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Isolation, Purification, and Biochemical Characterization of a Novel Water Soluble Protein from Inca Peanut (*Plukenetia volubilis* L.)

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A water soluble storage albumin from Inca peanut (IPA) accounted for ~25% (w/w) of defatted seed flour weight, representing 31% of the total seed protein. IPA is a 3S storage protein composed of two glycosylated polypeptides, with estimated molecular weights (MW) of 32800 and 34800 Da, respectively. IPA has an estimated sugar content of 4.8% ± 0.92% ($n = 6$). IPA is a basic protein (p*I* of ~9.4) and contains all of the essential amino acids in adequate amounts when compared to the FAO/WHO recommended pattern for a human adult. The tryptophan content of IPA is unusually high (44 mg/g of protein), whereas the phenylalanine content is low (9 mg/g of protein). IPA is a highly digestible protein in vitro.

KEYWORDS: Inca peanut; albumin; storage protein; in vitro protein digestibility; glycoprotein

INTRODUCTION

Inca peanut, *Plukenetia volubilis* L., is a plant that grows in the wild and is native to the rain forests of the Andean region of South America. The seeds of this plant are valued for their high oil and protein content, and the Chancas Indians and other native tribal groups eat roasted seeds either alone or mixed with corn meal and peppers. We have been investigating Inca peanut storage proteins over the past several years. During these investigations, we learned that the albumin fraction is mainly composed of a single storage protein that accounted for a substantial portion of total seed proteins. In the current paper we report a method to purify the water soluble major storage protein and some of the biochemical properties of the purified protein.

MATERIALS AND METHODS

Materials. Seeds were collected from multiple plants grown in an experimental plot at the University of San Martin, Tarapoto, Peru (*2*). Sources of electrophoresis chemicals, enzymes, salts, buffers, and DEAE DE-53 have been reported (*5*). Sephacryl S 300 HR was from Pharmacia Inc., Piscataway, NJ.

Methods. *Isolation and Purification of Inca Peanut Albumin (IPA).* Seed flour (60 mesh) was defatted for 6 h using a Soxhlet apparatus and 10 volumes of petroleum ether (boiling point range = 38.2−54.3 °C). The defatted flour was air-dried in a fume hood, homogenized using a Sorvall Omni-Mixer (Sorvall Inc., Newton, CT) with speed setting at 8, and stored in screw-capped plastic vials at −20 °C until further use. Defatted flour was extracted for 1 h at 4 °C with 20 volumes of distilled deionized water with constant magnetic stirring provided. The slurry was centrifuged for 15 min at 4 °C and 12600*g*. The residue was similarly re-extracted two more times, and the combined supernatants were dialyzed against distilled deionized water at 4 °C for 24 h with four water changes (5 L each). The molecular weight (MW) cutoff of the dialysis tubing was 6−8 kDa. Dialyzed sample was filtered through Whatman filter paper no. 4 and lyophilized. Lyophilized protein was dissolved in 20 mM Tris-HCl buffer, pH 8.1, and centrifuged (12600*g*, 4 °C, 10 min) to remove insoluble aggregates, and the supernatant was loaded onto an anion exchange (DEAE DE-53) column (2.6 × 34.0 cm) previously equilibrated in the same buffer. The column was flushed with ~2 volumes of equilibrium buffer followed by elution with a 0−0.5 M NaCl linear gradient in the equilibrium buffer (500 mL each). After completion of the gradient, the column was washed with 2 M NaCl in equilibrium buffer. Fractions were collected every 20 min. The column flow rate was set at 50 mL/h. Protein elution from the column was monitored by measuring the absorbance of fractions at 280 nm and electrophoresis.

Protein Determination. Soluble protein was determined according to the method of Lowry et al. (*4*) using bovine serum albumin as the standard protein. A standard curve was prepared in an appropriate solvent for every assay.

Ultraviolet (UV) Spectrum. UV spectra were recorded using a Perkin-Elmer λ 3 UV−VIS spectrophotometer (Oak Brook, IL) and appropriate solvent blanks. Distilled deionized water and 20 mM sodium phosphate buffer, pH 7.5, were used as solvents.

Ultracentrifugal Analysis. Samples were analyzed in a Beckman model E analytical ultracentrifuge (Palo Alto, CA) at 20−25 °C with a 30 mm double-sector cell at 48000 rpm. Potassium phosphate−sodium chloride buffer (0.033 M K_2HPO_4, 0.0026 M KH_2PO_4, 0.4 M NaCl; pH 7.6, $\mu = 0.5$) was used as solvent. Compositions were estimated

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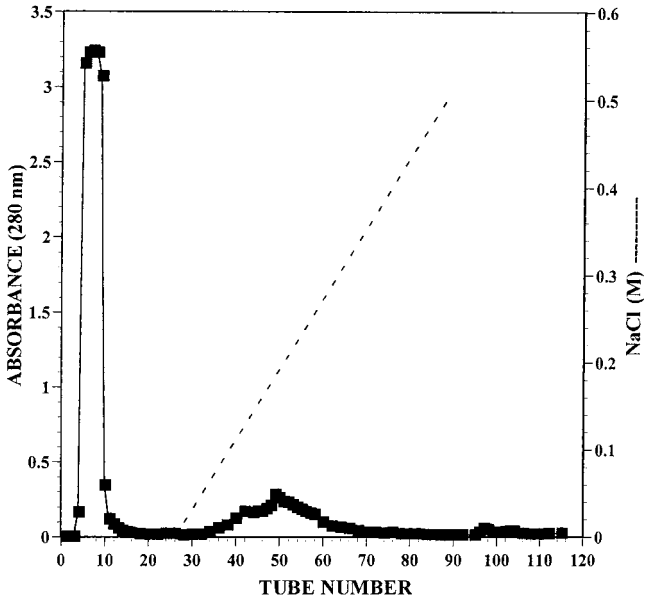

Figure 1. Elution profile for IPA off the DEAE DE 53 anion exchange column (2.6 × 34.0 cm). Column equilibrium buffer was 20 mM Tris-HCl, pH 8.1. Sample loading (tubes 1–4) was followed by flushing with the equilibrium buffer (tubes 5–26), 0–0.5 M NaCl linear gradient in equilibrium buffer, 500 mL each (tubes 27–88) (- - -), and washing with 2 M NaCl in equilibrium buffer (tubes 89–115). Fractions containing IPA (tubes 5–10) were collected.

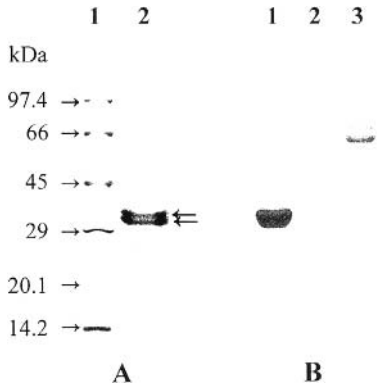

Figure 2. SDS-PAGE (A) and glycoprotein staining (B) for IPA: (A) (lane 1) protein standards (molecular weights indicated in the left margin), (lane 2) IPA (20 μg); (B) (lane 1) IPA, (lane 2) soybean 11S (negative control), (lane 3) soybean 7S (positive control); protein load in each lane was 40 μg.

by enlarging the ultracentrifuge patterns and measuring the area under each peak. Areas were corrected for radial dilution, and compositions were expressed as percentages of total area.

Electrophoresis, Amino Acid Composition, N-Terminal Amino Acid Sequencing, and in Vitro Digestibility. Sodium dodecyl sulfate (SDS)−polyacrylamide gel electrophoresis (PAGE) was done according to the method of Fling and Gregerson (*3*) as described earlier (*6*). Amino acid composition and in vitro digestibility were determined as described earlier (*6*). The N-terminal amino acid sequence was determined using an Applied Biosystems amino acid sequencer (Foster City, CA) and analyzed using the BLAST program (http://www.ncbi.nlm.nih.gov/BLAST).

RESULTS AND DISCUSSION

IPA Purification. A typical IPA elution profile for DEAE DE-53 column is shown in **Figure 1**. Because IPA is a basic protein, it eluted straight through the anion exchange column,

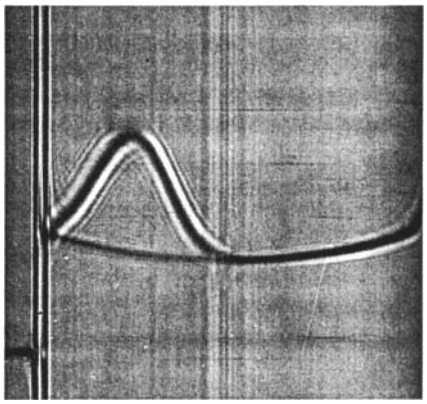

Figure 3. Ultracentrifuge pattern for IPA. Sedimentation is from left to right.

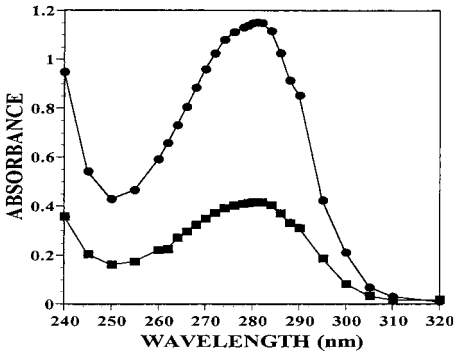

Figure 4. UV absorbance spectra for IPA: (■) distilled water (0.2 mg/mL); (●) 20 mM sodium phosphate buffer, pH 7.5 (0.5 mg/mL).

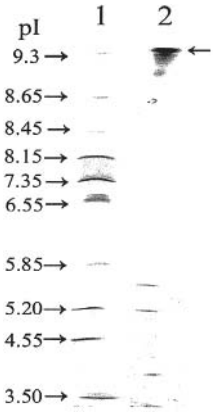

Figure 5. Isoelectric focusing for IPA: (lane 1) p*I* markers (p*I* values indicated in the left margin), (lane 2) IPA (~50 μg).

1 10 20 30

E-L-K-R-I-V-G-P-N-E-L-(-)-V/M-D-V-K-D-E-Q-Y-(-)-D-E-N-V-(-)-Q-L-W-(-)-(-)-K-K-

Figure 6. N-Terminal sequence of IPA.

as expected. Attempts to remove minor polypeptides from these preparations using gel filtration (Sephacryl S 300 HR) were not successful. Despite the inclusion of 0.1 M NaCl in the 20 mM Tris-HCl buffer, pH 8.1, used for Sephacryl S 300 HR column equilibration and elution, IPA consistently failed to elute as a sharp single peak and typically eluted as a trailing peak (data not shown). These data suggest that IPA either nonspecifically binds to Sephacryl S 300 HR or partially precipitates (or aggregates) during the gel filtration column run. We typically obtained 25 g of biochemically pure IPA from 100 g of defatted flour. The IPA obtained by this method was consistently >90%

Table 1. Amino Acid Composition of IPA[a]

amino acid	g/100 g of protein		amino acid	g/100 g of protein	
	IPA	FAO/WHO[b]		IPA	FAO/WHO[b]
Asx	12.7		Met	1.4	
Glx	14.6		Cys	4.3	
Ser	6.0		Met + Cys	5.7 (2.28)	2.5
Gly	4.8		Ile	5.0 (1.79)	2.5
His	1.0 (0.53)[c]	1.9	Leu	7.9 (1.20)	6.6
Arg	8.5		Phe	0.9	
Thr	5.7 (1.68)	3.4	Tyr	5.8	
Ala	3.7		Phe + Tyr	6.7 (1.06)	6.3
Pro	4.5		Lys	7.2 (1.24)	5.8
Tyr	5.8		Trp	4.4 (4.4)	1.1
Val	6.2 (1.77)	3.5	E/T (%)[d]	47.61	

[a] Data are expressed as grams of amino acid per gram of protein. [b] Recommended for children (2–5 years), although recently recommended for dietary protein quality evaluation for all age groups (except infants) by the joint FAO/WHO Expert Consultation (1990). [c] Figures in parentheses indicate amino acid scores. [d] Essential to total amino acid ratio.

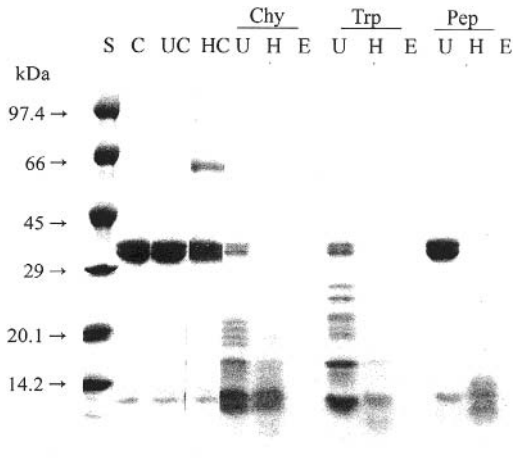

Figure 7. SDS-PAGE (8–25% linear monomer acrylamide gradient gel with 4% monomer acrylamide stacking gel) in the presence of β-ME for IPA digested with TLCK-chymotrypsin, TPCK-trypsin, and pepsin. S, Pharmacia low molecular weight standard proteins; C, no treatment control; UC, native protein incubated at 37 °C for 30 min without enzyme (control); HC, heat-denatured protein (100 °C for 30 min), incubated at 37 °C for 30 min without enzyme (control); U, native; H, heated (100 °C for 30 min); E, appropriate enzyme control (0.3 μg); Chy, TLCK-chymotrypsin; Trp, TPCK-trypsin; Pep, pepsin. IPA load = 30 μg.

pure judged on the basis of electrophoresis (bandwidth and staining intensity judged subjectively) and ultracentrifugal analysis.

Biochemical Properties. IPA is a dimeric protein composed of two polypeptides (**Figure 2A**), and both polypeptides are glycosylated (**Figure 2B**). Sedimentation analysis indicated that IPA is a 3S protein (**Figure 3**). The protein has a high UV absorbance (**Figure 4**) indicative of a high amount of tryptophan residues. $A^{1\%}$ at 280 nm was calculated to be 20.75 and 22.94, respectively in distilled deionized water and 20 mM sodium phosphate buffer, pH 7.5, as solvent, respectively. IPA is a basic protein (p$I \sim 9.4$, **Figure 5**). N-Terminal amino acid sequencing of the IPA (**Figure 6**) indicated that IPA has a high sequence homology (90% identity) with Nigerian walnut isolectins (*1*). Although many plant lectins are reportedly toxic, IPA does not appear to be toxic to humans as roasted Inca peanuts are commonly consumed by the tribal people and Chancas Indians in the Andean region.

Amino Acid Composition and in Vitro Digestibility. The amino acid composition of the IPA is summarized in **Table 1**. With the exception of histidine (an essential amino acid for infants and children under the age of 2), IPA has all of the essential amino acids in adequate amounts when compared with the FAO/WHO recommended amino acid pattern. IPA is therefore a complete protein with respect to the amino acid requirements of adult humans. To our knowledge, this is the first report of a nutritionally complete protein from plant seeds. Native IPA was not completely digested by TPCK-trypsin, TLCK-chymotrypsin, and pepsin; however, upon heat denaturation, IPA was readily digested by all of the proteases tested (**Figure 7**). Among the three proteases tested, pepsin was the most efficient in hydrolyzing the heat-denatured IPA as evidenced by complete hydrolysis of IPA within 30 min.

Conclusions. IPA is a dimeric, 3S, nutritionally complete plant storage glycoprotein that, upon heat denaturation, is easily digested by TLCK-chymotrypsin, TPCK-trypsin, and pepsin in vitro.

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