Isolation and Characterization of an Enzyme with Esterase Activity from *Micropolyspora faeni*

ELIZABETH N. BANNERMAN* AND J. NICOLET

Institute of Veterinary Bacteriology, University of Berne, CH-3012 Berne, Switzerland

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The isolation and the characterization of one of the enzymes of *Micropolyspora faeni* that hydrolyzes the substrate N-benzoyl-DL-phenylalanine-beta-naphthyl ester and that seems to be of medical importance are described. This enzyme (enzyme 1) was isolated with an 86-fold purification by using the following seven steps: ammonium sulfate precipitation, gel filtration through Sephadex G-150, heat treatment, chromatography on diethylaminoethyl-cellulose, rechromatography on diethylaminoethyl-Sephadex, gel filtration through Sephadex G-200, and affinity chromatography. Enzyme 1 has a molecular weight of approximately 500,000 and maximum activity at pH 7.8 to 8.0 and at 20°C. The enzyme is stable between pH 7.5 and 10.5 and at temperatures up to 60°C. Its activity is not inhibited by ethylenediaminetetraacetic acid. It is, however, sensitive to diisopropyl phosphofluoride and phenylmethyl sulfonil fluoride. These properties and the ability to hydrolyze the esters of phenylalanine, tyrosine, and tryptophan without endopeptidatic activity and no marked proteolytic activity suggest that the enzyme is an esterase.

Although several reports (32, 33, 38), with a comprehensive review from Singleton and Amelunxen (33), have been made on the enzymatic activities of thermophilic bacteria, only very few of these reports are on enzymes from thermophilic actinomycetes (8, 24).

*Micropolyspora faeni* is a facultative thermophilic actinomycete (growth temperature range between 37 and 60°C), which is actually classified in the family Nocardiaceae (7). This gram-positive branched bacterium with short chains of 5 to 10 spores is a saprophyte known to be involved, under well-defined conditions, in the molding of hay. This phenomenon raised considerable medical interest when it was recognized that inhalation of dust from moldy hay may cause an allergic respiratory disease called farmer's lung, an extrinsic allergic alveolitis in humans and animals.

The immunological response to the inhalation of moldy hay shows the formation of precipitins principally against metabolic products of the molding agent *M. faeni* (9, 10, 36).

Walbaum et al. (36) and Biguet et al. (2) reported that a large number of these metabolic antigens were enzymes, which they identified as aminopeptidases, beta-galactosidases, beta-glucuronidases, carboxypeptidases, chymotripsin-like enzymes, esterases, lipases, trypsin-like enzymes, catalases, malic dehydrogenase, and peroxidase. The most interesting group of these enzymes proved to be those that hydrolyze the substrate N-benzoyl-DL-phenylalanine-beta-naphthyl ester. This substrate is usually used in the estimation of chymotrypsin activity and, hence, these enzymes were called chymotripsin-like enzymes. These enzymes were found by the same authors (2, 36) to be highly immunogenic. In a serological survey they found that 30 out of 33 sera reacted with at least one and up to a maximum of four fractions present in their *M. faeni* extract. Later, Nicolet et al. (27), testing sera from cattle affected with farmer's lung, obtained similar results. These enzymes have also been shown to be released extracellularly on hay during molding and may thus be inhaled as free active enzymes with the moldy hay dust (26).

Some microbial enzymes are known to be of medical importance. Those of *Bacillus subtilis*, for example, may cause allergic reactions in detergent factory workers exposed to them (11, 12, 20, 28, 39). Other microbial enzymes may also cause the emphysema syndrome (14).

Thus, the present study was undertaken to isolate good immunogenic enzymes of *M. faeni*, known in the medical literature as chymotripsin-like enzymes, with the aim of acquiring a better understanding of the nature of allergens and their role in the pathogenesis of farmer's
lungs. In this paper, only the isolation, purification, and general properties of one of these enzymes are presented.

MATERIALS AND METHODS

Preparation of extract. M. faeni strain 9535 (origin, London School of Hygiene and Tropical Medicine), kindly supplied by S. Walbaum (Lille), was used.

Nineteen batches of culture were grown. For each batch, 15 to 30 petri dishes (diameter, 18 cm) were used. The organism was inoculated on V8 agar, using the method of Walbaum and Biguet (35), which was, however, slightly adapted by covering the agar surface aseptically with cellophane sheets before inoculation.

After incubation at 40°C for 6 days, the cultures were harvested and extracted with 10 times their weight in a volume of 1 M glycine, pH 7.5, by the method of Macaulay and Cowles (19). After centrifugation at 14,000 rpm for 20 min, the Supernatant of the glycine extract was then dialyzed against running tap water for 48 h. From a total wet weight of 989 g, 10,000 ml of dialyzed extract was obtained. The glycine extraction was used to enhance the yield of metabolic enzymes, since this proved to be superior to the method of Walbaum and Biguet (35).

Protein assay. Estimation of protein content in the extract and enzyme samples was based on the Hartree (16) modification of the Lowry method. Bovine serum albumin (Fluka, Buchs, Switzerland) was used to plot a standard curve.

Enzyme assays. Enzymatic activity was assayed with N-benzoyl-DL-phenylalanine-beta-naphthyl ester (Sigma Chemical Co., St. Louis, Mo.) as substrate by a modification of the method of Rabin et al. (29).

To 0.5 ml of a test solution appropriately diluted in 0.05 M veronal buffer, pH 7.8, 2.5 ml of the substrate (0.1 mg/ml) in the same buffer was added, and the reaction mixture was incubated at 25°C for 1 h. After incubation, 0.5 ml of a freshly prepared solution of Fast Blue B salt (Merck, Darmstadt, Germany) (4 mg/ml) was added. This was followed 3 min later by the addition of 80% trichloroacetic acid. The resulting redish pigment was extracted by shaking the reaction mixture with 5 ml of ethyl acetate. After centrifugation, the organic layer was measured at 550 nm with a Hitachi UV/Vis spectrophotometer. From a calibration curve of pure beta-naphthol, the color density was converted to milligrams of beta-naphthol. The unit of enzymatic activity was defined as micromoles of beta-naphthol released per minute at 25°C.

Esterase activity was assayed with beta-naphthyl acetate (Serva, Heidelberg, Germany) as substrate, using the method of Seligman and Nachlas (31). The unit of esterase activity was defined as micromoles of beta-naphthol released at 25°C in 1 min.

The hydrolytic activity of the purified enzyme on synthetic peptides and esters was determined by incubating 1 ml of peptide or ester solution (0.1 mg/ml of 0.05 M tris(hydroxymethyl)aminomethane (Tris)-hydrochloride buffer, pH 8.0) with 0.1 ml of the enzyme solution at 25°C for 1 h. After incubation, a suitable portion of the assay mixture was chromatographed on Merck cellulose thin-layer chromatography plates in the solvent system n-butanol-acetic acid-water (4:1.5, by volume) and developed with ninhydrin as well as Pauly reagent. Bovine beta-chymotrypsin was used as the model.

Electrophoresis. Analytical disk electrophoresis was carried out by the method of Bloemendal et al. (3,4), using a 5.6% gel. A current of 5 mA/tube was used, and the electrophoresis was carried out for 40 min. Then 20-μl amounts of the test solution were subjected to electrophoresis.

After electrophoresis, the gels were stained for enzymatic activity as well as for protein. The enzymatic activity staining was carried out by the method of Uriel (34), using N-acetyl-DL-phenylalanine-beta-naphthyl ester (Schwarz/Mann, Div. of Becton, Dickinson & Co., Orangeburg, N.Y.) as substrate. Protein staining was carried out with Coomassie brilliant blue R 250 (Serva, Heidelberg, Germany), using the method of Chrambach et al. (6). Enzyme purification. Step 1. Precipitation with ammonium sulfate. To 1.000 ml of the dialyzed M. faeni glycerol extract, 518 g of ammonium sulfate was added (to give a 75% ammonium sulfate concentration) with stirring and left overnight at 4°C. The precipitate was collected by centrifugation at 10,000 rpm for 20 min. The sediment was dissolved in 1/10 of its original volume of 0.025 M Tris-hydrochloride buffer, pH 7.2, and then dialyzed against the same buffer for 48 h.

Step 2. Fractionation on Sephadex G-150. A column (2.6 by 95 cm) of Sephadex G-150 was equilibrated in 0.025 M Tris-hydrochloride buffer, pH 7.2, containing 0.1 M KCl. A total of 50 ml of the dialyzed ammonium sulfate precipitate solution was introduced into the column and washed with the equilibrating buffer. Both test solution and buffer were introduced from below, by means of a flow adapter. A mariotte flask was used to maintain an operating pressure of 25 cm of water. The ultraviolet absorption was recorded at 280 nm on a Hitachi UV/Vis spectrophotometer, and the protein profile was recorded with a W + W recorder (series 1100). A total of 22 runs were carried out, and 6- to 8-ml fractions were collected at a flow rate of 30 ml/h.

Step 3. Heat treatment. The pools of the first G-150 peak containing the enzyme to be studied were divided into 545-ml portions, heated at 56°C for 60 min in a water bath, and then cooled in an ice bath. The cooled solutions were then centrifuged at 14,000 rpm for 20 min. The supernatant was retained and dialyzed against 0.05 M Tris-hydrochloride buffer, pH 7.2, for 48 h.

Step 4. Chromatography on DEAE-cellulose. A column (5 by 20 cm) of diethylaminoethyl (DEAE)-cellulose-SS (Serva, Heidelberg) was equilibrated with 0.05 M Tris-hydrochloride buffer, pH 7.2. A total of 545 ml of the dialyzed test solution was placed on it and washed with the buffer until the ultraviolet absorption at 280 nm was near zero. The active substance was then eluted with a linear NaCl gradient.

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gradient (500 ml of 0.05 M Tris-hydrochloride, pH 7.2, plus 0.7 M NaCl added to 500 ml of 0.1 M NaCl in the same buffer). Ten-milliliter fractions were collected at a rate of 40 ml/h.

Step 5. Rechromatography on DEAE-Sephadex. A column (5 by 20 cm) of DEAE-Sephadex A-50 (Pharmacia, Uppsala) was equilibrated in 0.05 M Tris-hydrochloride buffer, pH 7.2. The test solution (eluate from step 4) was dialyzed against 0.05 M Tris-hydrochloride, pH 7.2, and then placed on the column and washed with the equilibrating buffer until the absorption at 280 nm was near zero. The active fraction was eluted with a linear NaCl gradient (0.8 M NaCl in 500 ml of buffer added to 0.1 M NaCl in 500 ml of buffer), and 9-ml fractions were collected. The rate of flow was 45 ml/h.

Step 6. Gel filtration on Sephadex G-200. The required active fractions from step 5 were pooled and concentrated to 30 ml and dialyzed for 48 h against 0.05 M Tris-hydrochloride containing 0.1 M KCl. The dialyzed solution was placed on a Sephadex G-200 column (2.6 by 95 cm) that had been equilibrated in 0.05 M Tris-hydrochloride buffer, pH 7.2, containing 0.1 M KCl. This was then fractionated. Both enzyme and buffer solutions were introduced from below by means of a flow adapter. The rate of flow was 24 ml/h, and 4-ml fractions were collected.

Step 7. Affinity chromatography. Equilibration and chromatography on a column (1.5 by 8 cm) of agarose-epsilon-amino-caproyl-h-tryptophan methyl ester (Miles Yeda Ltd., Israel) was carried out per instructions (Miles Yeda Ltd.). Fractions (3 ml) were collected, and the rate of flow was 12 ml/h.

RESULTS

Glycine whole extract. The glycinic extract of M. faeni cultures revealed, after polyacrylamide gel disc electrophoresis, at least six fractions that reacted with the substrate N-acetyl-DL-phenylalanine-β-naphthyl ester. The bands were numbered 1 to 6, and the first slow-moving band (cathodic) numbered 1 was arbitrarily termed enzyme 1 (Fig. 1b). The glycinic extract also proved to be rich in other protein fractions as well (Fig. 1a), since about 25 bands could be counted.

Purification of enzyme 1. The quantitative results of the steps followed during the purification of the enzyme are shown in Table I. The eluate from the affinity chromatography (step 7) showed only one band in disc electrophoresis.

Step 1. Precipitation with ammonium sulfate. A 75% ammonium sulfate precipitation gave the maximum yield in enzymatic activity in the sediment and almost negligible yield in the supernatant. After the sediment had been resuspended, it was subjected to polyacrylamide gel electrophoresis, which subsequently revealed an increase in the number of bands that reacted with the substrate N-acetyl-DL-phenylalanine-β-naphthyl ester. At least one other fraction appeared that was not previously detected in the glycinic extract. Bands 1 and 6 also reacted with the acetyl esterase substrate β-naphthyl acetate.

Step 2. Gel filtration on Sephadex G-150. Initial separation of the enzyme 1 fraction was achieved at this step. Three peaks were obtained. Peak I (from 28 to 41) and II (from 42 to 100) fractions showed enzymatic activity with both N-benzoyl-DL-phenylalanine-β-naphthyl ester and beta-naphthyl acetate, whereas peak III (from 106 to 140) did not. The polyacrylamide gel patterns showed that the enzyme 1 fraction was in the first peak and that this fraction was eluted together with other fractions that also hydrolyze the substrate N-benzoyl-DL-alanine-β-naphthyl ester. Fractions 28 to 41 from all 22 runs, which contained among other enzymes the required enzyme 1 fraction,
were combined and subjected to temperature treatment (next step).

Step 3. Heat treatment. The protein content of the peak I pool, after heating at 60°C for 1 h, decreased from 1.6 to 1.45 mg/ml, whereas the units of activity increased slightly from 1.7 to 1.83/ml (Table 1). The disc electrophoretic pattern showed a slight decrease in the intensity of the protein bands, with the complete disappearance of about four bands.

Step 4. Chromatography on DEAE-cellulose-SS. Fractions 91 to 120 were found to be active when assayed with the substrate N-benzoyl-DL-phenylalanine-β-naphthyl ester. The disc electrophoretic pattern showed that most of the protein, as well as the required enzymatic fraction, was eluted at the same time although there was a decrease in protein content with a concomitant increase in the units of chymotrypsin activity per milliliter of eluate (Table 1).

Step 5. Rechromatography on DEAE-Sephadex A-50. Two active peaks (from 93 to 101 and from 102 to 140) were obtained. Fractions 105 to 136 contained the required enzyme 1. In the disc electrophoretic patterns, only four protein bands could be seen, and the enzymatic staining of gel revealed only the enzyme 1 band.

Step 6. Gel filtration on Sephadex G-200. Further purification from the accompanying proteins was achieved by gel filtration through Sephadex G-200. Only one other protein band was eluted together with enzyme 1. The required enzyme was found in fractions 18 to 32.

Step 7. Affinity chromatography. Purification was achieved by chromatography on agarose-episilon-amino-caproyl-d-tryptophan methyl ester. Enzyme 1 was found in fractions 6 to 15, and the contaminating protein was found in fractions 18 to 30.

The purification procedure is summarized in Table 1. The purified enzyme (enzyme 1) gave a final yield of 7.2%. From the 989 g, net weight, of M. faeni culture, 418 mg of lyophilized enzyme containing 1.7 mg of total protein was obtained. An 86-fold increase in specific activity over the ammonium sulfate precipitate fraction was obtained.

**Properties of enzyme 1. (i) Proteolytic properties.** Enzyme 1 slightly hydrolyzes hemoglobin and, to a much lesser extent, casein. Whereas 10 μg of chymotrypsin released 4 mmol of tyrosine from hemoglobin, 1 mg of enzyme released the same amount of tyrosine. In the case of casein, 1 mg of enzyme hydrolyzed the substrate to the same extent as did 2 μg of chymotrypsin. An increase in the amount of enzyme used did not produce any appreciable increase in the hydrolysis of casein.

(ii) Other enzymatic properties. Enzyme 1 hydrolyzes both N-acetyl- and N-benzoyl-DL-phenylalanine-beta-naphthyl ester. It also hydrolyzes the acetyl esterase substrate beta-naphthyl acetate. A 1-mg amount of enzyme produced 145 × 10³ U of esterase activity from naphthyl acetate.

(iii) Hydrolysis of synthetic peptides and esters revealed on thin-layer chromatography. Enzyme 1 was found to hydrolyze the esters of phenylalanine, tyrosine, and tryptophan but not the analogous amides. Bovine alphachymotrypsin, which was used as a model, was found to hydrolyze the substrate d,l-asparagine(NH₄)₂-valine⁵-d-histidine⁵-hypertensin II as well as the esters and amidates of tyrosine and phenylalanine but not those of tryptophan. The enzyme also did not hydrolyze the tripsin substrates N-benzoyl-arginine amide and its analogous ester nor the collagenase substrate 4-phenylazobenzoxyl-carbonyl-l-prolyl-l-leucylglycyl-l-prolyl-d-arginine. It also does not hydrolyze d,l-asparagine⁵-valine⁵-d-histidine⁵-hypertensin II.

(iv) Effect of pH. The optimum pH for enzyme 1 was determined with the N-benzoyl-DL-phenylalanine-β-naphthyl ester. Activity was assayed under standard conditions (see above) in 0.05 M Tris-maleate buffer, pH 4.0 to 8.0, and in 0.05 M Tris-hydrochloride buffer, pH 7.0 to

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**Table 1. Purification of enzyme 1**

<table>
<thead>
<tr>
<th>Step no.</th>
<th>Fraction</th>
<th>Vol (ml)</th>
<th>Total protein (mg)</th>
<th>U/ml (×10³)</th>
<th>Total units (×10³)</th>
<th>Yield in activity (%)</th>
<th>Sp act (U/mg of protein) (×10³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>75% ammonium sulfate precipitate</td>
<td>1,100</td>
<td>10,450</td>
<td>15.0</td>
<td>16,500</td>
<td>100</td>
<td>1.58</td>
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<tr>
<td>2</td>
<td>Sephadex G-150 pool I</td>
<td>2,180</td>
<td>3,488</td>
<td>1.7</td>
<td>3,706</td>
<td>100</td>
<td>1.06</td>
</tr>
<tr>
<td>3</td>
<td>Heating at 60°C</td>
<td>2,180</td>
<td>3,161</td>
<td>1.33</td>
<td>3,898.4</td>
<td>107.1</td>
<td>1.26</td>
</tr>
<tr>
<td>4</td>
<td>DEAE-cellulose</td>
<td>1,200</td>
<td>192</td>
<td>3.03</td>
<td>3,636.0</td>
<td>97.6</td>
<td>18.94</td>
</tr>
<tr>
<td>5</td>
<td>DEAE</td>
<td>290</td>
<td>38.5</td>
<td>4.8</td>
<td>1,392.0</td>
<td>37.4</td>
<td>36.16</td>
</tr>
<tr>
<td>6</td>
<td>Sephadex G-200</td>
<td>120</td>
<td>12.0</td>
<td>5.04</td>
<td>604.8</td>
<td>16.3</td>
<td>50.40</td>
</tr>
<tr>
<td>7</td>
<td>Agarose-episilon-amino-caproyl-d-tryptophan methyl ester</td>
<td>80</td>
<td>2.0</td>
<td>3.4</td>
<td>272.0</td>
<td>7.2</td>
<td>136.0</td>
</tr>
</tbody>
</table>
10.0. The optimal pH for the hydrolysis of the substrate was found at pH 7.8 and 8.0. Enzyme 1 was stable, with 100% activity, between pH 7.0 and 10.5 at 25°C.

(v) Effect of temperature. For the effect of temperature on the stability of enzyme 1, 1-ml portions of the enzyme solution were incubated at temperatures ranging from 20 to 80°C for 60 min. After incubation, the enzyme solutions were immediately cooled in an ice bath and then centrifuged at 10,000 rpm for 10 min. The supernatants were assayed. The maximal enzymatic activity of enzyme 1 was observed from 20 up to 60°C. At 70°C, about 61% of the activity was still present, and at 80°C about 100% was present.

(vi) Effect of known inhibitors. Ethylenediaminetetraacetic acid had negligible effect on the enzymatic activity. Only 1.8% of the total activity was inhibited by a 10⁻¹ M concentration of ethylenediaminetetraacetic acid. Diisopropyl phosphofluoridate totally inhibited the enzyme at a 10⁻¹ M concentration. Phenylmethyl sulfonyl fluoride totally inhibited the enzyme at a 10⁻³ M concentration. At a 10⁻⁴ M concentration, 87% of the enzyme was inhibited.

(vii) Estimation of molecular weight. By Sephadex G-200 gel filtration, using blue dextran 2000 and bovine β-chymotrypsinogen (molecular weight, 25,000), bovine serum albumin (molecular weight, 65,000 to 70,000), human gamma globulin (molecular weight, 150,000 to 170,000), apoferritin (molecular weight, 460,000 to 490,000), thyroglobulin (molecular weight, 660,000 to 680,000), and ferritin (molecular weight, 700,000 to 800,000) as marker proteins and the method of Andrews (1), the molecular weight was estimated to be approximately 720,000.

To determine the subunit composition of enzyme 1, the purified enzyme solution was heated at 37°C for 2 h in the presence of 1% sodium dodecyl sulfate and 1% β-mercaptoethanol and subjected to electroforeisis in acrylamide gels containing 0.1% sodium dodecyl sulfate (37). The migration distance relative to the migration distance of the above marker proteins was recorded. Four bands, one of which showed strong activity on staining for enzymatic activity, were observed. The molecular weight of the strongly reactive band was estimated to be 500,000. The molecular weights of the three inactive bands were estimated to be approximately 200,000, 20,000, and 18,000.

**DISCUSSION**

A few reports have been made on the presence of esterases in fungi (5) and in bacteria (17, 25, 30). Although the relationship of these esterases to our enzyme 1 from *M. faeni* is not known, some of the properties of the esterases from bacilli are, however, similar. Recently, Higerd and Spizizen (17) isolated two esterases from *B. subtilis*. These esterases, like enzyme 1, showed very low proteolytic activity. Likewise, other enzymes from *B. subtilis* (15, 22) and a cytoplasmic endopeptidase from *B. megaterium* (23) have been reported to be highly active on ester substrates but to have low activity on protein substrates.

Another property of enzyme 1 that is comparable with that of one of the esterases isolated by Higerd and Spizizen (17) is its thermostability. Esterase A of *B. subtilis*, like enzyme 1, retained all of its original activity after 10 min at 60°C. Enzyme 1, however, shows a higher thermal stability than does the esterase from *B. subtilis* in retaining 100% of its activity after 1 h at 60°C. Even after 1 h at 80°C, 10% of the activity was still retained.

The metal chelating reagent ethylenediaminetetraacetic did not inhibit enzyme 1, which indicates that the enzyme is not a metalloenzyme. It is, however, inhibited by the organophosphorus reagent diisopropyl phosphofluoridate and by phenylmethyl sulfonyl fluoride, suggesting that a serine residue is essential for the activity. The sensitivity towards these inhibitors and the ability to hydrolyze various esters, especially benzoyl or acetyl phenylalanine-β-naphthyl esters and the ethyl ester of benzoyl or acetyl tyrosine, ally enzyme 1 to the" chymotrypsin-like" enzymes of Walbaum et al. (36) and especially to the Chy 2 fraction. Like these enzymes, enzyme 1 is found in culture filtrates (36; E. S. Bannerman, Ph.D. thesis, Univ. of Berne, Berne, Switzerland, 1975) and thus seems to be extracellular. However, the enzyme is found in higher concentrations when the cells are disrupted with glycine. Since esterases are known to be mostly intracellular, this seems to suggest that the enzyme may be membrane bound and can be released during growth and, as such, is not a true extracellular enzyme.

Since enzyme 1 did not show any peptidase activity as did chymotrypsin, it must be regarded as an esterase. The enzyme probably belongs to the same group as the β-esterases of the carboxylic esters hydrolases (18). It is interesting to note that to this group belong several important endopeptidases, such as chymotrypsin, trypsin, elastase, subtilisin, and thrombin, all of which show esterase activity toward certain substrates and which have a serine residue at the active site.

A property unique to enzyme 1 is its high molecular weight. Higerd and Spizizen (17) re-
ported that the molecular weight of esterase A from *B. subtilis* was estimated at 160,000 by gel filtration but was subsequently found to be 31,000 after electrophoresis in sodium dodecyl sulfate gel. Matsubara and Feder (21) reported that the molecular weight of most microbial proteases with esterolytic activity lies in the range of 20,000 to 25,000, with very few exceptions, the highest recorded being 52,000 for a protease of *Aspergillus oryzae*. The molecular weight of enzyme 1 was estimated to be approximately 720,000 by gel filtration and 500,000 after sodium dodecyl sulfate-acrylamide gel electrophoresis. Like acetylcholinesterase (molecular weight, about 250,000), enzyme 1 was found to have unilateral subunit composition (19). Although the carboxylic ester hydrolases do possess subunits, the molecular weights are in the range of 162,000 to 168,000 (18).

The pH optimum for enzyme 1 falls within the range reported for most carboxyl esterases (pH 7.5 to 9.0) (18). Enzyme 1 has optimal activity at pH 7.8 and 8.0. It is stable between pH 7.5 to 10.5 and up to 60°C.

This enzyme has also been found to be highly immunogenic for rabbits and guinea pigs after experimental aerosol exposure with *M. faeni* (M. Wanner, DMV thesis, Univ. of Berne, Berne, Switzerland, 1975). It seems also that patients affected with farmer’s lung constantly harbor precipitins against this enzyme (36), also suggesting a high immunogenicity after natural exposure. On the basis of the present characterization of this enzyme, it seems that the molecular weight is one of the determinant factors for the stimulation of the immune response. The exact role played by enzyme I in the pathogenesis of the extrinsic allergic alveolitis due to inhalation of *M. faeni* and its products is, however, still not clear. With the purification of this seemingly important esterase of *M. faeni*, we seem to have a tool for further experimental investigations that could throw more light on the complex problems of the inhalation of biological substances.

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LITERATURE CITED


