Purification and Some Properties of Protease I Having Transfer Action from *Streptomyces griseus* var. *alcalophilus*

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Streptomyces griseus var. *alcalophilus* was selected because it secreted a unique protease (protease I) that catalyzed the transfer reaction forming the hydroxamic acids of various amino acids. Protease I was purified to the electrophoretically homogeneous state and an activity of more than 125-fold that of the culture broth. The molecular weight of the enzyme was estimated to be 25,000 by gel filtration. The enzyme was most active in neutral pH for the transfer reaction forming phenylalanine hydroxamic acid, although for the hydrolytic reaction with casein as substrate it was most active in alkaline pH. The enzyme was inhibited by diisopropylfluorophosphate. Protease I catalyzed the transfer reaction synthesizing the hydroxamic acids of hydrophobic, acidic, basic, and small aliphatic amino acids such as Phe, Tyr, Leu, Asp, Glu, Arg, Lys, Ala, and Gly. These results indicate that protease I has broad donor specificity. It is also considered that protease I is a unique enzyme with transfer activity, and distinct from the alkalophilic proteinase reported previously [Yamamoto *et al.*, *Agric. Biol. Chem.*, 38, 37 (1974)].

Proteases such as trypsin, papain, and α -chymotrypsin catalyze peptide bond hydrolysis via an acyl-enzyme intermediate. Although the equilibrium of the reaction overwhelmingly favors hydrolysis under physiological conditions, the reaction can be reversed by choosing appropriate conditions so that peptide bonds can be synthesized with a protease as catalyst. Since Bergmann and colleagues reported enzymatic peptide synthesis,^{1,2)} many papers have reported the potential of proteases as catalysts for peptide synthesis.^{3–8)} However, proteases used for peptide synthesis are enzymes obtained mainly from animals and plants, and the synthetic reaction is strongly dependent on the substrate specificities of the proteases used. Therefore, to synthesize various peptides, it is useful to obtain proteases that strongly catalyze the transfer reaction with a broad donor (or acceptor) specificity.

We screened for an enzyme effectively catalyzing the transfer reaction of amino acids from various commercial enzymes. An enzyme (protease I) having a strong transfer activity

was found in the crude enzyme prepared from *Streptomyces griseus* var. *alcalophilus*. Protease I catalyzed the transfer reaction forming hydroxamic acids of various amino acids from mixtures of amino acid esters and hydroxylamine. This study deals with the purification and some properties of protease I having transfer action from *S. griseus* var. *alcalophilus*.

Materials and Methods

Materials. Synthetic peptides and esters of various amino acids (or peptides) were purchased from the Peptide Institute, Inc. Osaka, Sigma Chemical Company, and Bachem Feinchemikalien AG. Monoiodo acetic acid, *p*-chloromercuribenzoate, and dithiothreitol were purchased from Nacalai Tesque, Inc. All other inhibitors were obtained from Sigma Chemical Company. All other chemicals used were of reagent grade.

Screening of an enzyme having transfer action. Enzymes having transfer action were screened from crude preparations of various commercial enzymes. Their transfer activities forming phenylalanine hydroxamic acid and proteolytic activities were measured at pH 7.0 and pH 7.2, respectively.

Assay of transfer activity. The transfer activity (TA) was assayed by a modification of the method of McDonald *et al.*⁹⁾ The assay medium was assembled by combining 0.1 ml of 2 M NH_2OH (prepared fresh and adjusted to pH 7.0), 0.1 ml of 0.2 M phosphate buffer (pH 7.0), and 0.1 ml of enzyme solution. After the mixture was kept at 40°C for 2 min, the reaction was started by adding 0.1 ml of 0.4 M phenylalanine ethyl ester (Phe-OEt) in 0.1 M phosphate buffer, pH 7.0. After an appropriate time of incubation at 40°C, the reaction was stopped by the addition of 0.4 ml of 20% trichloroacetic acid (TCA), followed by 0.5 ml of 5% $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ in 0.1 M HCl. The volume was adjusted to 2 ml with water, and the absorbance at 500 nm was measured with a Shimadzu UV-210A spectrophotometer. The zero time value was obtained by adding TCA and $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ before the enzyme, after Phe-OEt and NH_2OH solutions were incubated without enzyme. One unit is defined as the amount of enzyme that catalyzes the formation of 1 μmol of phenylalanine hydroxamic acid (Phe-NHOH) per min under the conditions of the assay.

Assay of proteolytic activity. The proteolytic activity (PA) was assayed by the method of Yamamoto *et al.*^{10,11)} A half milliliter of enzyme solution was added to 3.0 ml of 0.6% casein in 10 mM borate buffer (pH 11.0). After 10 min of incubation at 30°C, the reaction was stopped by the addition of TCA mixture solution (3.2 ml) containing 0.11 M TCA, 0.22 M sodium acetate, and 0.33 M acetic acid. The absorbance of the filtrate was measured at 275 nm using a Shimadzu UV-210A spectrophotometer. One unit is defined as the amount of enzyme that released 1 μmol of tyrosine equivalent per min from the substrate.

Assay of the hydrolytic activity of the enzyme against various peptides and amino acid esters. The reaction system for identification is almost the same as one of Yamamoto *et al.*¹⁰⁾ The mixture of the enzyme and each substrate was incubated in 0.05 M borate buffer (pH 10.0). The hydrolytic products were identified by thin layer chromatography (TLC). The chromatography was done with TLC plates silica gel 60 (Kieselgel 60) purchased from Merck Japan Ltd., by the ascending method, using phenol and water (3:1) as the solvent system.

For measurements, 0.5 ml of the enzyme solution was added to 0.5 ml of peptide solution in 0.05 M borate buffer (pH 10.0) containing 10% dimethyl sulfoxide. After an appropriate time of incubation at 40°C, the amino groups liberated in the reaction mixture were measured by the method of Yemm and Cocking.¹²⁾

The hydrolytic activity of the enzyme against amino acid esters was assayed by the method of Roberts.¹³⁾ A half milliliter of enzyme solution was added 0.5 ml of various amino acid esters in 0.1 M phosphate buffer (pH 7). After an appropriate time of incubation at 40°C, 0.6 ml of 1 M alkaline hydroxylamine was added to 0.1 ml of the reaction mixture. After 20–30 min, 0.4 ml each of 4 N HCl, 18% TCA, and 10% $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ were added to the above

solution. The amounts of ester remained were measured by the colorimetric method based on the formation of a ferric complex from the hydroxamic acid resulting in the reaction of an ester with alkaline hydroxylamine.

The hydrolytic activity of the enzyme against amides of peptides was assayed by measurement of the amounts of ammonia liberated, by the method of Snell and Snell.¹⁴⁾

Measurement of protein. Protein was measured spectrophotometrically by the absorbance at 280 nm using a Shimadzu UV-210A spectrophotometer. In the purified enzyme solution, the molar concentration of the enzyme was measured by using an absorbance at 280 nm for 1% solution in a 1-cm cell ($E_{280}^{1\%}$) of 10.3 and a molecular weight of 25,000.

Polyacrylamide gel electrophoresis. Disc electrophoresis was done by the methods of Davis¹⁵⁾ and Nagai¹⁶⁾ in a column of 5% polyacrylamide gel with a pH 4.0 buffer system. Electrophoresis was done at a current of 3 mA per column.

Sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis was done by the method of Weber and Osborn.¹⁷⁾ Electrophoresis was done at a current of 5 mA per gel.

Isoelectric focusing. Isoelectric focusing was done by the method of Matsuo¹⁸⁾ in a 110-cm column using carrier ampholites, (pH 3–10, LKB Product AB, Sweden) for 48 hr at 300 V and 4°C.

Results and Discussion

Screening of enzymes having transfer action

In the screening, transfer activities (TA) and proteolytic activities (PA) of the enzymes were measured. As shown in Table I, we found an enzyme having the largest ratio of TA to PA in the preparation of crude enzyme prepared from *S. griseus* var. *alcalophilus*. This is the strain that was screened as a microorganism secreting an alkalophilic proteinase by T. Yamamoto *et al.*^{10,11)} Therefore, to purify this enzyme, *S. griseus* var. *alcalophilus* was cultivated.

Cultivation and purification

S. griseus var. *alcalophilus*, donated by Dr. T. Yamamoto was first cultivated with shaking in a medium (70 ml) containing 2% potato starch, 1% milk casein, 0.5% corn steep liquor, 0.3% K_2HPO_4 , 0.05% $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, and 2% Na_2CO_3 at 30°C for 3 days. The same

Table 1. SCREENING OF ENZYMES HAVING THE TRANSFER ACTION

Enzymes	Transfer activity (TA) ^a (U/ml)	Proteolytic activity (PA) ^b (U/ml)	Ratio of TA/PA ($\times 10$)
1. Protease (<i>Streptomyces griseus</i> var. <i>alcalophilus</i>)	2.1	21	1.0
2. Prozyme (<i>Streptomyces</i> No. 1033)	0.065	31	0.021
3. Actinase E (<i>Streptomyces griseus</i>)	0.058	65	0.009
4. Protin (<i>Bacillus subtilis</i>)	1.7	60	0.28
5. Protease (<i>Bacillus subtilis</i>)	0.50	30	0.17
6. Orientase (<i>Bacillus subtilis</i>)	3.7	240	0.15
7. Protease (<i>Bacillus subtilis</i>)	0.16	16	0.10
8. Thermoase (<i>Bacillus subtilis</i>)	0.034	200	0.0017
9. Peptidase (<i>Aspergillus niger</i>)	0.27	65	0.042
10. Protease A (<i>Aspergillus oryzae</i>)	0	70	0
11. Glutase (<i>Rhizopus</i> sp.)	0	9	0
(Control)			
12. Subtilisin BPN'	2.0	110	0.18
13. α -Chymotrypsin	5.1	14	3.6
14. Papain	0.062	52	0.012

^a The transfer activity (TA) was measured as described in Materials and Methods.

^b The proteolytic activity (PA) was measured at pH 7.2 and 30°C, using milk casein as substrate.

Enzyme manufacturer: 1, 5, Toyobo; 2, Kyowa Hakko; 3, Kaken Pharmaceuticals; 4, 8, Daiwa Chemicals; 6, 7, 11, Hankyu Kyoei Bussan; 9, Nagase Biochemical; 10, Amano Pharmaceuticals; 12, 13, 14, Sigma Chemical.

medium, freshly autoclaved, was inoculated with 1 ml of the first culture solution (absorbance at 660 nm, 9.3) and then, the culture was continued at 30°C for 5 days. After centrifugation, the supernatant was used as the starting material for purification.

Step 1. Ammonium sulfate fractionation. The pH of the supernatant was adjusted to pH 7 by careful addition of acetic acid. To this

solution, solid ammonium sulfate was added up to 0.78 saturation. The resulting precipitate was collected by filtration and dissolved in a small amount of 0.05 M acetate buffer (pH 6.0) containing 2 mM calcium acetate. The solution was dialyzed sufficiently against the same buffer.

Step 2. SP-Sephadex C-50 column chromatography (First). The dialyzed solution was put on a SP-Sephadex C-50 column (4.4 $\times$ 51 cm), which had been equilibrated with 0.05 M acetate buffer (pH 6.0) containing 2 mM calcium acetate. The column was washed sufficiently with the same buffer and the proteases were eluted by a linear gradient of ionic strength from 0 to 0.7 M NaCl (Fig. 1). The fractions (I) from number 133 to 165 were combined and dialyzed against 0.05 M acetate buffer (pH 6.0) containing 2 mM calcium acetate and 0.1 M NaCl. It was found that fraction I had a strong transfer activity for forming phenylalanine hydroxamic acid (Phe-NHOH), but showed a weak hydrolysis of milk casein. This enzyme was tentatively named "protease I."

On the other hand, it was also observed that about 50% of the enzymes that had transfer activity forming Phe-NHOH were not adsorbed on SP-Sephadex C-50 under these conditions. Details will be reported in the future.

Step 3. Sephadex G-75 gel filtration. The dialyzed solution was concentrated by dialysis against polyethylene glycol (# 20,000). The concentrated solution (10 ml) was put on a Sephadex G-75 column (3.2 $\times$ 144 cm) that had been equilibrated with 0.05 M acetate buffer (pH 6.0) containing 2 mM calcium acetate and 0.1 M NaCl. Elution was done with the same buffer, and the active fractions were combined.

Step 4. SP-Sephadex C-50 column chromatography (Second). To remove NaCl, the active fractions were sufficiently dialyzed against 0.05 M acetate buffer (pH 6.0) containing 2 mM calcium acetate. The dialyzed solution was put on a SP-Sephadex C-50 column (3.1 $\times$ 40 cm) that had been equilibrated with 0.05 M acetate buffer (pH 6.0) containing 2 mM calcium acetate. The column was washed sufficiently with

Fig. 1. Elution Pattern of Protease I from *S. griseus* var. *alcalophilus* from a SP-Sephadex C-50 Column (First).

Elution was done with a linear gradient of NaCl at a flow rate of 90 ml/hr. Fractions of 10 ml were collected. ●, transfer activity; ○, proteolytic activity; ----, absorbance at 280 nm; ---, concentration of sodium chloride.

Table II. PURIFICATION PROCEDURE OF PROTEASE I FROM *S. griseus* var. *alcalophilus*.

Purification steps	Protein A_{280}	Transfer activity (TA) (U)	Specific activity (TA/ A_{280})	Yield (%)	Proteolytic activity (PA) (U)	Ratio (TA/PA)
Supernatant fluid of broth	117,000	3,710	0.032	100	79,300	0.047
$(\text{NH}_4)_2\text{SO}_4$	19,000	3,750	0.197	101	58,900	0.064
SP-Sephadex C-50	665	1,490	2.24	40	16,800	0.089
Sephadex G-75	234	867	3.71	23	7,540	0.115
SP-Sephadex C-50	154	616	4.00	17	5,130	0.120

the same buffer and protease I was eluted by a linear gradient of ionic strength from 0 to 0.5 M NaCl. The active fractions were combined and used as the purified enzyme preparation. This preparation was purified about 125-fold from the culture broth and the specific activity of transfer action was 4.0 units/Abs. at 280 nm. This purified enzyme was used in the following experiments. The results of the purification procedures are summarized in Table II.

Homogeneity of the purified enzyme preparation and isoelectric point measurement

Homogeneity of the purified enzyme preparation was tested by disc electrophoresis on polyacrylamide gels. The enzyme preparation was found to be homogeneous electrophoretically. The isoelectric focusing test found that the isoelectric point of the enzyme was 8.4.

Molecular weight

The molecular weight of protease I was esti-

mated by gel filtration and SDS-polyacrylamide gel electrophoresis. A column of Sephadex G-75 (1.76×76 cm) eluted with 0.05 M acetate buffer (pH 6.0) containing 2 mM calcium acetate and 0.1 M NaCl was used. The molecular weight of the enzyme was estimated to be 25,000 by gel filtration, using bovine serum albumin, ovalbumin, chymotrypsinogen A, and ribonuclease A as standard proteins. This value is distinct from that of alkalophilic proteinase ($M_w = 50,000$).¹⁰⁾

On SDS polyacrylamide gel electrophoresis, the enzyme preparation showed a single band. The molecular weight of the enzyme was estimated to be 28,000 from the mobility on SDS polyacrylamide gel electrophoresis, using α_2 -macroglobulin (reduced type), phosphorylase b, glutamate dehydrogenase, lactate dehydrogenase, and soybean trypsin inhibitor as standard proteins.

Effects of pH and temperature on stability and activity

Protease I was stable from pH 5.0 to 10.0 at 40°C for 1 hr, as shown in Fig. 2. Using casein as substrate, the optimum pH for the proteolytic reaction was 11.0, which is distinct from that of alkalophilic proteinase (Opt. pH at

Fig. 2. Effects of pH on Activity and Stability of Protease I from *S. griseus* var. *alcalophilus*.

The transfer activity (●, TA) and proteolytic activity (△, PA) of the enzyme were assayed at the indicated pH. The stability (○) of the enzyme was measured by assaying the residual activities (TA and PA) after incubation at the indicated pH and 40°C for 1 hr.

12.3 or higher).¹⁰⁾ On the other hand, protease I was most active from pH 7.0 to 7.2 in the transfer reaction forming phenylalanine hydroxamic acid (Phe-NHOH).

The enzyme was stable at temperature below 50°C at pH 6.0 for 15 min. The optimum temperature for the transfer reaction of the enzyme was 55°C.

Effects of various chemicals

In the study of the effects of inhibitors and metal ions, 1 ml of 1.06×10^{-5} M enzyme solution was incubated with 1 ml of each inhibitor

Table III. EFFECTS OF CHEMICAL REAGENTS ON PROTEASE I FROM *S. griseus* var. *alcalophilus*

Reagent	Concentration (mM)	Relative activity (%)
None		100
DFP	1	5
	2	0
Chloroquine	1	94
MIA	1	94
PCMB	1	100
DTT	1	97
DAN ^a	4.4	94
EPNP ^b	(10 mg/ml)	96
EDTA	10	97
<i>o</i> -Phenanthroline	1	98
N-Ethylmaleimide	1	94
BaCl ₂	1	103
CaCl ₂	1	104
CdCl ₂	1	98
CoCl ₂	1	99
CuCl ₂	1	85
FeCl ₃	1	93
HgCl ₂	1	81
MgCl ₂	1	94
MnCl ₂	1	103
NiCl ₂	1	105
SrCl ₂	1	100
ZnCl ₂	1	94

^a The reaction was done at 14°C and pH 6.0 for 1 hr by the method of Rajagopalan *et al.*¹⁹⁾

^b The reaction was done with 1 ml of 5.3×10^{-6} M enzyme solution and 10 mg of solid EPNP powder at 25°C and pH 6.0 for 72 hr by the method of Tang.²⁰⁾

DFP, diisopropylfluorophosphate; MIA, monoiodoacetate; PCMB, *p*-chloromercuribenzoate; DTT, dithiothreitol; DAN, diazoacetyl-DL-norleucine methyl ester; EPNP, 1,2-epoxy-3-(*p*-nitrophenoxy) propane; EDTA, disodium ethylenediamine tetraacetate.

at 30°C for 15 min, and with each metal ion at 40°C for 30 min in 0.05 M acetate buffer (pH 6.0). Then enzyme activity was measured as described in Materials and Methods. As shown in Table III, protease I was markedly inhibited by DFP, which is a typical inhibitor of serine proteinases. However, the enzyme was not inhibited by reagents that attack sulfhydryl residues of proteins such as MIA, PCMB, and *N*-ethylmaleimide. And the chelators and the inhibitors of aspartic proteinase such as DAN and EPNP did not cause inhibition. From these results, it is considered that protease I is a serine proteinase.

Most of metal ions had no effect on this enzyme, although Hg^{2+} inhibited the enzyme activity by about 20%.

Hydrolysis of peptides and amino acid esters by protease I

To examine the substrate specificity in the hydrolytic reaction, the enzyme was incubated

with each substrate at the indicated concentration. The results indicate that protease I strongly hydrolyzed poly-Lys, Leu-Leu-Leu, and Phe-OEt, and weakly the peptides (or amino acid esters) containing acidic amino acids such as poly-Glu, Cbz-Glu-Tyr, Asp-di-OBzl, and Glu-di-OEt, as shown in Table IV and V. Besides, the enzyme hydrolyzed the peptides (or amino acid esters) containing small aliphatic amino acids such as Gly, Ala, and Ser. However, it was not active on peptides containing D-amino acids or γ -Glu or on various N- and C-unprotected dipeptides. From this study, it can be considered that protease I is an enzyme having broad substrate specificity, although the affinity for amino acids such as Phe, Leu, Lys, Glu, Ala, and Gly may be different.

Interestingly, protease I hydrolyzed Phe-OEt, Gly-Gly-Phe, Cbz-Ala-Leu-NH₂, Cbz-Pro-Leu-NH₂, and Cbz-Gly-Leu-NH₂, although the alkalophilic proteinase¹⁰⁾ was not active

Table IV. HYDROLYTIC ACTION OF PROTEASE I ON VARIOUS SYNTHETIC PEPTIDES

Relative activities of the enzyme against each substrate were measured by the ninhydrin method of Yemm and Cocking.¹²⁾ The rate of L-Leu-L-Leu-L-Leu was taken as 100.

Peptide	Concentration (mM)	Relative activity	Peptide	Concentration (mM)	Relative activity
Leu-Leu-Leu	10	100	Gly-Gly	25	0
Gly-Gly $\downarrow$ Phe	10	27	Gly-Leu	25	0
Gly-Phe $\downarrow$ Phe	10	38	Gly-Phe	25	0
Phe-Phe-Phe	10	45	Gly-Pro	25	0
Ala-Ala-Ala	10	22	Gly-Tyr	25	0
Gly-Gly-Gly	17	0	Leu-Gly	25	0
Ala-Gly-Gly	17	0	Leu-Phe	12	0
Leu-Gly-Gly	17	0	Leu-Trp	12	0
γ -Glu-Cys-Gly	10	0	Glu-Glu	12	0
Cbz-Glu $\downarrow$ Tyr	1	8	Lys-Lys	12	0
Cbz-Tyr $\downarrow$ Gly $\downarrow$ NH ₂	1	57	poly-Lys	(0.5%)	351
Cbz-Gly $\downarrow$ Tyr $\downarrow$ NH ₂	1	21	poly-Glu	(0.5%)	7
Cbz-Ala-Leu $\downarrow$ NH ₂	1	48			
Cbz-Pro-Leu $\downarrow$ NH ₂	1	32			
Cbz-Gly-Leu $\downarrow$ NH ₂	1	26			
Cbz-Gly-D-Leu-NH ₂	10	0			

The hydrolytic products were identified by thin layer chromatography. The arrows show the bonds split. Cbz, carbobenzyloxy.

on them. From the substrate specificity results, it was also proved that protease I was distinct from the alkalophilic proteinase reported previously.¹⁰⁾

Table V. HYDROLYTIC ACTION OF PROTEASE I ON VARIOUS AMINO ACID ESTERS

The final concentration of the substrate was 0.1 M, except for 0.04 M Asp-di-OBzl. The rate of L-Phe-OEt was taken as 100.

Ester	Relative activity
Phe-OEt	100
Leu-OEt	39
Val-OMe	6
Ser-OMe	12
Gly-OEt	4
Asp-di-OBzl*	15
Glu-di-OEt	10
Glu- γ -OMe	0
Arg-OMe	12
Lys-OEt	25

* Dimethyl sulfoxide (25%) was contained in the reaction mixture.
-OBzl, benzyloxy.

Formation of hydroxamic acids by protease I

To identify the donor specificity in the transfer reaction, the enzyme reaction was done with various esters as donors. One-tenth of a milliliter of 1.5×10^{-5} M enzyme solution was incubated with 0.3 ml of a mixture containing each ester and hydroxylamine in 75 mM phosphate buffer (pH 7.0). After incubation for 10 min at 40°C, the amounts of hydroxamic acids synthesized were measured by the methods described in Materials and Methods. Table VI shows the relative rates of formation of various hydroxamic acids by protease I. This enzyme catalyzed the transfer reaction forming hydroxamic acids of various amino acids such as Tyr, Phe, Leu, Arg, Lys, Asp, Glu, Ala, and Gly, although the rates for each transfer reaction were different. However, it was not active on Glu- γ -OMe and Ac-Pro-OMe. From the above results, it was found that protease I had a broad donor specificity in the transfer reaction synthesizing hydroxamic acid.

There are several proteases that catalyze transpeptidation reactions,²¹⁻²³⁾ for example,

Table VI. FORMATION OF VARIOUS HYDROXAMIC ACIDS BY THE TRANSFER ACTION OF PROTEASE I FROM *S. griseus* var. *alcalophilus*

The reaction was done at 40°C and pH 7.0, using various esters (donors) containing 0.5 M NH_2OH as substrates. The rate of L-Phe-OEt was taken as 100.

Donor	Concentration (M)	Relative activity	Donor	Concentration (M)	Relative activity
Phe-OEt	0.1	100	Ac-Phe-OEt*	0.04	112
Phe-OMe	0.1	170	Ac-Tyr-OEt*	0.1	267
Tyr-OEt	0.1	297	Ac-Val-OMe	0.1	23
Leu-OEt	0.1	127	Ac-Leu-OMe*	0.1	299
Val-OMe	0.1	10	Ac-Arg-OMe	0.1	37
Arg-OMe	0.1	6	Ac-Lys-OMe	0.1	90
Arg(NO ₂)-OBzl*	0.1	21	Ac-Asp-OMe	0.1	43
Lys-OEt	0.1	13	Ac-Glu- α -OMe*	0.05	165
Asp-di-OBzl*	0.04	76	Ac-Pro-OMe	0.1	0
Glu- α -OBzl*	0.025	66	Bz-Arg-OEt	0.1	13
Glu-di-OBzl*	0.04	37	Bz-Lys-OMe*	0.1	27
Glu-di-OEt	0.1	23	Bz-Ala-OMe*	0.1	29
Glu- γ -OMe	0.1	0	Bz-Gly-OEt*	0.1	14
Ser-OMe	0.1	20	Ac-Gly-Lys-OMe	0.1	43
Gly-OEt	0.1	7	Cbz-Val-Phe-OMe*	0.02	22
Asp-Phe-OMe*	0.033	11	Cbz-Phe-Leu-OMe*	0.02	7
			Cbz-Ser-Leu-OMe*	0.03	88

* Dimethyl sulfoxide (25%) was contained in the reaction mixture.

Ac, acetyl; Bz, benzoyl; Cbz, carbobenzyloxy; -OEt, ethoxy; -OMe, methoxy; -OBzl, benzyloxy.

α -chymotrypsin, trypsin, papain, and cathepsin C. However, it is actually difficult to synthesize various peptides by a single protease, because the proteases so far reported have narrow substrate specificities. This study shows that protease I from *S. griseus* var. *alcalophilus* is a unique enzyme having broad donor specificity in transfer reactions and forming various hydroxamic acids from esters and hydroxylamine, and that is distinct from the alkalophilic proteinase reported previously.¹⁰⁾ It also suggests the utility of this enzyme for the synthesis of various peptides. At present we are investigating the acceptor specificity of protease I.

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