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Production and Some Properties of a New Type of Acid Carboxypeptidase of *Penicillium* Molds

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Among some 38 strains of the genus *Penicillium* we investigated seven wild-type strains (*P. daleae* IFO-6087, *P. frequentans* AHU-8328, *P. funiculosum* IAM-7013, *P. janthinellum* IFO-8070, IAM-7026, *P. lividum* IAM-7200, and *P. oxalicum* AHU-8336) that were found to be excellent strains for a new type of acid carboxypeptidase production in a surface koji culture at 25 C. The production of acid carboxypeptidase was determined in various culture conditions in a koji culture. The maximum yields of acid carboxypeptidase were obtained by *P. janthinellum* IFO-8070. Partial purification and isolation of the acid carboxypeptidase from strains of *Penicillium* were performed with gel filtration on Bio-Gel P-100. Characterization studies indicate that the acid carboxypeptidases from *P. daleae* IFO-6087, *P. funiculosum* IAM-7013, *P. janthinellum* IFO-8070, and *P. oxalicum* AHU-8336 have some properties similar to those of the enzyme of *Aspergillus saitoi* with regard to the hydrolysis of several peptides at acidic pH range but have other slightly different properties with regard to stability, pH optima, inhibitors, and molecular weights.

In recent years a new type of acid carboxypeptidase of *Aspergillus* has been found that catalyzes the hydrolytic liberation of amino acid from the carboxyterminal of the substrates at acidic pH range (4, 5). Previous studies (4) have demonstrated that the enzyme liberates basic, neutral, and acidic amino acids, including proline, from the carboxyterminal of the substrates. Specificity studies (4) have demonstrated that the enzyme exhibits a preference for aromatic and dicarboxylic amino acids in the position "X" when the X-Y bonds of small synthetic substrates of the type R-X-Y are hydrolyzed (R = peptide residue, benzyloxycarbonyl-, benzoyl-, or acetyl-). Arai et al. found that significant amounts of free leucine and phenylalanine were liberated by the action of *Aspergillus* acid carboxypeptidase from the nonapeptide (Ala, Arg, Asp, Gly, Val)-Gln-Tyr-Phe-Leu, which was isolated from the peptic hydrolysate of soybean as the compound having a bitter taste (1). The effect of culture conditions on the production of a new type of acid carboxypeptidase from molds of the *Aspergillus niger* group has been observed (7).

This paper describes the effects of culture conditions on the production of a new type of acid carboxypeptidase from *Penicillium* molds. Moreover, this paper describes the comparative studies of enzymatic properties of acid carboxypeptidases from *P. daleae* IFO-6087, *P. funiculosum* IAM-7013, and *P. janthinellum* IFO-8070.

MATERIALS AND METHODS

Materials. Benzyloxycarbonyl-L-glutamyl-L-tyrosine (Z-Glu-Tyr), benzyloxycarbonyl-glycyl-L-prolyl-L-leucyl-glycine (Z-Gly-Pro-Leu-Gly), and benzyloxycarbonyl-glycyl-L-prolyl-L-leucyl-glycyl-L-proline (Z-Gly-Pro-Leu-Gly-Pro) were purchased from the Protein Research Foundation, Osaka. Hammarsten milk casein was obtained from E. Merck. Bio-Gel P-100, Sephadex G-200, and Amberlite CG-50 were purchased from Bio-Rad Laboratories (Richmond, Calif.), Pharmacia, (Uppsala, Sweden), and Rohm & Haas (Philadelphia, Pa.), respectively. We obtained diisopropylfluorophosphate (DFP) and L-1-tosylamide-2-phenylethylchloromethyl ketone (TPCK) from Sigma Chemical Co., St. Louis, Mo., and Seikagakukogyo Co., Tokyo, respectively. *p*-Chloromecuribenzoate (PCMB) and ethylenediaminetetraacetate (EDTA) were from Tokyokasei Co., Tokyo.

Organisms. All cultures used in this investigation were subcultured from the culture collections. These strains were obtained as strains with acid proteinase production potency. *Penicillium daleae* IFO-6087, *P. frequentans* AHU-8328, *P. funiculosum* IAM-7013,

P. janthinellum IFO-8070, *P. janthinellum* IAM-7026, *P. lividum* IAM-7200, and *P. oxalicum* AHU-8336 were used for preliminary comparative studies. Stock cultures were maintained at 10 C on normal solid agar medium containing rice koji extract.

Koji culture. A wheat bran medium was prepared by mixing 3 g of wheat bran and 2.1 ml of tap water in a 100-ml Erlenmeyer flask and autoclaving at 15 lb/in^2 for 30 min. The sterilized medium was inoculated with one platinum loop of mold spores and then incubated at 25 C for at least 100 h. All strains tested grow satisfactorily on this medium. The flask was shaken twice daily to redistribute the medium and produce better temperature control.

Preparation of crude enzyme from koji culture filtrate. The major part of acid carboxypeptidase produced in koji culture was extracted with 10 volumes of 0.05 M acetate buffer (pH 3.0) at 30 C. The mixture was adjusted to pH 4.0 with 1 N HCl and was allowed to stand in the cold room for at least 2 h. After extraction, the culture filtrates were pooled at 5 C and the pH was adjusted to 4.0. The acid carboxypeptidase was then salted out by addition of 60.8 g of solid ammonium sulfate per 100 ml of solution at 5 C. The precipitate was removed by filtration and stored in the cold (5 C). The acid carboxypeptidase could be completely precipitated from the culture liquor by cold acetone to obtain a final acetone concentration of 60 to 65%. The precipitate was separated by centrifugation and dried in vacuo or lyophilized.

Partial purification of acid carboxypeptidase. Crude enzyme preparation obtained from ammonium sulfate or acetone precipitation was dissolved in a minimum amount of 0.01 M acetate buffer (pH 4.0) and filtered through Toyo no. 2 filter paper. Enzyme filtrate was applied to the bed surface of a Bio-Gel P-100 column (2 by 70 cm). The column was equilibrated for 24 h with the eluant (0.01 M acetate buffer, pH 4.0) before the sample was applied. The column was mounted on a fraction collector at 5 C, and 3 ml of eluate was collected per tube at a flow rate of 10 ml per h.

Enzyme filtrate was also applied to the Amberlite CG-50 ion-exchange chromatography. The column (2 by 50 cm) was equilibrated with 0.02 M acetate buffer (pH 3.5). A 30-ml portion of the enzyme solution was applied to the column. The column was eluted gradient to 0.15 M acetate buffer (pH 5.2).

Acid carboxypeptidase assay. The enzyme was assayed routinely with Z-Glu-Tyr in 0.05 M sodium acetate buffer (pH 3.7). One unit of Z-Glu-Tyr hydrolase activity was defined as the amount of enzyme required to liberate 1 μmol of tyrosine per min at pH 3.7 and 30 C. A 0.5-ml portion of 10^{-3} M substrate solution was added to 0.5 ml of the enzyme solution in a test tube (15 to 18 ml) at 30 C. After 20 min the reaction was stopped by acetic acid, and 2 ml of 0.5 M sodium citrate buffer (pH 5.0) was added to the reaction mixture. A 1-ml portion of the freshly prepared ninhydrin reagent (10) was added, and the mixture was heated at 100 C for 15 min and immediately cooled in an ice bath (0 C) for 3 to 10 min. After addition of 0.6 ml of 60% ethanol, the absorbance was measured at 570 nm in a Hitachi model 101 spectrophotometer, and the amount of tyrosine liberated was determined from the standard amino acid solution. The tyrosine standard (10^{-4} M) was prepared by dissolving 18.12 mg of pure, dry tyrosine in 1 liter of water containing 2 ml of 1 M HCl. Appropriate blanks were substituted, and the results were expressed as micromoles of tyrosine liberated per minute.

Acid proteinase assay. Endopeptidase assay was determined according to the previous method (3). One unit of acid proteinase activity was defined as the amount of enzyme that yields the color equivalent to 1 μmol of tyrosine per min at pH 3.0 and 30 C.

Protein determination. Protein concentration was estimated from the absorbance at 280 nm (A_{280}) using a Hitachi model 101 spectrophotometer.

Molecular-weight determination. The following preparations were used at a concentration of either 5 or 20 mg/ml: from the Tokyo Kasei Chemicals Co., Tokyo, bovine γ-globulin, fraction II (molecular weight 150,000); from the Nutritional Biochemicals Corp., Cleveland, Ohio, bovine hemoglobin (molecular weight 67,000); from the Sankyo Co., Tokyo, Taka-amylase A (molecular weight 51,200); from the Wako Pure Chemicals Co., Tokyo, egg albumin (molecular weight 45,000).

For gel filtration on a column (2 by 70 cm), the eluting buffer was 0.05 M sodium acetate buffer, pH 3.0, at 5 C.

RESULTS

Production of acid carboxypeptidase on koji culture. Almost all of the strains of the genus *Penicillium* screened contained measurable quantities of acid carboxypeptidase at 25 C. Table 1 shows distribution of potent strains of *Penicillium* molds for acid carboxypeptidase. Of 38 strains (30 species), seven strains (*P. daleae* IFO-6087, *P. frequentans* AHU-8328, *P. funiculosum* IAM-7013, *P. janthinellum* IFO-8070, *P. janthinellum* IAM-7026, *P. lividum* IAM-7200, and *P. oxalicum* AHU-8336) were found capable of producing a large amount of acid carboxypeptidase under the test system described (Table 1). *P. janthinellum* IFO-8070 gave the highest enzyme potency of any of the *Penicillium* organisms tested in the survey using the koji method. The specific activity of the enzyme in the culture filtrate was determined as 50 mU for A_{280}.

Effect of condition of enzyme production by P. janthinellum. Production of acid carboxypeptidase by *P. janthinellum* IFO-8070 was determined at various water contents of the medium (Fig. 1). The maximum enzyme production was reached after 4 days in a medium containing 3 g of wheat bran and 3 ml of tap water at 25 C; however, rapid inactivation of the enzyme was observed in the medium. A medium composed of 3 g of wheat bran and 2.1 ml of tap water in a koji culture supported substantial quantities of acid carboxypeptidase

TABLE 1. *List of strains of Penicillium molds for acid carboxypeptidase production by the koji method, inoculated at 25 C*[a]

Strain	Cultivation (h)	Activity (U × 10^3/ml of filtrate)
Penicillium cammenberti IFO-5855	189	123
P. chrysogenum OUT-2028	189	trace
P. citrinum OUT-2029	189	103
P. cyclopium IAM-7146	100	trace
P. daleae IFO-6087	189	836
P. decumbens IAM-7260	189	225
P. expansum IFO-6096	100	102
P. expansum IFO-5854	100	trace
P. frequentans AHU-8328	189	602
P. funiculosum IAM-7013	180	780
P. funiculosum IFO-6585	189	146
P. funiculosum OUT-2047	189	34
P. herquei IFO-4674	189	84
P. implicatum AHU-8332	189	64
P. italicum IAM-7247	189	25
P. janthinellum IFO-8070	180	1070
P. janthinellum IAM-7026	189	306
P. janthinellum IAM-7018	189	44
P. janthinellum IAM-7058	189	20
P. javanicum OUT-2044	189	34
P. lilacinum AHU-8333	189	15
P. lividum IAM-7200	189	454
P. luteum AHU-8022	189	trace
P. miczynski IAM-7165	189	127
P. nigricans IAM-7218	189	59
P. ochro-chloron IFO-4612	189	64
P. oxalicum AHU-8336	189	831
P. pallidum IFO-5758	189	84
P. piscarium IAM-7130	189	5
P. purpurogenum IFO-4684	189	136
P. restrictum IAM-7075	189	29
P. roqueforti IFO-4622	189	5
P. rubrum IFO-6580	180	88
P. simplicissimum IFO-5762	189	93
P. spinulosum IFO-6034	100	136
P. spinulosum IFO-5723	100	68
P. thomii OUT-2099	189	25
P. urticae IFO-7010	189	trace

[a] Activity was measured at pH 3.7.

production at 25 C. In this medium, the maximum production was reached after about 8 days. The production of acid carboxypeptidase by *P. janthinellum* IFO-8070 was determined at 25, 30, and 35 C; the maximum production was at 25 C. The maximum enzyme activities of *P. spinulosum* IFO-6034 and *P. funiculosum* IAM-7013 were reached after about 6 and 8 days of incubation after inoculation, respectively. The major part of the enzyme produced in koji culture was effectively extracted at least for 5 h or more at 5 C (Fig. 2). After extraction from cultures, the culture filtrates were pooled at 5 C and the pH was adjusted to 4.5. The enzyme filtrate was salted out with various amounts of solid $(NH_4)_2SO_4$ at 50 C. The best recovery (80%) demonstrated was at the final concentration of 80% ammonium sulfate (Fig. 3). The specific activity of the enzyme in ammonium sulfate precipitate was 500 mU for A_{280}.

Partial purification and isolation of acid carboxypeptidase. Partial purification and isolation of acid carboxypeptidases were performed with gel filtration on Bio-Gel P-100 at 5 C. Gel chromatograms on Bio-Gel P-100 of crude enzymes from *P. janthinellum* IFO-8070 and *P. funiculosum* IAM-7013 are shown in Fig. 4. As for the acid carboxypeptidases of *P. janthinellum*, gel chromatography showed that

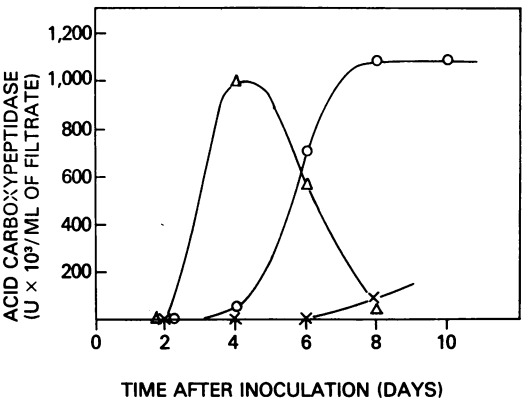

FIG. 1. *Effect of water content in the koji medium on the production of acid carboxypeptidase by P. janthinellum IFO-8070. Symbols: ×, 1.5 ml of tap water and 3 g of wheat bran; O, 2.1 ml of tap water and 3 g of wheat bran; Δ, 3 ml of tap water and 3 g of wheat bran.*

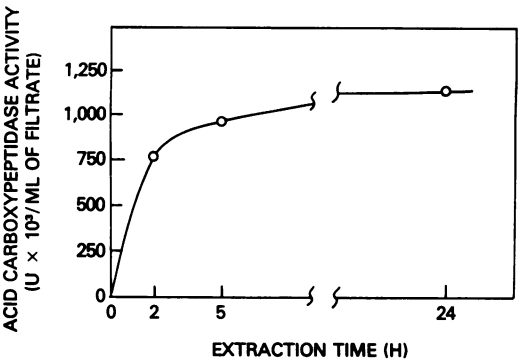

FIG. 2. *Effect of time on the enzyme extraction from koji culture of P. janthinellum IFO-8070. Incubated bran was obtained after 8 days of cultivation at 25 C on the medium containing 3 g of wheat bran and 2.1 ml of tap water.*

the strain produces a major active component with a low molecular weight of 51,000 and a minor active component with a high molecular weight of 160,000. The specific activity of a minor active component was 1,200 mU for A_{280}. Recovery of the activity in this step was about 90%. In further purification with Amberlite CG-50, a major active component was absorbed on the ion-exchange resin, whereas a minor active component was not absorbed. The specific activity of a major active component was 5,000 mU for A_{280}. The recovery of the enzyme in this step was about 70%. With regard to the specific activity of the acid carboxypeptidase activity, the value is approximately 100-fold that of the culture filtrate. No aggregated polymer of the major active component was observed in the presence of 0.2 and 1.5 M sodium chloride. The minor active component has similar enzymatic properties and a similar molecular weight to the acid carboxypeptidase of *Aspergillus saitoi*. Acid carboxypeptidases from *P. daleae* IFO-6087 and *P. oxalicum* AHU-8336 gave the same chromatographic patterns as that of *P. janthinellum* IFO-8070. Acid carboxypeptidase from *P. funiculosum* IAM-7013 was the only enzyme with a high molecular weight.

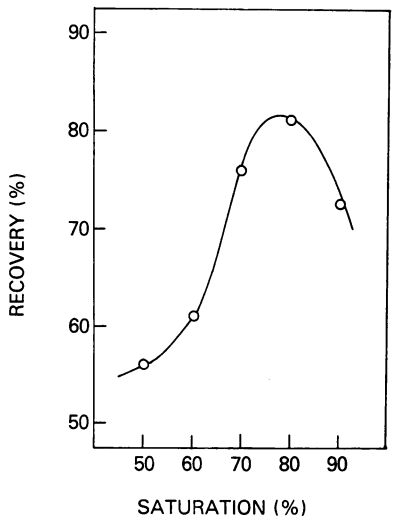

FIG. 3. *Effect of concentration of ammonium sulfate for precipitation of acid carboxypeptidase from P. janthinellum IFO-8070.*

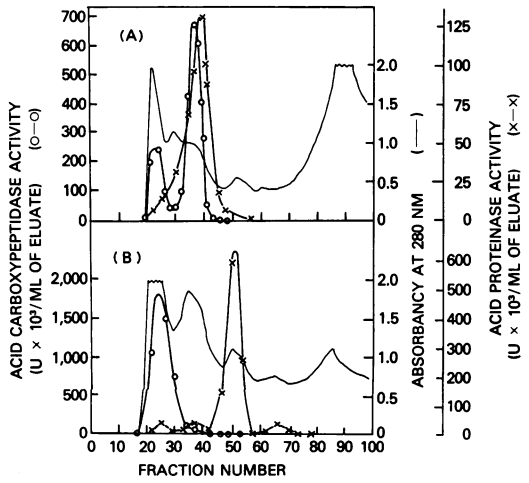

FIG. 4. *Gel filtration of acid carboxypeptidases from P. janthinellum IFO-8070 (A) and P. funiculosum IAM-7013 (B) on Bio-Gel P-100. Fraction size, 3 ml; flow rate, 10 ml/h; elution with 0.01 M acetate buffer (pH 4.0). Symbols: ——, absorbancy at 280 nm; ○, acid carboxypeptidase activity at pH 3.7; ×, acid proteinase activity at pH 3.0.*

Molecular-weight determination. Molecular-weight values of 160,000 and 51,000 from *P. janthinellum* IFO-8070 were obtained in Bio-Gel P-100 gel filtration for the enzymes with high and low molecular weights, respectively (Fig. 5). Molecular-weight values of 160,000 for the enzyme of *P. funiculosum* and 51,000 for the enzyme of *P. daleae* were obtained in the gel filtration (Table 2).

Optimum pH. Characteristic properties of enzymatic action for some synthetic peptides of acid carboxypeptidases from three strains of *Penicillium* molds are shown in Table 2. Maximum enzyme activities of a major active component with a low molecular weight from two strains, *P. daleae* IFO-6087 and *P. janthinellum* IFO-8070, were observed at pH 3.7 for Z-Glu-Tyr hydrolysis and pH 4.2 for Z-Gly-Pro-Leu-Gly hydrolysis. Enzymes with a high molecular weight from three strains, *P. daleae* IFO-6087, *P. funiculosum* IAM-7013, and *P. janthinellum* IFO-8070, gave a little lower pH optimum for Z-Glu-Tyr hydrolysis than that of low-molecular-weight enzymes. In the preliminary experiment, the enzyme can also release proline, glycine, and a small amount of leucine from Z-Gly-Pro-Leu-Gly-Pro at acidic pH range. Identification of amino acids liberated from the substrates was performed with paper chromatography. It was suggested that these enzymes are acid carboxypeptidases similar to those previously described (4).

Stability of acid carboxypeptidases. There was no appreciable difference in the residual activities of the low-molecular-weight acid carboxypeptidase from *P. daleae* IFO-6087, *P. funiculosum* IAM-7013, and *P. janthinellum* IFO-8070 kept for 30 min at pH 2.0 to 7.0 and 30 C (Fig. 6).

The enzymatic activities of these enzymes at pH 4.5 were stable at 60 C for 10 min and completely disappeared when the enzyme solution of pH 4.5 was maintained at 70 C for 10 min. The enzymes at pH 3.1 were stable at 50 C for 10 min, but rapid inactivation occurred at 60 C for 10 min (Fig. 7). The enzyme at pH 3.7 was rather stable at 60 C for 10 min, but complete inactivation occurred at 70 C for 10 min. After 10 days at −20 C, the enzymatic activities completely disappeared at pH 3.1, and no loss of enzymatic activities was observed at pH 4.5. In the absence of salt, no loss of enzymatic activity was detected through the lyophilization at pH 4.5, but 100% of enzymatic activity disappeared in the presence of 0.15 M sodium acetate.

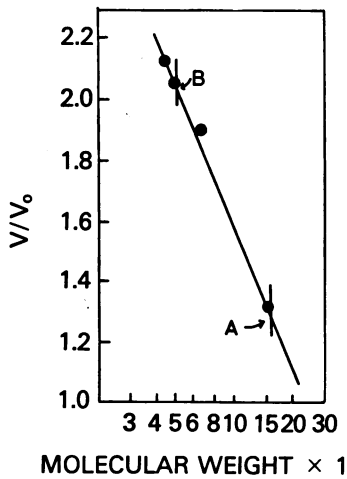

FIG. 5. *Molecular-weight determination of acid carboxypeptidase on Sephadex G-200. The column (2.0 by 70 cm) was eluted 0.05 M acetate buffer (pH 3.1). Symbols: A, acid carboxypeptidases with high-molecular-weight value; B, acid carboxypeptidase with low-molecular-weight-value; 1, egg albumin (molecular weight 45,000); 2, Taka-amylase A (molecular weight 51,200); 3, bovine hemoglobin (molecular weight 67,000); 4, bovine γ-globulin (molecular weight 150,000).*

Inhibitors. EDTA and *o*-phenanthroline have no effect on the enzymatic activities of *Penicillium* molds at acidic pH, suggesting that there is no requirement for metal ions. The inhibitory effects of monoiodoacetic acid, PCMB, hydrocinnamic acid, TPCK, and DFP to the acid carboxypeptidases were studied (Table 3). PCMB and monoiodoacetic acid were powerful inhibitors of the major active components with a low molecular weight from *P. daleae* IFO-6087, *P. janthinellum* IFO-8070, and *P. oxalicum* AHU-8336 (Table 3). A detailed account of the inhibitory effects of these enzymes will appear elsewhere.

DISCUSSION

Acid carboxypeptidase was observed in culture filtrates of *A. saitoi* by Ichishima in 1969 (2). In a previous paper (7), we found that a number of *Aspergillus* spp. did produce a new type of acid carboxypeptidase having an op-

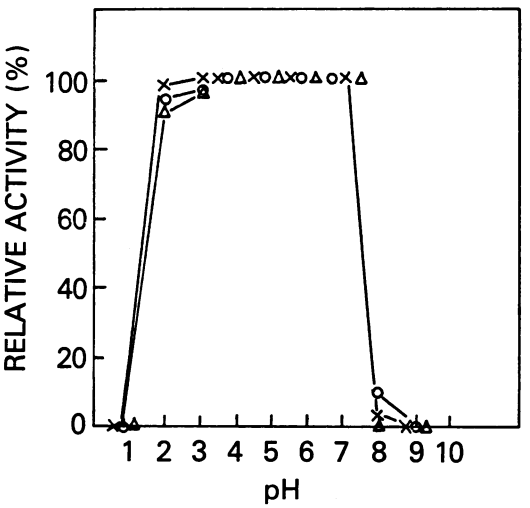

FIG. 6. *Effect of pH on the stability of acid carboxypeptidases from various strains of Penicillium (30 C, 20 min). Symbols: Δ, P. janthinellum IFO-8070; ○, P. daleae IFO-6087; ×, P. funiculosum IAM-7013.*

TABLE 2. *Enzymatic properties of acid carboxypeptidase of Penicillium molds*

Acid carboxypeptidase		Optimum pH for hydrolysis		
Fraction	Mol wt	Z-Glu-Tyr[a]	Z-Gly-Pro-Leu-Gly[a]	Bz-Gly-Lys[b]
P. daleae IFO-6087	160,000	3.5	4.3	
P. daleae IFO-6087	51,000	3.7	4.2	5.0
P. janthimellum IFO-8070	160,000	3.2	4.3	
P. janthinellum IFO-8070	51,000	3.7	4.2	5.2
P. funiculosum IAM-7013	160,000	3.4	4.1	5.0

[a] Reaction was performed in 0.1 M acetate buffer at 30 C.
[b] Reaction was performed in Sörensen citrate buffer.

timum pH of 3.1 for benzyloxycarbonyl-glutamyl-tyrosine. It was also found that *A. saitoi* R-3813 (ATCC no. 14332) and *A. usamii* IAM-2186 gave the highest enzyme potencies of any organisms belonging to *A. niger* group tested in the survey using the koji method (7).

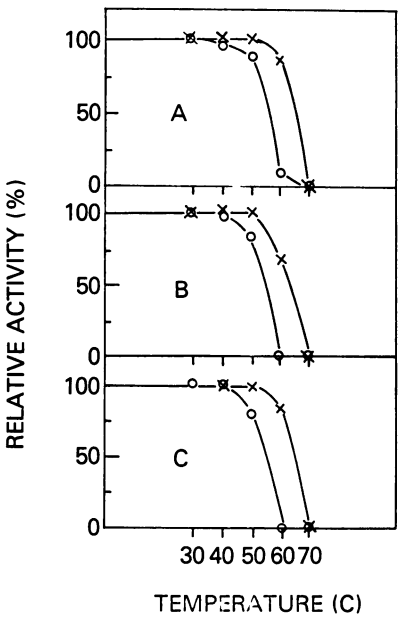

FIG. 7. *Effect of temperature on the stability of acid carboxypeptidases of Penicillium molds. (A) Acid carboxypeptidase with high-molecular-weight value from P. funiculosum IAM-7013; (B) acid carboxypeptidase with low-molecular-weight value from P. janthinellum IFO-8070; (C) acid carboxypeptidase with low-molecular-weight value from P. daleae IFO-6087. Symbols: O, pH 3.1 for 10 min; ×, pH 3.7 for 10 min.*

We applied a surface koji method for acid carboxypeptidase production to screen *Penicillium* molds that had been obtained as strains with a high potency of acid proteinase production. A survey of *Penicillium* molds for their ability to produce acid carboxypeptidase demonstrated that *P. janthinellum* IFO-8070, *P. daleae* IFO-6087, *P. oxalicum* AHU-8336, *P. funiculosum* IAM-7013, and *P. frequentans* AHU-8328 were better strains for acid carboxypeptidase production than *A. saitoi* (7), whereas only trace amounts of acid carboxypeptidases were produced by *P. chrysogenum*, *P. cyclopium*, *P. expansum*, *P. luteum*, and *P. urticae* (Table 1). The experiments reported indicate that there is no direct mutual dependence between the production of acid proteinase in *Penicillium* molds.

Maximum production of acid carboxypeptidase from *P. janthinellum* in a surface koji culture containing 3 g of wheat bran and 2.1 g of tap water was reached after about 8 days at 25 C. This strongly indicates that the conditions for acid carboxypeptidase production from *Penicillium* were for a much lower temperature and a longer time than the conditions for *A. saitoi* enzyme production (7).

By gel filtration of the main component of acid carboxypeptidases from *P. janthinellum* IFO-8070, *P. daleae* IFO-6087, and *P. oxalicum* AHU-8336 with Sephadex G-200, a molecular weight of 51,000 was obtained for these enzymes in the acetate buffer, pH 4.0, whereas the molecular weight of 160,000 for the enzyme of *P.*

TABLE 3. *Effect of various inhibitors on acid carboxypeptidase action of seven fractions of Penicillium molds toward 5 × 10^{-4} M Z-Glu-Tyr at pH 3.7 and 30 C*

Inhibitor		Relative activity of acid carboxypeptidase						
Compound	Final concn (mM)	*P. janthinellum* IFO-8070		*P. daleae* IFO-6087		*P. oxalicum* AHU-8336		*P. funiculosum* IAM-7013 (H_{MW})
		H_{MW}[a]	L_{MW}	H_{MW}	L_{MW}	H_{MW}	L_{MW}	
No inhibitor		100	100	100	100	100	100	100
Monoiodoacetic acid[b]	1.0	72	3	35	3	47	38	98
PCMB[c, d]	0.1	80	15	93	14	106	56	77
Hydrocinnamic acid[b]	5.0	29	42	34	47	45	67	13
TPCK[c]	1.0	73	74	59	57	107	105	86
DFP[e]	1.0	49	30	40	49	57	65	21

[a] H_{MW}, Enzyme with high molecular weight; L_{MW}, enzyme with low molecular weight.
[b] Incubated at pH 3.7 for 10 min and measured at pH 3.7.
[c] Incubated at pH 3.7 for 1 h and measured at pH 3.7.
[d] Partially insoluble at acidic pH range.
[e] Incubated at pH 3.7 for 2 h and measured at pH 3.7.

funiculosum IAM-7013, and the minor enzymes of the above-mentioned three strains, was obtained in the gel filtration technique. The aggregation to polymer in the presence of sodium chloride was not observed for either enzyme.

In a previous paper (4), molecular-weight values of 155,000 for the larger form or polymer and 51,000 for the smaller form or monomer of acid carboxypeptidase of *A. saitoi* were obtained in the absence of sodium chloride, whereas a molecular weight of 135,000 in the presence of 0.2 M sodium chloride was obtained on Sephadex G-200 gel filtration (4). These two fractions having the different molecular weights have the same K_m and V_{max} values toward Z-Glu-Tyr at pH 3.1 and 30 C (4).

The molecular weights of the enzymes from *A. oryzae* and *A. oryzae* var. *magnasporus* were found to be 80,000 (6).

The pH optima for small synthetic peptides of acid carboxypeptidases from the three strains of *Penicillium* are shown in Table 2. The acid carboxypeptidases with high-molecular-weight values from these strains of *Penicillium* have similar enzymatic properties and molecular-weight values to the acid carboxypeptidase from *A. saitoi* (4).

Characterization studies showed that the acid carboxypeptidase with the low-molecular-weight value, 51,000, from *P. janthinellum* IFO-8070, *P. daleae* IFO-6087, and *P. oxalicum* AHU-8336, has similar properties to the acid carboxypeptidase from *A. saitoi* R-3813 (4) with regard to hydrolytic reaction with several peptides at acidic pH range, but has slightly different properties with regard to stability, pH optima, inhibitors, and molecular-weight value.

These observations suggest that the enzymes studied in this paper differ from the known pancreatic carboxypeptidases A (EC 3.4.2.1) and B (EC 3.4.2.2). The enzymatic properties of

TABLE 4. *Comparison of acid carboxypeptidases from fungal origins*

Prepn	Hydrolysis			Mol wt ($\times 10^{-3}$)	Inhibitors	References
	Substrate	Optimal pH	$K_m \times 10^3$ M			
A. saitoi acid CPase[a]	Z-Glu-Tyr Z-Gly-Pro-Leu-Gly	3.1 3.2	1.25 3.3	139 (Yphantis) 155 ↔ 51 (Sephadex)	DFP, HA[a] IAA	4, 5
P. janthinellum peptidase B	Z-Glu-Tyr	4.7	0.5			9
Penicillocarboxypeptidase-S	Z-Glu-Tyr	4.0–4.5		48 (Yphantis) 48 (SDS-polyacrylamide)	DFP, *p*-hydroxymercury-benzoic acid	8
P. daleae acid CPase	Z-Glu-Tyr Z-Gly-Pro-Leu-Gly	3.7 4.2		51 (Sephadex)	DFP, PCMB, IAA[a]	This paper
P. daleae acid CPase	Z-Glu-Tyr Z-Gly-Pro-Leu-Gly	3.5 4.3		160 (Sephadex)	DFP, HA, IAA	This paper
P. janthinellum acid CPase	Z-Glu-Tyr Z-Gly-Pro-Leu-Gly	3.7 4.2		51 (Sephadex)	DFP, PCMB, IAA	This paper
P. janthinellum acid CPase	Z-Glu-Tyr Z-Gly-Pro-Leu-Gly	3.2 4.3		160 (Sephadex)	DPF, HA	This paper
P. funiculosum acid CPase	Z-Glu-Tyr Z-Gly-Pro-Leu-Gly	3.4 4.2		160 (Sephadex)	DFP, HA	This paper

[a] CPase, carboxypeptidase; HA, hydrocinnamic acid; IAA, monoiodoacetic acid.

these enzymes toward Z-dipeptides indicate that these fungal enzymes are similar to *P. janthinellum* peptidase B (9) and penicillocarboxypeptidase-S (8), which have higher optimum pH values of 4.7 and 4.0 to 4.5, respectively, for Z-Glu-Tyr hydrolysis (Table 4). Penicillocarboxypeptidase-S (8) has a molecular weight of about 48,000 and is not inhibited by the metal chelators EDTA, *o*-phenanthroline, and 8-hydroxyquinoline, or by DFP. The inhibition study for penicillocarboxypeptidase-S suggests that a thiol group is in or very near the active site of the enzyme.

Judging from preliminary experiments, the acid carboxypeptidases with the low-molecular-weight value of the *Penicillium* molds were inhibited by PCMB, hydrocinnamic acid, monoiodoacetic acid, and DFP, whereas treatments with EDTA and *o*-phenanthroline resulted in no loss of activity. These inhibitory effects are similar to those of the acid carboxypeptidases from *A. oryzae* and *A. oryzae* var. *magnasporus* (6).

The characteristic properties of the acid carboxypeptidase with low molecular weights were similar to penicillocarboxypeptidase-S reported by Jones and Hofmann (8) but showed important differences in the optimum pH for digesting substrates.

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