Purification and Characterization of a Novel Peptidase (IImes) from Mesquite (Prosopis velutina) Pollen*

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Although the mesquite plant (Prosopis velutina) is not as widely distributed as some other allergenic species, its pollen can induce serious pollinosis in areas where it is localized. We previously isolated and characterized a peptidase from mesquite pollen with trypsin-like specificity (peptidase IImes) (Matheson, N., Schmidt, J., and Travis, J. (1995) Am. J. Respir. Cell Mol. Biol. 12, 441–448). Now we have characterized a second enzyme with specificity for hydrophobic residues (mesquite pollen peptidase IImes). This enzyme has a molecular mass near 92 kDa and activity that was not affected by reducing or chelating agents but was inhibited by specific synthetic serine proteinase inhibitors and the aminopeptidase inhibitor bestatin. However, it was not inhibited by human plasma proteinase inhibitors, nor did it inactivate any of those tested. The enzyme possessed amidolytic activity against p-nitroanilide substrates most effectively after alanine residues and also displayed aminopeptidase activity against non-p-nitroanilide peptides with a preference for phenylalanine. This specificity for hydrophobic amino acid residues was corroborated by inhibition studies with chloromethyl ketone and organophosphonate inhibitors. More interesting from a physiological point of view is that the bioactive peptides, angiotensins I and II and vasoactive intestinal peptide, were also hydrolyzed rapidly, indicating an ability of peptidase IImes to act also as an aminopeptidase. Because these bioactive peptides play a role in the inflammatory responses in allergic asthma, our data suggest that the purified mesquite pollen peptidase IImes may be involved in the degradation of neuro- and vasoactive peptides during pollen-initiated allergic reactions.

Asthma is an allergic inflammation of the lungs which can occur after allergen sensitization. Such inflammatory responses are normally meant to defend against invading organisms or particulates or to effect tissue repair and are thus beneficial; however, in asthma, the response becomes exaggerated (perhaps because of a hereditary predisposition (1)), leading to adverse effects on the airways (2). Macrophages phagocytize the allergens introduced to the lungs by exposure to various environmental irritants such as dust, pollutants, and pollen, and process them to smaller fragments. As antigen-presenting cells, they then activate T-cells (3, 4) to stimulate B-cells to produce IgE. This immunoglobulin, when bound to a presenting cell, then activates T-cells (3, 4) to stimulate

Experimental Procedures

Materials

H-Val-pNA, H-Leu-pNA, N-Suc-Ala-Ala-Pro-Phe-pNA, N-Suc-Ala-Ala-Ala-Val-Leu-pNA, N-Suc-Ala-Ala-Ala-Val-pNA, N-Suc-Phe-pNA, benzoyl-stry-Ang-pNA, TPCK, TLCK, iodoacetate, active oxygen species, and chemotractants (5). The interaction of these mediators leads to the pathology of asthma, including bronchoconstriction, hypertrophy of airway smooth muscle, vasodilation, submucosal edema, and mucus hypersecretion (6). Also, the mucociliary apparatus becomes dysfunctional, reducing the clearance of inhaled particulates. Epithelial cells lining the airways are shed during this inflammatory response, removing a protective barrier (2) and are also a source of neutral endopeptidase (which normally degrades various bronchoconstrictor peptides (7)) while exposing nerve endings (8) that secrete neuropeptides such as vasoactive intestinal peptide (VIP)1 and substance P, and vasoactive peptides (e.g. angiotensin II). VIP, a neurotransmitter of the non-adrenergic inhibitory system (9), has an anti-inflammatory effect inhibiting lymphocyte proliferation and interleukin-2 release and is also a potent bronchodilator (10). Substance P, a neurotransmitter of the non-adrenergic excitatory system (11), in contrast, has a proinflammatory effect, increasing vascular permeability and bronchoconstriction, causing macrophages to release proinflammatory substances, and enhancing phagocytosis by neutrophils and macrophages (12). Angiotensin II is a strong vasoconstricting agent (13).

Pollen is one of the major initiators of allergic asthma. This gamete contains proteins (allergens) that are solubilized in the airway mucus and proceed to induce an immunological response. However, other proteins are also released, of which several have proven to be oligopeptidases (14–16). Because these latter enzymes appear to be members of a family of pollen oligopeptidases with varying specificities for peptide hydrolysis, we propose to name them, at least temporarily, as: peptidases IImes and Irag (trypsin-like specificity from both mesquite and ragweed pollens), peptidase IIrag (chymotrypsin-like specificity from ragweed pollen), and, as described in this report, peptidase IImes (hydrophobic amino acid specificity from mesquite pollen), an enzyme that rapidly degrades VIP, angiotensin II, and its precursor, angiotensin I. We suggest that through exo- and oligopeptidase activity, pollen may have the capability for participation in the inflammatory processes in allergic asthma by mechanisms other than those involving its immunological component.

The abbreviations used are: VIP, vasoactive intestinal peptide; pNA, p-nitroanilide; Suc, succinyl; TPCK, tosyl-L-phenylalanine chloromethyl ketone; TLCK, N′-tosyl-L-lysine chloromethyl ketone; AEBSF, 4,4′-aminozobenzyl benzzenesulfonyl fluoride; HIV, human immunodeficiency virus; Bis-Tris, 2-[bis(2-hydroxyethyl)aminol]-2-(hydroxymethyl)-propane-1,3-diol; FPLC, fast protein liquid chromatography; Tricine, N-[2-hydroxy-1,1-bis(hydroxymethyl)ethyl]glycine; HPLC, high performance liquid chromatography.

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amide, bestatin ([(2S,3R)-3-amino-2-hydroxy-4-phenylbutanoyl]-l-leucine), angiotensins I and II, VIP, atrial natriuretic peptide, Bradykinin, substance P, neurotensin, Phe-Gly-Leu-Met (substance P fragment) (peptide 1), Phe-Ser-Trp-Gly-Ala-Glu-Gly-Arg (active fragment of myelin basic protein) (peptide 2), Ala-Ser-Thr-Thr-Asn Tyr-Thr-Pro-Ile-Val-Glu-Aspartate T = HIV inhibitor) (peptide 3), and Leu-Pro-Pro-Pro-Ser-Arg (lymphocyte-activating pentapeptide from the F region of human IgG1) (peptide 4) were obtained from Sigma. H-Ala-Asp-NA, H-Ala-Ala-NA, Ac-Ala-NA, H-Ala-Ala-NA, H-Phe-NA, H-Ile-NA, H-Ala-Phe-NA, and TLCK) and organophosphonate inhibitors were kindly provided by Dr. Justin O. Schmidt (Carl Hayden Bee Research Center, Tuscon, AZ). All choloromethyl ketone (except TPCK and TLCK) and organophosphonate inhibitors were kindly provided by Dr. James Powers (Georgia Institute of Technology, Atlanta).

**Methods**

**Enzyme Extraction and Purification**—Mesquite pollen (100 g) was extracted by stirring in 400 ml of 0.02 M Bis-Tris, pH 6.5, 5 mM CaCl₂ (buffer A) overnight at 4 °C. Purification of the enzyme was performed using essentially the procedures described previously (14) with ammonium sulfate fractionation, precipitation of co-chromatins, and Cibacron blue-Sepharose, DEAE-Sephalac, and phenyl-Sepharose chromatography. The active elute from the phenyl-Sepharose column was dialyzed overnight at 4 °C against buffer A with two changes and concentrated to 20 ml using an Amicon P-30 membrane. The final step of purification involved the application of the dialyzed and concentrated enzyme solution to a Mono Q FPLC column (Amersham Pharmacia Biotech) equilibrated with buffer A. The column was washed with buffer A for 5 min, followed by a 0–0.05 M NaCl gradient for 5 min, then a 0.05–0.15 M NaCl gradient for 50 min during which the enzyme activity was eluted. The native conformation of the enzyme was obtained by polyacrylamide gel electrophoresis using a Tris-HCl/Tricine buffer system (17) omitting SDS.

**Molecular Weight Determination**—The molecular weight of the purified enzyme (peptidase IImes) was determined by both SDS-polyacrylamide gel electrophoresis using a Tris-HCl/Tricine buffer system (17) with or without reducing conditions and by gel filtration on a Sephadex G-150 column (2.2 × 90 cm).

**Enzyme Assays**—For routine assays during purification, pH optimum determination, temperature optimum determination, and the effects of inhibitors, the activity of peptidase IImes was determined by measuring the rate of release of pNA by spectrophotometry at 405 nm with H-Ala-pNA (1 mM, final concentration) in either 0.2 or 1.0 ml of 0.1 M Tris-HCl, pH 8.0, 0.15% dimethyl sulfoxide at 25 °C. Inhibitor studies, the enzyme was incubated with inhibitors for 25 min at 25 °C before the substrate (H-Ala-pNA) was added. Amidolytic activity of several substrates (1 mM, final concentration) was determined in 0.2 ml of the same buffer and temperature as above. Protein concentration was determined by the bicinchoninic acid-Cu(II) sulfate procedure with bovine serum albumin as the standard (18).

**Sequence Analysis**—Peptidase IImes (1.06 nmol) was denatured by boiling in 1% SDS followed by incubation with 0.017 nmol of high molecular weight Arg-gingipain from Porphyromonas gingivalis (19) in 0.2 ml of 0.02 M Tris-HCl, pH 7.6, and 1 mM fresh cysteine overnight at 37 °C. After SDS-polyacrylamide gel electrophoresis of the digest and electrophoretic transfer to a polyvinylidene difluoride membrane, sequence analysis was performed with an Applied Biosystems Procise Protein sequencer using the program designed by the manufacturer.

**Enzyme Specificity and Kinetics**—For specificity studies, the purified enzyme (peptidase IImes) was monitored for amino acid composition by mass spectrometry. Some of the cleavage products from peptidase IImes proteolysis were identified by HPLC as described above. The increase in the peak area of the product with time was used to determine the rate of peptide cleavage. Vmax and Km values for amino acid p-NA’s were determined using substrate concentrations ranging from 18.75 to 250 μM with the final concentrations of enzyme from 2.0 to 19.1 nm in 0.1 M Tris-HCl, pH 8.0, 0.125% dimethyl sulfoxide at 25 °C. Values for the bioactive peptides were determined with substrate concentrations ranging from 10 to 82 μM, with the final concentration of enzyme from 2.2 to 25.4 nm in 0.1 M Tris-HCl, pH 8.0, 5 mM CaCl₂ at 25 °C with peptidase IImes and 37 °C with peptidase IImes. Aliquots of 35 μl were removed at various times and added to 2 μl of 20% trifluoroacetic acid to stop the reaction. Each sample was subjected to HPLC as described above. The increase in the peak area of the product with time was used to determine the rate of peptide cleavage. Vmax and Km values were determined by using Hyperbolic Regression Analysis.

**RESULTS**

**Enzyme Purification**—Peptidase IImes was readily liberated from the pollen grains by gentle stirring with buffer at 4 °C, with 50% of the activity being released by 2.5 h, and maximum activity at 6 h (data not shown). However, because the enzyme was very stable, extraction was usually performed overnight as a matter of convenience.

As shown in Table I, several steps were required to purify peptidase IImes with the scheme utilized being essentially equivalent to that performed for the isolation of peptidase IImes (14). Although a single enzyme activity directed toward hydrolysis of H-Ala-pNA was obtained during all procedures up to the Mono Q FPLC step, three activities separated during this final

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<table>
<thead>
<tr>
<th>Fractionation step</th>
<th>Total activitya</th>
<th>Total protein</th>
<th>Specific activity</th>
<th>Purification Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude extract</td>
<td>81,700</td>
<td>10,200</td>
<td>8.0</td>
<td>1 100</td>
</tr>
<tr>
<td>(NH₄)₂SO₄ 30–60%</td>
<td>75,800</td>
<td>5,520</td>
<td>13.7</td>
<td>2 93</td>
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<tr>
<td>pH 4.5 supernatant</td>
<td>67,800</td>
<td>1,450</td>
<td>46.7</td>
<td>6 83</td>
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<tr>
<td>Monoclonal antibody-like Sepharose</td>
<td>36,400</td>
<td>374</td>
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<td>12 45</td>
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<tr>
<td>DEAE-Sephalac</td>
<td>34,600</td>
<td>122</td>
<td>284.0</td>
<td>35 42</td>
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<tr>
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<td>24,300</td>
<td>7.4</td>
<td>3280.0</td>
<td>410 30</td>
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<td>Mono Q FPLC</td>
<td>19,700</td>
<td>0.72</td>
<td>27,400.0</td>
<td>3,420 24</td>
</tr>
</tbody>
</table>

* Based on enzymatic activity using Cibacron blue-Sepharose where 1 unit = nmol of pNA released/min.

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The Hyperbolic Regression Analysis program, written by J. S. Eastbery (University of Liverpool, U. K.), was obtained through shareware.
chromatographic procedure. However, all forms exhibited identical specific activities against either H-Ala-pNA or H-Leu-pNA, all were 92 kDa, and all were inhibited by TPCK, 3,4-dichloroacoumarin, AEBSF, or the aminopeptidase inhibitor bestatin. A native polycrylamide gel revealed a single, diffuse, unresolvable band (data not shown). Because of these identical properties, we assumed that the various forms were isoforms of each other, pooled them together, and utilized the combined enzyme in the studies described below.

As in the case of peptidase I\textsubscript{mes}, peptidase II\textsubscript{mes} was also stable for at least several months at \(-20^\circ C\), although frequent freezing and thawing caused some loss. However, in comparison, Ca\textsuperscript{2+} was not required either for stability or activity.

Physical Properties—Treatment of the purified enzyme with SDS followed by gel electrophoresis revealed a major band with a molecular mass of 92 kDa and some very faint minor bands (Fig. 1). The molecular mass of the major band agreed very well with that determined by Sephadex G-150 gel filtration of active enzyme (96 kDa). Unfortunately, no amino-terminal sequence could be found, indicating that this enzyme has a blocked amino terminus. Utilizing the amidolytic activity assays with H-Ala-pNA, it was found that the enzyme had a broad pH optimum from pH 7.5 to 9.5 and was stable for at least 48 h at pH 8.0 and 25° or 37°C.

Amidase and Peptidase Specificities—Peptidase II\textsubscript{mes} activity was tested with several amino acid and peptide p-NAs (Table II). H-Ala-pNA was the preferred substrate by far (and thus was used in general assays), with the next best being H-Ala-Ala-pNA. Longer peptides were even less effective as substrates. An NH\textsubscript{2}-terminal blocking group nearly or completely abolished activity, with Suc-Ala-Ala-Ala-pNA, Suc-Phe-pNA, Ac-Ala-pNA, and Ac-Ala-Ala-pNA not acting as substrates at all; however, there was substantial activity against the corresponding non-succinylated or non-acetylated p-NAs. Ac-Ala-pNA and Ac-Ala-Ala-pNA, in fact, acted as inhibitors at 10 and 20 times the concentration of the substrate, H-Ala-pNA. These results indicate that the amidolytic activity of peptidase II\textsubscript{mes} requires a free amino group at the NH\textsubscript{2} terminus of a substrate, whereas a blocked NH\textsubscript{2} terminus can create a competitive inhibitor. It is possible that the enzyme may be sequentially removing the NH\textsubscript{2}-terminal amino acid or cleaving internally in the peptide pNA substrates since, as shown below utilizing non-pNA peptide substrates, both aminopeptidase and oligopeptidase activity could be detected.

The demonstration of exo- and oligopeptidase activity of peptidase II\textsubscript{mes} against both bioactive and randomly selected peptides is given in Table III. In peptides chosen because they contained unblocked phenylalanine, leucine, or alanine residues at the NH\textsubscript{2} terminus, hydrolysis at the amino terminus occurred at low E:S molar ratios: 1:8,000 for enzyme:FGLM, 1:2,000 for LPSP, and 1:100 for both FSWGAEQQR and ASTTNYT. Internal residues of alanine and leucine were untouched at these short times of incubation and low E:S ratios. The enzyme was particularly effective in cleaving after NH\textsubscript{2}-terminal phenylalanine residues, especially in the tetrapeptide, FGLM. Hydrolysis after either the NH\textsubscript{2}-terminal leucine or alanine residues was much slower (Fig. 2). Thus, peptidase II\textsubscript{mes} exhibited aminopeptidase activity. It is puzzling, however, why phenylalanine should be preferred rather than alanine, as was seen with the pNA substrates.

Non-pNA bioactive peptides of 8–28 amino acids were excellent substrates at E:S molar ratios of 1:400 to 1:600 (Table III). Angiotensins I and II were cleaved relatively rapidly (Fig. 3) with complete hydrolysis by 50 and 100 min, respectively, at these very low ratios. VIP was fragmented somewhat more slowly, 40% being cleaved by 90 min; atrial natriuretic peptide, bradykinin, substance P, and neurotensin were only slowly degraded. These results indicate that peptidase II\textsubscript{mes} also has oligopeptidase activity. This is not a novel concept because multiple reports indicate that many purified enzymes have both aminopeptidase and oligopeptidase activity. These include cathepsin H (20). In addition, many peptidylpeptidases, including cathepsin B (21), also have oligopeptidase activity. In all cases, this has been demonstrated with peptide substrates rather than with proteins, a result that is paralleled in this study.

The hydrolysis of all bioactive peptides occurred exclusively and internally after isoleucine, leucine, phenylalanine, alanine, and methionine residues (most rapidly after isoleucine) in the substrates tested but not after every such residue in every peptide. Six of the peptides had one to three cleavages, but VIP was cleaved at seven sites. Hydrolysis after methionine residues also occurred in the dipeptides Met-Phe and Met-Tyr, which are usually used as internal standards in determining kinetic constants by HPLC. Also, a small amount of inhibition
(20%) of the hydrolysis of the bioactive peptides occurred when Met-Phe or Met-Tyr were present. No preference for either hydrophilic or hydrophobic residues in either the P<sub>1</sub> or P<sub>2</sub> position was obvious. (The amino acid residues in substrates are numbered as P<sub>3</sub>,P<sub>2</sub>,P<sub>1</sub>, etc. toward the NH<sub>2</sub> terminus from the cleavage site and P<sub>1</sub>,P<sub>2</sub>,P<sub>3</sub>, etc. toward the COOH terminus (22).)

Although phenylalanine or alanine was the favored NH<sub>2</sub>-terminal residue for aminopeptidase activity and isoleucine for the oligopeptidase activity, cleavage was exhibited in both cases after all three residues. Significantly, no NH<sub>2</sub>-terminal amino acid cleavage occurred with the bioactive peptides tested because none had a suitable hydrophobic residue in that position, supporting our contention of a single enzyme with two activities of defined specificities.

As with mesquite pollen peptidase II<sub>mes</sub>, peptidase II<sub>mes</sub> only very slowly hydrolyzed proteins, such as azocasein and blue hide powder (a matter of days), whereas the plasma serpins, α<sub>1</sub>-proteinase inhibitor and α<sub>1</sub>-antichymotrypsin, were not hydrolyzed despite the known susceptibility to proteolytic attack within their respective reactive site loops. These results differ from data obtained recently with a chymotrypsin-like peptidase from ragweed pollen which rapidly inactivated human

<table>
<thead>
<tr>
<th>Peptide</th>
<th>Sequence</th>
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<tbody>
<tr>
<td>Angiotensin I</td>
<td>D-R-V-Y-I-H-P-F-H-L</td>
</tr>
<tr>
<td>Angiotensin II</td>
<td>D-R-V-Y-I-H-P-F</td>
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<tr>
<td></td>
<td>K-K-Y-L-N-S-I-L-N</td>
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<tr>
<td>Atrial natriuretic peptide</td>
<td>S-L-R-R-S-C-F-G-G-R-M-D-R-I-G-A-Q-S-G-</td>
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<tr>
<td></td>
<td>L-G-C-N-S-P-R-Y</td>
</tr>
<tr>
<td>Bradykinin</td>
<td>R-P-P-G-F-S-P-F-R</td>
</tr>
<tr>
<td>Substance P</td>
<td>R-P-K-P-Q-Q-F-F-G-L-M</td>
</tr>
<tr>
<td>Neurotensin</td>
<td>E-L-Y-E-N-K-P-R-R-P-Y-I-L</td>
</tr>
<tr>
<td>Peptide 1</td>
<td>F-G-L-M</td>
</tr>
<tr>
<td>Peptide 2</td>
<td>F-S-W-G-A-E-G-Q-R</td>
</tr>
<tr>
<td>Peptide 3</td>
<td>A-S-T-T-T-N-Y-T</td>
</tr>
<tr>
<td>Peptide 4</td>
<td>L-P-P-S-R</td>
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</table>

**Fig. 2.** Decrease in percent of peak area of various peptides with phenylalanine, leucine, or alanine in the NH<sub>2</sub>-terminal position from HPLC after incubation with mesquite pollen peptidase II<sub>mes</sub>. Purified enzyme was incubated with FGLM, FSWGAE-GQR, LPPSR, or ASTTTNTYT in 0.1 M Tris-HCl, pH 8.0, at 37 °C as described under “Methods.” At various times, aliquots were removed and acidified to stop the reaction, and peaks were separated on HPLC. Panel A: ■, FGLM, 1:8,000; ▲, FSWGAEQQR, 1:1,000. Panel B: ■, LPPSR, 1:2,000; ▲, ASTTTNTYT, 1:1,000

**Fig. 3.** Decrease in percent of peak area of various bioactive peptides from HPLC after incubation with mesquite pollen peptidase II<sub>mes</sub>. Purified enzyme was incubated with angiotensins I and II, VIP, atrial natriuretic peptide, bradykinin, substance P, or neurotensin in 0.1 M Tris-HCl, pH 8.0, at 37 °C as described under “Methods.” At various times aliquots were removed, acidified to stop the reaction, and peaks were separated on HPLC. ■, angiotensin I, 1:480; ▲, angiotensin II, 1:600; △, VIP, 1:570; ●, atrial natriuretic peptide, 1:450; ■, bradykinin, 1:510; □, substance P, 1:400; △, neurotensin, 1:500.
strates with P1 hydrophobic residues, a series of chloromethyl
M

It was, however, inactivated by low
dase activity exhibited by peptidase Imes, the aminopeptidase
whereas TLCK was not inhibitory. In support of the exopepti-
dicity on synthetic substrates. However, both 1,10-phenanthroline
inhibitor bestatin was very effective in reducing enzyme activ-
cally to the active site of some enzymes (23), which indicates
because the
greater affinity of the enzyme for these peptide substrates
H-Ile-

particularly angiotensins I and II, hydrolysis of the isoleucine-
were utilized in determining enzyme specificity (Table II). It
These results essentially parallel data shown earlier which

Peptidase IImes also cleaved both angiotensin II and atrial
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and 59% homology with aminopeptidase N from

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Haemophilus influenzae. The combined inhibitory activities of both 1,10- and 4,7-phenanthroline indicated the absence of a metal; however, the sequence found corresponded to residues 127–157 in aminopeptidase N from E. coli, whereas the zinc ion ligands in that enzyme were at residues 296, 300, and 319. Thus, this sequence was far from any of the zinc binding sites and may act like a mosaic protein as exemplified by the S8 serine peptidase from Vibrio alginolyticus (24), a member of the subtilisin family which acts as an endopeptidase with homologous domains similar to those found in metalloproteases, including an aminopeptidase from Vibrio proteolyticus (family M28) and an endopeptidase of the thermolysin family (M4).

DISCUSSION

Pollen is one of the well known triggers of bronchial hyperresponsiveness, or exaggeration of response to inflammation, observed in allergic asthma. Although mesquite does not have a widespread distribution, it has recently been cultivated extensively in the southcentral and southwestern United States, thereby increasing its contact with people and making its pollen a serious spring aeroallergen (25). Once the pollen grains come in contact with an aqueous environment, such as the mucus layer in the lung airways, they swell and split and release many proteins (26). Pollen proteins that are allergens and elicit an immunological response have been studied abundantly (27–31). However, some of these allergens in fact appear to have, in addition, enzymatic functions displaying lyase (32), esterase (33), and polygalacturonase (34) activities. Indeed, some dust mite allergens, such as Der p I and Der f I (from Dermatophagoides pteronyssinus and Dermatophagoides farinae, respectively), are cysteine proteinases, and Der p III and Der f III are serine proteinases (35).

Both mesquite and ragweed pollens have yielded peptidases with both trypsin-like and chymotrypsin-like specificities (14–16). This report concerns the results obtained in the study of a second mesquite pollen activity (peptidase \( \Pi_{mes} \)) that was quite different from the others. The enzyme manifested both aminopeptidase and oligopeptidase activity, based on results with blocked and unblocked peptide \( \alpha \)-NAs and with unblocked polypeptides. The data obtained suggested the importance of hydrophobic residues for both activities, with phenylalanine being preferred for aminopeptidase function and multiple hydrophobic residues required in the P1 position for internal cleavage. Such a combination of activities is not unusual and has been observed for cathepsin H (20). In addition, cathepsin B has been shown to have both peptidylpeptidase and oligopeptidase activities (21).

It is important to note that homology with aminopeptidases from other organisms was obtained readily in analysis of a single peptide fragment from peptidase \( \Pi_{mes} \). The complete amino acid sequence of peptidase \( \Pi_{mes} \) was shown previously (14) to be homologous with protease II from E. coli, a member of the prolylendopeptidase family. Recent results comparing structures of a trypsin-like oligopeptidase isolated from suspension-cultured soybean cells found that homology also existed between that peptidase and prolylendopeptidases (human or porcine) including protease II (E. coli) (36). In fact, the soybean oligopeptidase resembled peptidase I \(_{mes}\) in other ways as well; cleavage after arginine and, to a lesser extent, lysine residues, hydrolysis of peptide only, a serine peptidase specificity, and molecular mass of 90 kDa. Whether homologies exist between peptidase \( \Pi_{mes} \) and endopeptidases from other organisms remains to be established, but is likely.

The rapid hydrolysis of the bioactive peptides VIP and angiotensins I and II may be of potentially physiological significance. By rapidly degrading and inactivating VIP (a bronchodilator) while only slowly hydrolyzing substance P (a bronchoconstrictor), the peptidase could be expected to exacerbate the overall bronchoconstrictive effect detected in asthmatic lungs. In addition, angiotensin II is a potent vasoconstrictor (13), and its cleavage and inactivation by the peptidase could also be expected to contribute to the overall vasodilation observed in asthmatic lungs. Kinetic rate constants indicate that the rate of cleavage of two of these peptides (angiotensins I and II in vitro) was relatively rapid and comparable to the rates of hydrolysis of peptide bonds by other well known proteinases, such as chymotrypsin (37).

However, the fragmenting of both angiotensins I and II into smaller peptides could have important effects of their own. Macrophages appear rapidly in the lung after local allergen challenge, suggesting a rapid migration of monocytes (38). The two tetrapeptides (DRVY and IHPF) of angiotensin II are chemotactic factors for neutrophils and, particularly, monocytes (39), which exhibit 50% of the optimal response to C5a (a potent chemotactic factor) at very low concentrations of the peptides. The cleavage of angiotensin II by peptidase \( \Pi_{mes} \) produced a pentapeptide (DRVYI) and a tripeptide (HPF). Because the former peptidase contains the chemotactic \( \text{NH}_2 \)-terminal tetrapeptide it could be an effective chemotactic factor as well.

Many of the proteins extracted from pollen are enzymes that are no doubt normally involved in the germination of the plants (40). Under abnormal conditions, however, such as allergic asthma triggered by pollen, these enzymes may play a role in the pathology of the disease. Because the mesquite pollen peptidase \( \Pi_{mes} \) with both exo- and oligopeptidase specificity described here degrades both VIP and angiotensins I and II, this enzyme may have the potential for making a significant contribution to the pathological effects observed in allergy-related asthma.

REFERENCES
