An Aminopeptidase of Aspergillus sojae

Yoshinori OZAWA, Kazuo SUZUKI, Takeji MIZUNUMA and Koya MOGI

Central Research Laboratories, Kikkoman Shoyu Co., Ltd., 399 Noda-shi, Chiba-ken, Japan

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An aminopeptidase was purified from Aspergillus sojae X-816. The molecular weight of the enzyme was estimated to be 220,000. The isoelectric point was at pH 5.3. The optimum pH for L-leucylglycylglycine was 7.5. The enzyme was stable up to 37°C against temperature treatment for 15 min. Some chelating agents inhibited the enzyme activity. The Km value for L-leucylglycylglycine at pH 7.5 and 37°C was 45 mM. The Km value for L-leucyl-β-naphthylamide at pH 7.0 and 37°C was 2.2 mM.

In regard to proteinases from several species of Aspergillus many reports have appeared. However, there have been only a few reports about exopeptidase from Aspergillus. Johnson et al.1,2) attempted the isolation of aminopeptidase from Aspergillus sp. and Penicillium sp., long ago. Akatsuka and Sato3,4) purified a dipeptidase and an aminopeptidase from Asp. oryzae. Lehmann and Uhlig5) purified an aminopeptidase from Asp. oryzae and another aminopeptidase from Asp. parasticus. Recently, Ichishima6) highly purified an acid carboxypeptidase from Asp. saitoi. Nakadai et al.7) examined the action of various peptidases from Asp. oryzae and Asp. sojae on soy bean proteins. They also isolated three acid carboxypeptidases from culture filtrate of Asp. oryzae.8)

Isolation of an intracellular aminopeptidase from Asp. sojae which is widely used in soy sauce production in Japan, will be reported in the present paper. The purified aminopeptidase is different from those obtained previously from several species of Aspergillus in its substrate specificity and molecular weight.

METHODS AND MATERIALS

Enzyme substrates. Peptides and L-leucyl-β-naphthylamide were supplied by the Protein Research Foundation in the Institute for Protein Research, Osaka University.

Preparation of a crude enzyme extract. The microorganism used in this study was Asp. sojae X-816. A medium containing 3% skim milk and 3% glucose was prepared and adjusted to pH 7.0. Each 500 ml of the medium was taken into 5 liter Fernfach flasks and autoclaved (15 lbs. 15 min). The spores of the mold were inoculated and cultured at 30°C for 9 days. Each mycelial mat grown up on the surface of each medium was harvested. After being washed with water, the mats were treated with cold acetone for 5 min. The acetone-treated mats were dried up in vacuo and stored in a freezer. After the addition of 0.01 M phosphate buffer solution (pH 7.5) and sea sands, the mycelial mats were smashed for 30 min at 4°C by a mixing and grinding machine equipped with motor (type 24, Ishikawa Factory Co., Ltd.). The homogenate obtained was pressed by use of a hand press to collect the extract. The residue was extracted again by grinding in the same way described above. The combined extracts were centrifuged continuously (10,000 rpm) and the supernatant was used as a crude enzyme extract.

Precipitation with ammonium sulfate. Twenty liters of the crude enzyme extract obtained from 200 mats of surface culture were chilled below 4°C. Solid ammonium sulfate was added to the crude extract up to 70% saturation while the pH was adjusted to 7.5 with 1 N sodium hydroxide. After standing overnight at 4°C, the precipitate formed was collected by filtration with Hyflo Super-cel. The precipitate collected on the Hyflo Super-cel was extracted with 2 liters of 0.01 M phosphate buffer solution (pH 7.5). The extract was centrifuged at 33,000 g for 90 min, and the supernatant was concentrated to 200 ml by a Diaflo ultrafilter XM-300 (Amicon Corp.).

First gel filtration with Sephadex G-200. The concentrated supernatant was applied on a Sephadex G-200 column. Detailed methods are described in the legend to the Fig. 1. The enzyme fractions obtained by repetition of gel filtration were combined
and concentrated to half a volume by a Diaflo ultrafilter XM-50.

Column chromatography with DEAE Sephadex A-50. The enzyme fraction obtained by gel filtration was subjected to chromatography with DEAE Sephadex A-50. Details are described in the legend to the Fig. 2.

Second gel filtration with Sephadex G-200. Enzyme fraction obtained by chromatography on DEAE Sephadex A-50 was concentrated to one tenth of the initial volume by a Diaflo XM-50, and 2 ml of the concentrate was applied on a Sephadex G-200 column. Details are described in the legend to the Fig. 3. The enzyme fraction obtained was concentrated with a collodion bag (Sartorius-Membranfilter GmbH).

Assay of aminopeptidase activity. Automated measurement of the enzyme activity was carried out routinely using L-leucylglycylglycine (L-Leu-Gly-Gly) as a substrate. Procedure of the automated measurement was essentially the same as described previously about dipeptidase assay. About 0.3 ml of the enzyme solution was taken into the sample cup of the Auto Chemist. The enzyme solution (0.1 ml) from the sample cup and 1.5 ml of 1 mM L-Leu-Gly-Gly dissolved in 0.05 M phosphate buffer solution (pH 7.5) containing 0.5 mM ZnCl₂ were added into a reaction tube by the sampler unit of the Auto Chemist and incubated at 37 °C. After 15 min, 2 ml of 5% borate decahydrate containing 0.06% cupric sulfate was added by the first dispenser/stirrer unit to stop the enzyme reaction. In the control test, the copper reagent, enzyme and substrate were simultaneously transferred into the reaction tube. One and half minutes after the addition of copper reagent, 1 ml of sodium 2,4,6-trinitrobenzenesulfonate dihydrate (TNBS) solution (4 mg/ml) was added by the second dispenser/stirrer unit. After further 23 min incubation, the reaction mixture was introduced into a flow cell of 10 mm light path. The optical density at 420 nm was measured spectrophotometrically and recorded.

Definition of unit and specific activity. One unit of L-Leu-Gly-Gly hydrolase activity was defined as the amount of enzyme required to liberate 1 μmole of L-leucine per min at pH 7.5 and 37 °C. Specific activity was expressed as L-Leu-Gly-Gly hydrolase units at pH 7.5 and 37 °C per mg protein.

Determination of protein concentration. Protein concentration was determined according to the Lowry's method by the Auto Chemist. The concentrations of ‘A’, ‘B’, and ‘C’ solutions due to Lowry’s method were modified as follows, i.e., ‘A’ solution contained 35 g of sodium carbonate in one liter of 0.175 N sodium hydroxide; ‘B’ solution, 0.875 g of cupric sulfate tetrahydrate and 1.75 g of Rochelle salt in 100 ml of water; ‘C’ solution was prepared by mixing ‘A’ and ‘B’ solutions at the rate of 50:1 (v/v). Sample solution (0.1 ml) and 1.5 ml of distilled water were taken into a reaction tube by the sampler unit of the Auto Chemist and incubated at 30°C for 1.5 min. Then, 2 ml of ‘C’ solution was added by the first dispenser/stirrer unit. After 10 min, 1 ml of phenol reagent which was diluted 5.7-fold with water was added by the second dispenser/stirrer unit. After further 23 min incubation, the reaction mixture was introduced into a flow cell and the optical density at 750 nm was measured and recorded. Egg albumin was used as the referential standard protein.

Determination of Km values. In order to obtain Km value for L-Leu-Gly-Gly, increases of amino groups due to enzymatic hydrolysis were determined by the method of TNBS reaction. Fifty microliters of enzyme solution (177 μg protein/ml) and 0.5 ml of the substrate of various concentrations dissolved in 0.05 M phosphate buffer solution (pH 7.5) were incubated for 10 min at 37 °C. In the control test, heat-inactivated enzyme was used. After the incubation, enzyme reaction was stopped by adding 1.0 ml of 20 mM EDTA. Each reaction mixture was diluted with 20 mM EDTA to bring the initial substrate concentration to 4 mM. Automated measurement of the amino groups in the diluted reaction mixtures was performed by the method described previously.

In order to obtain Km value for L-leucine-β-naphthylamide, β-naphthylamine formed by the enzymatic hydrolysis of the substrate was determined according to the method of Matsutani et al. The enzyme solution (0.2 ml of 0.71 mg protein/ml) and 1.5 ml of substrate of various concentrations, which was dissolved in 0.2 M phosphate buffer solution (pH 7.0) were incubated for 25 min at 37 °C. Enzyme reaction was stopped by adding 3 ml of mixed p-dimethylaminocinnamaldehyde (DAC) solution by the first dispenser/stirrer unit of Auto Chemist. The mixed DAC solution was prepared by mixing equal volumes of 0.7% hydrochloric acid dissolved in 99.6% ethanol and 0.06% DAC dissolved in 99.6% ethanol. In the control test, the enzyme, substrate and mixed DAC solution were simultaneously introduced into the reaction tube. The reaction mixture was incubated for additional 10 min at 37 °C and introduced into a flow cell. The optical density at 540 nm was measured and recorded.

Preparation of water insoluble aminopeptidase. The aminopeptidase purified by chromatography on DEAE Sephadex A-50 was covalently bound to Sepharose 4B activated by cyanogen bromide according to the method of Axén et al. To 20 ml of activated Sepharose 4B, 20 ml of 0.1 M sodium bicarbonate and 2 ml of the enzyme (2.86 mg protein/ml) were added. The mixture
An Aminopeptidase of *Aspergillus sojae* was stirred for 24 hr at 3°C and then the gel was washed with 0.05 M phosphate buffer solution (pH 7.0) containing 0.5 mM ZnCl₂. No enzyme activity was found in the buffer solution with which the water insoluble enzyme was washed. Ninety % of the original enzyme activity remained in the preparation of the water insoluble enzyme.

**Determination of the activity of the water insoluble enzyme.** For the determination of the activity of the water insoluble enzyme, 3 ml of 0.1 M phosphate buffer solution (pH 7.5), 3 ml of 2 mM L-Leu-Gly-Gly, 0.2 ml of 2 mM ZnCl₂ and 0.2 ml of the suspension of the water insoluble enzyme were incubated at 37°C for 15 min. After the incubation, the reaction mixture was rapidly filtrated within 30 sec. Because the filtrate did not contain the water insoluble enzyme, no enzyme reaction proceeded further. By the method of Cu²⁺-TNBS reaction routinely used for the determination of the enzyme activity, L-leucine formed by enzyme reaction was determined using 1.6 ml of the filtrate.

**Disc electrophoresis.** Sodium chloride (0.4 M) was added to each stock solution described by Davis to prepare the gels. About 100 µg of the protein sample was applied and electrophoresis was carried out with a current of 5 mA per gel column (5% gel) for 4 hr at 4°C. The protein band was detected by staining the gel in 0.2% solution of amidoblack 10B dissolved in 7% acetic acid. Destaining was done by washing the gel repeatedly with 7% acetic acid.

**Preparation of antiserum.** A white rabbit was immunized by two injections into foot pads at one month intervals. Each injection contained 1 ml of 1% antigen solution of the purified aminopeptidase in phosphate buffer mixed with an equal volume of Freund's complete adjuvant (Miles). After the lapse of one month, the animal was given a 1 ml booster of the antigen (1 mg) through a marginal ear vein. Ten days after the booster the rabbit was bled, the serum was collected and stored in a freezer.

**Immunoelectrophoresis in agar gel.** The gel medium consisted of 1% agarose in barbital buffer (pH 8.6) containing 0.4 M sodium chloride. Electrophoresis was carried out for 2 hr with a current of 10 mA per microscope slide (2.5 × 7.5 cm). After the formation of precipitin band, the gel was washed with saline and dried. Staining of the precipitin arc was done with amidoblock 10B.

**RESULTS AND DISCUSSION**

The result of purification of aminopeptidase from *Asp. sojae* X–816 is summarized in Table I. In all the subsequent purification steps, enzyme activity was assayed using L-Leu-Gly-Gly as a substrate. The enzyme preparation obtained by ammonium sulfate precipitation was subjected to gel filtration with Sephadex G–200. As shown in Fig. 1, the aminopeptidase activity was eluted as two peaks at this stage of the separation. The major active fractions eluted near void volume were collected to obtain an aminopeptidase. In the gel filtration, a L-leucylglycine hydrolase activity appeared much behind the void volume. This dipeptidase is presumed to be the same enzyme as that reported by Akatsuka and Sato.⁴

For the comparison of the aminopeptidases from various species of *Aspergillus* and some of other molds, gel filtration of crude enzyme solutions obtained from various molds by the same method as that used for *Asp. sojae* X–816 was performed. The *Kₐᵥ* values of these aminopeptidases are shown in Table II. It appeared that *Asp. sojae* X–816, *Asp. flavus*, *Asp. japonicus*, and *Asp. awamori* had aminopeptidase of almost the same molecular size.

### Table I. Isolation of the Aminopeptidase

<table>
<thead>
<tr>
<th>Step</th>
<th>Total activity (units)</th>
<th>Total protein (mg)</th>
<th>Specific activity (units/mg protein)</th>
<th>Activity yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude extract</td>
<td>31,400</td>
<td>63,000</td>
<td>0.49</td>
<td>100</td>
</tr>
<tr>
<td>Ammonium sulfate fraction</td>
<td>24,800</td>
<td>43,000</td>
<td>0.58</td>
<td>79</td>
</tr>
<tr>
<td>First gel filtration with</td>
<td>18,500</td>
<td>2,300</td>
<td>8.0</td>
<td>59</td>
</tr>
<tr>
<td>Sephadex G–200</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DEAE Sephadex A–50 fraction</td>
<td>6,590</td>
<td>108</td>
<td>61</td>
<td>21</td>
</tr>
<tr>
<td>Second gel filtration with</td>
<td>4,320</td>
<td>15</td>
<td>288</td>
<td>14</td>
</tr>
<tr>
<td>Sephadex G–200</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
FIG. 1. Gel Filtration of Crude Enzyme Solution on Sephadex G-200 Column (3 × 70 cm).
Eluent; 0.01 M phosphate buffer solution (pH 7.5) containing 0.05 mM ZnCl₂. Load; 15 ml of crude enzyme solution. Flow rate; about 25 ml/hr. Fraction volume; 10 ml.
○—○, L-Leu-Gly-Gly hydrolase activity; •—•, L-Leu-Gly-Gly hydrolase activity which was determined by the method described previously;
 α×α×, protein concentration.

TABLE II. Kav VALUES OF PEPTIDASES FROM VARIOUS Aspergilli AND SOME OF OTHER MOLDS

Gel filtration was done with Sephadex G-200 column (3 × 70 cm). Eluent, 0.01 M phosphate buffer solution (pH 7.5). Load, 2 ml of the crude enzyme solution. Flow rate, 30 ml/hr. Fraction volume, 2 ml. Activity of peptidases and protein concentration of each fraction were determined as described in the text. Kav values were calculated by the formula defined by Gelotte.²²

<table>
<thead>
<tr>
<th>Aspergillus</th>
<th>Kav value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asp. sojae X-816</td>
<td>0.17 (0.36)*</td>
</tr>
<tr>
<td>Asp. flavus</td>
<td>0.14</td>
</tr>
<tr>
<td>Asp. japonicus</td>
<td>0.16</td>
</tr>
<tr>
<td>Asp. awamori</td>
<td>0.12 (0.42)*</td>
</tr>
<tr>
<td>Asp. oryzae</td>
<td>0.05</td>
</tr>
<tr>
<td>Asp. oryzae, miso</td>
<td>0.08</td>
</tr>
<tr>
<td>Asp. niger</td>
<td>0.05</td>
</tr>
<tr>
<td>Asp. saitoi</td>
<td>0.08</td>
</tr>
<tr>
<td>Penicillium pallidus</td>
<td>0.05</td>
</tr>
<tr>
<td>Rhizopus oryzae</td>
<td>0.36</td>
</tr>
<tr>
<td>Abisidia ranosa</td>
<td>0.38</td>
</tr>
</tbody>
</table>

* peak of minor activity.

Penicillium pallidus had much larger aminopeptidase in molecular size than Asp. sojae X-816. On the other hand, the aminopeptidases from Rhizopus oryzae and Abisidia ranosa were much smaller in molecular size than that of Asp. sojae X-816.

The aminopeptidase fraction obtained by gel filtration from Asp. sojae X-816 was run through a column of DEAE Sephadex A-50. As shown in Fig. 2 the aminopeptidase frac-

Fig. 2. Column Chromatography of Aminopeptidase on DEAE Sephadex A-50.
The column (3 × 10 cm) was equilibrated with 0.01 M phosphate buffer solution (pH 7.5) containing 0.05 mM ZnCl₂. Active fraction of L-Leu-Gly-Gly hydrolyzing activity was obtained by gel filtration shown in Fig. 1 and the concentrate (40 ml) was applied on to the column. Elution was performed with the above buffer solution with increasing concentration of NaCl using a mixing chamber of 1 liter. To the mixing chamber, 0.3 M NaCl dissolved in the above buffer solution stored in the reservoir was tightly connected. Flow rate; 25 ml/hr. Fraction volume; 15 ml.

Asp. oryzae, Asp. saitoi, Asp. niger and
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**Fig. 3.** Second Gel Filtration of Aminopeptidase on Sephadex G–200 Column (2 × 240 cm).

Eluent; 0.01 M phosphate buffer solution (pH 7.5), containing 0.05 mM ZnCl₂. Load; 2 ml of aminopeptidase preparation purified by chromatography on DEAE Sephadex A–50 (Fig. 2). Flow rate; 2 ml/hr. Fraction volume; 2 ml.

○—__, L-Leu-Gly-Gly hydrolase activity; ——●, protein concentration; —, specific activity.

monodisperse sedimentary boundary as illustrated in Fig. 4. The determination of sedimentation constant, \( s_{20,w} \) at a protein concentration of 0.316% gave a value of 11.5 S. According to the method described by Yphantis, the extrapolated value of molecular weight was estimated to be 220,000, assuming the partial specific volume of the enzyme to be 0.75 ml/g. The molecular weight of the aminopeptidase obtained from yeast by Johnson was reported to be 670,000. Lehmann and Uhlig reported that the molecular weight of the aminopeptidase from *Asp. oryzae* and *Asp. parasiticus* were 60,000 and 32,000, respectively. Molecular weight of a thermophilic aminopeptidase obtained from *Talomyces duponti* by Chapuis and Zuber was reported to be approximately 400,000. Blaich presumed the existence of an aminopeptidase of high molecular weight produced by *Basidiomycetes*. The aminopeptidase purified from *Asp. sojae* X–816 in the present experiment had a distinctly different molecular weight from the other fungal aminopeptidase so far reported.

The homogeneity of the purified enzyme fraction was further examined by disc electrophoresis and immunoelectrophoresis. A single band was obtained by disc electrophoresis as shown in Fig. 5. A single precipitin band was obtained by immunoelectrophoresis (Fig. 6).

By the isoelectric focusing method with Ampholine Carrier Ampholites, the isoelectric point of the aminopeptidase was found to lie at pH 5.3 as shown in Fig. 7. This value was different from that of a leucine aminopeptidase from *Asp. oryzae* (pH 4.5).

**Fig. 4.** Schlieren Diagrams of an Ultracentrifuge Run of the Purified Aminopeptidase.

The concentration of the enzyme was 3.16 mg/ml in 0.01 M phosphate buffer solution (pH 7.5), containing 0.1 M NaCl. The photographs were taken at a phase-plate angle of 65° at 0, 5, 10, 15, 25 and 35 min after 47,300 rev/min was reached. The direction of sedimentation was toward right.
Experimental details are described in the legend to Materials and Methods. In the upper and lower wells, 85 μg and 34 μg of the antigen protein were set.

The aminopeptidase of dialyzed DEAE Sephadex A-50 fraction (4 ml, 436 μg protein/ml) was charged. Electrophoresis was done at 700 V for 112 hr at low temperature. After electrophoresis, 1.5 ml of each fraction of the Ampholine was taken and its pH was determined. After the dialysis of each fraction for overnight, the aminopeptidase activity was determined.

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The effect of pH on the activity of the purified aminopeptidase is shown in Fig. 8. The optimum pH on the activity of the water insoluble aminopeptidase was almost the same as that of the original enzyme itself. The effect of pH on the stability of the enzyme is shown in Fig. 9. The effect of temperature on the activity of the enzyme is shown in Fig. 10. The optimum temperature of the enzyme reaction was about 30°C. Thermal stability of the enzyme is shown in Fig. 11. The Km values for L-Leu-Gly-Gly at pH 7.5

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The enzyme had no endo-type activity at pH 7.5 when milk casein was used as a substrate. However, when casein partially hydrolyzed by an alkaline proteinase from Asp. sojae X-816 was used as a substrate, the degree of peptide bond hydrolysis reached 85% by the incubation with the aminopeptidase. For the determination of the degree of peptide bond hydrolysis, TNBS reaction was used.

The Km values for L-Leu-Gly-Gly at pH 7.5
An Aminopeptidase of *Aspergillus sojae*

**FIG. 9.** Effect of pH on the Stability of the Aminopeptidase.

The aminopeptidase was incubated for 45 hr at 4°C. Then the remaining activity was determined by the usual method (pH 7.5, 37°C). The activities remaining after the incubation was expressed as percentage of the initial activity.

**FIG. 10.** Effect of Temperature on the Activity of the Aminopeptidase.

The aminopeptidase and the substrate, L-Leu-Gly-Gly were incubated for 15 min at various temperature. After the incubation, the enzyme reaction was stopped by adding 5% borate decahydrate containing 0.06% cupric sulfate. The degree of hydrolysis of the substrate due to enzyme reaction was determined by the routine Cu²⁺-TNBS reaction. 

- ---, aminopeptidase; O---O, water insoluble aminopeptidase.

**FIG. 11.** Thermal Stability of the Aminopeptidase.

The aminopeptidase was incubated at pH 7.5 and various temperatures for 15 min. After the incubation the enzyme solution was cooled in ice cold water. Then the remaining activity was determined by the usual method (pH 7.5 and 37°C).

- ---, aminopeptidase; O---O, water insoluble aminopeptidase.

The substrate specificity of the aminopeptidase was studied (Table III). An aminopeptidase from *Asp. oryzae* isolated by Sato and Akatsuka did not split L-leucylglycine and L-leucyl-β-naphthylamide at pH 7.0 obtained from Lineweaver-Burk’s plots (Fig. 12) were found to be 45 mM and 2.2 mM, respectively.

**TABLE III. SUBSTRATE SPECIFICITY OF THE AMINOPÉPTIDASE**

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Activity Δ O.D./mg protein/min</th>
<th>Relative activity %</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-Leu-Gly-Gly</td>
<td>1.13</td>
<td>100</td>
</tr>
<tr>
<td>L-Leu-Gly</td>
<td>0.21</td>
<td>9.0</td>
</tr>
<tr>
<td>Gly-Gly-Gly</td>
<td>0.05</td>
<td>4.4</td>
</tr>
<tr>
<td>Gly-Gly</td>
<td>0.00</td>
<td>0.0</td>
</tr>
<tr>
<td>L-Leu-NH₂</td>
<td>0.00</td>
<td>0.0</td>
</tr>
</tbody>
</table>
FIG. 12. Lineweaver-Burk’s Plots for the Determination of \( K_m \) Values in the Hydrolysis of L-Leu-Gly-Gly and L-Leu-β-naphthylamide.

Details of the experiment are described in the legend to Materials and Methods.


at all.3) To the contrary, the aminopeptidase reported here possessed an activity to the above substrate. Furthermore, though the aminopeptidases from Asp. oryzae and Asp. parasiticus isolated by Lehmann and Uhlig5) split L-leucineamide, the aminopeptidase described here did not split the substrate. From these data the aminopeptidase obtained from Asp. sojae appears to be clearly distinguishable in its substrate specificity from the enzymes of the other species of Aspergilli.

Effect of various reagents on the enzyme is shown in Table IV. The aminopeptidase was remarkably inhibited by metal chelating reagents.

The enzyme described above was extracted from mycelial mats of the fungi obtained by surface culture. When a submerged culture of the fungi was performed with airation, the enzyme appeared little in the culture filtrate before the autolysis of the fungi began. After the autolysis of the mycelia, the enzyme was obtained from the culture filtrate. From these results the aminopeptidase is seemed to be an intracellular enzyme.

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### Table IV. Effect of Various Reagents on the Aminopeptidase Activity

<table>
<thead>
<tr>
<th>Reagent added</th>
<th>Concentration during enzyme incubation (mM)</th>
<th>Activity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EDTA</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>o-Phenanthroline</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>p-Chloromercuribenzoate</td>
<td>2.5</td>
<td>43</td>
</tr>
<tr>
<td>Iodoacetate</td>
<td>5</td>
<td>97</td>
</tr>
<tr>
<td>Dipyridyl</td>
<td>5</td>
<td>135</td>
</tr>
<tr>
<td>Disopropyl fluorophosphate</td>
<td>5</td>
<td>139</td>
</tr>
<tr>
<td>Control</td>
<td>---</td>
<td>100</td>
</tr>
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REFERENCES