selectivity for proteins directly related to diseases. Here, we report the first peptide-cleaving catalyst meeting those criteria designed by using peptide deformylase (PDF) as the target.

PDF is involved in deformylation of the formyl−methionyl derivative of proteins formed in the prokaryotic translational systems, and thus, its inhibitors are searched as candidates for new antibiotic drugs.[5] The active PDF has an Fe(II) ion in the active site, which reacts readily with oxygen. To obtain a stable variant, the Fe(II) ion is often substituted with Zn(II), although Zn(II)−PDF has reduced activity by 2−3 orders of magnitude. *Escherichia coli* Zn(II)−PDF provided by LG Life Sciences, Ltd. was used as the target enzyme in the present study.

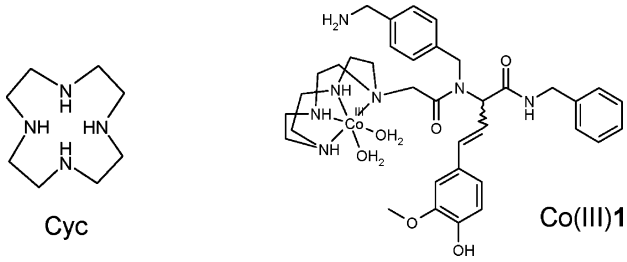
The PDF-cleaving catalyst was searched with a library of catalyst candidates synthesized by the Ugi reaction[6] (eq 1). The catalyst candidates are *N*-acylamino acid amides with various polar and nonpolar pendants as well as the Co(III) complex of cyclen (Cyc). The Co(III)Cyc moiety was chosen as the proteolytic center in view of the results of the previous study.[1c] Cyc with three secondary amines protected with *t*-boc groups was incorporated in either the carboxyl or the amine component of the Ugi reaction. Later, the *t*-boc groups were removed, and Co(III) ion was inserted to the Cyc portion (see Supporting Information).



(1)

By using various compounds listed in Supporting Information, about 7500 combinations of the four components were employed for the Ugi reaction. As each Ugi condensation reaction produces two enantiomers, the total number of catalyst candidates prepared in the present study was about 15 000. The number of compounds to be placed in one reaction vessel was adjusted to produce nine different combinations and, consequently, 18 catalyst candidates in each vessel. As checked randomly by MALDI-TOF MS before incorporating Co(III) ion into the Cyc moieties, formation of more than 80% of the condensation products was confirmed.

A mixture of 18 catalyst candidates generated in one vessel was screened together for the peptide-cleaving activity toward PDF. In a typical screening condition, the concentration of PDF was 5 μM, whereas that of each catalyst candidate was 1.5−3 μM assuming that the overall yield for the synthetic steps was 100%. After the solution was incubated overnight at pH 7.5 and 37 °C, whether new protein fragments was formed by the cleavage of PDF was examined by MALDI-TOF MS. When the formation of some protein fragments was indicated by a batch of the catalyst candidates, each of the Co(III)Cyc derivatives contained in the batch was individually generated and then incubated with PDF to identify the active compound. The only compound that manifested PDF-cleaving activity positively was the Co(III) complex of **1** (Co(III)-**1**), which was subsequently synthesized on a large scale as described in Supporting Information.



MALDI-TOF MS (Figure 1) of a reaction mixture obtained by incubation of PDF (peak a; m/z = 19 198) with Co(III)**1** disclosed that PDF was dissected to produce a new peak (peak b) with m/z value of 17 236. Peak b may be formed by cleavage of PDF at either Gln(152)−Arg(153) or Ala(17)−Lys(18), which produces a protein fragment with m/z of 17 236 or 17 243, respectively.

The cleavage site was identified as Gln(152)−Arg(153) by treating the cleavage product with carboxypeptidase A, which releases amino acid residues from the C-terminus in a sequential manner. When treated with carboxypeptidase A, peak b produced new MALDI-TOF MS peaks[3c] with m/z reduced by 128, 256, and 385. These are consistent with the theoretical values of 128, 256, and 384 for the C-terminal amino acid residues of Lys(150)−Gln(151)−Gln(152).[7]

The MALDI-TOF MS data indicated that the optimum pH was 7.5.[8] The relative amount of proteins, such as a and b of Figure 1, can be estimated fairly accurately by taking the MALDI-TOF MS several times.[9] On the basis of 10 different MALDI-TOF MS measurements, it appears that about one-half of PDF was cleaved

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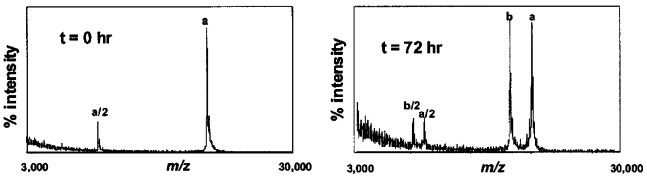

Figure 1. MALDI-TOF MS of PDF (5.2 μM; peaks a and a/2) before and 72 h after incubation with Co(III)**1** (1.0 μM; calculated by assuming that only one enantiomer of **1** is active) at pH 7.5 (0.05 M Hepes) and 37 °C.

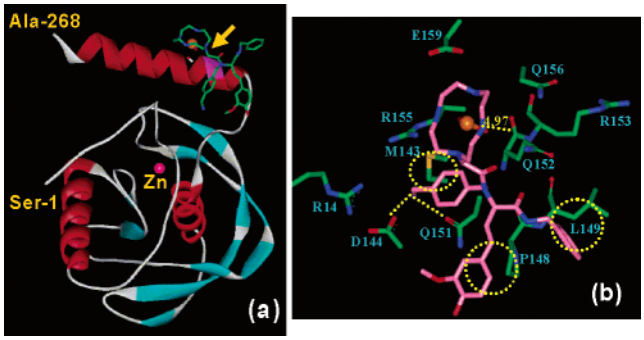

Figure 2. The lowest-energy conformation of the Co(III)**1**−PDF complex predicted by the docking simulations. Full view, (a) arrow indicates the cleavage site, and expanded view, (b) P148−E159 come from the helix and M143 and D144 from the loop; the catalyst is shown in pink.

on incubation with 0.19 equiv of the catalyst for 72 h (Figure 1), which corresponds to k_o of 0.01 h^{-1}. Here, the initial fraction of PDF complexed with the catalyst cannot exceed 20%. The k_o observed when PDF is fully bound to the catalyst is k_{cat}. The lower limit of k_{cat} is, therefore, estimated to be 0.05 h^{-1}. For the Mb-cleaving catalyst, k_{cat} was 0.02 h^{-1} under the same conditions.[1a,c]

To gain insights into the mechanism of the PDF cleavage by Co(III)**1**, docking experiments were performed for the complex formed between PDF and Co(III)**1**.[10] The docking simulations indicated that the *S* isomer of Co(III)**1** produces a complex with PDF that is more stable than that with the *R* isomer.[12] Figure 2a shows the lowest-energy conformation of (*S*)-Co(III)**1** in the PDF surface thus predicted. In the complex, the catalytic head of the Co(III)Cyc and the central acyclic chain of the catalyst interact with the C-terminal α-helix, while the three aromatic tails make contact with the helical and the loop structures residing above the active site. Thus, the catalyst would not recognize other proteins that do not have these helical and loop structures.[13] An expanded view (Figure 2b) disclosed several modes of interactions between the catalyst and the side chains of PDF; the hydroxo ligand of the Co(III) ion, the putative nucleophile attacking the peptide group of Gln(152)−Arg(153), is situated in proximity of Gln(152) and Gln(156). Each of the three phenyl rings of the catalyst forms independent van der Waals contact with the side chain of Met-(143), Pro(148), or Leu(149). Hydrogen bonds are formed between Cyc N−H of Co(III)**1** and the carboxylate group of Glu(159) and between the ammonium group of the catalyst and the side chains of both Asp(144) and Gln(151).[14]

The high selectivity observed between Co(III)**1** and PDF can be attributed to the multiple modes of interaction. The Co(III) center, an effective Lewis acid catalyst[2c] for peptide hydrolysis, can be located in a highly productive position in the PDF−catalyst complex. The high effective molarity of the Co(III) center thus achieved would lead to effective peptide cleavage.

The present study reports the first peptide-cleaving catalyst selective for a protein related to a disease. Moreover, mechanistic analysis of the protein cleavage was performed for the first time owing to the moderate size (MW of **1** = 644) of the catalyst. It is expected that new catalysts with therapeutic potential can be designed on the basis of peptide-cleaving catalysts, especially with regard to cancer-related, viral, and bacterial proteins as well as toxic proteins lacking active sites.

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Supporting Information Available: Structures of compounds used for construction of the library, experimental procedures for construction of the library, synthesis of Co(III)**1**, details of theoretical analysis, and a list of proteins tested with Co(III)**1**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(7) Expected values are 71, 227, and 298 if the cleavage site is Ala(17)−Lys(18).

(8) In the study on Mb-cleaving catalysts,[1a,c] kinetic data were collected by electrophoresis, which requires a much larger amount of the protein than the total amount (1.5 mg) of PDF used in this work. Kinetic analysis was, therefore, mainly performed with MALDI-TOF MS data in this study.

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(10) The geometry of Co(III)**1** was first optimized using the JAGUAR 4.1 program. The optimized structure was then docked onto the surface of PDF around the C-terminal α-helix including the cleavage site. The docking simulation was first carried out with the AutoDock 3.0.5 program. Here, the protein structure was fixed, but the program allowed torsional flexibility of Co(III)**1**. The coordinates of the protein atoms were taken from the X-ray structure reported in the literature.[11] To examine the dynamic stability of the PDF−Co(III)**1** complex, we carried out 1.0 ns molecular dynamic simulation in aqueous solution with the SANDER module of AMBER 7. Used in this calculation was an HP GS320 SMP machine in Supercomputing Center of Korea Institute of Science and Technology Information. The results showed that the time evolutions of root-mean-square deviation from the starting structure ($RMSD_{init}$) remained <2.0 Å for all C_α atoms of PDF and 0.7 Å for all heavy atoms of Co-(III)**1**. Furthermore, the $RMSD_{init}$ values of Co(III)**1** were maintained lower than those of PDF C_α atoms during the entire course of simulation, indicating that the movement of Co(III)**1** on the protein surface is highly restricted compared to the conformational change of the protein. See Supporting Information for details of theoretical analysis.

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(12) Attempts to isolate (*R*)-**1** or (*S*)-**1** by resolution of **1** or enantioselective stepwise synthesis were unsuccessful.

(13) When tested with 15 other proteins listed in the Supporting Information, Co(III)**1** did not cleave the proteins.

(14) Docking simulation of Co(III)**1** in the active site of PDF indicated that the active site was too narrow to accommodate Co(III)**1** properly, and thus, the binding in the active site causes bad van der Waals contacts between protein and catalyst atoms.

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