Purification and Characterization of Crystalline Microbial Alkaline Proteinase Inhibitors (MAPI), Produced by *Streptomyces nigrescens* WT-27[†]

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The three microbial alkaline proteinase inhibitors (alpha, beta and gamma-MAPI) have been obtained as needle crystalline forms by a combination of various adsorption and ion-exchange column chromatographies.

The crystalline inhibitors showed the same inhibitory spectrum but different inhibitory potential. They strongly inhibited various microbial alkaline proteinases, such as fungal alkaline proteinases, and also inhibited α -chymotrypsin, papain, ficin, bromelain and cathepsin B.

The three MAPI gave positive Rydon-Smith, Sakaguchi and 2,4-dinitrophenylhydrazine reactions but were negative to ninhydrin reaction. From the amino acid analysis, three MAPI contained arginine, phenylalanine and some aliphatic amino acids; aliphatic amino acid of alpha-MAPI was valine only, that of beta-MAPI was probably valine (small amount of leucine and isoleucine was detected) and that of gamma-MAPI was leucine plus isoleucine (trace amount of valine was also detected).

As reported in the previous communications,^{1,2)} a novel proteinase inhibitor, having a potent inhibitory action against various microbial alkaline proteinases, was discovered in a culture filtrate of an actinomycete. From the taxonomic studies, the strain was identified as *Streptomyces nigrescens*. The cultural conditions for the accumulation of the inhibitor were also examined in detail.

The present paper deals with the purification procedure and general properties of the inhibitor from *S. nigrescens* WT-27, together with the inhibitory activity against various proteolytic enzymes.

MATERIALS AND METHODS

Microorganism and cultural conditions. *Streptomyces nigrescens* WT-27 was maintained on an agar medium containing 1% malt extract, 0.4% yeast extract, 0.4% glucose and 1.5% agar, pH 7.3.

The strain was cultured in the best conditions as previously reported.²⁾

Estimation of inhibitor activity. Purification of inhibitor was monitored by measuring inhibitory activity against subtilisin BPN[†], the assay system of inhibitor and inhibitor unit calculation were the same as described in the previous paper.²⁾

Enzymes and substrates. Subtilisin Carlsberg (crystallized and lyophilized), elastase (hog pancreas, 2 times crystallized), bromelain, proteinase of *Aspergillus oryzae* and acetone powder of bovine spleen were purchased from Sigma Chemical Co. Human thrombin and human plasmin for medical use were purchased from Green Cross Co., Osaka. Ficin was purchased from Wako Pure Chemical Co., Osaka. Crystalline alkaline proteinase and metallo proteinase of *Bacillus subtilis* var. *amylosacchariticus*, carboxyl proteinase of *Rhizopus chinensis* were a kind gift from Dr. D. Tsuru, Faculty of Pharmaceutical Science, Nagasaki University. Carboxypeptidase Y was a kind gift from Dr. K. Inouye, Faculty of Agriculture, Kyoto University. Alkaline proteinase of *Candida lipolytica*³⁾ and metallo proteinase of *B. subtilis* YT-25⁴⁾ were kindly supplied by Ajinomoto Co., Kawasaki. Alkaline proteinase of *B. subtilis* SO4,⁵⁾ alkaline proteinase (I) of *Penicillium lilacinum*,⁶⁾ metallo proteinase of *Pseudomonas aeruginosa* IFO 3455,⁷⁾ carboxyl proteinase of *Rhodotorula glutinis*,⁸⁾ carboxyl proteinase of *Cladosporium* sp.⁹⁾ and carboxyl proteinases (A & B) of *Scytalidium lignicolum*¹⁰⁾ were purified in this laboratory. Other various proteinases employed in the present investigation were

[†] A part of this investigation was presented at the Annual Meeting of the Agricultural Chemical Society of Japan at Nagoya, April, 1978.

the same as described previously.²⁾

The substrates, *p*-toluensulfonyl-L-arginine methyl ester (Tos-Arg-OMe), N α -benzoyl-L-arginine ethyl ester (Bz-Arg-OEt), N α -benzoyl-L-argininamide (Bz-Arg-NH₂), N α -benzoyl-L-arginine *p*-nitroanilide (Bz-Arg-NPhNO₂) and N α -benzoyl-L-tyrosine *p*-nitroanilide (Bz-Tyr-NPhNO₂) were purchased from the Foundation for Promotion of Protein Research, Institute for Protein Research, Osaka. Fibrinogen (bovine, plasminogen free) was a product of Pentox. Casein according to Hammarsten was purchased from Wako Pure Chemical Co., Osaka.

Assay system for inhibitory action of MAPI against various proteinases

1) *Microbial alkaline proteinases, microbial metallo proteinases, microbial carboxyl proteinases and elastase (caseinolytic activity).* Inhibitory action of MAPI against above proteinases was estimated by the same procedure of subtilisin BPN' assay system described previously,²⁾ excepting the buffer system and enzyme concentration, *i.e.*, in the optimum conditions of each enzyme.

2) *α -Chymotrypsin (amidase activity).* Mixture containing 0.25 ml of the enzyme solution (100 μ g/ml in 0.05 M phosphate buffer pH 7.8) and 0.25 ml of the same buffer with or without inhibitor was preincubated for 10 min at 37°C before addition of 2.5 ml of 0.0006 M Bz-Tyr-NPhNO₂ (in the same buffer containing 20% (v/v) dimethylformamide). After a further 15 min incubation at 37°C, the reaction was terminated by addition of 0.5 ml of 30% acetic acid and read the absorbance at 410 nm.

3) *Thrombin and plasmin (esterolytic activity).* Esterolytic activity of thrombin and plasmin toward Tos-Arg-OMe was determined by the same method of kallikrein, described previously.²⁾

4) *Plasmin (fibrinolytic activity).* Fibrin clot lysis activity of plasmin was measured by the method described previously.¹¹⁾

5) *Thrombin (fibrinogen clotting activity).* Human thrombin activity was measured by bovine fibrinogen clotting activity according to the method of Magnusson.¹²⁾

6) *Bromelain and ficin (caseinolytic activity).* Caseinolytic activity of bromelain and ficin was estimated by the same method of papain, as described previously.²⁾

7) *Cathepsin B.* Acetone powder (50 mg) of bovine spleen was extracted with 5 ml of 0.05 M phosphate buffer (pH 6.0) and insoluble material was removed by centrifugation (8000 rpm for 10 min), the resulting supernatant was used as cathepsin B assay. Mixture consisting of 0.2 ml enzyme solution, 0.1 ml of

activator solution (0.05 M cysteine-HCl and 0.02 M ethylenediamine tetraacetic acid, pH 6.0) and 0.2 ml of the same buffer with or without inhibitor was preincubated for 5 min at 37°C before addition of 0.25 ml of 0.001 M Bz-Arg-NPhNO₂ solution (in the same buffer containing 1% dimethyl sulfoxide) and incubated for 6 hr at 37°C. The reaction was terminated by addition of 1 ml of 30% acetic acid and read the absorbance at 410 nm.

8) *Carboxypeptidase Y.* Amidase activity of carboxypeptidase Y toward Bz-Tyr-NPhNO₂ was determined by the method described by Aibara *et al.*¹⁸⁾

Miscellaneous analytical methods. Ultraviolet absorption spectrum of inhibitor was measured with a recording spectrophotometer (Hitachi 200-10). High-speed liquid chromatograph employed in preparative and analytical works was Hitachi 635 liquid chromatograph. Amino acids were analyzed with an amino acid analyzer (Hitachi KLA-5).

Peptides in the eluates of column chromatography were measured by ninhydrin after alkaline hydrolysis method.¹⁴⁾

Resin. Amberlite XAD-2 (20~50 mesh), XAD-7 (20~50 mesh), CG-50 (200~400 mesh) and IR-45 were purchased from Organo Co., Tokyo. Dowex 1 $\times$ 2 (50~100 mesh) from Muromachi Kagaku, Osaka. Aluminium oxide and silica gel from Merck Japan.

RESULTS

Purification of MAPI

Step 1. Purification source. After cultivation, as described in MATERIALS AND METHODS, the cultured broth was centrifuged (8000 rpm for 15 min) to remove mycelium and the supernatant (7.45 liters) was used as purification source.

Step 2. Amberlite XAD-2 column chromatography. The active culture supernatant obtained in Step 1 was applied to an Amberlite XAD-2 column (5 $\times$ 20 cm) equilibrated with deionized water. After washing the column with about 5 liters of deionized water, the adsorbed inhibitor was eluted with 80% methanol solution at a flow rate of 100 ml/hr, the adsorbed inhibitor emerged from the column immediately after changing of solvent. The active fractions (1420 ml) were evaporated to syrup *in vacuo* at 40°C by a rotary evaporator.

Step 3. *n*-Butanol extraction. The resulting syrup (about 300 ml) was extracted with 500 ml of *n*-butanol, and then the *n*-butanol layer was washed with an equal volume of water twice and concentrated to syrup *in vacuo*.

Step 4. Amberlite XAD-7 column chromatography. The active material obtained in Step 3 was dissolved in 74 ml of 50% methanol, and the solution was applied to an Amberlite XAD-7 column (3.5 × 20 cm) equilibrated with 50% methanol. After the column was washed with 1.2 liters of 50% methanol and 1 liter of 80% methanol, inhibitor was eluted with 80% methanol containing 0.05 N HCl. Fractions having inhibitory activity were pooled. The bulked eluate (450 ml) was neutralized by passing an Amberlite IR-45 column (5 × 3.2 cm), and then evaporated dryness *in vacuo*.

Step 5. Dowex 1X2 column chromatography. The dry active material was dissolved in 100 ml of 80% methanol, and then applied to a column of Dowex 1X2 (acetate form) (5 × 6.4 cm) equilibrated with 80% methanol. Inhibitor was eluted with the same solvent, a large amount of colored impurities was removed by this step.

Step 6. Amberlite CG-50 column chromatography. The bulked eluate (300 ml) from Dowex 1X2 column was evaporated to dryness *in vacuo*, and then active material was dissolved in 120 ml of 0.1 M pyridine-acetic acid buffer (pH 4.8)–30% methanol solution. The active solution was applied to an Amberlite CG-50 column (3.5 × 16 cm) equilibrated with 0.1 M pyridine-acetic acid buffer (pH 4.8)–30% methanol and developed with the same buffered solvent. Inhibitor was eluted with 0.1 M acetic acid–80% methanol. The active fractions were pooled.

Step 7. Aluminium oxide column chromatography. The pooled eluate (620 ml) from Step 6 was evaporated to dryness *in vacuo*, the dry active material was suspended in 50 ml of *n*-butanol: H₂O (19: 1) and applied to an aluminium oxide column (5 × 9 cm) equilibrated with *n*-butanol: H₂O (19: 1). After

developing the column with the same solvent mixture and *n*-butanol: H₂O (9: 1), the inhibitor was eluted with a linear gradient from *n*-butanol: H₂O (9: 1) to *n*-butanol: methanol: H₂O (8: 1: 1). The active fractions were collected.

Step 8. Silica gel column chromatography. The pooled eluate (920 ml) from Step 7 was evaporated to dryness *in vacuo*. The dry active material was dissolved and/or suspended in 40 ml of ethylacetate: *n*-butanol: acetic acid (5: 5: 1) and applied to a silica gel column (2 × 38 cm), previously equilibrated with the same solvent mixture. The inhibitor was eluted with a linear gradient from the starting solvent mixture to *n*-butanol: H₂O (9: 1). The active fractions were collected.

Step 9. Preparative high-speed liquid chromatography. The pooled eluate (850 ml) from Step 8 was evaporated to dryness *in vacuo*. Drying the material in a vacuum desiccator gave an active white amorphous powder (292 mg).

This preparation had the same homogeneity as the sample described in the previous communication,¹¹ when examined by a thin-layer chromatography. However, detailed investigations on the conditions of analytical high-speed liquid chromatography revealed an apparent chromatographic heterogeneity of this preparation. Accordingly the present investigation was made to separate each component in this preparation by a preparative high-speed liquid chromatography.

Ten mg of the preparation obtained in Step 8 was dissolved in 10 ml of 50% methanol, insoluble material was removed by filtration and applied to a chromatograph. A typical elution profile is shown in Fig. 1. A good separation into three fractions, followed after first emerging of solvent or some impurities was achieved. In order of their retention time, each fraction was designated as alpha-MAPI (fractions having retention time from 48 min to 65 min), beta-MAPI (84 min ~ 112 min), gamma-MAPI (148 min ~ 200 min), respectively. In this chromatographic conditions, gamma-MAPI fractions were seemingly inactive

FIG. 1. Preparative High-speed Liquid Chromatogram of MAPI.

Ten mg of sample was dissolved in 10 ml of 50% methanol and applied to an apparatus. Chromatographic conditions are as follows: packing material, Hitachi gel #3019; column size, 8×500 mm dual; column temperature, 50°C; eluent, 50% methanol; flow rate, 3.0 ml per min; pressure, 10 kg per cm²; detector, UV monitoring at 210 nm. Eluate from the column was fractionated at each 2 min intervals. ○—○, inhibitory activity against subtilisin BPN'; -----, absorbance recording at 210 nm.

but showed a weak inhibitory activity in higher concentration.

Twenty five time's run in the same conditions were carried out and obtained three fractions. Evaporating the pooled eluate gave a white amorphous powder (alpha-MAPI, 39.4 mg;

beta-MAPI, 103.4 mg; gamma-MAPI, 24.1mg).

Step 10. Crystallization. The white powder of Step 9 was dissolved in a minimum volume of 80% methanol. Needle crystals of each MAPI appeared after standing the solution at room temperature for a few days. Crystals were collected by filtration on a glass filter, and washed with small volume of acetone. After drying in a vacuum desiccator for overnight, 27.8 mg of alpha-MAPI, 60.2 mg of beta-MAPI and 8.2 mg of gamma-MAPI were obtained.

A summary of the purification of MAPI from the culture medium of *Streptomyces nigrescens* WT-27 is shown in Table I.

Homogeneity of MAPI

The crystals of three MAPI gave a single spot on a thin-layer chromatography, while three MAPI represented same *R_f* value. On a thin-layer chromatography using Silica gel 60 (E. Merck); *R_f* 0.17 in *n*-butanol: H₂O (9:1), *R_f* 0.40 in *n*-butanol: methanol: H₂O (4:1:2), *R_f* 0.63 in *n*-propanol: methanol: H₂O (5:1:2).

On a paper electrophoresis using a buffer consisting of 80% formic acid: acetic acid: H₂O (50:15:800) pH 1.9 (10 v/cm for 2 hr

TABLE I. SUMMARY OF PURIFICATION PROCEDURE OF MAPI

Step	Vol. (ml)	Total peptide ^a	Total activity ^b	Specific activity ^c	Recovery (%)
Culture medium	7,450	912,000	52,500	0.058	100
Amberlite XAD-2	1,420	27,000	79,000	2.9	151
<i>n</i> -Butanol extraction	420	8,900	50,700	5.7	97
Amberlite XAD-7	450	1,520	43,900	28.9	84
Dowex 1X2	300	1,380	40,000	29.0	76
Amberlite CG-50	650	1,090	32,700	30.0	62
Aluminium oxide	920	937	31,500	33.6	60
Silica gel	850	561	19,700	35.1	38
Preparative HSLC ^d					
α-MAPI	1,260	231	10,100	43.7	19
β-MAPI	2,140	401	4,840	12.1	9
γ-MAPI	3,900	118	656	5.6	1
Crystallization					
α-MAPI	—	99	5,590	56.5	11
β-MAPI	—	166	2,050	12.3	4
γ-MAPI	—	25	176	7.0	0.3

^a Absorbance at 570 nm of ninhydrin after alkaline hydrolysis.

^b Inhibitor activity against subtilisin BPN'.

^c Total activity/total peptide.

^d High-speed liquid chromatography.

FIG. 2. Analytical High-speed Liquid Chromatogram of MAPI.

Five μ l of each MAPI solution (ca. 0.1% concentration in 80% methanol) was applied to an apparatus. The chromatographic conditions are as follows; packing material, LiChrosorb RP-18 (5 μ m); column size, 4 $\times$ 150 mm; column temperature, ambient; eluent, 0.02 M phosphate buffer pH 7.0 containing 60% methanol; flow rate, 0.5 ml per min; pressure, 100 kg per cm^2 ; detector, UV monitoring at 210 nm; absorbance unit full scale, 0.16.

A, alpha-MAPI; B, beta-MAPI; C, gamma-MAPI.

on a Toyo filter paper No. 51), three MAPI moved toward cathode with same R_m value of 0.74 (relative movement to glycine).

However, three MAPI had different retention times on an analytical high-speed liquid chromatography, as shown in Fig. 2.

General properties of MAPI

Ultraviolet absorption spectrum. The ultraviolet absorption spectrum of three MAPI in 80% methanol solution showed maximum at 268 nm, 264 nm, 258 nm, 252 nm and 247 nm with a shoulder at 260 nm, the spectrum could be superimposed to the absorption spectrum of phenylalanine, representing that three MAPI have phenylalanine residue in the molecule. A 1.0% solution of alpha-MAPI in 80% methanol solution had an absorbance of 5.1 at 268 nm, 6.75 at 264 nm, 8.55 at 258 nm,

7.4 at 252 nm and 6.05 at 247 nm. Similar results were observed in the case of beta-MAPI and gamma-MAPI.

Chemical analysis. Three MAPI gave positive Rydon-Smith, chlorine-tolidine, sodium nitroprusside-ferricyanide, phenanthrenequinone, Sakaguchi, α -naphthol-diacetyl, Tollens, 2,4-dinitrophenylhydrazine, 2,3,5-triphenyl-tetrazolium chloride reactions but negative Pauly, Ehrlich, Folin-Ciocalteu and ninhydrin reactions. These results suggested that three MAPI were a type of peptides, containing arginine and some reducing group.

TABLE II. AMINO ACID COMPOSITION OF MAPI

Amino acid	Molar ratio ^a		
	α -MAPI	β -MAPI	γ -MAPI
Phenylalanine	1.00	1.00	1.00
Valine	9.92	9.16	0.24
Leucine	0.00	0.37	2.25
Isoleucine	0.00	0.60	5.51
Arginine	0.89	0.92	1.04

^a Relative molar ratio compared to phenylalanine.

After acid hydrolysis of three MAPI in evacuated tube (6 N HCl, 110°C for 44 hr), amino acid constituents were analyzed with amino acid analyzer, the results are shown in Table II. Three MAPI contained phenylalanine, arginine and some aliphatic amino acids.

Inhibitory spectrum of MAPI

As reported in the previous papers,^{1,2)} mixtures of three MAPI had inhibitory actions against various microbial alkaline proteinases, α -chymotrypsin and papain. The present paper investigated the inhibitory effect of three MAPI against various proteinases including serine, metallo, thiol and carboxyl proteinases, the results are summarized in Table III. Three MAPI had the same inhibitory specificities, they inhibited microbial alkaline proteinases of various origins, α -chymotrypsin and thiol proteinases (papain, ficin, bromelain and cathepsin B) but not trypsin, plasmin, kallikrein, thrombin, elastase, metallo proteinases and carboxyl proteinases. However, three MAPI had different inhibitory potential. These

TABLE III. INHIBITORY SPECTRUM OF MAPI ON VARIOUS PROTEINASES

Inhibitory action of MAPI was estimated by measuring residual enzyme activity after incubation of each enzyme with inhibitor (50 μ g).

Inhibitory ratings used are as follows: +, more than 80% inhibition; —, less than 10% inhibition of enzyme activity.

Enzyme [E] μ g	Inhibition		
	α -MAPI	β -MAPI	γ -MAPI
Subtilisin BPN' (25 μ g)	+	+	+
Subtilisin Carlsberg (25 μ g)	+	+	+
Subtilisin amylosacchariticus (20 μ g)	+	+	+
<i>B. subtilis</i> SO4 alkaline proteinase (25 μ g)	+	+	+
<i>Streptomyces</i> sp. alkalophilic proteinase (19 μ g)	+	+	+
<i>Penicillium lilacinum</i> alkaline proteinase (25 μ g)	+	+	+
<i>Aspergillus oryzae</i> alkaline proteinase (25 μ g)	+	+	+
<i>A. melleus</i> semialkaline proteinase (25 μ g)	+	+	+
<i>Cephalosporium</i> sp. alkaline proteinase (25 μ g)	+	+	+
<i>Candida lipolytica</i> alkaline proteinase (25 μ g)	+	+	+
Carboxypeptidase Y (10 μ g)	—	—	—
Bovine trypsin (25 μ g)	—	—	—
Bovine α -chymotrypsin (25 μ g)	+	+	+
Human thrombin (12.5 NIH unit)	—	—	—
Human kallikrein (50 μ g)	—	—	—
Human plasmin (1.25 mg)	—	—	—
Hog elastase (45 μ g)	—	—	—
Papain (25 μ g)	+	+	+
Ficin (100 μ g)	+	+	+
Bromelain (100 μ g)	+	+	+
Cathepsin B	+	+	+
Thermolysin (8 μ g)	—	—	—
<i>B. subtilis</i> var. <i>amylosacchariticus</i> metallo proteinase (10 μ g)	—	—	—
<i>B. subtilis</i> var. <i>amylolyquefaciens</i> metallo proteinase (23 μ g)	—	—	—
<i>B. subtilis</i> YT-25 metallo proteinase (20 μ g)	—	—	—
<i>Pseudomonas aeruginosa</i> metallo proteinase (12.5 μ g)	—	—	—
<i>A. oryzae</i> metallo proteinase (20 μ g)	—	—	—
Porcine pepsin (100 μ g)	—	—	—
<i>Scytalidium lignicolum</i> carboxyl proteinase (50 μ g)	—	—	—
<i>Rhizopus chinensis</i> carboxyl proteinase (25 μ g)	—	—	—
<i>Rhodotorula glutinis</i> carboxyl proteinase (25 μ g)	—	—	—
<i>Cladosporium</i> sp. carboxyl proteinase (25 μ g)	—	—	—

TABLE IV. INHIBITION OF SERINE AND THIOL PROTEINASE BY MAPI

Enzyme	Substrate	[E] μ g	ID ₅₀ ^a		
			α -MAPI	β -MAPI	γ -MAPI
Subtilisin BPN'	Casein	25	1.5	3.8	18
<i>Streptomyces</i> sp. alkalophilic proteinase	Casein	25	0.058	0.061	0.085
<i>Cephalosporium</i> sp. alkaline proteinase	Casein	25	0.15	0.32	0.85
α -Chymotrypsin	Casein	25	0.32	0.82	6.2
Papain	Bz-Tyr-NPhNO ₂ ^b	25	0.31	0.80	4.5
	Casein	25	0.13	0.36	2.7
	Bz-Arg-NPhNO ₂ ^c	50	0.26	0.34	2.6
	Bz-Arg-NH ₂ ^d	50	0.25	0.23	0.41

^a Amount of inhibitor (μ g) necessary for 50% inhibition of activity.

^b N α -Benzoyl-L-tyrosine *p*-nitroanilide.

^c N α -Benzoyl-L-arginine *p*-nitroanilide.

^d N α -Benzoyl-L-argininamide.

enzymes were preincubated with different amount of MAPI and the changes in residual enzyme activities were measured using appropriate substrates. The results are shown in Table IV, indicating that alpha-MAPI had more potent inhibitory actions than the other two. In addition, it is noteworthy that inhibition against alkaline proteinases of actinomycetes, fungal or yeast origin was more potent than that of bacterial origin.

DISCUSSION

Peptidic inhibitors, MAPI, were finally purified by a preparative high-speed liquid chromatography into three components (alpha, beta and gamma-MAPI) as needle crystals from the culture filtrate of *Streptomyces nigrescens* WT-27.

Three MAPI had the same inhibitory spectrum but different inhibitory potential. MAPI strongly inhibited caseinolytic activity of various microbial alkaline proteinases, such as fungal alkaline proteinases. In addition, α -chymotrypsin and thiol proteinases (papain, ficin, bromelain and cathepsin B) were also inhibited by three MAPI.

The three MAPI gave positive Rydon-Smith, Sakaguchi and 2,4-dinitrophenylhydrazine reactions but were negative to ninhydrin reaction. From the amino acid analysis, three MAPI contained arginine, phenylalanine and some aliphatic amino acid; aliphatic amino acid of alpha-MAPI is valine only, that of beta-MAPI is probably valine (small amount of leucine and isoleucine was detected) and that of gamma-MAPI is leucine plus isoleucine (trace amount of valine was also detected).

Recently, several proteinases inhibitors of peptide aldehyde type, having the C-terminal aldehyde structure, have been discovered and reviewed by H. Umezawa *et al.*^{15,16)} As discussed in the previous communication,²⁾ MAPI had similar inhibitory specificities to chymotrypsin inhibitor "chymostatin," having inhibitory actions against α -chymotrypsin and papain.¹⁷⁾ As reported by H. Umezawa,¹⁷⁾ chymostatin weakly inhibits papain-hydrolysis

of casein but not papain-hydrolysis of Bz-Arg-NH₂. The three MAPI apparently differ from chymostatin, as they inhibited not only caseinolytic activity of papain but also amidase activity of papain, their inhibitory rate against papain was of similar order to that of anti-pain¹⁸⁾ or leupeptin.¹⁹⁾ It is noteworthy that basic amino acid residue of chymostatin is a diastereomer of capreomycinidine,²⁰⁾ but that of the three MAPI is arginine.

The detailed descriptions on the structural elucidation of MAPI will be presented elsewhere.

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