HPLC ISOLATION AND NUTRITIONAL VALUE OF A MAJOR TUBER PROTEIN

B.E. Liedl, T. Kosier, and S.L. Desborough[1]

Abstract

A predominant protein complex from the albumin fraction of potato (*Solanum tuberosum* L.) tuber has been isolated by size exclusion high performance liquid chromatography. The isolate has an estimated native molecular weight of 60 kD. Anionic gel electrophoresis of the isolate separated it into several polypeptides, all of which were glycosylated and had non-specific esterase activity. Two polypeptides between 35 and 42 kD were identified with SDS electrophoresis, thus the native form is postulated to be a dimer. The proteins in the isolate are antigenic to an antibody developed against 'patatin,' a tuber glycoprotein. Nutritional values for the isolate were extremely high, which suggests that it is at least partly responsible for the excellent nutritional value of potato protein.

Compendio

Mediante cromatografía líquida de exclusión de alta precisión (HPLC), se aisló de la fracción albuminoidea de tubérculos de papa (*Solanum tuberosum* L.) un complejo proteico predominante. El aislamiento tiene un peso molecular natural estimado de 60 kD. La electroforesis separó al aislamiento en varios polipectidos, todos ellos glicosilatados y con actividad de esterasa no específica. Por medio de electroforesis (SDS) se identificaron dos polipectidos, entre 35 y 42 kD, por lo que se considera que la forma natural del aislamiento es un dímero. Las proteínas del aislamiento son antigénicas a un anticuerpo desarrollado contra la 'patatina,' una glicoproteína del tubérculo. Los valores nutricionales del aislamiento fueron extremadamente altos, lo que sugiere que este complejo es por lo menos parcialmente responsable del excelente valor nutricional de la proteína de la papa.

Introduction

As a food, potato ranks second to soybean in amount of protein produced per hectare, and second to sugarcane in carbohydrate production (11). The nutritional quality of potato proteins is decidedly higher than that of cereals and legumes (12, 28). Potato carbohydrate is complex, rather than simple,

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as it is in sugarcane. Tetraploid hybrid potato lines have been selected for higher tuber protein content as well as for enhanced protein nutritional quality (3, 6, 7).

Potato tuber proteins have been found to be of high nutritional value (7, 14, 18). Nutritional quality of a protein is related to both the quantity and bioavailability of essential amino acids. Prediction of protein quality by chemical score calculations does not accurately assess quality (7). Feeding studies, while more definitive, have several limitations (2, 23, 26). Recently methods have been developed that are relatively inexpensive, rapid and correlate well with results from feeding studies (10). These include *in vitro* digestability (IVD), calculated PER (C-PER) and discriminant computed, PER (DC-PER) which are based, not only on the amino acid content, but also on amino acid availability related to enzymatic cleavage. Nutritional values of potato albumin and two acid subfractions with IVD, C-PER and DC-PER correlated with the PER values determined by rat feeding studies (3).

Much of the present knowledge about plant proteins has come from the study of storage in seeds such as corn and beans (4, 9). Isolation of potato tuber proteins began in 1896 when use of differential solubility resulted in isolation of a globulin protein, named 'tuberin' (19). More recently, Racusen and Foote (24) isolated a potato tuber glycoprotein with an apparent molecular weight of 45 kD, and gave it the trivial name 'patatin.' Park and his coworkers (20, 22) stated that patatin can constitute up to 45 percent of soluble tuber protein. Both groups used conventional column chromatography based on ion exchange and affinity separation to purify the protein.

To help understand the genetic control and physiological role of tuber proteins, techniques must be developed to study individual proteins or protein families. Our choice of the albumin fraction from potato proteins for further study was based on the following observations: 1) it is the predominant soluble protein fraction in potato tubers, 2) the essential amino acid values are high in this fraction, and 3) there is a positive correlation between the amount of albumin and the amount of total protein in tubers (5). The objectives of this study were to isolate the predominant tuber protein of the albumin fraction and to assess its contribution to the overall nutritional value of tuber protein.

Materials and Methods

Tuber Material—Tubers of three high protein hybrids (2526, 2628, and 82082) with Groups Andigena, Phureja and Tuberosum parentage (7) were potted in 15 cm styrofoam pots with a medium of 3 parts peat, 3 parts vermiculite, 2 parts sand. Plants were grown in a greenhouse with 21±3 C day and 15±3 C night temperatures and fertilized weekly with 400 ppm 20-20-20. Plants were fully senescent at harvest. Tubers were stored at 5-8 C. Proteins were extracted from tubers within one month following harvest.

Albumin Isolation—Albumin was prepared from 50 g of tuber material that had been peeled and sliced into 200 ml of 0.05 M phosphate buffer with 5 mg/l $Na_2S_2O_4$ and 2 ul/l NP-40 at 4 C. Infusion was carried out *in vacuo* for five min in an ice bath, followed by homogenization in a pre-chilled Waring blendor. The slurry was then suction-filtered through two layers of Miracloth and the filtrate was centrifuged at 12,000 x g at 4 C for 40 min. Membranes with a cutoff of 12 kD were used for dialysis of the supernatant at 4 C for 12 h against three changes of double distilled water. The dialysate was subsequently centrifuged for 20 min at 12,000 x g, and the resulting supernatant was frozen to -80 C and lyopholized.

Size Exclusion HPLC—Chromatography was performed with a Dupont model 870 pump, a Rheodyne model 7120 injector with a 500 ul loop, a Waters model 440 UV absorbance detector with a 280 nm filter and a Spectra Physics SP4270 integrator. The columns from Kratos, Inc. were used in the following order: a guard column, a 60 cm TSK3000SW and a 60 cm TSK2000SW. All separations were done at room temperature.

Albumin (50 mg) was dissolved in 500 ul of mobile phase buffer (0.1 M sodium phosphate, pH 6.8, and 0.1 M ammonium sulfate). The injection volume was 500 ul and a flow rate of 1 ml/min was used in the system. The diluted proteins were monitored at 280 nm and consecutive fractions were collected at 1 min intervals.

Molecular Size Calibration—The HPLC apparatus was calibrated by injecting protein standards at a concentration of 12.5 mg/500 ul mobile phase buffer. The following protein standards from Sigma Chemical Co. were used: gamma globulin (96 kD), bovine serum albumin (66 kD), ovalbumin (45 kD), pepsin (35 kD) gamma chymotrypsin (23 kD), and cytochrome C (13 kD).

Electrophoretic Techniques—Electrophoreses were performed using 12.5% SDS acrylamide gels as described by Laemmli (17) and 7.5% anionic acrylamide as described earlier for potato proteins (8). Gels were stained for protein with 0.25% (w/v) Coomassie Blue R250 in methanol:water:acetic acid (22.7:22.7:4.6) and destained overnight with acetic acid:methanol:water (1.5:1.0:17.5). Esterase activity was detected by use of the following stain solution: 100 mg alpha naphthyl acetate was dissolved in 5 ml acetone to which 100 ml 0.1 M sodium phosphate buffer pH 7.0 and 200 mg Fast Blue RR salt were added. Following visualization of the bands, the gels were stored in 7% acetic acid. Schiff's reagant was used to stain glycoproteins using the method of Zacharius (29). Western blotting was done following the protocol provided by Bio-Rad Laboratories (1) and polyclonal antibody to patatin was available.

Amino Acid Analysis—Amino acid analyses were done in the laboratory of Dr. I. Liener, Biochemistry Department, University of Minnesota. For

the determination of tryptophan, the protein was hydrolyzed with mercaptoethane sulfonic acid. Cysteic acid values were determined by hydrolysis with dimethyl sulfoxide (27). Essential amino acid (EAA) indices were calculated based on the FAO/WHO reference protein. The discriminant computed-protein efficiency ratio (DC-PER) calculations were performed as described by Jewell, *et al.* (10).

Results

A representative HPLC chromatogram of tuber albumin proteins with an apparent void volume of 23 ml is shown in Figure 1. The largest peak on the chromatogram, with a retention time of approx. 40 min was collected. This peak, hereafter referred to as the isolate, accounted for 43.5 percent of the albumin from 2628. The isolated peak resulted in a simple symmetrical peak with a retention time of 40 min, representing 99% of the protein injected when rechromatographed under identical conditions. The other genotypes, 2526 and 82082, had isolates which were 41.1 and 56.5 percent of the albumin (Table 1). The native molecular weight of the isolate was estimated to be 60 kD from a HPLC calibration plot of standard molecular weight proteins (Figure 2).

Anionic electrophoretic analyses of the isolates from the three high protein hybrids revealed phenotypic differences in their protein banding patterns. The protein patterns observed and the variation among them appeared to be similar to those observed for esterase isozyme banding patterns (8). To determine whether the isolate had non-specific esterase activity, electrophoretic separation of total soluble protein and the isolate was done in duplicate gels. One gel of each was stained with the general protein dye, and the other gel was stained for non-specific esterase activity. Each of the protein bands of the isolate had esterase activity (Figure 3) and all were glycosylated (data not presented).

To accurately assess the efficiency of HPLC protein separation, consecutive fractions were collected during size separation and subjected to SDS electrophoresis. The proteins that had a molecular weight between 35 and 42 kD were present in the fractions corresponding to the isolated peak (Figure 4).

Western blots of the albumin proteins and the isolate separated in either anionic or SDS electrophoresis were probed with antibody to 'patatin,' a potato tuber glycoprotein corresponded to bands with esterase activity in anionic separation, or to the subunits in SDS electrophoresis (Figure 5).

Amino acid compositions of both the albumin and the isolate for hybrid 2628 were determined (Table 2). The amino acid profile of the albumin fraction corresponds to that of Kapoor, *et al.* (13) and has an EAA score of 98. The recovery of tryptophan was low in the isolate, but the remaining essential amino acids were present in sufficient quantities to result in a very high EAA score of 99. Minor variations did occur among the

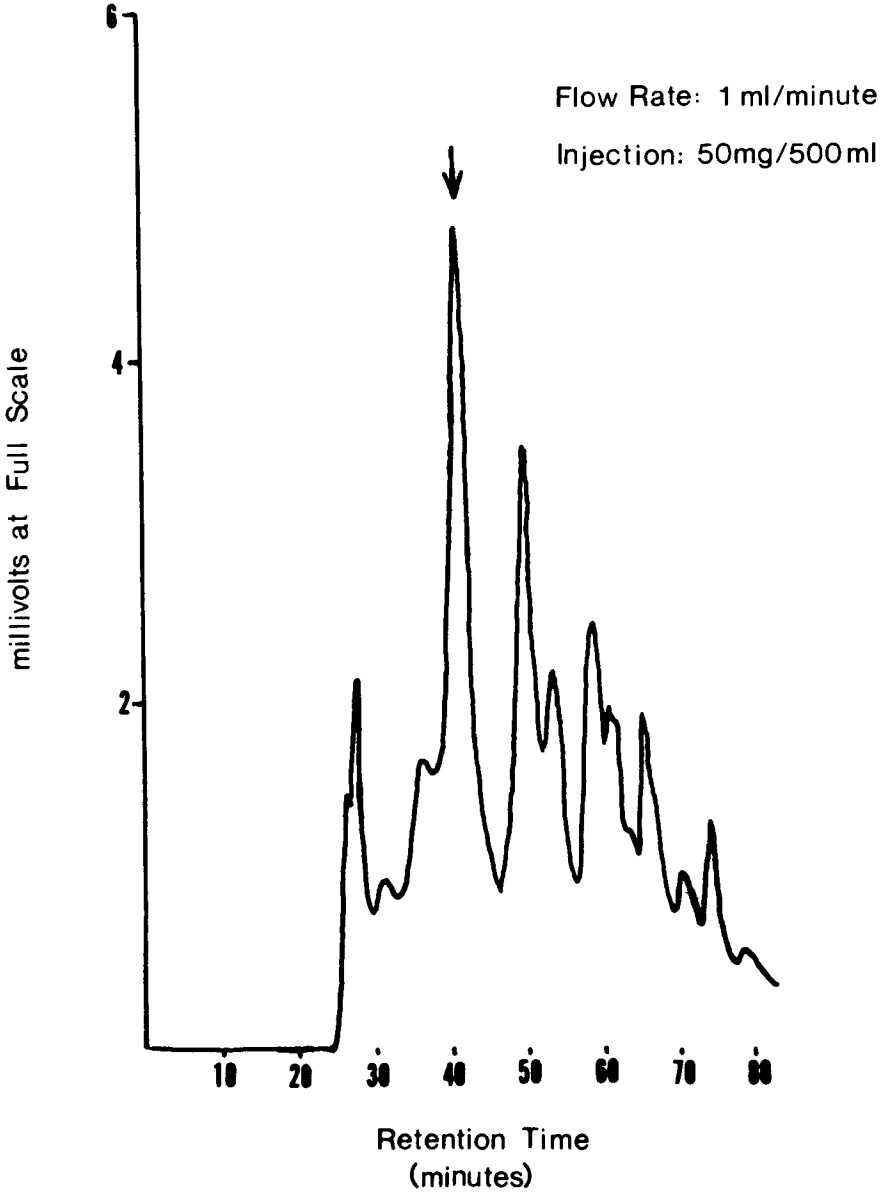

FIG. 1. HPLC chromatogram of potato tuber albumin (genotype 2628) monitored at 280 nm.

genotypes, but overall, the amino acid composition of the isolates was similar (Table 3).

TABLE 1. — *The nutritional values from the isolates of three genotypes and the control, casein.*

	Genotype of the Isolate			
	2526	2628	82082	Casein*
PER**	2.51	2.46	2.77	2.5
EAA	84.6	99.3	80.2	89.8
cal IVD	85.22	88.98	83.67	86.67
DC-PER	2.53	2.67	2.41	2.45
Percent	41.1	43.5	56.5	—

*control
**Desborough, *et al.* 1981
PER=Protein Efficiency Ratio for albumin
EAA=Essential Amino Acid Index
IVD=In Vitro Digestibility
DC-PER=Discriminant Computer-Protein Efficiency Ratio
Percent=Percent of isolate in the albumin

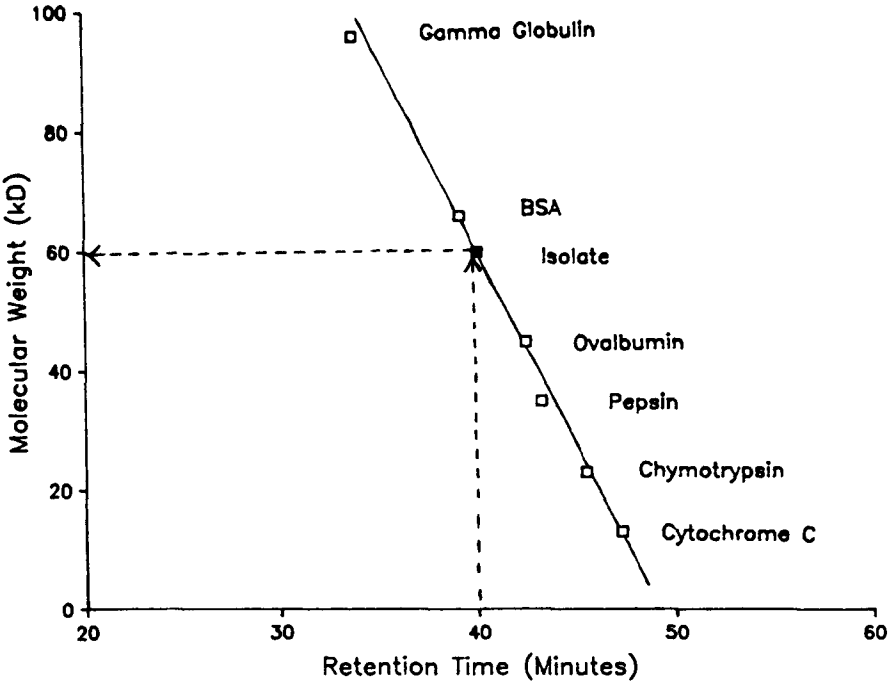

FIG. 2. The molecular weight calibration plot for six standard proteins. Molecular weight estimation for the isolate, with a retention time of 40 minutes, is 60 kD.

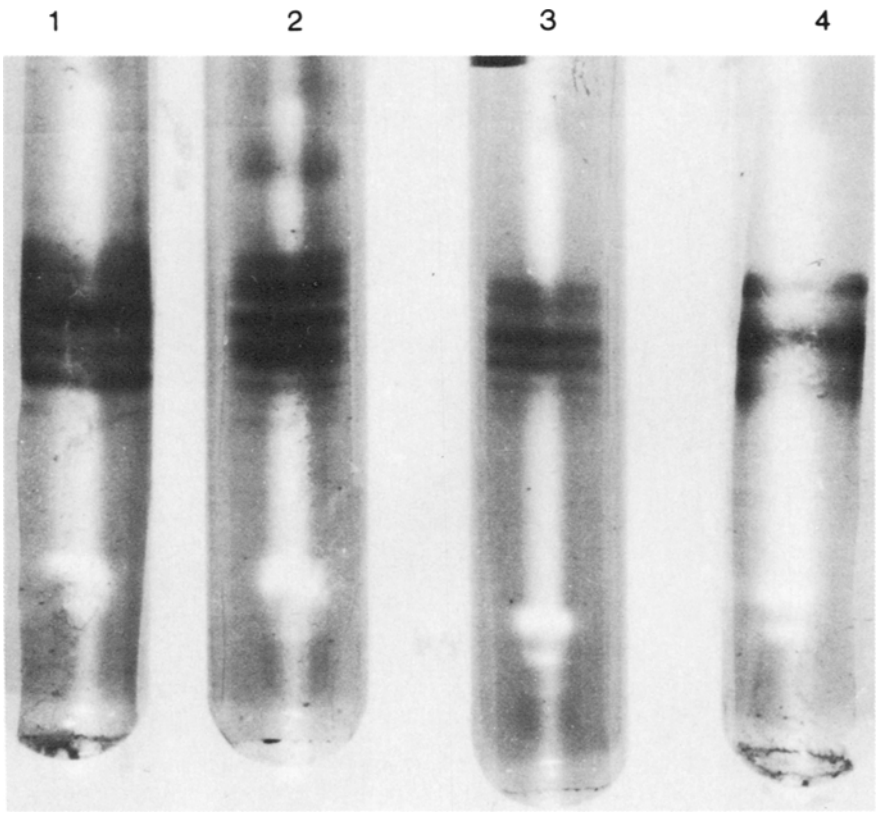

FIG. 3. Anionic 7.5% polyacrylamide gel electrophoresis (pH 8.3) of albumin and isolate preparations stained for total protein and esterase activity. The proteins were applied to the tubes as follows: 1) the albumin fraction of tuber proteins in genotype 2628 stained for esterase activity; 2) the albumin proteins stained for total protein; 3) HPLC isolate of genotype 2628 stained for total protein; 4) HPLC isolate of genotype 2628 stained for esterase activity.

The nutritional quality of the protein isolates from three genotypes was predicted by calculation of the IVD value and the DC-PER (Table 1). Within the limits (±.24 PER units) of DC-PER calculations there were no significant differences between the three genotypes and the casein standard. The calculated *in vitro* digestibility (IVD) values were also similar to the standard, casein.

Discussion

Potato albumin was composed of many proteins as visualized with electrophoretic separation. However, chromatographs of albumins separated by HPLC have a smaller number of peaks, suggesting that each peak may be a

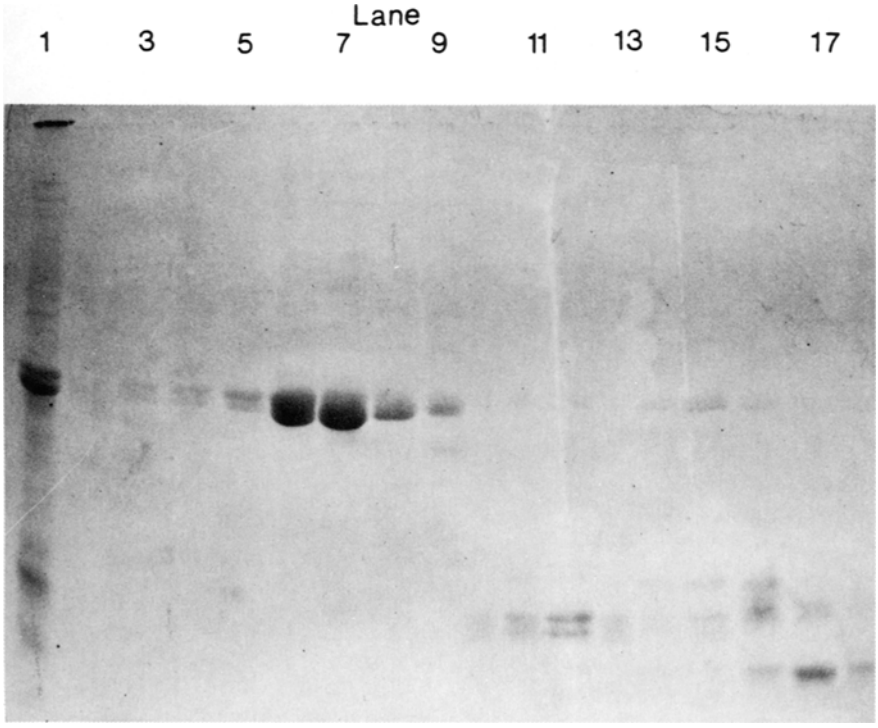

FIG. 4. SDS 12.5% PAGE of tuber albumin (Lane 1) and fractions taken from HPLC separation. The isolate is located in Lanes 5-9.

mixture of proteins. One peak, referred to as the isolate and approximately 43.5% of the total area, was collected and analyzed by electrophoresis. The proteins in the isolates were glycosylated and had different banding patterns dependent on genotype. Banding patterns of the isolates corresponded to the same esterase banding patterns observed for albumin of crude samples. The isolate had two polypeptides when subjected to SDS electrophoresis. Antibody to the tuber glycoprotein 'patatin' was available. The proteins in the isolate were found to be immunologically related to patatin in both native and subunit forms. In spite of charge heterogeneity and small differences in apparent molecular weight, Park, *et al.* (22) found that patatins were immunologically identical both within and between cultivars, and were sufficiently homologous that these proteins should be considered as a group. Therefore the isolate appears to be a related subset of tuber proteins that could be referred to as 'patatin.'

Based on results obtained from HPLC molecular size calibration and SDS electrophoresis, we propose that patatin is a dimer with a native

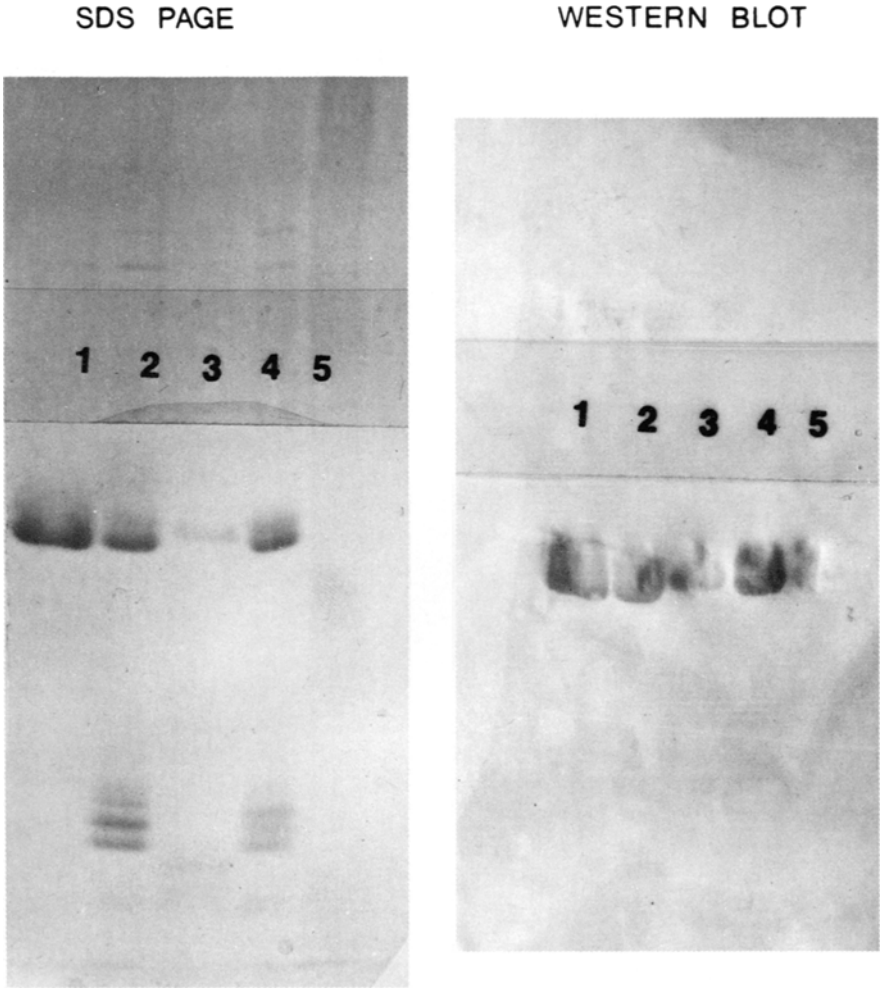

FIG. 5. SDS 12.5% PAGE and Western blot of a similar gel using antibody to patatin which identified the proteins in the isolate. The proteins in the lanes are as follows: 1) HPLC isolate of genotype 2628; 2) the albumin fraction of tuber proteins in genotype 2628; 3) HPLC isolate of genotype 82082; 4) the albumin fraction of tuber proteins in genotype 82082; 5) molecular weight standards.

molecular weight of 60 kD composed of two lower molecular weight subunits. Racusen and Weller (25) proposed that patatin exists as an 88±4 kD dimer, based on the molecular weight values of non-dissociated patatin ranging from 78 to 100 kD. In all size-exclusion chromatography systems, the separation is based on the hydrodynamic volume of the molecules to be separated and not on the molecular weight (15). Therefore, the apparent

TABLE 2. — *Amino acid compositions of albumin and isolate from tubers of genotype 2628 compared with the composition of patatin.*

	ALBUMIN	ISOLATE	PATATIN**
		mg/gm protein	
Amino Acid			
ala	4.4	3.3	5.1
arg	5.4	2.7	2.9
asp	10.6	6.5	9.5
cys*	0.9	0.5	ND
glu	10.9	7.8	10.8
gly	4.0	2.1	2.7
his	1.9	1.1	2.3
ile*	4.6	2.6	3.5
leu*	9.0	6.2	9.2
lys*	7.8	4.2	6.6
met*	2.0	1.7	2.4
phe*	5.3	3.5	5.3
pro	8.8	3.6	3.2
ser	4.4	2.6	4.9
thr*	5.0	3.8	7.0
trp*	3.5	0.6	ND
tyr*	4.6	3.6	5.2
val*	5.7	2.7	4.1
amm	1.1	3.3	ND
EAA	98.1	99.3	ND

*essential amino acid
**Racusen and Foote, 1980 (24)
ND=not determined
amm=ammonium
EAA=Essential Amino Acid Index

TABLE 3. — *Essential Amino Acid Comparison of the three potato protein isolates with casein as the control.*

		Genotype of the Isolate		
Amino Acids	Casein	2628	2526	82082
lys	7.46*	6.73	7.40	7.15
m+c	2.90	3.52	4.05	3.75
thr	4.28	6.09	6.18	6.24
iso	4.98	4.17	4.52	4.03
leu	8.75	9.94	10.01	9.93
val	6.20	4.33	4.47	4.34
p+t	10.17	11.38	11.15	11.01
trp**	1.37	0.90	0.76	0.70

*relative % of amino acid
**low recovery

molecular weight reported here for the isolate may be severely underestimated and must be regarded as a working estimate of the true molecular weight.

Moreover, this HPLC isolation method is based on size differences and may fail to include related members of a protein family, such as monomers and dimers. A protein isolation technique for patatin based on ion exchange and affinity chromatography was developed by Racusen and Foote (24). A limitation of affinity chromatography is that if related members of a protein family are glycosylated to varying degrees this may not be detectable. This technique would fail to bind those members that have few or no mannosyl or glucosyl residues. Racusen and Foote (24) suggest about 6% of patatin is neutral sugar and hexosamine. The isolate reported here may contain more than 6% carbohydrate because only about 70 percent of it is accounted for by amino acid quantitation.

One reason for the isolation of the predominant tuber protein was to assess its contribution to the overall nutritional value of potato protein. Nutritional quality of a protein is related to both the quantity and bioavailability of essential amino acids. The ranking of PER values from rat feeding studies (7) for total protein is not directly reflected in the DC-PER values for the isolates. This may be in part due to the percentage of the isolate in the total protein of a particular genotype, rather than the percent in the albumin.

The cal IVD and DC-PER values are qualitative measures of nutritional value derived from the amino acid composition of the protein. These values for the isolates were not significantly different from casein, indicating that the predicted nutritional values of the isolates are extremely high. Therefore, the quality of the isolates is partly responsible for the characteristically high nutritional value of potato protein.

The genotype differences among isolates separated by SDS electrophoresis were primarily related to molecular weight differences. However, Kosier (16) has shown that a two-dimensional electrophoretic gel separation reveals more charge variation in the polypeptides than size variation. Whether the observed variations of molecular weight correspond to subunits controlled by different alleles or genes, or post-transcriptional or -translational modification of the same or different gene products is unknown.

The speed and preparative nature of the HPLC method and the cross reactivity of the proteins with antibody will make subsequent studies on the predominant tuber proteins more efficient and precise. Phenotype comparisons both qualitative (isozymes) and quantitative (enzymatic activity) can be made rapidly for inheritance studies. Patatin can be isolated from various hybrids and varieties to determine whether the phenotype differences in protein bands are due to the carbohydrate and/or protein moieties.

The physiological role of patatin is not known. It may serve as a storage protein (21) or an active enzyme system, as reported here. If 'patatin' serves as a storage protein for the tuber, both the quantity and quality will be vital to its role in plant development. However, if it is active as an enzyme system,

then measurements of its quantity and quality may not be meaningful, unless the physiological state of the tuber is considered. The role of this major tuber protein is of interest because this is the first report of a predominant protein with enzymatic properties in a storage organ used as food.

Since the esterase activity of patatin is retained during HPLC isolation, this method will be valuable for the study of the functional role of the esterase isozymes. While the initial goal for isolation of the predominant tuber protein was to assess its contribution to the overall nutritional value of potato protein, the significance of the enzymatic activity of patatin deserves and is receiving further scrutiny.

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