Isolation of Three Isoinhibitors of Trypsin from Garden Bean, *Phaseolus vulgaris*, Having Either Lysine or Arginine at the Reactive Site*

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**SUMMARY**

Three isoinhibitors of trypsin have been isolated from the garden bean, *Phaseolus vulgaris*, in a highly purified state, as indicated by disc electrophoresis, equilibrium chromatography, and amino acid analysis. The amino acid composition is characterized by the absence of free -SH groups, high half-cystine, 14 residues, absence of tryptophan in all three, and absence of methionine in isoinhibitors, I and IIIb. Isoinhibitor I contains 74, II -77, and IIIb -81 amino acid residues. Isoinhibitor I has lysine in the reactive site, while isoinhibitor II has arginine. Isoinhibitor II is significantly displaced from the trypsin complex by 1 mm substrate, isoinhibitors I and IIIb are not. Isoinhibitors I and II inhibit α-chymotrypsin weakly (0.2 units per mg). Isoinhibitor IIIb inhibits strongly (2.87 units per mg) and has an independent site for each enzyme: trypsin and chymotrypsin.

Protein inhibitors of trypsin are widespread in the plant kingdom, with the seeds of the *Leguminosae* being particularly rich sources (1). A number of these have been purified and characterized (2), including their primary structures (3-6). The use of these inhibitors as models for the study of structure-function relationships is particularly attractive due to several of their properties. Two major avenues may be taken to evaluate the relationship between structure and function. In the first, most direct approach, the protein may be specifically modified, either with chemical reagents or enzymatically, and the effect upon activity determined. In the second, more indirect approach, the protein inhibitors of trypsin are widespread in the plant kingdom, with the seeds of the *Leguminosae* being particularly rich sources (1). A number of these have been purified and characterized (2), including their primary structures (3-6). The use of these inhibitors as models for the study of structure-function relationships is particularly attractive due to several of their properties. Two major avenues may be taken to evaluate the relationship between structure and function. In the first, most direct approach, the protein may be specifically modified, either with chemical reagents or enzymatically, and the effect upon activity determined. In the second, more indirect approach, the structure of several or many homologous proteins having similar activity determined. In the second, more indirect approach, the protein inhibitors may be specifically modified, either with chemical reagents or enzymatically, and the effect upon activity determined.
from Calbiochem, Pierce Chemical, and Sigma, respectively. Sephadex G-50 medium and SP-Sephadex\(^1\) C-25 were obtained from Pharmacia Fine Chemicals. DEAE-cellulose, type 70, was from Schleicher and Schuell, Inc., Keene, N. H. Prior to use, cellulose casings (Union Carbide, Sizes 20 and 40) were boiled in 1% sodium carbonate, and extensively washed in distilled water.

Urea was recrystallized from 95% ethanol, and solutions were deionized immediately before use by passage through a mixture of Dowex 50-X8 and 1-X8. All other chemicals were reagent grade or better, and glass-distilled water was used throughout.

**Determination of Inhibitor Activity**—Trypsin inhibitor activities were determined by the method of Schwert and Takenaka (12) as modified by Kassell et al. (13). Chymotrypsin inhibitor activities were determined by a modification of the method of Hummel (14), as previously described (15). A unit of inhibitor is defined as that amount of inhibitor that will inhibit the enzymatic activity of 1 mg of active trypsin (or chymotrypsin) in the above assays.

Concentrations of trypsin and chymotrypsin solutions were determined spectrophotometrically with optical factors of 0.67 and 0.49, respectively, to convert absorbance at 280 nm to milligrams of protein per ml. Concentrations of purified inhibitor solutions were determined using optical factors described under "Results."

**Amino Acid Analyses**—Samples of approximately 1 to 2 mg of protein were hydrolyzed for 20 and 48 hours at 110° in constant boiling, glass-distilled HCl in sealed, evacuated tubes. Analyses were carried out on a Beckman-Spinco analyzer, model 120B, by the method of Spackman et al. (16). Half-cystine was determined as cysteic acid after oxidation with performic acid (17).

**Trypsin Digestions**—Tryptic digests were prepared by adding 10 \(\mu\)l of trypsin solution (1-tosylamido-2-phenylethly chloro-methyl ketone-treated, 0.46 mg per ml in 2.5 mM HCl containing 0.02 M CaCl\(_2\)) to 3.5 mg of each of the performic acid-oxidized inhibitors in 0.7 ml of 0.1 M ammonium bicarbonate. After 3 hours at 37°, another 5 \(\mu\)l of trypsin solution were added. After another 3 hours, the digestion was terminated by freezing and lyophilization.

**Peptide Maps**—Peptide mapping was performed on Whatman No. 3MM paper. The sample (usually approximately 1.5 mg of digested protein) was applied at the origin in 0.1 M ammonium bicarbonate. The maps were developed in the first direction by descending chromatography for 20 hours in 1-butanol-acetic acid-water-pyridine (30:6:24:20 by volume). They were then dried in an oven at 80° for 30 min. Electrophoresis in the second direction was carried out in a Gilson Medical Electronics model D high voltage electrophorator in pyridine-acetic acid-water buffer (1:10:300 by volume), pH 3.6, for 75 min at 2000 volts. Single dimensional peptide separations were carried out in a similar manner, except that the electrophoresis was performed for 100 min. Peptides were visualized by spraying with 0.2% ninhydrin in acetone and developing for 10 min at 80°.

**Disc Gel Electrophoresis**—Disc electrophoresis at pH 9.5 was performed as described by Davis (18). Electrophoresis at pH 2.3 in glycine-acetate buffer was carried out as described in Canal Industrial Corp. literature. In each case 10% monomer gels were used. The gels were stained for protein with Amido schwarz, and for trypsin and chymotrypsin inhibitory activity by the method of Uriel and Berges (19).

**Chromatography**—Sephadex G-50 and SP-Sephadex columns

\(^1\) The abbreviation used is: SP-Sephadex, sulfopropyl-Sephadex.

were poured in accord with the instructions of the manufacturer. DEAE-cellulose was cycled through 0.5 mM NaOH and 0.5 mM HCl, washed extensively with water (with decanting of fines) and thoroughly equilibrated with the appropriate buffer. The equilibration of all ion exchange columns was ascertained by effluent pH and conductivity. Sodium chloride concentration was determined from plots of conductivity. The standard buffers with variable NaCl were prepared and conductivity was plotted against NaCl concentration.

Material to be charged on an ion exchange column was dissolved in the starting buffer and then dialyzed at least 4 hours against 20 volumes of the same buffer. Pools from ion exchange chromatography were subjected to rotary evaporation at 37°, extensive dialysis against water in cellulose casings, and lyophilization.

Inhibitory activity is expressed in milligrams of trypsin inhibited by 1 \(A_{280}\) unit of inhibitor. An \(A_{280}\) unit is an amount of protein that gives an absorbancy of 1.0 at 280 nm when dissolved in 1 ml of solvent and read in a cuvette with a 1-cm light path.

**RESULTS**

**Extraction and Initial Fractionation Steps**

Beans were processed in batches of 3.5 kg. They were first ground to a fine meal in a blender. The meal was extracted with 14 liters of 0.05 mM HCl at room temperature with stirring. After 2 hours the mixture was passed through several layers of cheesecloth on a Buchner funnel to remove the larger particles. Several milliliters of toluene were added, and the filtrate was held overnight at 4°. The extract was clarified by centrifugation at 9500 rpm at 0° for 30 min in the CSA rotor of a Sorvall RC-2B centrifuge (the same conditions applied to other centrifugations below).

The clarified extract (approximately 9 liters) was adjusted to pH 5.0 with 10 mM NaOH, and solid ammonium sulfate was added to attain 65% saturation at 0° (407 g per liter). After standing overnight at 4°, the suspension was centrifuged and the supernatant was discarded. The precipitate was dissolved in a minimal volume of water (approximately 1 liter) and a sufficient amount of 25% (w/v) trichloroacetic acid was added to attain a final concentration of 5%. After 5 min at room temperature, the inactive precipitate was removed by centrifugation. The supernatant was immediately adjusted to pH 5.0 with 10 mM NaOH and the precipitation with 65% saturation ammonium sulfate was repeated. The resulting precipitate was recovered by centrifugation and dissolved in a minimal volume of water. The inhibitor solution was then transferred to cellulose casings and dialyzed against three 8-liter changes of water. The resulting precipitate was removed by centrifugation and discarded. The salt-free crude inhibitor solution was lyophilized and stored as a powder at -20° until needed.

The results of the extraction and selective precipitation steps are outlined in Table I. Treatment of the bean meal with 0.05 mM HCl extracts approximately 1000 units of trypsin inhibitor from 1 kg of meal. The subsequent ammonium sulfate and trichloroacetic acid treatment result in an 8 fold purification with an over-all yield of approximately 60% of the initial inhibitory activity. The largest single loss of activity occurs in the first ammonium sulfate precipitation, where difficulty is encountered in obtaining complete sedimentation of the precipitate. This is due to the high viscosity of the crude extract.
Examination of the crude inhibitor by disc gel electrophoresis (Fig. 1) reveals a minimum of 10 components staining with Amido Schwarz, with both trypsin and chymotrypsin being inhibited by two or more of these.

### Table I

**Summary of the initial purification procedure**

Starting material was 3.5 kg of bean meal.

| Steps                       | Protein (mg) | Inhibitor (μg) | Specific activity | Recovery | Purification factor |
|-----------------------------|--------------|----------------|------------------|----------|--------------------|
| I. Crude extract           | 76,000       | 3,520          | 0.046            | 100      | 1                  |
| II. 65% Ammonium sulfate   | 21,170       | 2,730          | 0.128            | 77.5     | 2.8                |
| III. 2.5% Trichloroacetic acid | 10,200       | 2,190          | 0.215            | 62.1     | 4.7                |
| IV. 65% Ammonium sulfate   | 5,420        | 2,040          | 0.375            | 57.8     | 8.2                |
| V. Sephadex G-50           | 436          | 1,925          | 4.42             | 54.7     | 96                 |

**Gel Filtration**

The crude inhibitor was next subjected to gel filtration on Sephadex G-50 (Fig. 2). The trypsin inhibitory activity is well separated from a large quantity of several inactive high molecular weight components, and a smaller amount of low molecular weight contaminants. A purification of approximately 11-fold was achieved in this step, with a recovery of activity of 95%. The specific activity curve exhibits a distinct peak with a shoulder, corresponding to an ascending arm and a main peak in the absorbance curve.

**Purification of Isoinhibitors**

**Ion Exchange Chromatography on DEAE-cellulose**—This step and subsequent steps were aimed at the separation of the various iso inhibitors. The inhibitor pool from the gel filtration was next subjected to ion exchange chromatography on DEAE-cellulose at pH 7.0 (Fig. 3). A large quantity of inactive material emerged at the void volume of the column. Subsequent elution with a linear sodium chloride gradient resulted in four major peaks of absorbing material, all possessing some degree of inhibitory activity. The overall yield of activity from the column was 90%. The peaks were pooled separately as shown. Further fractionation of Pools II and IV is described below.
Pool II—The material recovered in Pool II from DEAE-cellulose was next chromatographed on SP-Sephadex at pH 3.80. The resulting complex elution pattern is shown in Fig. 4. Inhibitory activity was found throughout the eluted material, indicating a large number of isoinhibitor species. The single major peak, eluting at 0.2 M NaCl, was pooled and chosen for further purification. Rechromatography under the same conditions (not shown) indicated the presence of two minor active contaminants emerging on either side of the main inhibitor peak. The main inhibitor peak was again pooled and subjected to equilibrium chromatography on SP-Sephadex at pH 3.80 (Fig. 5). A single major peak, eluting at approximately 5 column volumes, was found with constant specific activity across its width. This peak was pooled and designated isoinhibitor I.

Pool IV of Fig. 3—The material from DEAE-cellulose Pool IV was chromatographed on SP-Sephadex in a manner analogous to Pool II. Two major peaks, A and B, eluting at 0.24 and 0.30 M NaCl, were obtained (Fig. 6). Peak A was subjected to equilibrium chromatography on SP-Sephadex at pH 3.75 (Fig. 7). A major symmetrical peak, with a constant specific activity across its width, was obtained. This material was designated isoinhibitor II.

Equilibrium chromatography of Peak B on SP-Sephadex at pH 3.75 gave partial resolution of the material into two active components (not shown). The material was repooled and rechromatographed under similar conditions, with the exception that 4 M urea was included in the buffer (Fig. 8). The material was resolved into two overlapping peaks, 1 and 2, which were pooled separately. Pools 1 and 2 were rechromatographed under identical conditions (Fig. 8, A and B) and the main portions of the peaks were pooled as indicated. These pools were designated isoinhibitors IIIA and IIIB. Isoinhibitor IIIA was subsequently found to be highly heterogeneous by disc electrophoresis and will not be considered further.

Criteria for Purity of Inhibitor Components—Components I and II both exhibit single bands upon gel electrophoresis at pH 2.3 and 9.5 (Fig. 9). This, together with the constant specific activities across the peaks in equilibrium chromatography, and the near integral residue values found in the amino acid analyses, point to the homogeneity of I and II. While IIIB shows a single band in electrophoresis at pH 2.3, a second minor band is observed at pH 9.5. On the basis of staining density, IIIB may
Amino Acid Composition—The amino acid compositions of isoinhibitors I, II, and IIIb are shown in Table II. All three have high contents of aspartic acid, serine, and half-cystine. Valine, methionine, tyrosine, and phenylalanine are present in small amounts that vary characteristically among the isoinhibitors. Tryptophan is absent from all three components as judged from the ultraviolet absorption spectra (20). The minimum molecular weights calculated from the amino acid analysis for the inhibitors are approximately 8100 to 9000, with 74, 77, and 81 amino acid residues per molecule.

In the 20-hour hydrolysate of isoinhibitor II, two unknown peaks were observed, the first cutting between leucine and tyrosine on the long column of the analyzer, and the second in the position of phenylalanine. A peak similar to or identical with the first was also present in the 20-hour hydrolysate of isoinhibitor I. Both peaks were absent in the 4-hour hydrolysates, and are presumed to have been peptides.

The inhibitors do not appear to be glycoproteins. The phenolsulfuric acid test (21) revealed less than 0.60% (by weight) total carbohydrate in inhibitor I and less than 0.25% in inhibitor II. No amino sugars were observed in any of the hydrolysates for amino acid analysis. Furthermore, the inhibitors are totally unreactive to periodic acid-Schiff staining in disc electrophoresis gels (while several other bean proteins stain strongly).

Optical factors for the inhibitors may also be calculated from the amino acid analyses. By assuming the correctness of the minimum molecular weights, the weight of anhydrous salt-free protein may be calculated from the molar recovery of amino acids. From this, and the known $A_{280}$ of the protein stock solu-

| Table II | Amino acid compositions of Phaseolus vulgaris inhibitors |
|-----------------|-----------------|-----------------|-----------------|
|                | Isoinhibitor I  | Isoinhibitor II | Isoinhibitor IIIb |
| Amino acid      | 70 hrs | 48 hrs | Integer a | 70 hrs | 48 hrs | Integer a | 70 hrs | 48 hrs | Integer a |
| Aspartic acid   | 10.9   | 11.1   | 11         | 10.2   | 10.4   | 10         | 11.0   | 11.2   | 11         |
| Threonine       | 3.1    | 3.0    | 3          | 1.4    | 0.8    | 0          | 2.0    | 1.9    | 2          |
| Serine          | 10.5   | 10.4   | 11         | 4.8    | 4.7    | 5          | 4.8    | 4.8    | 5          |
| Glutamic acid   | 6.0    | 6.0    | 6          | 5.0    | 5.0    | 5          | 7.0    | 7.0    | 7          |
| Proline         | 6.2    | 6.0    | 6          | 5.0    | 5.0    | 5          | 6.4    | 6.3    | 6          |
| Glycine         | 1.0    | 1.1    | 1          | 1.8    | 1.9    | 2          | 1.8    | 1.8    | 2          |
| Alanine         | 3.0    | 3.1    | 3          | 2.0    | 2.1    | 2          | 2.9    | 3.0    | 3          |
| Half-cystine    | 12.7   | 13.3   | 14         | 11.4   | 13.2   | 14         | 12.4   | 13.2   | 14         |
| Valine          | 0.8    | 0.9    | 1          | 1.3    | 1.9    | 2          | 0      | 0      | 0          |
| Methionine      | 0      | 0      | 0          | 0      | 0      | 0          | 0      | 0      | 0          |
| Isoleucine      | 2.9    | 2.9    | 3          | 2.4    | 2.9    | 3          | 3.4    | 3.9    | 4          |
| Leucine         | 2.8    | 2.8    | 3          | 1.8    | 2.0    | 2          | 2.0    | 2.0    | 2          |
| Tyrosine        | 1.0    | 0.9    | 1          | 1.7    | 1.8    | 2          | 1.0    | 0.9    | 1          |
| Phenylalanine   | 1.2    | 1.1    | 1          | 0      | 0      | 0          | 2.1    | 2.0    | 2          |
| Lysine          | 4.0    | 3.8    | 4          | 3.8    | 3.9    | 4          | 4.0    | 4.0    | 4          |
| Histidine       | 2.9    | 2.8    | 3          | 2.8    | 2.8    | 3          | 5.0    | 4.9    | 5          |
| Arginine        | 3.5    | 2.9    | 3          | 2.6    | 2.7    | 3          | 2.7    | 2.9    | 3          |
| Total           | 74     | 77     | 81         |
| Molecular weight| 8086   | 8371   | 8884       |

a Integer values for glutamic acid of 6, 5, and 7 residues per molecule assumed for Components I, II, and IIIb, respectively.

b Determined as cysteic acid after performic acid oxidation.

be estimated to be approximately 70% pure. An insufficient amount of IIIb did not allow further purification attempts.

Characterization

The inhibitors are approximately 70% pure. An insufficient amount of IIIb did not allow further purification attempts.
tion used to prepare the hydrolysis, the optical factor was calculated. Thus, for isoinhibitors I, II, and IIIb at pH 8.0, optical factors of 3.04, 2.31, and 3.40 mg per A280 unit, respectively, were found. The optical factors at pH 2 were somewhat higher, measuring 3.69, 2.38, and 3.40, respectively.

Reactive Site Residue—All trypsin inhibitors may be divided into two groups, depending upon whether arginine or lysine is involved in the reactive site (22). The involvement of lysine in the reactive site is readily tested through the use of 2,4,6-trinitrobenzene sulfonic acid. Such tests were performed on I and II by the method of Haynes et al. (23), with the exception that the reactions were performed at 37° rather than 40° (Fig. 10). Isoinhibitor I shows rapid inactivation upon treatment with trinitrobenzene sulfonate, while II is fully active even after all of the amino groups in the molecule have reacted. Isoinhibitor I is, therefore, presumably a “lysine” trypsin inhibitor, while II is an “arginine” trypsin inhibitor.

Confirmation of this conclusion was afforded by another experiment. Isoinhibitors I and II, 0.35 mg each separately in 0.05 M sodium formate, pH 3.75, were incubated for 2 days at room temperature with 28 µg of 1-tosylamido-2-phenylethylchloromethylketone-treated trypsin (2.8 moles/100 moles of inhibitor). The mixtures were then adjusted to pH 8.0 with sodium borate buffer and 96 µg of carboxypeptidase B were added to each. After an additional 24 hours at room temperature, 1 ml of 0.2 M sodium citrate buffer, pH 2.2, was added, and the mixtures were analyzed for free amino acids. Lysine, 0.46 mole per mole of I, and arginine, 0.85 mole per mole of II, were released, confirming the presence of these residues in the reactive site bonds of I and II, respectively. No arginine with I, or lysine with II, was released above background.

Inhibitory Activities—Isoinhibitors I, II, and IIIb all have similar trypsin inhibitory activities (2.57, 2.65, and 2.87 units per mg, respectively). However, they do vary in the character of their inhibition. When I and IIIb are assayed in the standard system, linear plots of substrate hydrolysis versus time are obtained. However, when II is assayed, nonlinear, upward curving plots are obtained, particularly at inhibitor to trypsin ratios approaching 1:1 (not shown). Thus, II is significantly displaced from the trypsin-inhibitor complex by the I mM substrate, while I and IIIb are not.

Fig. 11 shows the pH versus dissociation curves of the three inhibitor-β-trypsin complexes. As might be anticipated from the above results, the β-trypsin complex with II is more dissociated at all pH values than the complexes of either I or IIIb. Thus, at pH 5.0, the complexes of tryptic and I and IIIb are only slightly dissociated, while that of II is over 50% dissociated. Furthermore, even at pH 8.0 (where the dissociation curves have apparently leveled off) the trypsin-component II complex is approximately 10% dissociated. This is in sharp contrast to I and IIIb, whose complexes are less than 1% dissociated at pH values above 6.

The inhibitors also vary in their activity toward α-chymotrypsin. Both I and II only weakly inhibit chymotrypsin, each with a specific activity of 0.20 units per mg. However, IIIb is as potent a chymotrypsin inhibitor (specific activity, 2.84 units per mg) as it is a trypsin inhibitor (2.87 units per mg). This inhibitor has two independent sites for trypsin and chymotrypsin. The trypsin inhibitor-tryptic complex still inhibits chymotrypsin, and vice versa the chymotrypsin complex inhibits trypsin.

Peptide Maps—The peptide maps of I and IIIb show striking similarity between these two inhibitors. Three of the five strongly staining tryptic peptides in each of the inhibitors appear to be common, with seemingly identical chromatographic and electrophoretic mobilities. However, much less similarity is apparent between II and either I or IIIb. Isoinhibitors I and II have no peptides in common (although several of their peptides are of similar electrophoretic, but not chromatographic, mobilities), while II and IIIb share one slightly acidic peptide in common (again with several others of similar electrophoretic, but not chromatographic, mobility). A one-dimensional electrophoretic separation of the tryptic peptides of the three inhibitors is shown in Fig. 12.

**DISCUSSION**

The isoinhibitors described here closely resemble the low molecular weight trypsin inhibitors isolated from lima (9), mung...
The occurrence of both arginine- and lysine-type trypsin inhibitors in the same plant has previously been observed in soybean (30), but II is relatively a much weaker inhibitor than either I or IIIb. The dissociation constant of II with bovine $\alpha$-trypsin is two to three orders of magnitude greater than those of I or IIIb. While all three isoinhibitors described here conform to the general legume inhibitor pattern, they do vary significantly among themselves. All three strongly inhibit bovine trypsin, chymotrypsin, and $\alpha$-chymotrypsin. The comparison is particularly interesting in view of the major similarities between I and IIIb with regard to tryptophan inhibitory activity and primary structure (trypsin peptides), suggesting that I and IIIb differ by only relatively minor structural changes, affecting particularly the chymotryptic reactive site.

REFERENCES

1. LASKOWSKI, M., and LASKOWSKI, M., Jr. (1954) Advan. Protein Chem. 9, 203-242
2. VOGEL, R., TRAUTSCHILD, L., and WERLE, E. (1968) Natural Proteinase Inhibitors, p. 9, Academic Press, New York
3. HOCHSTEIN, K., ILCHEIMANN, K., and WERLE, E. (1970) Hoppe-Seyler's Z. Physiol. Chem. 351, 1503-1512
4. TAN, C. G. I., and STEVENS, F. C. (1971) Eur. J. Biochem. 18, 515-523
5. KOIDE, T., TSUNASAWA, S., and IKENAKA, T. (1972) J. Biochem. 71, 165-167
6. ODANI, S., and IKENAKA, T. (1972) J. Biochem. 71, 839-848
7. KRESS, L. P., MARTIN, S. R., and LASKOWSKI, M., Sr. (1971) Biochim. Biophys. Acta 229, 836-841
8. JONES, G., MOORE, S., and STEIN, W. H. (1963) Biochemistry 2, 66-71
9. HAINES, R., and FRENCH, R. E. (1967) J. Biol. Chem. 242, 5378-5385
10. SCHROEDER, D. D., and SHAW, E. (1968) J. Biol. Chem. 243, 2943-2949
11. WANG, S.-S., and CARPENTER, F. H. (1965) J. Biol. Chem. 240, 1619-1625
12. SCHWERT, G. W., and TAKEAKA, Y. (1955) Biochim. Biophys. Acta 16, 570-575
13. KASSELL, R., RANDECOVIC, M., BERLOW, S., PEANASKY, R. J., and LASKOWSKI, M., Sr. (1963) J. Biol. Chem. 238, 3274-3279
14. HUMMEL, B. C. W. (1959) Can. J. Biochem. Physiol. 37, 1392-1399
15. KRESS, L. P., WILSON, K. A., and LASKOWSKI, M., Sr. (1968) J. Biol. Chem. 243, 1768-1772
16. SPACKMAN, D. H., STEIN, W. H., and MOORE, S. (1958) Anal. Chem. 30, 1190-1296
17. HIRS, C. H. W. (1966) J. Biol. Chem. 241, 611-621
18. DAVIS, B. J. (1964) Ann. N. Y. Acad. Sci. 121, 404-427
19. URIEL, J., and BERGES, J. (1968) Nature 218, 578-580
20. BEAVEN, G. H., and HOLIDAY, E. R. (1982) Advan. Protein Chem. 37, 319-386
21. HIRS, C. H. W. (1967) Methods Enzymol. 11, 411-413
22. LASKOWSKI, M., Jr., and SEALOCK, R. W. (1971) in The Enzymes (BOYER, P. D., ed), Vol. 3, pp. 375-473, Academic Press, New York
23. HAYNNS, R., OSTUGA, D. T., and FRENKEL, R. F. (1967) Biochemistry 6, 541-547
24. CHU, H-M., and CHI, C-W. (1965) Sci. Sinica 14, 1441-1453
25. WAGNER, L. P., and RITH, J. P. (1967) Arch. Biochem. Biophys. 121, 672-677
26. PUSZTAI, A. (1969) Biochim. J. 101, 379-384
27. BIK, Y., GENTLER, A., and KHALEY, S. (1983) Biochem. J. 211, 281-284
28. KRAHN, J., and STEVENS, F. C. (1972) Biochemistry 11, 1801-1808
29. SUZUKI, D. S., and LIEBER, I. E. (1971) Biochim. Biophys. Acta 251, 83-95
30. OZAWA, K., and LASKOWSKI, M., Jr. (1966) J. Biol. Chem. 241, 3955-3961
31. OZAWA, T., HIGASA, T., and HATA, T. (1971) Agr. Biol. Chem. 35, 712-716
32. FRATTALY, V., and FEINBERG, R. W. (1969) Biochim. Biophys. Res. Commun. 24, 480-487

and in Japanese radish seeds (31). However, in each case, the two inhibitors differ significantly in both molecular weight and chemical composition. This is, to our knowledge, the first instance where two similar inhibitors with different reactive site residues have been isolated from the same plant tissue.

In many instances the low molecular weight legume inhibitors are double-headed, being capable of simultaneous and independent inhibition of both trypsin and chymotrypsin (9, 32). This is the case for IIIb, which possesses independent sites for trypsin and chymotrypsin. The comparison is particularly interesting in view of the major similarities between I and IIIb with regard to tryptophan inhibitory activity and primary structure (trypsin peptides), suggesting that I and IIIb differ by only relatively minor structural changes, affecting particularly the chymotryptic reactive site.
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