The C-S Lyases of Higher Plants

DIRECT COMPARISON OF THE PHYSICAL PROPERTIES OF HOMOGENEOUS ALLIIN LYASE OF GARLIC (ALLIUM SATIVUM) AND ONION (ALLIUM CEPA)

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LINDA P. NOCK and MENDEL MAZELIS*
Department of Food Science and Technology, 1480 Chemistry Annex, University of California, Davis, California 95616

ABSTRACT

Garlic and onion alliin lyases, although from closely related species, have many differences. The two enzymes differ in their $K_m$ values, pK optima, and isoelectric points. There is a major difference in their molecular weight and subunit structure. The garlic holoenzyme has a molecular weight of 85,000 and consists of two subunits of molecular weight 42,000. The onion enzyme has a holoenzyme molecular weight of 290,000 composed of four subunits of molecular weight 50,000. The onion enzyme is much more difficult to dissociate into its subunits which suggests differences in subunit interaction between the two enzymes. The dimeric structure of the garlic and the tetrameric structure of the onion enzyme is consistent with a coenzyme content (pyridoxal-5'-phosphate) equivalent to one mole per subunit. The two enzymes vary vastly in their spectra, the onion enzyme having a lower pyridoxal-5'-phosphate absorbance at 430 nanomoles and an inability to react with L-cysteine. Both enzymes are glycoproteins and bind to concanavalin A-Sepharose columns. The onion alliin lyase binds more tightly than the garlic enzyme. The amino acid content of both enzymes is similar as is the carbohydrate content. However, upon hydrolysis the onion lyase does yield more mannose units than the garlic enzyme which is consistent with the former's stronger affinity for concanavalin A.

Many higher plant families possess nonprotein amino acids as secondary metabolites (5). These amino acids are often characteristic of a particular species or genus. Several plant families which are widely divergent taxonomically, are characterized by the presence of a significant amount of $S$-alkyl-L-cysteines or their sulfoxides. Much of the odor and flavor characteristic of these plants after tissue rupture is due to the degradation of these amino acids by C-S lyases which are separated from the substrate in undamaged tissue. Well known examples of these plants are garlic and onion in the Amaryllidaceae; cabbage, cauliflower, broccoli of the genus Brassica in the Cruciferae; and Acacia sp. in the Leguminosae.

The enzymes from onion bulbs (24), garlic cloves (11, 14, 17), broccoli buds (8), and acetone powders of hypocotyls from Acacia farnesiana seedlings (13, 23) have all been purified to homogeneity by a variety of procedures. Significant differences were observed in the physical properties and kinetic behavior of these enzymes, even from such closely related species as garlic (Allium sativum) and onion (Allium cepa). The primary endogenous substrates differ for the garlic and onion alliin lyases (EC 4.4.1.4). $S$-Allyl-l-cysteine sulfoxide is dominant in the former and $S$-propenyl-l-cysteine sulfoxide in the latter. However both enzymes catalyze the same basic beta elimination reaction (Fig. 1). This report directly compares the alliin lyases from garlic and onion after purification by essentially the same method. Marked differences have been found in physical structure such as mol wt, subunit interaction, carbohydrate moiety composition, and spectral properties.

MATERIALS AND METHODS

Chemicals. The standard substrate used was $S$-ethyl-l-cysteine sulfoxide. This was synthesized from $S$-ethyl-l-cysteine purchased from Sigma Chemical Co. by oxidation with hydrogen peroxide as described previously (22). Hydroxylapatite (fast flow) was obtained from Calbiochem-Behring Corp. Bio-Gel A 0.5 m. the gel filtration and SDS-PAGE protein standards were purchased from Bio-Rad. Isoelectric focusing carrier ampholites (broad range Pharmalyte [pH 3–10]) and the broad pl kit (pH 3–10) protein standards were obtained from Pharmacia Inc. Ammonium sulfate (Ultra Pure) was purchased from Schwarz-Mann Biotech. The following chemicals were obtained from the Sigma Chemical Company: PVPP, PMSF, ConA-Sepharose 4B, urea, pyridoxal-5'-phosphate, methyl-$\alpha$-$\beta$-mannopyranoside (grade 3), L-lactic dehydrogenase, NADH, blue dextran, BSA, bovine $\gamma$-globulin, ovalbumin, horse myoglobin, L-arabinose, D-xylene, D-mannose, D-galactose, D-glucose, myo-inositol, N-acetyl-d-glucosamine, N-acetyl-d-galactosamine. EDTA was purchased from MCB Manufacturing Chemists.

Plant Materials. Garlic cloves and yellow onions were pur-

\[
\begin{align*}
2 \text{R}S\text{CH}_2\text{CH}=\text{CHCOO}^- + \text{H}_2\text{O} & \quad \text{NH}_3^+ \\
\text{S-CH}_2\text{CH}=\text{CHCOO}^- + \text{H}_2\text{O} & \quad \text{NH}_3^+ \\
\text{R-S-S-R} + 2 \text{CH}_3\text{COO}^- + 2 \text{NH}_3^+ & \quad \text{CH}_3\text{COO}^- \\
\text{Preferred Natural Substrates.} & \\
\text{For Garlic Alliin lyase} & \quad \text{R=CH}_2\text{CH}=\text{CH}^- \\
\text{For Onion Alliin lyase} & \quad \text{R=CH}_3\text{CH}=\text{CH}^- \\
\end{align*}
\]

FIG. 1. Overall reaction catalyzed by garlic and onion alliin lyase.

1 Abbreviations used: PVPP, polystyrylpolypropylene; PMSF, phenylmethylsulfonyl fluoride; ConA, concanavalin A.

2 Supported in part by National Science Foundation Grant DMB84-04182.
chased from local markets.

**Purification of the Garlic and Onion Lyases.** The purification procedures for both enzymes were essentially identical except for slight variations in the buffers used at different steps. Phosphate buffers were obtained by mixing varying proportions of equimolar solutions of NaH$_2$PO$_4$ and K$_2$HPO$_4$ to obtain the desired final pH. The concentration of the primary phosphate solutions was varied to obtain buffers of different molarity. Regardless of the buffer phosphate concentration, each buffer had a final concentration of 10% glycerol, 1 mM PMSF, and 5 mM EDTA. Buffers used in the garlic enzyme isolation were at pH 7.0, and for the onion isolation at pH 7.5. The latter buffers also contained 0.05% 2-mercaptoethanol since this helped stabilize the onion enzyme activity for several weeks.

Standard purification procedures are summarized in Table I. A typical purification started with 250 g of peeled and chopped garlic cloves or with 1 kg of peeled onion bulbs. The plant material was blended for 1 min in a cold Waring Blender in 0.02 M of the appropriate buffer as described above. One ml of buffer per g wet weight of tissue was used. The blending buffers also contained 5% NaCl, 5% PVPP, and 0.05% 2-mercaptoethanol in each case. The homogenate was filtered through two layers of cheesecloth and the filtrate centrifuged at 16,300 g for 1 h at 4°C. The supernatant solution was brought to 35% (for garlic) and 65% (for onion) saturation with (NH$_4$)$_2$SO$_4$ and stirred slowly for 30 min at 4°C. The slurry was centrifuged at 16,300 g for 30 min at 4°C. The pellet was resuspended in approximately 100 ml of the respective 0.05 M standard buffers and then dialyzed against the same buffer. The dialyze was centrifuged at 12,000 g to remove insoluble material. The supernatant solution was then placed on a fast flow hydroxylapatite column (2.2 × 50 cm) and washed with 0.05 M buffer to remove inactive material. The active peak was eluted using 3 volumes of 0.3 M buffer. Tubes containing the enzyme peak were combined, concentrated by ultrafiltration, and then dialyzed. The concentrate was placed on a conA-Sepharose 4B column (50 ml). Unbound inactive protein was eluted with the standard 0.05 M buffers. The enzyme was eluted using a gradient of 0 to 100 mM (for garlic) and 0 to 300 mM (for onion) methyl α-D-mannopyranoside in the respective buffers. Active fractions were combined and used for detailed characterization studies.

**Enzyme Assay.** The standard assay mixtures consisted of the following in a final volume of 1 ml:

<table>
<thead>
<tr>
<th></th>
<th>Garlic</th>
<th>Onion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phosphate buffer (pH 6.5)</td>
<td>100 mM</td>
<td>100 mM</td>
</tr>
<tr>
<td>Tricine buffer (pH 8.0)</td>
<td>100 mM</td>
<td>100 mM</td>
</tr>
<tr>
<td>Pyridoxal 5'-P</td>
<td>0.025 mM</td>
<td>0.025 mM</td>
</tr>
<tr>
<td>S-Ethyl-L-cysteine sulfoxide</td>
<td>40 mM</td>
<td>40 mM</td>
</tr>
</tbody>
</table>

Enzyme was added to start the reaction.

The reaction mixtures were incubated at room temperature (23°C) for the desired time, usually 5 min, and then terminated by addition of 2 ml of 10% (w/v) TCA. Aliquots of this final solution were assayed for pyruvate by the total keto acid method of Friedemann and Haugen (6).

**Protein Determination.** Protein was located in column eluates by its A at 280 nm and determined quantitatively by the procedure of Lowry et al. (12) using ovalbumin as the standard.

**Electrophoretic Analysis of Protein Samples.** Protein samples were analyzed using dissociating SDS-PAGE buffer systems. The gels were prepared by the methods described by Harries (9) with the addition of 8 M urea to the stacking and resolving gels and to the sample buffers.

Following electrophoresis, proteins were visualized either by staining with Coomassie blue (9) or by silver staining (15, 18). Location of glycoproteins on the gels was determined by use of a periodic acid-Schiff base stain (20). Mol wt were obtained by comparison to a series of standard proteins used as SDS/PAGE mol wt standards. The location of proteins staining with Coomassie blue on tube gels was determined by using a linear transport gel scanner accessory on a Gilford spectrophotometer at 600 nm. Slab gels were scanned by means of an LKB Ultrascan XL.

**Isoelectric Focusing of Proteins.** Isoelectric focusing of the garlic and onion lyases was carried out by the horizontal slab gel method of Giulian et al. (7) in 0.5 mm thick gels made of 5.5% acrylamide, 0.5% bisacrylamide, 10% glycerol and 0.66 ml amphotelyes (pH 3–10). The gels were focused, in conjunction with proteins of known isoelectric points for 3.5 h at 20 W. Following isoelectric focusing the gels were stained with Coomassie blue.

**Molecular Weight Determinations.** The mol wt of the garlic and onion holoenzymes and subunits were determined by gel filtration column chromatography (2.2 × 47 cm) using Bio-Gel A 0.5 M equilibrated with the respective buffers. For all onion aIlin lyase determinations, 0.05% 2-mercaptoethanol was also included. The column was standardized with a gel filtration standard consisting of bovine thyroglobulin, γ-globulin, ovalbumin, myoglobin, and vitamin B12.

The subunit mol wt were obtained after prior dissociation of the enzymes in 6 M urea at 65°C for 45 min. Columns were equilibrated with the respective buffers in 6 M urea. The onion enzyme could be further dissociated by the addition of 0.1% SDS to the 6 M urea in both the sample and equilibrating buffer. Blue dextran, γ-globulin, serum albumin, ovalbumin, and myoglobin were used as protein standards.

**Carbohydrate Analysis.** The carbohydrate content of garlic alliinase was determined by means of the phenol-sulfuric acid method of Dubois et al. (3). The amount of pentose was estimated by the orcinol method of Dische (2). Carbohydrate was also examined by the GLC of the alditol acetate derivatives (10) of sugars released after acid hydrolysis with 3 N HCl (1). L-Arabinoose, D-xylene, D-mannose, D-galactose, D-glucose, N-acetylated-glucosamine, and N-acetylated-galactosamine, were used as sugar standards.

**Amino Acid Analysis.** Samples for amino acid analysis were obtained by hydrolysing portions of the purified enzymes in 6 N HCl in sealed tubes for 24 h at 110°C followed by separation using a Beckman model 6300 amino acid analyzer (16). The tryptophan content was estimated in a denatured unhidrolyzed sample by a modification of the procedure of Edelhoch (4) in which samples in 8 M guanidine·HCl were heated at 85°C for 45 min before determining the absorbance.

**Pyridoxal-5'-Phosphate Estimation.** Enzyme samples were assayed for bound pyridoxal 5'-P by the colorimetric procedure of Wada and Snell (25). The spectra of the pure enzymes were also obtained between 280 and 800 nm in the presence and absence of 3 mM L-cysteine.

**RESULTS**

By the purification procedure described previously and summarized in Table I, homogeneous enzyme was obtained by a relatively simple five-step procedure from both garlic and onion extracts. The enzyme obtained from each source was analyzed by the different procedures described below and direct comparisons made.

**Gel Electrophoresis Analysis.** Twenty μg samples of garlic and onion alliin lyase (Table I, fraction 5), in conjunction with the low mol wt standards, were subjected to SDS/urea PAGE using 12.5% vertical slab gels (Fig. 2). Gels were stained with both Coomassie blue and a silver stain (latter not shown). By either procedure only one band was visualized for either the garlic or onion samples. After scanning the gel and comparing the positions of the purified enzymes to standards, a subunit mol wt of 50,000 was obtained for the garlic lyase and 56,000 for the onion
Table I. Summary of Enzyme Purification

<table>
<thead>
<tr>
<th>Step</th>
<th>Protein mg</th>
<th>Total units*</th>
<th>Specific Activity units/mg protein</th>
<th>Recovery % Purification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Garlic</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. Homogenate</td>
<td>2,856</td>
<td>38,842</td>
<td>13.6</td>
<td>100</td>
</tr>
<tr>
<td>2. (NH₄)₂SO₄</td>
<td></td>
<td></td>
<td></td>
<td>1.0</td>
</tr>
<tr>
<td>fraction</td>
<td>1,582</td>
<td>35,753</td>
<td>22.6</td>
<td>92</td>
</tr>
<tr>
<td>3. Dialysis</td>
<td>1,659</td>
<td>34,839</td>
<td>21.0</td>
<td>90</td>
</tr>
<tr>
<td>4. Hydroxyapatite</td>
<td>140</td>
<td>11,060</td>
<td>79.0</td>
<td>28</td>
</tr>
<tr>
<td>5. ConA Sepharose</td>
<td>44</td>
<td>4,796</td>
<td>109</td>
<td>12</td>
</tr>
<tr>
<td>Onion</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. Homogenate</td>
<td>2,660</td>
<td>3,697</td>
<td>1.4</td>
<td>100</td>
</tr>
<tr>
<td>2. (NH₄)₂SO₄</td>
<td></td>
<td></td>
<td></td>
<td>1.0</td>
</tr>
<tr>
<td>fraction</td>
<td>852</td>
<td>912</td>
<td>1.1</td>
<td>25</td>
</tr>
<tr>
<td>3. Dialysis</td>
<td>750</td>
<td>810</td>
<td>1.1</td>
<td>22</td>
</tr>
<tr>
<td>4. Hydroxyapatite</td>
<td>73</td>
<td>504</td>
<td>6.9</td>
<td>14</td>
</tr>
<tr>
<td>5. ConA Sepharose</td>
<td>12</td>
<td>279</td>
<td>23.3</td>
<td>8</td>
</tr>
</tbody>
</table>

* One unit of enzyme activity produces one μmol pyruvate per min.

Fig. 2. Fraction 5 samples (20 μg) of garlic (lanes A + B) and onion (lanes C + D) alliin lyase were subjected to discontinuous SDS/urea PAGE. The gels all contained 8 M urea and 0.1% SDS (w/v). The proteins were stacked using a constant 120 V in a 4% (w/v) acrylamide plus 0.1% (w/v) bisacrylamide gel and were resolved at 200 V constant voltage in a 12.5% (w/v) acrylamide, 0.3% bisacrylamide gel. Protein standards of known mol wt were also run in this gel and their positions are marked in the figure.

enzyme.

Fraction 5 samples were also subjected to SDS-PAGE in 10% tube gels. Following electrophoresis the gels were stained with Coomassie blue and scanned at 600 nm. Recorder plots showed that only one protein was present in the gels of both garlic and onion samples (Fig. 3, A and B).

Isoelectric Focusing of Garlic and Onion Enzymes. Thirty μg samples of both garlic and onion fraction 5 were subjected to isoelectric focusing in a broad (pH 3–10) pH gradient. The samples were run alongside a set of protein standards of known isoelectric points for 3.5 h at 20 W. The gels were then stained with Coomassie blue. Both enzyme samples exhibited some charge heterogeneity but their major bands differed greatly. The major band for garlic alliin lyase was at pH 7.0 and that for the onion at pH 8.0.

Molecular Weight Determinations. Mol wt were determined after chromatography of fraction 5 samples (4 mg) on Biogel A 0.5 m and use of a standard curve based on the elution volumes of a standard protein mixture of known mol wt on the same column. A mol wt of 85,000 was obtained for the garlic lyase and 200,000 for the onion. On equilibration of the column in 6 M urea, the subunit mol wt of the garlic enzyme was found to be 42,000 while that of the onion enzyme was 100,000. If 6 M urea plus 0.1% SDS was used, the subunit mol wt of the garlic enzyme

Fig. 3. Recorder tracings (10% SDS/PAGE tube gels) of purified fraction 5 (20 μg) from garlic (A) and onion (B). The gels after staining with Coomassie blue were scanned at 600 nm by use of a linear transporter.

Table II. Sugars Recovered on Acid Hydrolysis and GLC Analysis

<table>
<thead>
<tr>
<th>Plant</th>
<th>Arabinose</th>
<th>Xylose</th>
<th>Mannose</th>
<th>Galactose</th>
<th>Glucose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Garlic</td>
<td>9</td>
<td>5</td>
<td>43</td>
<td>8</td>
<td>34</td>
</tr>
<tr>
<td>Onion</td>
<td>1</td>
<td>1</td>
<td>78</td>
<td>10</td>
<td>10</td>
</tr>
</tbody>
</table>

Table III. Amino Acid Composition of Garlic and Onion Alliin Lyase

<table>
<thead>
<tr>
<th></th>
<th>Garlic</th>
<th>Onion</th>
<th>Garlic</th>
<th>Onion</th>
</tr>
</thead>
<tbody>
<tr>
<td>%*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Asp</td>
<td>11.6</td>
<td>11.1</td>
<td>Met</td>
<td>0.6</td>
</tr>
<tr>
<td>Thr</td>
<td>6.8</td>
<td>5.5</td>
<td>Ile</td>
<td>3.4</td>
</tr>
<tr>
<td>Ser</td>
<td>7.3</td>
<td>7.7</td>
<td>Leu</td>
<td>6.8</td>
</tr>
<tr>
<td>Glu</td>
<td>11.0</td>
<td>9.5</td>
<td>Tyr</td>
<td>5.5</td>
</tr>
<tr>
<td>Pro</td>
<td>4.3</td>
<td>4.2</td>
<td>Phe</td>
<td>4.3</td>
</tr>
<tr>
<td>Gly</td>
<td>7.0</td>
<td>8.2</td>
<td>Lys</td>
<td>7.2</td>
</tr>
<tr>
<td>Ala</td>
<td>6.9</td>
<td>6.0</td>
<td>His</td>
<td>2.0</td>
</tr>
<tr>
<td>Val</td>
<td>6.4</td>
<td>6.9</td>
<td>Arg</td>
<td>4.9</td>
</tr>
<tr>
<td>Cys</td>
<td>1.6</td>
<td>0.4</td>
<td>Trp</td>
<td>2.2</td>
</tr>
</tbody>
</table>

* Percentages were based on the mol amino acid per mol protein values.
was still 42,000; however, that of the onion enzyme was now 50,000.

Carbohydrate Analysis. Following gel electrophoresis, glycoproteins could be located on gels by staining with the periodic acid-Schiff base stain (20). In the case of both garlic and onion, the single fraction 5 band (corresponding to that visualized by Coomassie blue in Fig. 2) stained positively by this method.

Total carbohydrate was assayed colorimetrically by the phenolsulfuric acid method (3), and pentoses by the orcinol method (2). The garlic alliinase contained 5.5% carbohydrate (40% as pentose), and the onion lyase 4.6% carbohydrate also 40% as pentose.

Glycoprotein samples were also hydrolyzed in 3 N HCl for 3 h at 100°C under N2. Released monosaccharides were derivatized to alditol acetates and detected by GLC. Retention times were compared to alditol acetate derivatives of standard monosaccharides. There were significant differences in the amount of mannose present between the two enzymes and also the amount of glucose. The level of pentoses present in the carbohydrate moiety was appreciably higher in the garlic enzyme compared to the onion lyase (Table II).

Amino Acid Analysis. A direct comparison of the alliin lyases from garlic and onion, prepared by the above procedure, was obtained by simultaneous acid hydrolysis followed by analysis as described in "Materials and Methods." In addition, the tryptophan content of each enzyme was estimated by the modified method described in "Materials and Methods." Results are given in Table III.

Pyridoxal Phosphate Determination. The amount of endogenous pyridoxal-5'-phosphate in each enzyme was determined chemically (25) on known weights of fraction 5 protein. Using the mol wt as estimated by gel filtration, 1.68 mol of pyridoxal-5'-phosphate were present per mol of garlic lyase compared to 3.76 mol per mole onion enzyme.

The absorbance spectra of both the fraction 5 garlic and onion enzymes were obtained by use of an automatic scanning spectrophotometer. Figure 4a is the spectrum of the garlic enzyme in the presence and absence of L-cysteine. The results confirm our previous findings (17) in that the native enzyme has a sharp absorption peak at 430 nm due to the bound pyridoxal-5'-phosphate. In the presence of L-cysteine this absorption maximum is lost and a new one appears at about 330 nm (14, 17). The onion enzyme also shows a maximum at 430 nm but not as marked for the same weight of enzyme (Fig. 4b). In addition, there appears to be very little effect of added L-cysteine.

Although onion and garlic are very close taxonomically, this present work reports a surprising number of differences in the enzyme responsible for the characteristic flavor and odor associated with these two species as foods. Previous work (14, 22, 24) had shown a considerable difference in the pH optima for activity between these two enzymes. This present report details some further variations of their physical and structural nature.

For convenience the substrate used for isolation and assay purposes was S-ethyl-L-cysteine sulfodiide. This is not the natural substrate for either enzyme and a marked difference in $K_m$ values was obtained, that for garlic being 5.7 mM and that for onion 9.4 mM. However, the $V_{\text{max}}$ was almost identical with this substrate for both enzymes. This difference suggests that the active site configuration might be different. Evidence supporting this contention would be the spectral studies of the purified enzyme in the presence and absence of cysteine (Fig. 4a and b). The garlic enzyme maximum at 430 nm due to the bound pyridoxal phosphate disappeared in the presence of L-cysteine because of the interaction to form a thiazolidine ring with an absorbance maximum at 330 nm (17). The onion enzyme on the other hand showed no change in the presence of cysteine. This suggests very strongly that the environment surrounding the co-factor binding site between the two enzymes is quite different.

There is a considerably greater amount of the enzyme present in the soluble, expressed protein from garlic than in onion. Since only an 8-fold purification is necessary to obtain a homogeneous garlic lyase compared to a 17-fold purification for onion (Table I), this suggests that the enzyme comprises about 12% of the soluble protein in garlic claves, and about 6% of that in onion bulb. The isolation procedure described here had PVPP and PMSF in the extraction buffer. This increased the specific activity of the original homogenates of both garlic and onion from 3- to 5-fold over that originally observed (14, 24).

It was not possible to determine the purity or mol wt of either enzyme by nondenaturing PAGE analysis. Gel filtration, however, showed the onion lyase had a holoenzyme mol wt of 200,000 and the garlic 85,000. SDS slab and tube gel analysis showed only one band after staining corresponding to 56,000 mol wt for onion and 50,000 for garlic. Use of gel filtration columns with buffers containing 6 M urea and 0.1% SDS, however, yielded subunit mol wt of 50,000 and 42,000, respectively, for onion and garlic. The variability between the results from the slab gels and gel filtration may be explained by the anomalous behavior of glycoproteins on SDS gels in that reaction with the gel matrix gives incorrect larger values (20). The subunit values obtained by gel filtration studies show that the onion enzyme is a tetramer and the garlic enzyme is a dimer. The results of the pyridoxal phosphate analysis would suggest that both enzymes have sufficient cofactor to correspond to 1 mol/subunit.

Both the garlic and onion enzymes show some charge heterogeneity on isoelectric focusing and multiple bands are observed. Since both enzymes are glycosylated, this may be due to carbohydrate microheterogeneity. Removal of the carbohydrate moiety could determine if these were indeed the case and future studies into this possibility are planned. Under present circumstances, the major bands are visualized at pH 7.0 for garlic (a double band) and pH 8.0 for the onion. The more basic isoelectric point for onion lyase may reflect some conformity with the differences in their pH optima, 6.5 for the garlic enzyme (14) and 8.5 for onion (24).

The fact that both of these enzymes are glycoproteins was utilized in their purification. They both bind to a ConA-Sepharose 4B column and can be eluted with α-methyl mannoside. The garlic enzyme was eluted at a lower concentration of α-methyl mannoside than the onion enzyme. This suggested that more of the carbohydrate associated with the onion enzyme
consisted of oligosaccharides with a stronger affinity for this lectin (e.g. \(\alpha-D\)-mannopyranose or \(\alpha-D\)-glucopyranose). Colorimetric analysis showed both enzymes had effectively the same amount of total carbohydrate and pentose. However, acid hydrolysis of the enzymes and GLC analysis of the released sugars (Table II) showed that only 14% of the released sugars from garlic lyase and 2% from the onion alliin lyase was pentose. This was inconsistent with the colorimetric analysis and pointed to one of two possibilities; first, that the orcinol method above also detects hexoses or that total hydrolysis of the carbohydrate moiety was not 100% and Table II represents the sugars most available for hydrolysis. On repeating the orcinol method using D-mannose no color products were formed for up to 100 \(\mu\)g of sugar. In addition, sugar recovery values from the GLC analysis were very low (around 10%) which seemed to indicate that the latter possibility was the most likely. Many plant glycoproteins which bind to ConA are of the mannose rich type (21) and have mannose residues readily available for binding to the lectin. Table II shows that almost twice as much mannose was available for hydrolysis (and therefore for binding to ConA) in the onion enzyme as in garlic. The garlic enzyme, however, contains an appreciable amount of hydrolyzable glucose which presumably is either not available to bind to the ConA Sepharose or which does so less tightly.

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LITERATURE CITED
1. CARTER WG, ME ETZLER 1975 Isolation and characterization of cyanogen bromide fragments and a glycopeptide from the Dolichos biflorus lectin. Biochemistry 14: 5118-5122
10. KATO Y, DJ NEVINS 1984 Enzymic dissociation of Zea shoot cell wall polysaccharides II. Dissociation of (1\(\rightarrow\)3), (1\(\rightarrow\)4)-\(\beta\)-D-glucan by purified (1\(\rightarrow\)3):(1\(\rightarrow\)4)-\(\beta\)-D-glucan 4-glucanohydrolase from Bacillus subtilis. Plant Physiol 75: 745-752
19. Deleted in proof.
22. STOLL A, E SEEBRICK 1951 Chemical investigations of alliin, the specific principle of garlic. Adv Enzymol 11: 377-400
23. SWEET WJ, M MAZELIS 1987 Homogeneous alkylcysteine lyase of Acacia farnesiana: fresh seedlings vs acetone powders. Phytochemistry 26: 945-948