Food Chemistry 44 (1992) 227–233
Analytical Methods Section

Isolation and partial characterization of a porcine thyroglobulin-binding *Phaseolus vulgaris* L. lectin

William R. Bonorden* & Barry G. Swanson

Food Science and Human Nutrition, Washington State University, Pullman, Washington 99164-6376, USA

(Received 5 May 1991; accepted 26 September 1991)

An albumin lectin from *Phaseolus vulgaris* L. cultivar Black Turtle Soup (BTS) was purified with porcine thyroglobulin (PTG)-Sepharose affinity chromatography and Sephadex SP-C50-120 ion exchange chromatography. The PTG-binding lectin concentration of BTS bean is 22·5 ± 0·9 mg g^{-1} dry flour. The lectin is a tetrameric protein with an estimated molecular weight of 140,000 ± 9,000, and a sedimentation coefficient of 6·7 S at pH 6·0. The estimated molecular weights of the polypeptide subunits were 37,500, 35,000, 34,500 and 33,500. Isoelectric focusing of the purified lectin revealed three major bands and two minor bands. The isoforms of the lectin focused in a pI range of 5·7–6·3. The partition coefficient of the lectin in a Waters I-250 protein column was 0·25. The lectin contained small concentrations of methionine and no detectable cysteine.

INTRODUCTION

Legumes are important dietary constituents in tropical and subtropical countries, especially in the areas where animal protein consumption is low (Fernandez *et al.*, 1982). Investigations on the nutritional quality of legumes have revealed that they contain a number of antinutrients, including polyphenols, phenolic acids, flavonoids, tannins, alkaloids, sapogens and lectins.

Lectins are non-enzymatic proteins capable of binding to complex carbohydrate moieties and are perhaps the most hazardous antinutritional factor present in legumes. In animal feeding studies, lectins have proved lethal when consumed in the non-denatured state (Honaver *et al.*, 1962; Antunes & Sgarbieri, 1980; Noah *et al.*, 1980; Bender, 1983; Pusztai & Greer, 1984). Lectins bind to and inflict damage upon the proximal small intestine luminal surface, particularly the non-crypt regions of the microvilli and brush border cells of the duodenum and jejunum (Evans *et al.*, 1973; Etzler & Branstrator, 1974; King *et al.*, 1980*a*,*b*, 1986; Pusztai, 1980; Banwell *et al.*, 1985; Greer *et al.*, 1985).

*Food Research Institute, Department of Food Microbiology and Toxicology, University of Wisconsin-Madison, 1925 Willow Dr., Madison, Wisconsin 53706, USA.

Food Chemistry 0308–8146/92/$05.00 © 1992 Elsevier Science Publishers Ltd, England. Printed in Great Britain

The primary foci of this research were to develop lectin purification and quantification procedures and to chemically and physically define a purified BTS bean (*Phaseolus vulgaris* L.) PTG-binding lectin.

MATERIALS AND METHODS

Protein extraction and fractionation

BTS beans were obtained from the Institute of Nutrition for Central America and Panama (INCAP), Guatemala. Seed protein was extracted according to Osborn *et al.*, (1983). Flour was prepared by milling seed in a UDY cyclone mill (UDY Corp., Fort Collins, Colorado, USA) equipped with a screen containing 0·5 mm diameter perforations. Bean flour, 4 g, was stirred overnight at 4°C in 100 ml phosphate-buffered saline (PBS), pH 7·0 (0·15 M NaCl, 1·1 mM NaH_2PO_4 and 2·5 mM Na_2HPO_4; PBS-1) containing 4·0 mM β-mercaptoethanol (β-ME), 1·0 mM phenylmethylsulphonyl fluoride (PMSF) and 1·0 mM *N*-α-*p*-tosyl-L-lysine chloromethyl ketone (TLCK). A 100 ml volume of cold, deionized, distilled water was added to the flask and the contents stirred for 2 h, reducing the NaCl concentration to 0·075 M and precipitating the phaseolin fraction. The final concentration of the bean flour in the extracting solution was 20 mg ml^{-1}. The bean flour–0·075 M NaCl PBS mixture was cen-

trifuged at 31,300 × *g* for 15 min at 4°C in a Beckman Model J-21C centrifuge equipped with a type JA-20 rotor. The supernatant was first filtered through Whatman No. 4 filter paper, then through a combination of a spun glass prefilter and a 0·45 μm nitrocellulose filter.

Albumin and globulin-2 (G2) fractions were isolated by dialysis of the filtered protein extract against three 5-litre volumes of deionized, distilled water at 4°C. The supernatant was centrifuged at 31,300 × *g* for 45 min. The resulting supernatant, which contained the albumin fraction, was retained for further analysis. The pellet was placed in a 100 ml Erlenmeyer flask and the G2 proteins extracted with 75 ml of pH 7·0 PBS (0·075 M NaCl, 1·1 mM NaH_2PO_4 and 2·5 mM Na_2HPO_4; PBS-2). The mixture was stirred for 2 h, centrifuged at 31,300 × *g* and filtered through a glass prefilter and a 0·45 μm nitrocellulose filter. Albumin, G2 and G2/albumin protein extracts were immediately used as a lectin source for affinity chromatography, or lyophilized and stored at −10°C.

Affinity matrix synthesis

Cyanogen bromide-activated Sepharose was prepared according to the method described by Cuatrecasas (1970). Cross-linking of the PTG to the cyanogen bromide-activated Sepharose was completed according to Felsted *et al.* (1975).

Affinity chromatography

Protein extract, 250 ml of the centrifuged supernatant containing the albumin fraction or 75 ml of pH 7·0 PBS-2 containing the G2 extract, was added to 100 ml of packed PTG-Sepharose and mixed for 1 h at 4°C on a gyrotory shaker. The slurry was filtered over Whatman No. 4 filter paper in a Buchner funnel and washed with pH 7·0 PBS-2 until the absorbance at 280 nm of the washing solution stabilized. Lectins were eluted from the affinity matrix by the addition of three 50 ml volumes of pH 3·0 glycine buffer (0·05 M glycine and 0·5 M NaCl). Each volume was added to the funnel, stirred, and removed by vacuum filtration after a 5 min holding period. The eluents were pooled, filtered and lyophilized or assayed for protein. The affinity chromatography procedure parallels that of Felsted *et al.* (1975).

Lectin quantification

The concentration of the PTG-binding BTS lectin in the flour was determined by preparing serial dilutions of a BTS flour protein extract, isolating the lectins with the PTG affinity matrix and determining the protein concentrations of the pH 3·0 glycine buffer–lectin eluent with the Folin–Ciocalteau assay. Affinity chromatography-isolated BTS PTG-binding lectin was used for protein standards. Results indicated the assay was reliable and the affinity matrix was not saturated with lectin.

Ion exchange chromatography

A 2·5 cm i.d. × 45 cm length column was packed with Sephadex SP-C50-120 (Sigma Chem. Co., St. Louis, Missouri, USA) in pH 6·0 PBS (0·05 M KH_2PO_4; PBS-4). The lectin–pH 3·0 glycine buffer solution prepared with affinity chromatography was placed in an Amicon Model 202 ultrafiltration device fitted with an Amicon Diaflo membrane filter, 10,000 molecular weight (MW) cutoff (Amicon Corp., Lexington, Massachusetts, USA). Pressure was supplied by gaseous nitrogen at 65 psi. The ultrafiltration device was used to exchange the pH 3·0 glycine buffer with pH 6·0 PBS-4. The volume of the PBS-4 pH 6·0–lectin solution was reduced to 10 ml, 20–30 mg of protein, and loaded on the column. The ion exchange procedure follows that described by Osborn *et al.* (1983). The column was washed with 250 ml of pH 6·0 PBS-4 prior to running a 0–0·188 M NaCl linear salt gradient in PBS-4. The linearity of the salt gradient was monitored by comparing the conductivity of standard NaCl solutions with that of specific column fractions (conductivity detector model 21511000; Wescan Instruments Inc., Santa Clara, California, USA). Flow rate was maintained at 0·5 ml min^{-1} with a Manostat Junior Cassette Pump (Manostat, New York, USA). Lectin-containing fractions were frozen at −10°C.

Protein quantification

The Folin–Ciocalteau assay was used for the determination of protein concentrations (Clark & Switzer, 1977).

Discontinuous polyacrylamide gel electrophoresis

The native protein cathodic discontinuous polyacrylamide gel electrophoresis (DISC-PAGE) procedure was a modified version of the procedure described in Reisfeld *et al.* (1962). The modification involved the substitution of the Reisfeld stacking gel with a gel recommended by Pharmacia (1982).

Protein solutions were prepared according to Reisfeld *et al.* (1962). Gels were run at a constant 180 V at 4°C and fixed for 1 h in a solution described by Chen and Whitaker (1986). The staining and destaining formulas and times were taken from a Bio-Rad isoelectric focusing procedure (Bio-Rad Laboratories, Richmond, California, USA).

Isoelectric focusing

The isoelectric focusing (IEF) method is described in Pharmacia (1982). Isoelectric points (pI) of the lectin polypeptides were determined from linear least-squares regressions of plots of the pI values of protein standards (Pharmacia, Piscataway, New Jersey, USA) versus the distance the protein migrated from the anode.

Sodium dodecyl sulphate polyacrylamide gel electrophoresis

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) was performed at 15°C using the Tris–glycine buffer system of Laemmli (1970) and the 8–16% linear gradient polyacrylamide resolving gel of O'Farrell (1975).

Molecular weights of unknowns were estimated by a linear least-squares regression of plots of the logarithm of the molecular weight standards (Bio-Rad Laboratories) versus the distance the protein migrated from the anode.

Sedimentation velocity

Sedimentation velocity analysis was performed by the Washington State University Bioanalytical Laboratory with a Beckman Model E analytical ultracentrifuge (Beckman Instrum. Inc., Spinco Div., Palo Alto, California, USA). Analysis was performed in an An-F rotor at 20°C. Lectin extract protein concentration was 2·3 mg ml^{-1} in pH 6·0 PBS-4, density 1·008 g cm^{-3}. To prevent aggregate formation, the lectin-containing ion exchange fractions were not frozen or lyophilized. Ion exchange chromatography fractions were pooled and concentrated in an ultrafiltration device.

Equilibrium sedimentation

Equilibrium sedimentation studies were performed by the Washington State University Bioanalytical Laboratory with a Beckman model E analytical ultracentrifuge equipped with an An-H rotor. Molecular weight was determined by using the high-speed meniscus-depletion sedimentation equilibrium method (Yphantis, 1964). Lectin concentration was 0·78 mg ml^{-1} in pH 6·0 PBS-4, density 1·008 g cm^{-3}.

An additional preparatory step involved ultracentrifugation of the lectin–pH 6·0 PBS-4 solution at 45,000 × *g*. Ultracentrifugation was required to remove lectin aggregates formed during concentration in the ultrafiltration device. The aggregate-free supernatant was filtered through a glass prefilter and a 0·45 μm nitrocellulose filter immediately prior to analytical ultracentrifugation.

Partial specific volume estimation

An estimate of the partial specific molar volume of the BTS lectin was determined from the density of a solution of lectin and the density of the solvent used to prepare the lectin solution. Density was measured with a mechanical oscillator type densitometer (Model DMA 40, Mettler/Paar, Graz, Austria).

An unknown percentage of the BTS lectin formed insoluble aggregates during purification and lyophilization; hence, the mass of lectin could not be determined gravimetrically. The mass was estimated by preparing a BTS lectin solution, filtering the solution through a glass prefilter and a 0·45 μm nitrocellulose filter, and assaying the solution for protein with the Folin–Ciocalteau method.

Partition coefficient determination

The partition coefficient of the BTS bean lectin was determined with a Waters I-250 high-pressure liquid chromatography column (HPLC; Waters Associates, Inc., Milford, Massachusetts, USA). The mobile phase was pH 7·0 PBS-2. The flow rate was maintained at 1 ml min^{-1}. Protein standards were from Sigma Chem. Co.

Blue dextran (2 × 10^6 MW; Bio-Rad Chem. Div.) was used to estimate the void volume of the column. Tyrosine (181 MW; Sigma Chem. Co.) was used to determine the column volume. Partition coefficients were determined from the mean of four replicate injections.

Amino acid analysis

Amino acid analysis was conducted by the Washington State University Bioanalytical Laboratory. Amino acids were analysed according to the method of Spackman *et al.* (1958). The analyses were performed on a Beckman 121 MB automatic amino acid analyser equipped with a model 126 data system integrator (Beckman Instrum. Inc., Spinco Div.). Cysteine and methionine were analysed using performic acid oxidation, as described in Moore (1963). Tyrosine and tryptophan concentrations were estimated by the method of Edelhoch (1967).

Statistical analyses

Experimental error is reported as the standard deviation of the mean.

RESULTS AND DISCUSSION

Ion exchange chromatography and DISC-PAGE electrophoresis

Despite electrophoretic data indicating a single BTS PTG-binding lectin eluted from the affinity column (data not shown), initial ion exchange chromatography of the isolated BTS lectin produced a chromatogram with four non-distinct minor peaks adjacent to a major peak. To reduce the number of minor peaks associated with the BTS lectin, modifications were made in the lectin isolation procedure. The modifications avoided dialysis, freezing and lyophilizing; that is, procedures thought to promote dissociation of lectin protomers or metal ions from the lectin monomers. The alternative

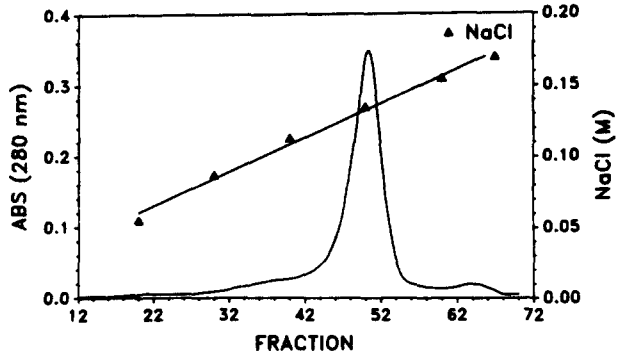

Fig. 1. Ion exchange chromatography of BTS PTG-binding lectin.

technique used ultrafiltration to exchange the pH 3·0 glycine buffer with pH 6·0 PBS-4 and to concentrate the PBS-4 pH 6·0–lectin solution prior to ion exchange chromatography. The ion–exchange chromatogram of the BTS PTG-binding lectin isolated by the modified procedure is depicted in Fig. 1.

Native protein cathodic DISC-PAGE electrophoretic patterns of the G2/albumin fraction, ion exchange purified BTS PTG-binding lectin, and albumin and G2 fractions are presented in Fig. 2. The data indicates that the BTS flour albumin fraction contained two major and one minor basic proteins (lane A), while the G2 fraction contained one major and two minor basic proteins (lane G). The purified BTS PTG-binding lectin (lane L) was the slower migrating protein when compared with the two major albumin proteins (lane A).

Sodium dodecyl sulphate polyacrylamide gel electrophoresis

SDS-PAGE of the purified BTS PTG-binding lectin produced four distinct polypeptides, with estimated molecular weights of 37,500, 35,500, 34,500 and 33,500.

Brown *et al.* (1981, 1982) reported the V_{G2} type G2/albumin group contained five polypeptides; designated 6, 8, 9, 10 and 11, the polypeptides had molecular weights of 41,000, 38,000, 36,500, 36,500 and 39,000, respectively. The difference in the number of poly- peptides reported by Brown *et al.* (1981, 1982) and the number of peptides observed in this study, five versus four, can be attributed to two-dimensional electrophoresis of a G2/albumin fraction that contained both of the V_{G2} albumin basic proteins (lane A, Fig. 2). The SDS-PAGE analysis involved the single PTG-binding V_{G2} lectin. Based on molecular weights, the 41,000 molecular weight polypeptide (6) is a peptide unique to the non-PTG-binding V_{G2} albumin basic protein.

The 1500–2500 difference in molecular weights of lectin subunits in the V_{G2} cultivars Viva and BTS is not understood. The difference in molecular weight indicates subtle differences in the G2/albumin group peptides of individual cultivars. Discrepancies in the estimated molecular weight values of lectin subunits are common. The erythroagglutinating (E) and leucoagglutinating (L) subunits of the T_{G2} G2/albumin group cultivars range in molecular weight from 29,000–34,000 (Goldstein & Hayes, 1978). The L subunit has reported molecular weights of 31,000 (Rasanen *et al.*, 1973), 34,000 (Yachnin & Svenson, 1972; Bollini & Chrispeels, 1978) and 33,000 (Leavitt *et al.*, 1977).

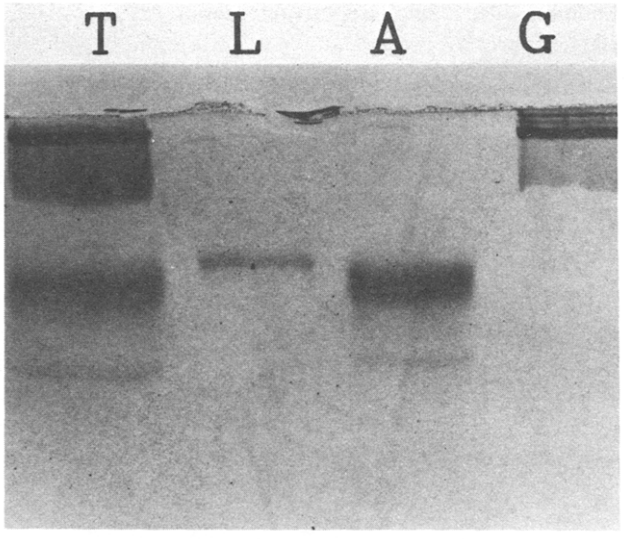

Fig. 2. Cathodic DISC-PAGE electrophoretic patterns of non-denatured BTS seed proteins: lane T — total G2/albumin fraction; lane L — purified BTS lectin; lane A — albumin fraction; lane G — G2 fraction. The anode was at the top of the gel, the cathode at the bottom.

Isoelectric focusing

Initial IEF studies of purified BTS lectin produced three major and five minor bands. In an attempt to reduce the number of bands in the subsequent IEF experiments β-ME, TLCK and PMSF were added to the BTS flour protein extraction buffer and ultrafiltration/concentration was substituted for dialysis and lyophilization prior to ion–exchange chromatography. The resulting IEF gels produced three major bands, pI values 5·9, 6·0 and 6·3, and two minor bands, pI values 5·7 and 5·8. Assuming the BTS lectin protomer did not dissociate, the presence of five electrophoretic isoforms is difficult to understand. Variable staining intensity of the four polypeptides in the SDS-PAGE gel suggests isoforms of the BTS lectin exist. If isoforms of the BTS lectin exist, cathodic DISC-PAGE gel electrophoresis failed to resolve the isoforms. The lack of IEF homogeneity among lectins is a common occurrence resulting in the reporting of pI ranges (Pusztai & Stewart, 1978).

Manen *et al.* (1984) offered an explanation for the lack of IEF homogeneity among lectins. Five purified lectins from the cultivar Contender were analysed by DISC-PAGE and IEF. Manen *et al.* (1984) demon-

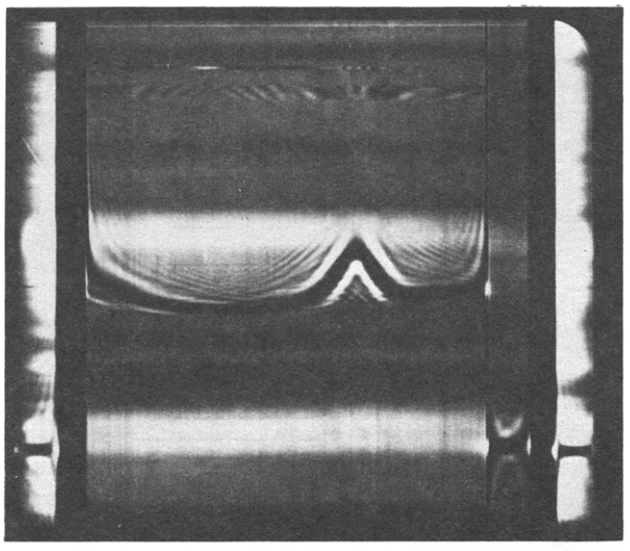

Fig. 3. Sedimentation pattern of the BTS PTG-binding lectin.

strated that purified tetrameric lectins can exist in five electrophoretic forms, depending on the concentration of Mn^{2+} bound to the tetramer, and suggested that Mn^{2+} ions dissociate from *P. vulgaris* lectins at pH values of 4·5 or lower. The existence of forms of the BTS lectin differing in Mn^{2+} concentrations is possible, considering a pH 3·0 glycine buffer (i.e. a pH below 4·5) was used to elute the lectin from the affinity matrix.

Sedimentation velocity

Sedimentation patterns of the BTS PTG-binding lectin indicate that the BTS lectin is a single component (Fig. 3). The calculated sedimentation coefficient was 6·7 S at pH 6·0. Lectins from the cultivars Greensleeves (Bollini & Chrispeels, 1978) and Red Kidney beans (Leavitt *et al.*, 1977) and commercially prepared phytohaemagglutinin (Oh & Conrad, 1971) have sedimentation coefficients of 6·4 (pH 7·0), 6·9 (pH 7·4) and 7·0 S (pH 7·0), respectively.

Sedimentation equilibrium

During sedimentation equilibrium analysis, the BTS lectin migrated as a single component. Initial calculations using an estimated partial specific volume of 0·735, based on the amino acid composition, resulted in an estimated molecular weight that did not correlate with the SDS-PAGE molecular weight data. The absence of carbohydrate analysis precluded a more accurate recalculation of the amino acid-based partial specific volume; hence, the partial specific volume was physically determined via density measurements. The partial specific volume was 0·772. The molecular weight range estimated from sedimentation equilibrium with the partial specific volume of 0·772 was 133,000–146,000, or 140,000 ± 9,000. The molecular weight range of the BTS lectin, based on SDS-PAGE electrophoresis, assuming the lectin protomer is a tetrameric molecule, is estimated as 134,000–150,000.

Partition coefficient

BTS lectin eluted as a single component when analysed by HPLC on a Waters I-250 protein column. The partition coefficient of the lectin was 0·25. The molecular weight was not estimated by partition chromatography because two of the seven protein standards used for the molecular weight standard curve failed to elute in order of decreasing molecular weight, indicating that chromatographic interactions other than molecular sieving were occurring.

Amino acid analysis

The amino acid analysis of the BTS lectin is presented in Table 1. The moles of amino acid per mole of lectin were calculated assuming an average estimated molecular weight of 140,000. Edelhoch's (1967) tyrosine and tryptophan analysis correlated well with the acid hydrolysis amino acid analysis. The Edelhoch (1967) technique estimated a tyrosine content of 14·58 moles $mole^{-1}$ BTS lectin, while the acid hydrolysis data estimated a tyrosine concentration of 14·93 moles $mole^{-1}$ of lectin.

The amino acid analysis of the BTS lectin is consistent with literature data (Oh & Conrad, 1971; Leavitt *et al.*, 1977; Itoh *et al.*, 1980). The BTS lectin is void of detectable concentrations of cysteine, common among T_{G2} G2/albumin type *P. vulgaris* lectins (Oh & Conrad, 1971; Leavitt *et al.*, 1977) and the *P. vulgaris* cultivar Tora-mame (Itoh *et al.*, 1980).

Methionine was present in small concentrations in the BTS lectin. Methionine was detected in T_{G2} G2/albumin type lectins by Oh and Conrad (1971), but not by Leavitt *et al.* (1977). Methionine was not detected in the cultivar Tora-mame (Itoh *et al.*, 1980). In many instances where methionine was detected, it represented a small percentage of the total amino acid residues.

Table 1. Amino acid analysis of the BTS PTG-binding lectin

Amino acid	Mole amino acid $mole^{-1}$ lectin	Amino acid	Mole amino acid $mole^{-1}$ lectin
Asp	131	Thr	88
Ser	102	Glu	62
Pro	44	Gly	122
Ala	93	Val	73
Met	2	Ile	53
Leu	93	Tyr	15
Phe	44	His	8
Lys	41	Cys	0
Arg	16	NH_3	1025
Trp	21		

The most prevalent amino acid residues in the BTS lectin were Asp, Thr, Ser, Gly, Ala and Leu. Evidently, there is conservation of structure or amino acid composition among lectins. The four amino acids Asp, Thr, Ser and Leu represent 41–44% of the total amino acid residues (not including Asn and Gln) of BTS, Toramame (Itoh *et al.*, 1980) and T_{G2} G2/albumin type lectins (Oh & Conrad, 1971; Leavitt *et al.*, 1977). The number of moles of ammonia liberated during the acid hydrolysis of the BTS lectin indicates that a substantial percentage of the amino acid residues are Asn and Gln or that the lectin contains a significant concentration of amine-containing sugars.

Lectin concentration

The concentration of the PTG-binding lectin in BTS bean flour was 22·5 ± 0·9 mg g^{-1} dry flour.

CONCLUSIONS

BTS bean contains isoforms of a single PTG-binding albumin lectin. The lectin resolves as a single component during HPLC, ion–exchange chromatography, analytical ultracentrifugation and cathodic DISC-PAGE analyses. The BTS lectin is a tetrameric protein composed of combinations of four polypeptides, molecular weights 37,500, 35,000, 34,500 and 33,500, with an estimated molecular weight of 140,000 ± 9,000, a sedimentation coefficient of 6·7 S at pH 6·0 and an amino acid composition similar to other *P. vulgaris* lectins.

SDS-PAGE, IEF and the large molecular weight standard deviation suggested isoforms of the BTS PTG-binding lectin exist, but were not resolved by HPLC, analytical ultracentrifugation, ion–exchange and cathodic DISC-PAGE techniques. The staining intensity of the 33,500 molecular weight protein in the SDS gel indicated that the isoforms are primarily composed of the 33,500 subunit.

Whether the isoforms are generated by dissociation of metal ions from the lectin subunits or are a function of differing peptide or carbohydrate composition remains unknown. Partial proteolysis would produce isoforms, but the addition of protease inhibitors to the protein extraction buffer should have minimized artifact generation. The possibility of the formation of demetallized variants of the lectin cannot be ignored, especially when considering the number of isoforms present in the IEF gel. Five isoforms would represent five tetramers, each with a different number of metal-containing subunits.

Analytical ultracentrifugation did not confirm the presence of lectin isoforms. However, a large standard deviation was associated with the sedimentation equilibrium molecular weight estimation. The standard deviation represented a range that matched the isoform molecular weight range predicted from SDS-PAGE data.

Amino acid analysis demonstrated that the BTS PTG-binding lectin is a poor protein source, due to the deficiency in essential sulphur-containing amino acids. The large concentration of lectin in the dry flour further increases the lectin's contribution to the low protein quality of the BTS seed.

ACKNOWLEDGEMENTS

Partial financial support for this research was provided by USAID Title XII Dry Bean/Cowpea CRSP, and the Agricultural Research Center, College of Agriculture and Home Economics, Washington State University, USA (10A–3057–0560).

REFERENCES

Antunes, P. L. & Sgarbieri, V. C. (1980). Effect of heat treatment on the toxicity and nutritive values of dry bean (*Phaseolus vulgaris* var. Rosina G2) proteins. *J. Agric. Food Chem.*, **28**, 935–6.

Banwell, J. G., Howard, R., Cooper, D. & Costeron, J. W. (1985). Intestinal microbial flora after feeding phytohemagglutinin lectins (*Phaseolus vulgaris*) to rats. *Appl. Environ. Microbiol*, **50**(1), 68–80.

Bender, A. E. (1983). Haemagglutinins (lectins) in beans. *Food Chem.*, **11**, 309–20.

Bio-Rad Isoelectric Focusing Standards Instruction Pamphlet, Bio-Rad Chemical Division, 2200 Wright Ave., Richmond, California 94804, USA.

Bollini, R. & Chrispeels, M. J. (1978). Characterization and subcellular localization of vicilin and phytohemagglutinin, the two major reserve proteins of *Phaseolus vulgaris* L. *Planta*, **142**, 291–8.

Brown, J. W. S., Osborn, T. C., Bliss, F. A. & Hall, T. C. (1981). Genetic variation in the subunits of globulin-2 and albumin seed proteins of French bean. *Theor. Appl. Genet.*, **60**, 245–50.

Brown, J. W. S., Osborn, T. C., Bliss, F. A. & Hall, T. C. (1982). Bean lectins. Part 1: relationships between agglutinating activity and electrophoretic variation in the lectin-containing G2/albumin seed proteins of French bean (*Phaseolus vulgaris* L.). *Theor. Appl. Genet.*, **62**, 263–71.

Chen, A. D. & Whitaker, J. R. (1986). Purification and characterization of a lipoxygenase from immature English peas. *J. Agric. Food Chem.*, **34**, 203–11.

Clark, J. M. Jr. & Switzer, R. L. (ed.) (1977). *Experimental Biochemistry*, 2nd ed. W. H. Freeman and Co., New York, p. 12.

Cuatrecasas, P. (1970). Protein purification by affinity chromatography. Derivatizations of agarose and polyacrylamide beads. *J. Biol. Chem.*, **245**(12), 3059–65.

Edelhoch, H. (1967). Spectroscopic determination of tryptophan and tyrosine in proteins. *Biochem.*, **6**, 1948–54.

Etzler, M. E. & Branstrator, M. L. (1974). Differential localization of cell surface and secretory components in rat intestinal epithelium by use of lectins. *J. Cell Biol.*, **62**, 329–43.

Evans, R. J., Pusztai, A., Watt, W. B. & Bauer, D. (1973).

Isolation and properties of protein fractions from navy beans (*Phaseolus vulgaris*) which inhibit the growth of rats. *Biochim. Biophys. Acta*, **303**, 175–84.

Felsted, R. L., Leavitt, R. D. & Bachur, N. R. (1975). Purification of the phytohemagglutinin family of proteins from Red Kidney beans (*Phaseolus vulgaris*) by affinity chromatography. *Biochim. Biophys. Acta*, **405**, 72–81.

Fernandez, R., Elias, L. G., Braham, J. E. & Bressani, R. (1982). Trypsin inhibitors and hemagglutinins (*Phaseolus vulgaris*) and their relationship with the content of tannins and associated polyphenols. *J. Agric. Food Chem.*, **30**, 734–9.

Goldstein, I. J. & Hayes, C. E. (1978). Lectins: carbohydrate-binding proteins. In *Advances in Carbohydrate Chemistry and Biochemistry*, Vol. 35, ed. R. S. Tipson & D. Horton. Academic Press, New York, pp. 127–340.

Greer, F., Brewer, A. C. & Pusztai, A. (1985). Effect of kidney bean (*Phaseolus vulgaris*) toxin on tissue weight and composition and some metabolic functions of rats. *Brit. J. Nutr.*, **54**, 95–103.

Honavar, P. M., Shih, C. & Liener, I. R. (1962). Inhibition of the growth of rats by purified hemagglutinin fractions isolated from *Phaseolus vulgaris*. *J. Nutr.*, **77**, 109–14.

Itoh, M., Kondo, K., Komada, H., Izutsu, K., Shimabayashi, Y. & Takahashi, T. (1980). Purification and characterization of a lectin from *Phaseolus vulgaris* seed. *Agric. Biol. Chem.*, **44**(1), 125–33.

King, T. P., Pusztai, A. & Clarke, E. M. W. (1980*a*). Immunocytochemical localization of ingested kidney bean (*Phaseolus vulgaris*) lectins in rat gut. *Histochem. J.*, **12**, 201–8.

King, T. P., Pusztai, A. & Clarke, E. M. W. (1980*b*). Kidney bean (*Phaseolus vulgaris*) lectin-induced lesions in rat small intestine: 1. Light microscope studies. *J. Comp. Path.*, **90**, 585–95.

King, T. P., Pusztai, A., Grant, G. & Slater, D. (1986). Immunogold localization of ingested kidney bean (*Phaseolus vulgaris*) lectins in epithelial cells of the rat small intestine. *Histochem. J.*, **18**, 413–20.

Laemmli, U. K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T_4. *Nature*, **227**, 680–5.

Leavitt, R. D., Felsted, R. L. & Bachur, N. R. (1977). Biological and biochemical properties of *Phaseolus vulgaris* isolectins. *J. Biol. Chem.*, **252**(9), 2961–6.

Manen, D., Manen, J. F. & Morgan, M. R. (1984). *Phaseolus vulgaris* lectin heterogeneity related to their metal content. *Plant Sci. Lett.*, **37**, 105–10.

Moore, S. (1963). On the determination of cystine as cysteic acid. *J. Biol. Chem.*, **238**, 235–7.

Noah, N. D., Bender, A. E., Reaidi, G. B. & Gilbert, R. J. (1980). Food poisoning from raw Red Kidney beans. *Brit. Med. J.*, **19**, 236–7.

O'Farrell, P. H. (1975). High resolution two-dimensional electrophoresis of proteins. *J. Biol. Chem.*, **250**, 4007–21.

Oh, Y. H. & Conrad, R. A. (1971). Some properties of mitogenic components isolated from phytohemagglutinin by a preparative gel electrophoresis. *Arch. Biochem. Biophys.*, **146**, 525–30.

Osborn, T. C., Ausloos, K. A., Brown, J. W. S. & Bliss, F. A. (1983). Bean lectins III: evidence for greater complexity in the structural model of *Phaseolus vulgaris* lectin. *Plant Sci. Lett.*, **31**, 193–203.

Pharmacia (1982). *Isoelectric Focusing Principles and Methods*. Pharmacia Fine Chemicals, Sept., Ljungforetagen AB, Orebro.

Pusztai, A. (1980). Nutritional toxicity of the kidney bean (*Phaseolus vulgaris*). *Rep. Rowett Inst.*, **36**, 110–8.

Pusztai, A. & Greer, F. (1984). Effect of dietary legume proteins on the morphology and secretory response of the rat small intestine. In *Protides of the Biological Fluids* ed. H. Peter. Pergamon, New York, pp. 347–50.

Pusztai, A. & Stewart, J. C. (1978). Isolectins of *Phaseolus vulgaris*. Physicochemical studies. *Biochim. Biophys. Acta*, **536**, 38–49.

Rasanen, V., Weber, T. H. & Grasbeck, R. (1973). Crystalline kidney-bean leucoagglutinin. *Eur. J. Biochem.*, **38**, 193–200.

Reisfeld, R. A., Lewis, U. J. & Williams, D. E. (1962). Disk electrophoresis of basic proteins and peptides on polyacrylamide gels. *Nature*, **195**, 281–3.

Spackman, K. H., Stein, W. H. & Moore, S. (1958). Automatic recording apparatus for use in the chromatography of amino acids. *Anal. Chem.*, **30**, 1190–206.

Yachnin, S. & Svenson, R. H. (1972). The immunological and physicochemical properties of mitogenic proteins derived from *Phaseolus vulgaris*. *Immunology*, **22**, 871–83.

Yphantis, D. A. (1964). Equilibrium ultracentrifugation of dilute solutions. *Biochemistry*, **3**(3), 297–317.