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, benzoylcarbonyl-, benzyol-, 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 pectic 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. Benzylxoycarbonyl-L-glutamyl-L-tyrosine (Z-Glu-Tyr), benzylxoycarbonyl-glycyl-L-prolyl-L-leucyl-glycine (Z-Gly-Pro-Leu-Gly), and benzylxoycarbonyl-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 Seikagaku Kogyo Co., Tokyo, respectively. p-Chloromercuribenzoate (PCMB) and ethylenediaminetetra-acetate (EDTA) were from Tokyo Kasei 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/in² 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 grew 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 4.0) 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 mixture was adjusted to pH 4.0 and filtered through glass fiber. The filtrate was then applied to 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.

**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 hydrolyase 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 (A280) 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 0 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. fuscus* IFO-6087, *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 A280.

**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 2.4 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.
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$_4$)$_2$SO$_4$ 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

| Strain                  | Cultivation (h) | Activity ($U \times 10^{4}$/ml of filtrate) |
|-------------------------|-----------------|-------------------------------------------|
| *Penicillium camemberti*| 189             | 123                                       |
| IFO-5855                | 189             | trace                                     |
| *P. chrysogenum*        | 189             | 103                                       |
| OUT-2028                | 189             | 836                                       |
| *P. citrinum*           | 100             | 225                                       |
| OUT-2029                | 100             | 102                                       |
| *P. cyclopium*          | 189             | 34                                        |
| IAM-7146                | 189             | 84                                        |
| *P. daeleae*            | 189             | 64                                        |
| IAM-6087                | 189             | 25                                        |
| *P. decumbens*          | 189             | 34                                        |
| IAM-7260                | 189             | 25                                        |
| *P. expansum*           | 189             | 107                                       |
| IFO-6096                | 189             | 306                                       |
| *P. frequens*           | 189             | 44                                        |
| AHU-8328                | 189             | 800                                       |
| *P. fumicola*           | 189             | 145                                       |
| IAM-7013                | 189             | 34                                        |
| *P. fumicola*           | 189             | 34                                        |
| IAM-6586                | 189             | 15                                        |
| *P. fumicola*           | 189             | 5                                         |
| IAM-7013                | 189             | 64                                        |
| *P. fumicola*           | 189             | 831                                       |
| IAM-7013                | 189             | 5                                         |
| *P. fumicola*           | 189             | 84                                        |
| IAM-7013                | 189             | 25                                        |
| *P. fumicola*           | 189             | 136                                       |
| IAM-7013                | 189             | 29                                        |
| *P. fumicola*           | 189             | 5                                         |
| IAM-7013                | 189             | 88                                        |
| *P. fumicola*           | 189             | 93                                        |
| IAM-7013                | 189             | 5                                         |
| *P. fumicola*           | 189             | 5                                         |
| IAM-7013                | 189             | 5                                         |
| *P. fumicola*           | 189             | 5                                         |
| IAM-7013                | 189             | 5                                         |
| *P. fumicola*           | 189             | 5                                         |
| IAM-7013                | 189             | 5                                         |
| *P. fumicola*           | 189             | 5                                         |
| IAM-7013                | 189             | 5                                         |
| *P. fumicola*           | 189             | 5                                         |
| IAM-7013                | 189             | 5                                         |
| *P. fumicola*           | 189             | 5                                         |
| IAM-7013                | 189             | 5                                         |
| *P. fumicola*           | 189             | 5                                         |
| IAM-7013                | 189             | 5                                         |

* Activity was measured at pH 3.7.
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 A280. 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 A280. 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.

**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).

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**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.
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.

**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 monooiodoacetic acid, PCMB, hydrocinnamic acid, TPCK, and DFP to the acid carboxypeptidases were studied (Table 3). PCMB and monooiodoacetic 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. saatoi* 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-

![Graph](http://aem.asm.org/)

**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-amyase A (molecular weight 51,200); 3, bovine hemoglobin (molecular weight 67,000); 4, bovine γ-globulin (molecular weight 150,000).

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

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

| Acid carboxypeptidase | Optimum pH for hydrolysis |
|-----------------------|---------------------------|
|                       | Z-Glu-Tyr⁸ | Z-Gly-Pro-Leu-Gly⁸ | Bz-Gly-Lys⁸ |
| *P. daleae* IFO-6087  | 160,000    | 3.5               | 4.3       |
| *P. daleae* IFO-6087  | 51,000     | 3.7               | 4.2       | 5.0       |
| *P. janthinellum* 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       |

*Reaction was performed in 0.1 M acetate buffer at 30°C.  
Reaction was performed in Sörensen citrate buffer.
timum pH of 3.1 for benzylxocarbonyl-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).

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. urticace (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.

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**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.

**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         | Final concn (mM) | P. janthinellum IFO-8070 |                  | P. daleae IFO-6087 |                  | P. oxalicum AHU-8336 |                  | P. funiculosum IAM-7013 (H_Mw) |
|-------------------|------------------|--------------------------|------------------|-------------------|------------------|---------------------|------------------|-----------------------------|
|                   | H_Mw             | L_Mw                     | H_Mw             | L_Mw             | H_Mw             | L_Mw             | H_Mw             | L_Mw             | H_Mw             |
| No inhibitor      |                  |                          |                  |                  |                  |                  |                  |                  |                 |
| Monoiodoacetic acid a | 1.0            | 72                       | 3                | 35               | 3                | 47               | 38               | 100             |                 |
| PCMB b c          | 0.1              | 80                       | 15               | 93               | 14               | 106              | 56               | 77              |                 |
| Hydrocinnamic acid a | 5.0           | 29                       | 42               | 34               | 47               | 45               | 67               | 13              |                 |
| TPCK c d          | 1.0              | 73                       | 74               | 59               | 57               | 107              | 105              | 86              |                 |
| DFP e             | 1.0              | 49                       | 30               | 40               | 49               | 57               | 65               | 21              |                 |

* H_Mw, Enzyme with high molecular weight; L_Mw, enzyme with low molecular weight.
* Incubated at pH 3.7 for 10 min and measured at pH 3.7.
* Incubated at pH 3.7 for 1 h and measured at pH 3.7.
* Partially insoluble at acidic pH range.
* 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* was 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 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 Substrate                  | Optimal pH | *K*ₘ (× 10⁻⁹ M) | Mol wt (× 10⁻⁹) | Inhibitors      | References       |
|------------------------|---------------------------------------|------------|------------------|-----------------|----------------|-----------------|
| *A. saitoi* acid CPase | Z-Glu-Tyr                             | 3.1        | 1.25             | (139 (Yphantis) | DFP, HA*       | 4, 5            |
|                        | Z-Gly-Pro-Leu-Gly                     | 3.2        | 3.3              | (155 → 51 (Sephadex) | IAA          |                 |
| *P. janthinellum* peptidase B | Z-Glu-Tyr                             | 4.7        | 0.5              |                 |                 | 9               |
| Penicillocarboxypeptidase-S | Z-Glu-Tyr                             | 4.0-4.5   |                  | 48 (Yphantis)   | DFP, p-hydroxy-mercury-benzoic acid | 8          |
|                        | Z-Gly-Pro-Leu-Gly                     | 3.7        | 51 (Sephadex)    |                 |                 |                 |
| *P. daleae* acid CPase  | Z-Glu-Tyr                             | 3.7        | 51 (Sephadex)    |                 |                 |                 |
|                        | Z-Gly-Pro-Leu-Gly                     | 3.5        | 4.2              |                 | DFP, PCMB, IAA | This paper      |
| *P. daleae* acid CPase  | Z-Glu-Tyr                             | 3.5        | 4.3              |                 |                 |                 |
| *P. janthinellum* acid CPase | Z-Glu-Tyr                             | 3.7        | 51 (Sephadex)    |                 |                 |                 |
|                        | Z-Gly-Pro-Leu-Gly                     | 3.2        | 4.2              |                 | DFP, HA        | This paper      |
| *P. janthinellum* acid CPase | Z-Glu-Tyr                             | 3.2        | 4.3              |                 |                 |                 |
| *P. funiculosum* acid CPase | Z-Glu-Tyr                             | 3.4        | 4.2              | 160 (Sephadex)  | DFP, HA        | This paper      |
|                        | Z-Gly-Pro-Leu-Gly                     | 4.2        |                  |                 |                 |                 |

*CPase, carboxypeptidase; HA, hydrocinnamic acid; IAA, monooiodoacetic 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). Penicillo-
carboxypeptidase-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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