Characterization of Trypsin Inhibitor in Florunner Peanut Seeds (Arachis hypogaea L.)

E. M. Ahmed* and J. A. Applewhite

ABSTRACT

Florunner peanut seeds contained five trypsin isoinhibitors. Amino acid profiles of the trypsin inhibitors fraction showed high levels of aspartic acid, half-cystine and serine and low levels of histidine and tyrosine. The molecular weight of the inhibitor was 8.3 KDa.

The presence of multiforms of this inhibitor, its low molecular weight and the high amount of half-cystine indicate that peanut trypsin inhibitor is of the Bowman-Birk type.

Key Words: Antinutrients, Groundnuts, Arachis hypogaea L.

Proteinaceous inhibitors of trypsin (TI) have been found in various legume seeds, including those of the peanut. Peanut TI is localized in the albumin protein fraction (5). Trypsin inhibitors (TI) of legumes are generally composed of several protein components: lima beans contained six (8), navy beans contained five (1), soybeans contained eight (13), Lathyrus sativus contained five protein components (15), and winged beans contained eight (3). However, there have been few reported studies of TI in peanut seeds. Two inhibitors of molecular weight 17 KDa were found in peanuts and it was assumed that these were tetramers of a subunit containing 48 amino acid residues (9). Tur-Sinai, et al. (19) have described a single inhibitor of molecular weight 7.5 KDa that inhibited equally well trypsin and chymotrypsin. Most recently two sets of anti-tryptic factors were separated into a total of five iso inhibitors which inhibited chymotrypsin to a larger extent than the trypsin (14). Their molecular weights ranged from 6.7 to 7.6 KDa. Amino acid analysis of the isomers showed high levels of cysteine.

The objective of this study was to characterize the trypsin inhibitor(s) present in Florunner peanut seeds.

Materials and Methods

Blanched Florunner peanut seeds were obtained from Pert Labs, Edenton NC. All chemicals used in this study were obtained from Sigma Chemical Co., St. Louis Mo., unless otherwise stated.

Extraction

The seeds were pressed in a hydraulic press at 30,000 kg of pressure per 0.122 sq. meter for thirty min to partially remove the oil and then were soaked in hexane for four hr to remove any remaining expressed oil distributed throughout the press cake. Two hundred g of the solvent free defatted seeds were suspended in 0.02 M HCl and stirred with magnetic stirrer for at least three hr at room temperature. The suspension was then centrifuged at 6000 g for 20 min at room temperature. The yellow supernatant was then filtered through Whatman #4 filter paper. The pH of the filtrate was then raised to 7.0 using concentrated NaOH and the mixture was again filtered through Whatman #4 filter paper. Ammonium sulfate was added to the filtrate to 70% saturation (at room temperature). The gray mixture was allowed to precipitate overnight at 3.5 C, centrifuged at 6000 g for twenty min and the supernatant was discarded. The gray precipitate was then desalted by either dialysis or by gel filtration chromatography on Sephadex G-15. Dialysis was performed for one day against magnetically stirred distilled water at room temperature with frequent changes. Dialysis tubing was obtained from Fisher Scientific Company (3.5 KDa cutoff). The salt-free solution was lyophilized in a Virtil Model 25 BRC bulk freeze dryer. The sample was poured into clean metal trays and frozen to -50 C. followed by sublimation of water at a shelf heat of 0 C and a vacuum of 100 microns. The yield was 1.6 mg of lyophilized TI obtained from 200 g defatted peanut seeds.

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Analytical Methods

Protein was determined by the method of Bradford (2). The absorbance was measured at 595 nm.

Trypsin inhibition activity was measured by the test developed by Kakade and Backis (11), which employs benzoyl-DL-arginine p-nitroaniline HCl (BAPA) as a substrate for trypsin. Trypsin from bovine pancreas (Type III) was used in this assay. Digestion of BAPA produced a yellow color due to the release of p-nitroaniline. The absorbance was measured at 410 nm.

Chromatography

Affinity chromatography was carried out on a trypsin-agarse column (U. S. Biochemicals). The column was equilibrated with a pH 8.0 buffer containing 0.05 M Tris, 0.1 M KC1, 0.02 M CaCl2 and 0.02 M NaNO3. After sample introduction the column was washed with the equilibrating buffer until non-specific proteins were removed as indicated by the negative Bradford assay (2). The inhibitors were eluted with 0.2 M KCl, pH 2. This stage was monitored by measuring the absorbance was measured at 410 nm.

Proline was determined using Beckman single column amino acid analysis (Beckman Application Notes No. 118/119 CL-AN001). Proline is expressed as % of total amino acids present in TI hydrolysate. Trypsophan was determined by the method of Edelhoch (6).

Amino acids analyses

HPLC analysis of amino acids were carried out on Perkin-Elmer liquid chromatograph (Series 400) equipped with Pecosphere 5C C18 column, fluorescence detector model LS-1 and Laboratory Computing Integrator model LCI-100. The TI protein was hydrolyzed with 6 N HCl for 24 hr at 110 C. The HCl contained 2% thiglycolate to protect tryptophan (10) and 0.25 M dimethyl sulfoxide (DMSO) for the conversion of half-cystine to cysteic acid (17) during the hydrolysis. The hydrolyzed sample was lyophilized and resuspended in 1.0 mL of 0.1 N HCl, filtered and derivatized using 50 pL sample with 200 pL 0-phthaldialdehyde-thiol (OPT) solution, incubated for 2 min at room temperature and immediately injected (20 pL) on column. The buffer used contained 2% tetrahydrofuran (THF) for the separation of threonine and glycine (10).

Non-denaturing gel electrophoresis of the TI also resulted in the resolution of five bands (Fig 2). The trypsin inhibitors consistently ran well ahead of the lowest molecular weight standard (alpha-lactoalbumin, bovine milk, MW 14,200) available for the non-denaturing gel electrophoresis. Denatured gel standards, with molecular weights ranging from 2.5 to 16.9 KDa, were used to determine the molecular weight of the isoinhibitors. The denatured trypsin isoinhibitors were too close to resolve on the gel, but the center of the band was at a molecular weight of 8.3 KDa (Fig 3).

Amino acid analysis of Flourrunner TI indicated high levels of aspartic acid, half-cystine and serine and low levels of histidine and tyrosine and an absence of tryptophan (Table 1). These findings agree with the characteristic amino acid composition of Bowman-Birk type inhibitors (20). Other characteristics of these inhibitors are their low molecular weight and the multiple forms of the inhibitor, isoinhibitors (20). This agrees with the results obtained in the present study. Amino acid analysis obtained in the present study contained more glycine, serine and valine but less half-cystine and the results obtained in the present study.
arginine than those reported by other investigators (9,14,19) as shown in Table 2.

Calculations of the isoelectric point (pI) from the net electrical charges of specific amino acids at different pH's showed a value of 4.1 (Fig 4). This pI calculations from the reported amino acid analysis (19) indicated a value of 4.67 of the Indian peanuts, although the value reported was 8-9 (19). However, this value of 4.67 is in much closer agreement with that found in the present study and those calculated for the German and Japanese peanuts (9,14). Various proteins possess isoelectric points ranging from 1.9 to 11.0 (13).

Results obtained in the present study (Table 2) agree to a great extent with those found by other workers (9,14,19). Peanut seed TI is of low MW, present in several forms (isoinhibitors), and its amino acid profile shows a high content of aspartic acid, half-cystine and serine and low levels of tyrosine. These properties classify TI in peanut seed as of Bowman-Birk type.

### Acknowledgement

The authors acknowledge with gratitude the HPLC amino acid analysis by Dr. C. McGowan's Laboratory and the stimulating discussions during the progress of this research by Mr. E. R. Mason of the Food Science and Human Nutrition Department, University of Florida.
Table 2. Characterization of TI in peanut seeds grown in different parts of the world.

<table>
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<tr>
<th>Location</th>
<th>JAPAN (10)</th>
<th>GERMANY (3)</th>
<th>INDIA (11)</th>
<th>USA</th>
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<tbody>
<tr>
<td>Amino acid</td>
<td></td>
<td></td>
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<tr>
<td>Aspartic acid</td>
<td>13</td>
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<td>7</td>
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<tr>
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<td>Alanine</td>
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</table>

aReference number
bPresent study
cValues reported as % of total amino acids to the nearest integers
dTetramer (TI complex)
eCalculated from % aspartic acid, glutamic acid, lysine, histidine and arginine of total amino acids and dissociation constants for each acid at pH's 2, 3, 4, 5, 6 and 7.

Literature Cited


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