Supplementary Material (ESI) for Chemical Communications

Electronic Supplementary Information

Zn(II) Complex for Selective and Rapid Scission of Protein Back Bone

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Chemical Communications

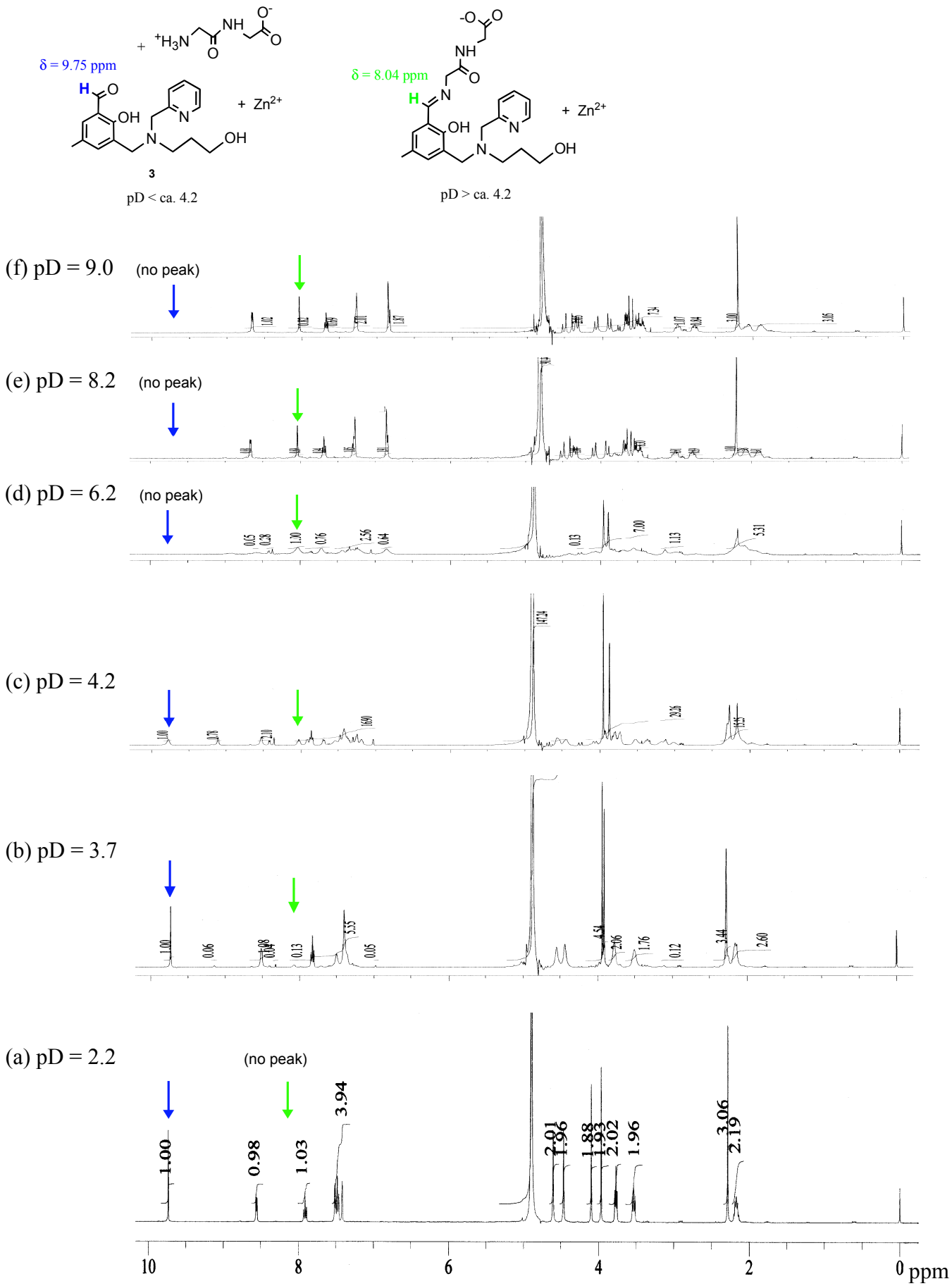

Fig. S4 ^{1}H NMR spectra (400 MHz, D_2O, r.t.) of a 1:1:1 mixture of GlyGly, **3** and $ZnSO_4$ (ca. 0.1 M). No hydrolysis of GlyGly to form Gly was observed during the measurements. The spectra show an equilibrium of the Schiff base formation between Zn^{II}-**3** and GlyGly depending on pD.

(a) Bovine Serum Albumin

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  1 DTHKSEIAHR FKDLGEEHFK GLVLIAFSQY LQQCPFDEHV KLVNELTEFA KTCVADESHA  60
 61 GCEKSLHTLF GDELCKVASL RETYGDMADC CEKQEPERNE CFLSHKDDSP DLPKLKPDPN 120
121 TLCDEFKADE KKFWGKYLYE IARRHPYFYA PELLYYANKY NGVFQECCQA EDKGACLLPK 180
181 IETMREKVLT SSARQRLRCA SIQKFGERAL KAWSVARLSQ KFPKAEFVEV TKLVTDLTKV 240
241 HKECCHGDLL ECADDRADLA KYICDNQDTI SSKLKECCDK PLLEKSHCIA EVEKDAIPEN 300
301 LPPLTADFAE DKDVCKNYQE AKDAFLGSFL YEYSRRHPEY AVSVLLRLAK EYEATLEECC 360
361 AKDDPHACYS TVFDKLKHLV DEPQNLIKQN CDQFEKLGEY GFQNALIVRY TRKVPQVSTP 420
421 TLVEVSRSLG KVGTRCCTKP ESERMPCTED YLSLILNRLC VLHEKTPVSE KVTKCCTESL 480
481 VNRRPCFSAL TPDETYVPKA FDEKLFTFHA DICTLPDTEK QIKKQTALVE LLKHKPKATE 540
541 EQLKTVMENF VAFVDKCCAA DDKEACFAVE GPKLVVSTQT ALA  583
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(b) Elastases

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1       10          20          30              40          50          60
.......... .....VVGGT EAQRNSWPSQ ISLQYRSGSSWAH TCGGTLIRQN WVMTAAHCVD
.......... .....IVGGR RARPHAWPFM VSLQLA....GGH FCGATLIAPN FVMSAAHCVA

            70          80          90          100         110         120
RE..LTFRVVVGE HNLNQNNGTE QYVGVQKIVV HPYWNTDDVAAG YDIALLRLAQ SVTLNSYVQL
NVNVRAVRVVLGA HNLSRREPTR QVFAVQRIFE DG.YD..PVNLL NDIVILQLNG SATINANVQV

        130         140         150         160           170         180
GVLPRAGTIL ANNSPCYITG WGLTRTNGQ LAQTLQQAYL PTVDYAICSSSS YWGSTVKNSM
AQLPAQGRRL GNGVQCLAMG WGLLGRNRG IASVLQELNV .TVVTSLC.... ......RRSN

          190         200         210         220           230         240
VCAGGD.GVRSG CQGDSGGPLH CLVNGQYAVH GVTSFVSRLGC NVT.RKPTVFTR VSAYISWINN
VCTLVRGRQAGV CFGDSGSPLV C..NG..LIH GIASFVR.GGC A.SGLYPDAFAP VAQFVNWIDS


VIASN       (240mer)
IIQ         (218mer)
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Fig. S5 (a) Amino acid sequence of bovine serum albumin.[11] (b) Amino acid sequences of porcine pancreatic elastase[12] (PDB ID: 1B0E, above) and human elastase[13] (PDB ID: 1B0F, below). The amino acid residue numbers of elastase are those employed by PDB. All lysine residues (K) are highlighted.

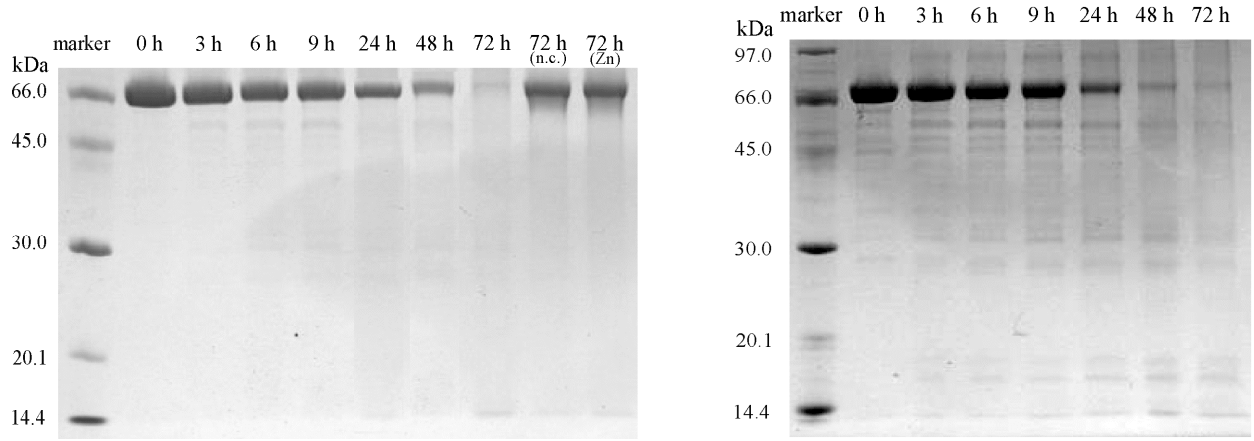


Fig. S6 SDS-PAGE analysis (12.5% gel) of the reaction mixture of BSA (9.0 μM) and Zn^{II}-**3** (0.91 mM) at 50 °C and pH 11.0 (CAPS, 50 mM) (two independent experiments conducted under identical conditions are shown). LEFT Lane 1: molecular weight marker; Lanes 2-8: with Zn^{II}-**3**; Lane 9: without Zn^{II}-**3**; Lane 10: with Zn^{II} but without **3**. RIGHT Lane 1: molecular weight marker; Lanes 2-8: with Zn^{II}-**3**.

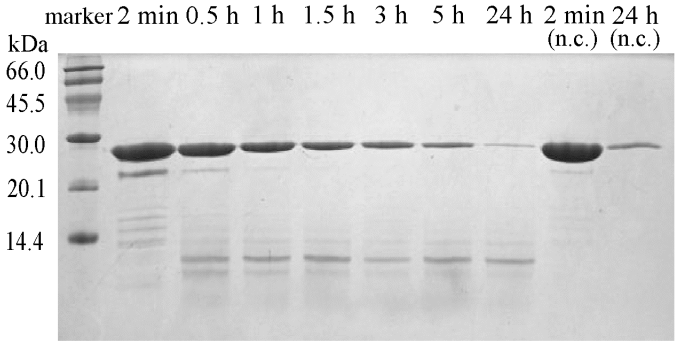


Fig. S7 SDS-PAGE analysis (20% gel) of the supernatant of the reaction mixture of porcine pancreatic elastase (62.4 μM) with Zn^{II}-**3** (3.0 mM) at pH 8.0 (HEPES, 50 mM) and 50 °C. Lane 1: molecular weight marker; Lanes 2-8: with Zn^{II}-**3**; Lanes 9, 10: without Zn^{II}-**3**.

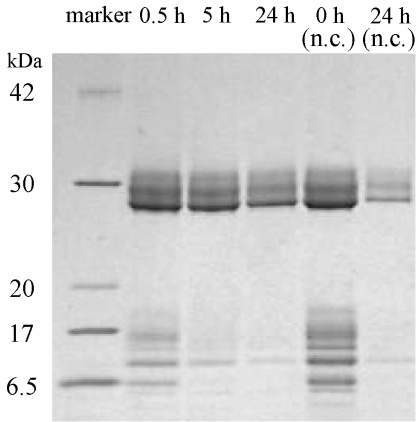
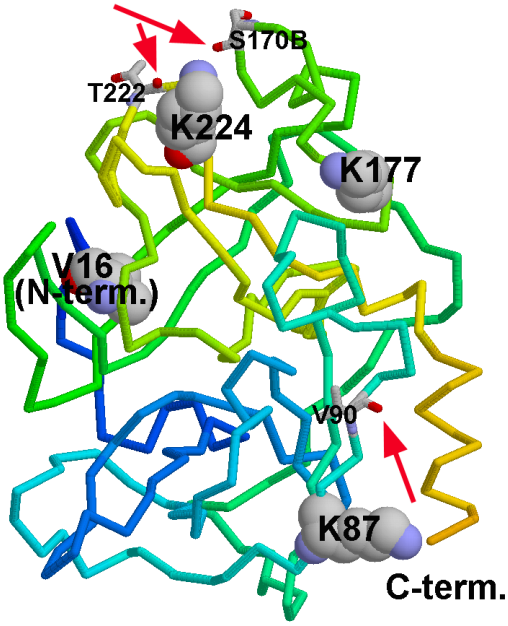
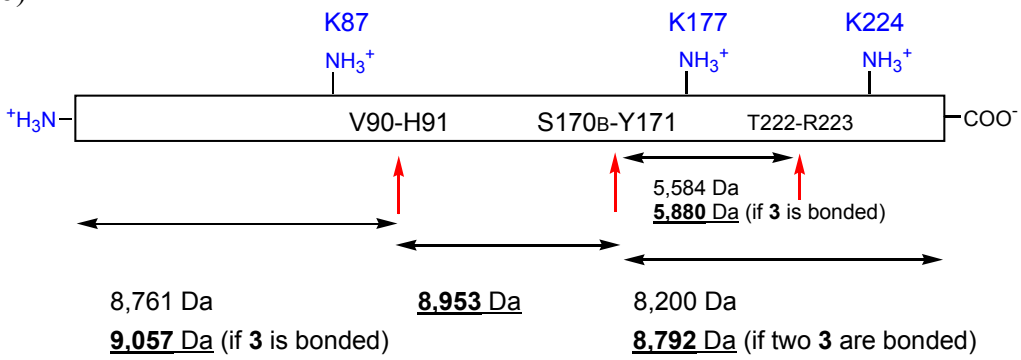


Fig. S8 SDS-PAGE analysis (10-20% gradient gel) of the reaction mixture of human elastase (62.4 μM) with Zn^{II}-**3** (3.0 mM) at pH 8.0 (HEPES, 50 mM) and 50 °C. This analysis shows all species involved in the reaction mixture, because precipitates were not formed during the reaction, in contrast to the case of porcine pancreatic elastase. Lane 1: molecular weight marker; Lanes 2-4: with Zn^{II}-**3**; Lanes 5, 6: without Zn^{II}-**3**.

(a)



(b)



possible target site by Zn^{II}-**3** linked to K87 : **V90**[a]
to K177 : A99, A166, S174
to K224 : **S170B**,[a] **T222**[a]
The N-term. (V16) forms a salt bridge with D194.
[a] Only those with bold letters are shown in the above scheme.

Fig. S9 (a) The steric structure of porcine pancreatic elastase (protein data bank (PDB) ID: 1B0E). The structure was drawn using RasMol. Whole structure is shown as a backbone representation, with color gradient from the blue *N*-terminus to the yellow *C*-terminus. The *N*-terminus valine, V16, and three lysine residues, K87, K177 and K224, are shown as a spacefill representation. The amino acid residue numbers are those employed by PDB.[12] Possible scission sites are indicated by arrows. (b) The possible scission site analysis of porcine pancreatic elastase by Zn^{II}**-3:** On the basis of the X-ray structure, all possible target sites of the protein backbone by Zn^{II}**-3** are listed (selected ones are shown in the box), assuming that the complex is linked to the protein by a Schiff base formation at NH_2 sites, i.e. N-terminus V16, and/or K87, K177 and/or K224. Among all expected fragments, those whose molecular weights are consistent with the MS observation are selected. They are indicated by red arrows in the structure. For fragments involving lysine residues, molecular weights were calculated both for simple polypeptide fragments and for Schiff bases involving **3**.

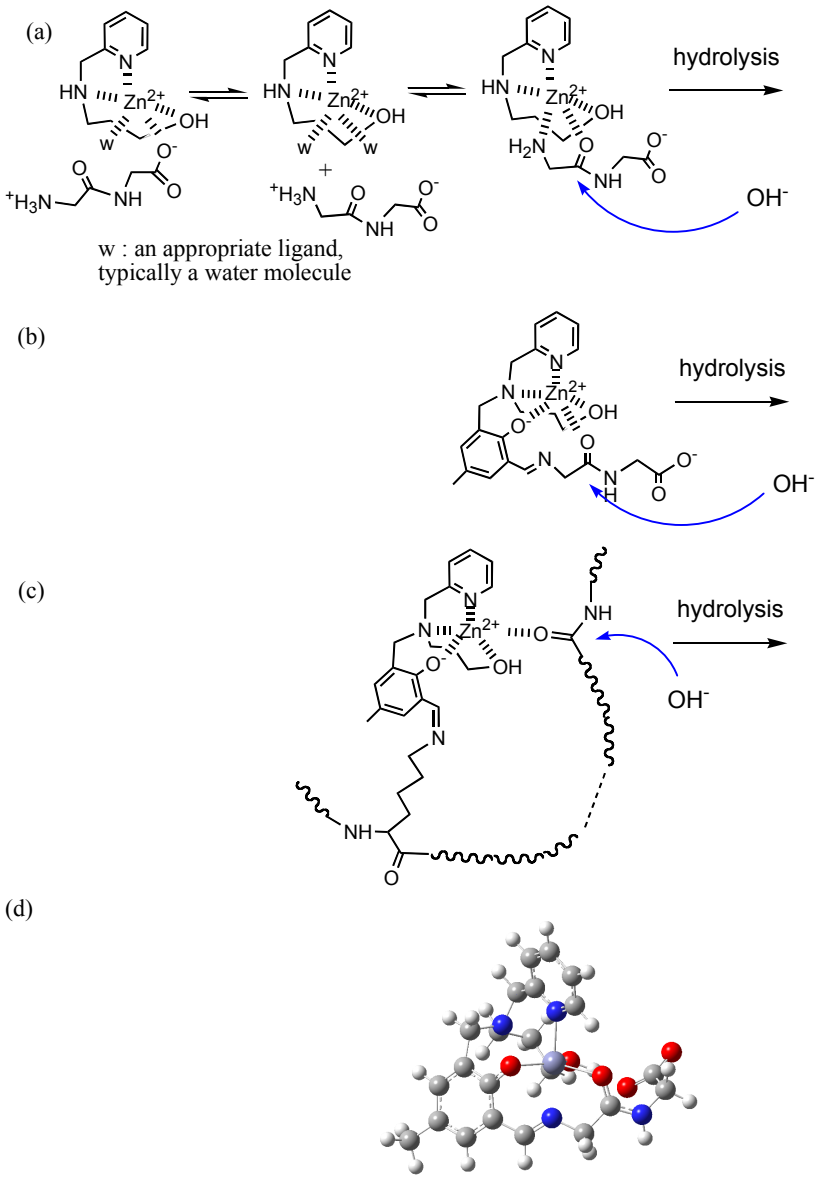


Fig. S10 A plausible reaction mechanism for the peptide hydrolysis promoted by a Zn^{II} complex. (a) The GlyGly hydrolysis by a simple Zn^{II} complex, Zn^{II}-**2**. Multiple way of coordination of Zn^{II} to GlyGly is possible, and the stability constant is low (two of possible coordination modes are shown). Hydrolysis is accelerated when the Zn^{II} --- O=C coordination is formed.[7,10] (b) The GlyGly hydrolysis by a Schiff base-forming Zn^{II} complex, Zn^{II}-**3**. The Zn^{II} --- O=C coordination is more preferentially formed compared with the case of Zn^{II}-**2**. As a result, Zn^{II}-**3** shows higher activity toward peptide hydrolysis than Zn^{II}-**2**. (c) A protein hydrolysis by Zn^{II}-**3**. Zn^{II}-**3** binds to an NH_2 group in the *N*-terminus or in a side chain of a lysine residue, and form a Zn^{II} --- O=C coordination with a sterically accessible carbonyl group. Hydrolysis is selectively promoted at the Zn^{II} --- O=C coordination site. (d) A theoretically optimized structure of the Schiff base formed from Zn^{II}-**3** and GlyGly. The softwares used were Gaussian 03W and GaussViewW 3.0, and the structure was optimized using the density functional theory, B3LYP, with the 6-31+G(d) basis set. In the reaction schemes, OH^- is depicted as a nucleophile. On the basis of previous studies, a possible role of the OH group in **3** as an intracomplex nucleophile is also suggested.[7,10]

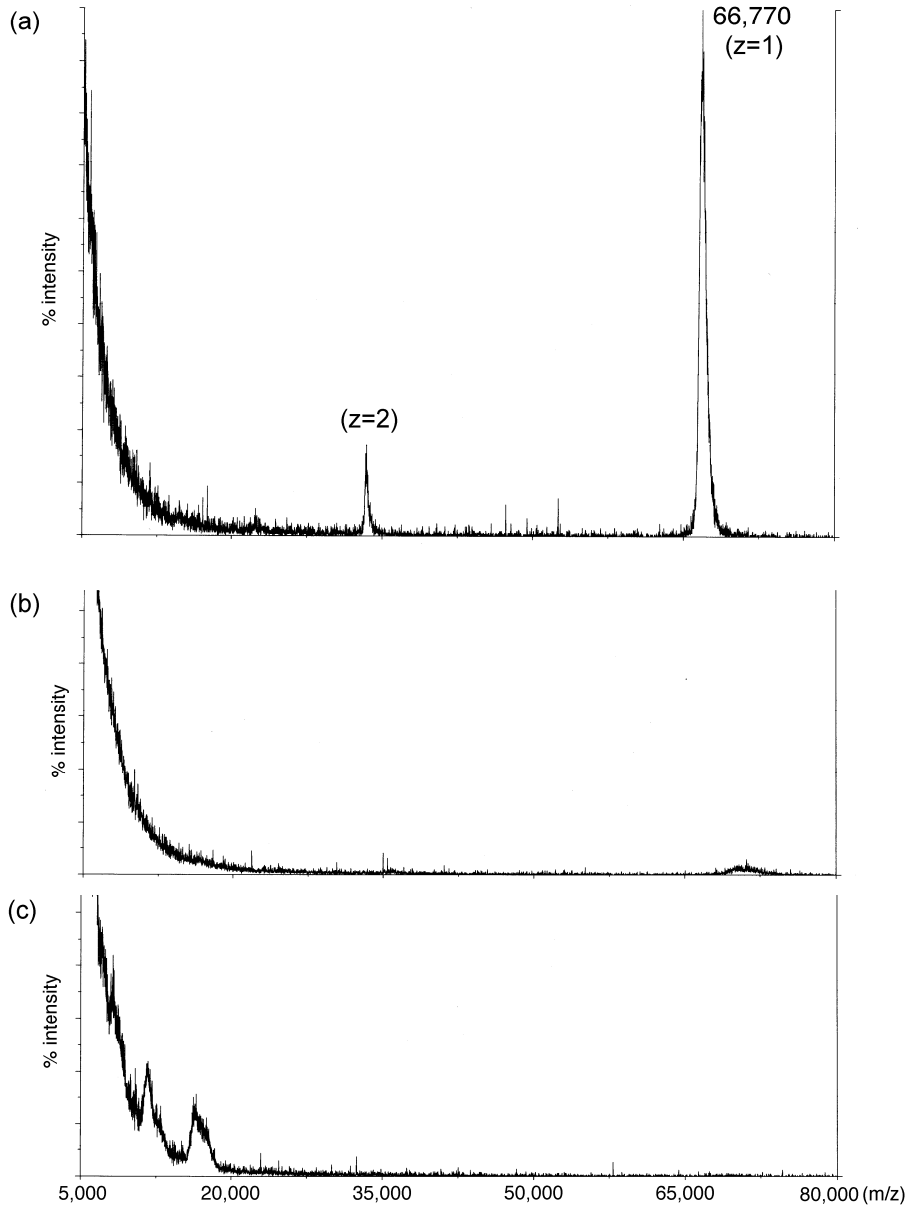


Fig. S11 MALDI-TOF/MS analysis. (a) BSA, (b) a mixture of BSA and Zn^{II}-**3** (1/100 mol/mol) at pH 11 (CAPS, 50 mM), (c) a mixture of BSA and Zn^{II}-**3** (1/100 mol/mol) treated at pH 11 (CAPS, 50 mM) and 50 °C for 72 h.

Details of Experimental Procedures

Preparations

2 was prepared by the reaction of 2-chloromethylpyridine hydrochloride (2 mmol) with 3-amino-1-propanol (40 mmol) in methanol. **3** was prepared by the reaction of 3-chloromethyl-5-methylsalicylaldehyde (0.40 mmol) with **2** (0.40 mmol) in the presence of triethylamine (1.6 mmol) in absolute methanol, and was isolated as a dihydrochloride salt (83 mg, 66%):

Anal. Found: C, 6.33; H, 53.08; N, 6.88. Calcd for $C_{18}H_{24}N_2O_3Cl_2 \bullet H_2O$: C, 6.47; H, 53.34; N, 6.91%. ^{1}H NMR δ_H(400 MHz, D_2O, pD 0.7, DSS) 9.76 (1 H, s, CHO), 8.79 (1 H, d, *J* 5.3, CH), 8.44 (1 H, t, *J* 8.2, CH), 8.04 (1 H, d, *J* 7.2, CH), 7.95 (1 H, t, *J* 6.7, CH), 7.57 (1 H, s, CH), 7.50 (1 H, s, CH), 4.85 (2 H, s, CH_2), 4.59 (2 H, s, CH_2), 3.81 (2 H, t, *J* 5.3, CH_2), 3.65 (2 H, t, *J* 7.2, CH_2), 2.33 (3 H, s, CH_3) and 2.24 (2 H, m, CH_2).

4 was prepared similarly by using 2-propylaminomethylpyridine instead of **2**, and was isolated as a dihydrochloride salt:

Anal. Found: C, 6.41; H, 56.80; N, 7.34. Calc. for $C_{18}H_{24}N_2O_2Cl_2 \bullet 1/2H_2O$: C, 6.63; H, 56.85; N, 7.37%.

Materials

GlyGly was purchased from Tokyo Chemical Industry Co., Ltd.

BSA was purchased from Sigma-Aldrich.

Elastases from porcine pancreas and from human sputum were purchased from Wako Pure Chemical Industries, Ltd., catalog no. 058-05361 and 531-24084, respectively.

SDS-PAGE analyses

To a 30 μl portion of the reaction mixture were added 29 μl of 0.12 M EDTA, 10 μl of 10% SDS, 1 μl of 2-mercaptoethanol, 20 μl of glycerin and 10 μl of 0.5 M Tris buffer (pH 6.8). The resulting mixture was treated at 80°C for 2 min, and its 10 μl portion was applied to the SDS-PAGE analysis. For the analysis of the precipitates formed during the reaction, the precipitates were separated from the supernatant, and added EDTA, SDS, 2-mercaptoethanol, glycerin and Tris buffer as described above, and then treated at 80°C for 2 min. A 10 μl portion of the resulting solution was applied to the analysis. Protein band intensities were determined by using a Kodac1D2.0 software.

MALDI-TOF/MS Measurements

MALDI-TOF/MS were observed by using Applied Biosystems VOYAGER Pro. Sinapic acid was used as a matrix. Observed mass accuracy was ca. 0.1 %. For the MALDI-TOF/MS analysis of the precipitates formed during the reaction, the precipitates separated from the supernatant by centrifugation were suspended in a HEPES buffer (pH 8.0), and were treated at 50 °C with excess urea. The resulting homogeneous solution was analyzed.