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Purification and identification of interacting components in a wheat starch–soy protein system

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Abstract

The objective of this research was to quantify the solubility, hydrophobicity and interaction characteristics of wheat–starch proteins (puroindoline, gliadin and glutenin) and protein-containing soy fractions (soy flour isolate [SFI], SFI 7S and 11S fractions, hexane-extracted textured soy flour [TVP] isolate, TVP 7S and 11S fractions, expelled, extruded soy flour [TSP] isolate, TSP 7S and11S fractions). Functional characteristics were assessed in aqueous sucrose solutions at pH 5.5 and 7.5 after heating to 25, 50, and 100 °C. Textured soy protein fractions were more soluble and had higher surface hydrophobicity profiles than their untextured counterparts. Sucrose addition decreased hydrophobicity in the textured proteins but increased it in untextured proteins. Characteristics of the isolate, as a whole, appear to be dictated by those of its 11S moiety. Dissociation constants (K_d values) for soy protein and starch-derived puroindoline were determined and indicated an extremely short association in all cases. The 11S fractions formed a complex with puroindoline in solution; however 7S fractions did not.

Keywords: Wheat-starch granule; Gliadin; Glutenin; Soy fractions; Puroindoline

1. Introduction

Incorporation of proteins, other than wheat, into baked wheat systems disrupts the starch–protein interaction (Fleming & Sosulski, 1978; Kulp & Lorenz, 1981). This disruption may be due to a lack of interaction among the proteins which may be because the extreme hydrophilicity of soy proteins interrupts the formation of a starch–protein complex. However, the exact interaction mechanism of soy protein and starch has not been fully elucidated. Dahle (1971) examined the binding characteristics of soy protein and wheat starch, but suggested no mechanism for protein adhesion. Research involving the adsorption of several different proteins onto the surface of wheat starch granules has indicated that neither molecular weight of the adsorbing protein nor electrostatic differential between the starch and the binding polypeptide are solely responsible for differences in interaction kinetics (Eliasson & Tjerneld, 1990). A complete understanding of the interactions involved in protein adhesion to wheat starch granule surfaces could allow development of texturally acceptable soy protein-containing baked products.

The internal molecular organization of wheat-starch granules has been thoroughly examined (French, 1984; Gallant, Bouchet, & Baldwin, 1997). Larsson and Eliasson (1997) conjectured that the wheat-starch granule surface plays an important role in starch–protein interactions. They indicated that the starch granule surface components influence the rheological characteristics of resultant doughs. Starch granule surface topography and the interactions of components present thereon have not been comprehensively investigated. Ten major granule surface proteins, ranging from 5 to 149 kDa, have been identified (Baldwin, 2001). Puroindoline is the most studied because of its proposed connection with wheat softness and because it is present on the exterior of the granule only. Greenwell and Schofield (1986) suggested that starch granule interactions were due to a

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group of M_r 15,000 proteins (i.e., puroindoline) on the starch granule surface. Malouf and Hoseney (1992) found that puroindolines gave reconstituted wheat tablets high tensile strength. Puroindolines have been linked to the hardness locus (*Ha*) on the same chromosome (5D) that controls endosperm texture (Law, Young, Brown, Snape, & Worland, 1978; Simmons, Barlow, & Wrigley, 1973). Puroindoline and other granule-associated components appear to be required for starch–protein interaction in dough systems (Delcour et al., 2000).

Incorporation of puroindoline into model French bread systems suggested that this starch granule protein increased loaf volume and stabilized crumb texture through a starch–wheat protein interaction (Igrejas et al., 2001). The action of puroindoline in systems containing proteins other than wheat, such as soy, has not been thoroughly examined. Studies focussed on soy protein adhesion to wheat-starch granules have failed to account for the large variety of soy proteins currently available for incorporation into wheat-based products. Previous studies (Ryan, 2003) indicated that heat- and pressure-treated soy proteins adsorb to wheat-starch granules to a greater degree than do unprocessed soy proteins. Wagner, Sorgentini, and Anon (2000) reported that laboratory and commercial methods of processing and extracting soy proteins significantly alter interactive behaviours.

The objective of this research was to quantify the interaction between puroindoline and other protein fractions, including bovine serum albumin (BSA), gliadin, glutenin, soy flour solate, soy flour 7S and 11S protein fractions, hexane-extracted textured soy flour (TVP) isolate, TVP 7S and 11S fractions, expelled, extruded soy flour (TSP) isolate, TSP 7S and11S fractions. Solubility and surface hydrophobicity were determined over a range of pH values and sucrose concentrations as a measure of overall protein functionality. In vitro dissociation constants between puroindoline and several soy protein fractions were determined to assess the significance of puroindoline in the binding of soy proteins to wheat starch granules.

2. Materials and methods

2.1. Materials

All chemicals were purchased from Fisher Scientific (Hanover Park, IL) and were of reactant grade, unless otherwise noted.

2.2. Wheat flour fractionation

Commercially milled hard (15.1% protein, 0.45% ash, 5.6% damaged starch, 70.2% water retention capacity) and soft (12.3% protein, 4.5% ash, 3.4% damaged starch, 58.2% water retention capacity) wheat flours were donated by Archer-Daniels Midland Milling (Overland Park, KS). Prime starch was isolated using the dough-ball method of Wolf (1964). Flour (500 g) was hand mixed with distilled water (315 ml) to form a ball. Starch was washed away from the gluten using distilled water (22 °C) until the water ran clear; then the gluten fraction was lyophilized. Starch suspension was allowed to stand overnight (~8 h, 4 °C) to sediment, then centrifuged (5000*g*, 10 min; Sigma 2–5 Benchtop Centrifuge, Sigma International, Osterode and Harz, Germany). Supernatant containing the water-soluble fraction was decanted, shell frozen and lyophilized. The top layer of precipitate (tailings) was removed and discarded. The bottom white layer (prime starch) was removed and air-dried (~8 h). All fractions were ground in a burr mill (Mr. Coffee coffee grinder IDS55, Sunbeam Products, Hattisburg, MS), sieved (#40 sieve, Endecotts Limited, Lombard Road, London SW193TZ, England) and stored in polyethylene bags at 22 °C until used (<30 days).

Glutenins were extracted as described by Melas, Morel, Autran, and Feillet (1994) and Uthayakumaran, Gras, Stoddard, and Bekes (2000). Soft wheat flour (16 g) was extracted three times for 30 min with 190 ml of 50% propan-2-ol at 22 °C. Suspension was centrifuged (20,600*g*, 15 min) and discarding the supernatant each time. The resulting pellet was resuspended in 100 ml of dithiothreitol (DTT; 1 g DTT, 50 ml propan-2-ol, 50 ml 160 mM Tris–HCl, pH 8.0), shaken at 65 °C in a shaking water bath for 4 min; then centrifuged (29,600*g*, 20 min). Supernatant was decanted, acetone was added (40% v/v), the solution was allowed to precipitate overnight (4 °C); then centrifuged (29,600*g*, 20 min). The pellet was resuspended in 100 ml distilled water and centrifuged (20,600*g*, 10 min). This procedure was repeated and the final pellet (glutenin) was suspended in 0.1% acetic acid (v/v), lyophilized, ground using a mortar and pestle to pass a #40 Taylor sieve, then stored in polyethylene bags at 22 °C until used (<30 days).

Gliadins were extracted as described by Wieser, Antes, and Seilmeier (1998). Soft wheat flour (10 g) was extracted twice with a salt solution (67 mM $HKNaPO_4$, pH 7.6, +0.4 M NaCl, 100 ml) at 22 °C. After centrifugation (20,000*g*, 10 min, 20 °C), the combined supernatants were dialyzed against distilled water (25 °C) using a semi-permeable membrane (14,000 MW cut-off; Sigma Chemical Company, St. Louis, MO), lyophilized, then ground to pass a #40 Taylor sieve, using a mortar and pestle.

Flour fractions were loaded onto a 4–12% gradient Tris–Bis SDS–PAGE system (Invitrogen Corp., Carlsbad, CA) to determine approximate molecular weight distributions.

2.3. Soy isolate and fraction production

Defatted, SF (SF) (53% protein, 9.7% moisture; sieved to pass a #40 Taylor sieve) and solvent-extracted textured soy flour (TVP; 50% protein, 10.2% moisture) were obtained from Archer Daniels Midland (Decatur, IL). Extruded–expelled soy flour (TSP; 49% protein, 10.8% moisture) was provided by Insta-Pro International (Des Moines, IA).

Soy protein isolates of textured, ground (#40 sieve) and non-textured soy flour were produced by extracting the flour with alkaline water (pH 8.0, adjusted with 2 N NaOH) for 2 h at room temperature (~22 °C; water: flour 10:1 mass/v). Suspension was centrifuged (1000*g*, 30 min) and the resulting supernatant adjusted to pH 4.5 with 2 N HCl. Precipitate was separated by centrifugation (5000*g*, 15 min), suspended in water (5% protein mass/v) and adjusted to pH 8.0 with 1 N NaOH (Ortiz & Anon, 2001). Isolates were lyophilized and stored in polyethylene bags at room temperature until used (<30 days).

Major soy protein fractions (11S and 7S) of non-texturized soy flour and textured soy flour were separated via isoelectric precipitation (Than & Shibasaki, 1979). Flour (100 g) was extracted with 0.03 M Tris buffer (flour:buffer 1:20, pH 8.0) containing 0.01 M mercaptoethanol at room temperature for 1 h. Solution was centrifuged (20,000*g*, 20 min), adjusted to pH 6.4 with 2 N HCl, then re-centrifuged (20,000*g*, 20 min, 4 °C). Precipitate (11S globulin) was suspended in 50 mM Tris–HCl (pH 7.8) containing 20 mM NaCl and dialyzed against 20 mM NH_4HCO_3 (6 h). Aggregated globulins were removed by gel filtration (Sepharose CL-6B; flow rate = 0.5 ml/min). The resulting protein was lyophilized. Supernatant was adjusted to pH 4.8 with 2 N HCl, then centrifuged (20,000*g*, 20 min, 4 °C). Resulting pellet was suspended in 0.03 M Tris–HCl with addition of 2 N NaOH until dissolution (pH 7.6). Supernatant (7S globulin) was dialyzed against 20 mM NH_4HCO_3 (6 h), and lyophilized. Fractions were checked for purity using 4–12% gradient Bis–Tris SDS–PAGE (NOVEX Xcell SureLock Mini-Cell System, NOVEX USA, San Diego, CA).

2.4. Puroindoline purification

Puroindolines were separated from soft wheat-starch granules using the detergent method described by Bloch, Darlington, and Shewry (2001). Soft wheat flour (100 g) was extracted for 8 h at 4 °C with 500 ml of 100 mM Tris–HCl buffer (5 mM EDTA, 100 mM potassium chloride and 4% (w/v) Triton X-114, pH 7.8). The solution was centrifuged (5000*g*, 15 min), incubated at 37 °C until phase separation occurred, then re-centrifuged (5000*g*, 15 min). Upper phase of supernatant was removed, replaced with an equal volume of Tris–HCl buffer minus Triton X-114 and the phase separation procedure was repeated. The lower phase was precipitated for 8 h at −18 °C, followed by addition of 200 ml of ice-cold diethylether and ethanol (1:3). Solution was centrifuged (2000*g*, 15 min) and the resulting pellet was washed with solvent and dried under vacuum for 8 h. The pellet was resuspended in 5 ml of 50 mM acetic acid, centrifuged (20,000*g*, 5 min; Sanyo/Harrier 18–80 Refrigerated Centrifuge, Belton Park, Loughborough, Leics, LE115XG, UK), and the supernatant loaded onto a Sephadex G50 column equilibrated with the Tris–HCl buffer. Column fractions were analyzed using 4–12% Bis–Tris gels (NOVEX Xcell SureLock Mini-Cell System, NOVEX USA, San Diego, CA). Eluted fractions containing M_r 15,000 proteins were pooled and lyophilized.

2.5. Solubility

Protein solubilities in water were determined by a modification of the method of Mohammed, Hill, and Mitchell (2000). Samples (0.1 g) of lyophilized protein (SFI, soy flour 7S, soy flour 11S, TVP isolate, TVP 7S, TVP 11S, TSP isolate, TSP 7S, TSP 11S, BSA, puroindoline, gliadin, glutenin) were mixed individually with 10 ml of 10 mM phosphate buffer (pH 7.5), 10 mM acetate buffer (pH 5.5) or a sucrose solution (2.5%, 5.0%, or 10%) at pH 7.5 or 5.5 for 8 h. Solutions were heated in a water bath (0, 25, 50, or 100 °C) for 5 min, centrifuged (1500*g*, 10 min) and filtered (Whatman No. 4). An aliquot (0.4 ml) of the solution was diluted to 10 ml with the appropriate buffer and the protein content determined using the Lowry method (Lowry, Rosenbrough, Farr, & Randall, 1951). Results are expressed as a percentage of the total protein content in the original sample.

2.6. Surface hydrophobicity

Individual aliquots of 0.1% (w/v) of each protein and 200 μl of 1% SDS were stirred for 30 min at 300 rpm using a magnetic stirrer. Protein solutions were heated to 25, 50, or 100 °C in a water bath for 5 min. Samples were dialyzed against 25 volumes of 10 mM phosphate or acetate buffer for 24 h. $CHCl_3$ (6 ml) and 1 ml of 0.5% (w/v) methylene blue, diluted 100-fold in phosphate buffer or acetate pH 5.5 or 7.5, were added to each 1 ml sample and vortexed for 30 s. Absorbance of the SDS–methylene mixture in the lower layer was determined at 655 nm (Beckman Spectrophotometer, DU 640, Fullerton, CA; Hayashi 1975). Absorbance represented μg of SDS bound to 500 μg of protein. Protein samples were evaluated under the pH, sucrose and heat conditions described for solubility determination. Blanks included all pH levels, sucrose levels and heat treatments minus protein.

2.7. Statistical analyses

Data were treated as a protein × pH × sucrose × heat factorial design subjected to analysis of variance to determine significant ($p < 0.05$) effects and LSD was used for means separation (SAS, 2002). Means for solubility and surface hydrophobicity were derived using three independent determinations, with three replications each.

2.8. Binding constant determination

An 18-ml gel filtration column (Kontes Flex Column, Kontes-Kimble, Vineland, NJ) was loaded with Sepharose CL-6B and gravity flow equilibrated with 100 mM Tris–HCl buffer (pH 7.8, flow rate of 0.5 ml/min). Samples of puroindoline (20 μM) and each of the soy fractions (20 μM) were injected into the column to establish normal retention times, which were checked against molecular weight standards. Individual mixtures were prepared containing puroindoline and each of the soy fractions (20 μM each) in Tris–HCl buffer to determine whether a strong association between the proteins formed that travelled with a retention time shorter than either of the two proteins individually. The equilibrium binding technique of Hummel and Dryer (1962) was used to determine the binding constant (K_d) between puroindoline and soy protein fractions. The filtration column was equilibrated with Tris–HCl buffer containing 20 μM *puroindoline*. A range of concentrations (20, 30, 40 μM) of each soy flour fraction (soy flour 7S, soy flour 11S, TVP 7S, TVP 11S, TSP 7S, TSP 11S) plus puroindoline (20 μM) in buffer were applied to the column. Protein filtration traces were determined by continuous monitoring of absorption at 280 nm (Beckman Coulter DU 640 Spectrophotometer, Beckman Inc., Fullerton, CA). The cross-point of protein concentration for the stoichiometry of the interactions between the proteins was confirmed by repeating the gel filtration run using different levels of puroindoline in the sample (20, 30, 40, 50, 75 μM). Interactions between each of the soy fractions and puroindoline were triplicated.

3. Results and discussion

3.1. Solubility and hydrophobicity

Solubility data are presented in Table 1. Comparison of the soy isolates indicates that each has a characteristic solubility profile at the pH, sucrose, and temperature levels evaluated. Soy flour, TVP and TSP isolate solubilities were not significantly ($p < 0.05$) altered by sucrose concentration alone (data not shown). However, these proteins were more soluble in solutions containing 5–10% sucrose at 50 and 100 °C than in solutions containing no sucrose. Overall, solubility of the textured proteins was greater than that of the untextured proteins, with the greatest solubility for all three occurring at pH 5.5, in a solution containing the maximum amount of sucrose, then subjected to the maximum heat treatment. The distinct difference between each of the soy isolates is due, in part, to the complex interaction between the two main globulins of which each isolate is composed. Previous work has shown that isolates produced from processed soy respond with a constant increase or decrease in solubility when exposed to environmental changes (Hermansson, 1978). TVP and TSP are composed of globulins that have been previously pressure- and heat-treated. This causes formation of strong inter- and intra-molecular disulfide bonds between the fragments of the globulin subunits. Therefore, isolates derived from these proteins are composed of aggregated globulins with fixed and constant reactions to environmental alterations. In contrast, SFI reacted in a more complex manner, a result of greater mobility of the globulin fraction in the surrounding solution. Protein solubility in SFI, similar to that of textured SFI, was achieved only through the combined use of high temperatures and high sucrose concentrations. As with the textured soy flour, high temperature probably caused unfolding of SFI globulin components, increasing the protein–solvent interactions and subsequent solubility.

The 11S fractions had solubility profiles similar to their parent isolates (Table 1). Maximum sucrose and heat treatment produced solubilities near 100% in soy flour 11S fractions. The high solubility of the 11S fraction from non-textured soy at pH 5.5, despite being near its p*I*d (4.64), may be due to the shielding of several of the 11S subunits by the sucrose moiety (Yagasaki, Takagi, Sakai, & Kitamira, 1997). This type of shielding may not have been possible in processed 11S forms because of its altered shape and size.

Surface hydrophobicity of the soy isolates (Table 2) followed a different trend from did solubility. Surface hydrophobicity of the isolates was unaffected by the addition of sucrose alone (data not shown). On the other hand, heat treatment caused a significant increase in hydrophobicity. In addition, sucrose reduced the impact of heat on the protein system. As sucrose levels increased, surface hydrophobicity was lower at higher temperatures, with the 100 °C/10% sucrose samples having a hydrophobicity less than or equal to samples subjected to no sucrose or heat treatment. Numerous groups have documented this thermal stabilization effect of sucrose on proteins (Baier & McClements, 2001; Lee & Timasheff, 1981). Kulmyrzaev, Bryant, and McClements (2000) reported that sugars alter the gelation mechanism and thermal stability of protein systems by increasing the viscosity of the continuous phase, thereby

Table 1
Solubility of soy and wheat fractions after heat and sucrose treatment

Heat treatment				Sucrose concentration				pH	
None	25 °C	50 °C	100 °C	0%	2.5%	5%	10%	5.5	7.5
Soy flour protein isolate									
	X			X				47.5 (0.04)[a]	56.4 (0.02)[b]
		X		X				61.3 (0.14)[c]	69.0 (0.21)[cd]
			X	X				76.3 (0.10)[d]	81.9 (0.02)[d]
	X				X			49.3 (0.03)[ab]	54.3 (0.01)[b]
		X			X			71.0 (0.13)[cd]	74.2 (0.14)[cd]
			X		X			80.8 (0.34)[d]	88.5 (0.06)[e]
	X					X		56.9 (0.12)[b]	59.4 (0.03)[c]
		X				X		77.3 (0.01)[d]	76.0 (0.09)[d]
			X			X		83.3 (0.02)[de]	88.1 (0.09)[e]
X							X	52.4 (0.12)[ab]	59.2 (0.01)[c]
	X						X	58.1 (0.03)[bc]	60.2 (0.17)[c]
		X					X	78.1 (0.18)[d]	80.9 (0.13)[d]
			X				X	86.7 (0.15)[e]	91.7 (0.02)[e]
Soy flour 7S									
	X			X				84.3 (0.07)[ab]	87.4 (0.01)[b]
		X		X				84.1 (0.06)[a]	87.1 (0.09)[b]
			X	X				87.0 (0.02)[b]	88.0 (0.01)[b]
	X				X			83.1 (0.01)[a]	85.3 (0.12)[b]
		X			X			84.2 (0.04)[ab]	84.2 (0.09)[ab]
			X		X			84.3 (0.06)[ab]	85.6 (0.03)[b]
	X					X		88.9 (0.07)[c]	85.1 (0.01)[ab]
		X				X		83.1 (0.04)[a]	86.3 (0.11)[b]
			X			X		83.7 (0.12)[a]	87.2 (0.09)[b]
X							X	89.5 (0.05)[c]	84.2 (0.01)[ab]
	X						X	85.2 (0.14)[b]	84.9 (0.06)[ab]
		X					X	91.1 (0.34)[c]	85.4 (0.05)[b]
			X				X	87.2 (0.14)[b]	87.7 (0.05)[b]
Soy flour 11S									
	X			X				77.2 (0.08)[cd]	53.1 (0.02)[a]
		X		X				79.3 (0.10)[d]	56.3 (0.12)[ab]
			X	X				82.7 (0.04)[d]	69.3 (0.37)[c]
	X				X			79.0 (0.19)[d]	54.2 (0.11)[b]
		X			X			84.0 (0.11)[de]	57.8 (0.07)[b]
			X		X			89.2 (0.22)[e]	74.0 (0.03)[c]
	X					X		81.9 (0.07)[d]	56.7 (0.03)[ab]
		X				X		87.3 (0.04)[a]	57.5 (0.44)[b]
			X			X		92.2 (0.19)[e]	78.4 (0.34)[d]
X							X	78.4 (0.11)[d]	59.3 (0.03)[b]
	X						X	82.4 (0.28)[d]	57.1 (0.03)[b]
		X					X	89.4 (0.02)[e]	57.2 (0.18)[b]
			X				X	95.4 (0.32)[e]	78.3 (0.23)[d]

Table 1 (*continued*)

Heat treatment				Sucrose concentration				pH	
None	25 °C	50 °C	100 °C	0%	2.5%	5%	10%	5.5	7.5
TSP isolate									
	X			X				94.1 (0.04)[b]	91.2 (0.14)[b]
		X		X				96.1 (0.05)[c]	91.2 (0.02)[b]
			X	X				97.3 (0.12)[c]	92.3 (0.19)[b]
	X				X			95.1 (0.02)[bc]	91.8 (0.07)[b]
		X			X			96.3 (0.04)[c]	86.3 (0.03)[a]
			X		X			97.2 (0.13)[c]	90.3 (0.02)[b]
	X					X		96.3 (0.03)[c]	88.4 (0.05)[ab]
		X				X		97.1 (0.04)[c]	84.2 (0.02)[a]
			X			X		98.2 (0.02)[c]	85.6 (0.03)[a]
X							X	95.9 (0.01)[c]	87.1 (0.04)[ab]
	X						X	96.0 (0.05)[c]	87.3 (0.32)[ab]
		X					X	97.5 (0.02)[c]	84.1 (0.19)[a]
			X				X	99.8 (0.66)[d]	86.4 (0.03)[a]
X				X				82.1 (0.03)[d]	79.4 (0.04)[d]
TSP 7S									
			X	X				85.3 (0.12)[ab]	87.4 (0.05)[b]
	X				X			88.2 (0.03)[b]	89.4 (0.24)[bc]
		X			X			85.8 (0.05)[ab]	90.8 (0.06)[c]
			X		X			83.0 (0.06)[a]	88.3 (0.02)[b]
	X					X		87.9 (0.41)[b]	87.3 (0.34)[b]
		X				X		84.8 (0.29)[a]	86.5 (0.09)[b]
			X			X		80.7 (0.35)[a]	88.4 (0.12)[b]
X							X	90.6 (0.12)[c]	86.5 (0.03)[b]
	X						X	88.4 (0.09)[b]	88.0 (0.14)[b]
		X					X	84.9 (0.03)[a]	89.2 (0.06)[b]
	X						X	90.6 (0.23)[c]	90.4 (0.22)[bc]
		X					X	96.3 (0.03)[d]	82.5 (0.07)[a]
			X				X	98.9 (0.09)[e]	80.7 (0.04)[a]
TSP 11S									
		X		X				72.1 (0.08)[b]	70.9 (0.01)[b]
			X	X				70.8 (0.17)[b]	69.3 (0.09)[b]
	X				X			75.4 (0.07)[c]	74.3 (0.02)[c]
		X			X			73.4 (0.15)[bc]	76.2 (0.03)[c]
			X		X			72.1 (0.05)[b]	75.8 (0.07)[c]
	X					X		73.1 (0.03)[b]	74.1 (0.19)[c]
		X				X		74.5 (0.05)[c]	75.4 (0.05)[c]
			X			X		75.7 (0.04)[c]	74.1 (0.09)[c]
X							X	63.9 (0.45)[a]	72.5 (0.09)[b]
	X						X	61.8 (0.36)[a]	73.8 (0.17)[bc]
		X					X	60.8 (0.16)[a]	74.9 (0.21)[c]
			X				X	65.1 (0.01)[a]	77.9 (0.19)[cd]
			X				X	78.9 (0.09)[a]	87.3 (0.05)[cd]

TVP 11S											
	X				X					78.5 (0.05)[d]	79.6 (0.09)[d]
		X			X					70.8 (0.09)[b]	69.6 (0.13)[b]
			X		X					67.8 (0.33)[b]	68.1 (0.12)[b]
	X					X				72.3 (0.49)[c]	71.9 (0.10)[bc]
		X				X				69.0 (0.02)[b]	73.7 (0.08)[c]
			X			X				68.4 (0.27)[b]	73.4 (0.21)[c]
	X						X			70.4 (0.24)[b]	71.5 (0.10)[bc]
		X					X			70.6 (0.26)[b]	70.4 (0.23)[b]
			X				X			72.4 (0.16)[c]	72.3 (0.06)[c]
X								X		60.9 (0.09)[a]	69.5 (0.09)[b]
	X							X		58.9 (0.07)[a]	70.8 (0.25)[b]
		X						X		56.8 (0.06)[a]	71.2 (0.68)[b]
			X					X		61.2 (0.03)[a]	75.2 (0.33)[c]
			X					X		67.0 (0.31)[b]	83.2 (0.18)[e]
TVP 7S											
	X				X					89.5 (0.17)[d]	87.9 (0.09)[d]
		X			X					85.7 (0.06)[bc]	85.4 (0.13)[b]
			X		X					83.5 (0.09)[b]	84.9 (0.03)[b]
	X					X				86.9 (0.07)[c]	86.0 (0.05)[c]
		X				X				83.5 (0.32)[b]	86.9 (0.43)[e]
			X			X				80.7 (0.11)[a]	84.0 (0.31)[b]
	X						X			84.9 (0.22)[b]	83.7 (0.08)[b]
		X					X			81.9 (0.17)[a]	83.9 (0.03)[b]
			X				X			77.4 (0.19)[a]	84.5 (0.09)[b]
X								X		86.8 (0.06)[c]	85.7 (0.05)[bc]
	X							X		83.7 (0.28)[b]	84.7 (1.09)[b]
		X						X		81.9 (0.17)[a]	86.0 (0.31)[c]
			X					X		78.9 (0.09)[a]	87.3 (0.05)[cd]
	X				X					75.9 (0.06)[f]	83.9 (0.04)[f]
		X			X					78.4 (0.19)[f]	70.8 (0.09)[e]
			X		X					81.7 (0.05)[f]	68.4 (0.27)[d]
	X					X				60.7 (0.31)[c]	72.9 (0.06)[e]
		X				X				66.7 (0.21)[d]	64.9 (0.13)[d]
			X			X				74.9 (0.06)[e]	66.9 (0.03)[d]
	X						X			52.9 (0.17)[b]	69.8 (0.23)[e]
		X					X			55.0 (0.02)[bc]	50.7 (0.01)[b]
			X				X			78.9 (0.18)[f]	56.9 (0.07)[c]
X								X		83.5 (0.08)[f]	64.9 (0.28)[d]
	X							X		45.9 (0.34)[a]	60.9 (0.09)[c]
		X						X		50.9 (0.32)[b]	50.9 (0.28)[b]
			X					X		69.9 (0.28)[e]	55.0 (0.09)[bc]
Gliadin											
	X				X					77.0 (0.04)[a]	86.8 (0.03)[c]
		X			X					81.9 (0.23)[b]	83.9 (0.05)[b]
			X		X					83.4 (0.09)[b]	81.2 (0.04)[b]
	X					X				79.0 (0.05)[a]	86.9 (0.05)[c]
		X				X				83.8 (0.18)[b]	84.5 (0.11)[b]
			X			X				86.9 (0.23)[c]	82.8 (0.19)[b]

Table 1 (*continued*)

Heat treatment				Sucrose concentration				pH	
None	25 °C	50 °C	100 °C	0%	2.5%	5%	10%	5.5	7.5
	X					X		81.6 (0.09)[b]	87.0 (0.04)[c]
		X				X		85.6 (0.04)[c]	85.0 (0.13)[c]
			X			X		91.2 (0.17)[d]	81.9 (0.06)[b]
X							X	79.6 (0.28)[a]	87.4 (0.47)[cd]
	X						X	83.0 (0.07)[b]	88.6 (0.05)[d]
		X					X	89.7 (0.26)[d]	84.0 (0.12)[b]
			X				X	93.9 (0.09)[d]	83.9 (0.28)[b]
Glutenin									
	X			X				75.9 (0.08)[a]	87.0 (0.09)[c]
		X		X				79.5 (0.28)[b]	89.0 (0.45)[d]
			X	X				82.0 (0.04)[b]	88.9 (0.07)[cd]
	X				X			76.0 (0.34)[a]	87.4 (0.12)[c]
		X			X			87.0 (0.24)[c]	86.9 (0.05)[c]
			X		X			88.4 (0.10)[c]	87.1 (0.07)[c]
	X					X		79.0 (0.19)[b]	88.8 (0.23)[c]
		X				X		88.5 (0.10)[c]	85.8 (0.06)[bc]
			X			X		91.2 (0.54)[d]	87.2 (0.01)[c]
X							X	76.3 (0.04)[a]	88.2 (0.32)[c]
	X						X	81.2 (0.02)[b]	86.2 (0.11)[c]
		X					X	92.1 (0.19)[d]	89.1 (0.32)[d]
			X				X	96.1 (0.43)[e]	91.0 (0.71)[d]
Soy flour protein isolate									
	X			X				2.71 (0.32)[b]	2.68 (0.07)[b]
		X		X				9.78 (0.18)[d]	15.38 (0.26)[e]
			X	X				8.76 (0.06)[d]	9.04 (0.17)[d]
	X				X			2.56 (0.09)[b]	2.65 (0.07)[b]
		X			X			14.98 (0.04)[e]	14.17 (0.84)[e]
			X		X			7.98 (0.31)[d]	6.98 (0.05)[d]
	X					X		3.58 (0.01)[bc]	2.32 (0.09)[b]
		X				X		12.98 (0.41)[d]	13.17 (0.06)[d]
			X			X		3.89 (0.30)[c]	5.04 (0.07)[cd]
X							X	3.77 (0.01)[c]	1.76 (0.06)[a]
	X						X	2.56 (0.21)[b]	2.23 (0.07)[b]
		X					X	3.29 (0.03)[b]	2.94 (0.15)[b]
			X				X	2.11 (0.02)[b]	5.04 (0.05)[c]

Protein solubility (%) after treatment. Results are means ± standard deviation of three determinations.

[a–f] Means within a fraction with the same superscripts are not significantly different ($p < 0.05$).

Table 2
Surface hydrophobicity of soy and wheat fractions after heat and sucrose treatment

Heat treatment				Sucrose concentration				Surface hydrophobicity	
None	25 °C	50 °C	100 °C	0%	2.5%	5%	10%	pH 5.5	pH 7.5
Soy flour 7 S									
	X			X				32.44 (0.21)^{b}	13.28 (0.06)^{a}
		X		X				31.99 (0.30)^{b}	13.17 (0.05)^{a}
			X	X				35.99 (0.49)^{c}	13.50 (0.06)^{a}
	X				X			32.88 (0.01)^{bc}	13.02 (0.02)^{a}
		X			X			33.61 (0.13)^{c}	12.99 (0.08)^{a}
			X		X			33.34 (0.11)^{c}	12.87 (0.06)^{a}
	X					X		36.02 (0.07)^{cd}	12.79 (0.10)^{a}
		X				X		32.09 (0.03)^{b}	12.45 (0.05)^{a}
			X			X		33.01 (0.04)^{bc}	12.43 (0.09)^{a}
X							X	38.91 (0.27)^{d}	12.45 (0.05)^{a}
	X						X	35.37 (0.79)^{c}	12.76 (0.04)^{a}
		X					X	39.09 (0.12)^{d}	12.41 (0.01)^{a}
			X				X	36.56 (0.12)^{d}	12.47 (0.06)^{a}
Soy flour 11S									
	X			X				30.47 (0.32)^{b}	32.72 (0.15)^{b}
		X		X				39.01 (0.55)^{c}	39.35 (0.60)^{c}
			X	X				30.19 (0.02)^{b}	33.30 (0.06)^{b}
	X				X			30.11 (0.91)^{b}	31.83 (0.21)^{b}
		X			X			25.99 (0.98)^{a}	31.66 (0.33)^{b}
			X		X			31.61 (0.30)^{b}	32.84 (0.11)^{b}
	X					X		29.90 (0.06)^{b}	31.68 (0.06)^{b}
		X				X		28.57 (0.06)^{ab}	31.54 (0.06)^{b}
			X			X		39.09 (0.01)^{c}	41.37 (0.54)^{c}
X							X	40.10 (0.44)^{c}	41.67 (0.05)^{c}
	X						X	40.19 (0.67)^{c}	41.65 (0.11)^{c}
		X					X	25.91 (0.02)^{a}	31.46 (0.08)^{b}
			X				X	39.78 (0.04)^{c}	41.33 (0.18)^{c}
TSP soy isolate									
	X			X				30.77 (0.14)^{c}	31.00 (0.15)^{c}
		X		X				27.59 (0.07)^{b}	29.70 (0.12)^{c}
			X	X				27.23 (0.28)^{b}	30.64 (0.47)^{c}
	X				X			26.99 (0.01)^{b}	30.19 (0.81)^{c}
		X			X			21.99 (0.31)^{a}	27.43 (0.52)^{b}
			X		X			24.01 (0.51)^{ab}	29.52 (0.40)^{c}
	X					X		26.10 (0.06)^{b}	28.10 (1.07)^{bc}
		X				X		22.06 (0.07)^{a}	25.48 (0.40)^{b}
			X			X		23.09 (0.17)^{a}	25.35 (0.58)^{b}
X							X	22.00 (0.02)^{a}	27.13 (0.16)^{b}
	X						X	26.91 (0.39)^{b}	28.47 (0.48)^{bc}
		X					X	21.09 (0.02)^{a}	24.48 (0.57)^{ab}
			X				X	21.54 (0.43)^{a}	25.71 (0.52)^{b}

Table 2 (*continued*)

Heat treatment				Sucrose concentration				Surface hydrophobicity	
None	25 °C	50 °C	100 °C	0%	2.5%	5%	10%	pH 5.5	pH 7.5
TSP 7S									
	X			X				49.09 (0.76)^{e}	46.34 (1.06)^{e}
		X		X				25.98 (1.54)^{b}	13.64 (0.99)^{a}
			X	X				19.02 (2.12)^{b}	12.96 (0.17)^{a}
	X				X			30.91 (0.54)^{c}	30.85 (0.58)^{c}
		X			X			39.01 (0.01)^{d}	31.56 (0.50)^{c}
			X		X			32.09 (0.33)^{c}	31.96 (0.06)^{c}
	X					X		31.00 (0.87)^{c}	29.87 (0.10)^{c}
		X				X		44.91 (0.43)^{de}	39.96 (0.09)^{d}
			X			X		44.89 (0.07)^{de}	41.34 (0.98)^{d}
X							X	35.98 (0.32)^{cd}	31.60 (0.55)^{c}
	X						X	22.09 (0.09)^{b}	19.79 (0.07)^{b}
		X					X	33.97 (0.65)^{c}	29.49 (0.03)^{c}
			X				X	19.90 (0.91)^{b}	11.10 (0.84)^{a}
TSP 11S									
	X			X				60.06 (0.33)^{e}	57.25 (0.67)^{d}
		X		X				41.09 (0.02)^{a}	39.96 (0.08)^{a}
			X	X				39.00 (0.33)^{a}	40.54 (0.48)^{a}
	X				X			56.99 (0.02)^{cd}	57.44 (0.58)^{d}
		X			X			57.01 (0.02)^{d}	56.68 (0.57)^{c}
			X		X			58.33 (0.76)^{d}	58.40 (0.52)^{d}
	X					X		55.98 (0.45)^{c}	53.53 (0.49)^{c}
		X				X		56.07 (0.09)^{c}	52.49 (1.20)^{c}
			X			X		56.21 (0.32)^{c}	55.44 (0.24)^{c}
X							X	43.08 (0.01)^{a}	42.97 (0.16)^{a}
	X						X	44.00 (0.01)^{b}	43.14 (0.41)^{a}
		X					X	41.46 (0.22)^{a}	40.46 (0.69)^{a}
			X				X	43.08 (0.19)^{a}	42.01 (0.96)^{a}
TVP isolate									
	X			X				27.09 (0.03)^{b}	31.20 (0.30)^{c}
		X		X				31.56 (0.45)^{c}	36.24 (0.61)^{d}
			X	X				31.09 (0.59)^{c}	34.37 (0.54)^{d}
	X				X			24.71 (0.03)^{ab}	29.40 (1.46)^{c}
		X			X			25.09 (0.06)^{b}	31.45 (0.50)^{c}
			X		X			30.07 (0.02)^{c}	34.41 (0.52)^{d}
	X					X		21.09 (0.31)^{a}	28.27 (0.34)^{c}
		X				X		22.90 (0.04)^{a}	28.12 (0.17)^{c}
			X			X		22.19 (0.56)^{a}	29.28 (0.05)^{c}
X							X	21.09 (0.41)^{a}	28.78 (0.39)^{c}
	X						X	23.54 (0.17)^{a}	28.96 (0.18)^{c}
		X					X	27.01 (0.09)^{b}	27.80 (0.68)^{b}
			X				X	22.09 (0.78)^{a}	28.07 (1.00)^{bc}

TVP 7S												
	X				X						49.13 (1.92)[g]	46.36 (0.44)[f]
		X			X						39.89 (0.23)[e]	34.23 (0.53)[cd]
			X		X						37.66 (0.32)[d]	33.73 (0.22)[c]
	X					X					37.65 (0.21)[d]	35.39 (0.63)[d]
		X				X					35.77 (0.01)[d]	33.78 (0.61)[c]
			X			X					38.09 (0.09)[de]	32.45 (0.37)[c]
	X						X				28.91 (0.21)[c]	24.21 (0.13)[b]
		X					X				43.10 (0.49)[f]	39.74 (0.60)[e]
			X				X				33.91 (0.32)[c]	32.77 (1.56)[c]
X								X			37.18 (0.43)[d]	32.14 (0.14)[c]
	X							X			38.83 (0.77)[e]	33.52 (0.56)[c]
		X						X			16.02 (0.79)[ab]	9.67 (0.34)[a]
			X					X			21.09 (0.04)[b]	11.82 (0.84)[a]
TVP 11S												
	X				X						49.13 (1.92)[g]	46.36 (0.44)[f]
		X			X						39.89 (0.23)[e]	34.23 (0.53)[cd]
			X		X						37.66 (0.32)[d]	33.73 (0.22)[c]
	X					X					37.65 (0.21)[d]	35.39 (0.63)[d]
		X				X					35.77 (0.01)[d]	33.78 (0.61)[c]
			X			X					38.09 (0.09)[de]	32.45 (0.37)[c]
	X						X				28.91 (0.21)[c]	24.21 (0.13)[b]
		X					X				43.10 (0.49)[f]	39.74 (0.60)[e]
			X				X				33.91 (0.32)[c]	32.77 (1.56)[c]
X								X			37.18 (0.43)[d]	32.14 (0.14)[c]
	X							X			38.83 (0.77)[e]	33.52 (0.56)[c]
		X						X			16.02 (0.79)[ab]	9.67 (0.34)[a]
			X					X			21.09 (0.04)[b]	11.82 (0.84)[a]
Puroindoline												
X					X						17.01 (0.03)[c]	16.96 (0.86)[c]
X						X					18.35 (0.11)[cd]	18.20 (0.56)[cd]
X							X				22.91 (0.43)[d]	20.44 (0.46)[d]
	X				X						17.89 (0.13)[c]	16.51 (0.60)[c]
		X			X						32.95 (0.54)[e]	13.97 (0.17)[b]
			X		X						42.09 (0.21)[f]	9.74 (0.21)[ab]
	X					X					13.96 (0.42)[b]	16.01 (0.11)[c]
		X				X					22.91 (0.27)[d]	7.74 (0.46)[a]
			X			X					37.49 (0.77)[f]	11.3 (0.57)[b]
	X						X				15.61 (0.63)[c]	18.20 (0.16)[d]
		X					X				17.81 (0.91)[c]	7.62 (0.37)[a]
			X				X				30.92 (0.12)[e]	10.66 (0.78)[b]
X								X			41.91 (0.76)[f]	19.72 (2.04)[d]
	X							X			12.71 (0.29)[b]	16.91 (0.14)[c]
		X						X			15.23 (1.56)[c]	7.35 (0.06)[a]
			X					X			24.21 (2.34)[d]	6.44 (0.22)[a]
Gliadin												
X					X						21.08 (0.07)[c]	20.77 (0.29)[c]
X						X					19.86 (0.65)[c]	20.74 (1.01)[c]

Table 2 (*continued*)

Heat treatment				Sucrose concentration				Surface hydrophobicity	
None	25 °C	50 °C	100 °C	0%	2.5%	5%	10%	pH 5.5	pH 7.5
X						X		21.33 (0.34)^{cd}	19.56 (0.50)^{c}
	X			X				20.81 (0.04)^{c}	19.86 (0.78)^{c}
		X		X				23.98 (0.96)^{d}	18.56 (0.23)^{c}
			X	X				17.09 (0.11)^{b}	14.38 (0.49)^{a}
	X				X			24.65 (0.15)^{d}	20.39 (0.06)^{c}
		X			X			15.99 (0.72)^{b}	17.06 (0.05)^{b}
			X		X			16.87 (1.44)^{b}	16.18 (0.17)^{b}
	X					X		19.55 (0.86)^{c}	19.01 (1.00)^{c}
		X				X		14.09 (1.87)^{a}	13.74 (0.63)^{a}
			X			X		15.32 (0.23)^{ab}	14.48 (0.48)^{a}
X							X	20.53 (0.32)^{c}	19.24 (1.06)^{c}
	X						X	19.98 (0.14)^{c}	19.89 (0.06)^{c}
		X					X	16.02 (0.44)^{b}	11.12 (1.16)^{a}
			X				X	15.00 (2.01)^{ab}	13.60 (0.50)^{a}
Glutenin									
X				X				21.08 (0.07)^{c}	20.77 (0.29)^{c}
X					X			19.86 (0.65)^{c}	20.74 (1.01)^{c}
X						X		21.33 (0.34)^{cd}	19.56 (0.50)^{c}
	X			X				20.81 (0.04)^{c}	19.86 (0.78)^{c}
		X		X				23.98 (0.96)^{d}	18.56 (0.23)^{c}
			X	X				17.09 (0.11)^{b}	14.38 (0.49)^{a}
	X				X			24.65 (0.15)^{d}	20.39 (0.06)^{c}
		X			X			15.99 (0.72)^{b}	17.06 (0.05)^{b}
			X		X			16.87 (1.44)^{b}	16.18 (0.17)^{b}
	X					X		19.55 (0.86)^{c}	19.01 (1.00)^{c}
		X				X		14.09 (1.87)^{a}	13.74 (0.63)^{a}
			X			X		15.32 (0.23)^{ab}	14.48 (0.48)^{a}
X							X	20.53 (0.32)^{c}	19.24 (1.06)^{c}
	X						X	19.98 (0.14)^{c}	19.89 (0.06)^{c}
		X					X	16.02 (0.44)^{b}	11.12 (1.16)^{a}
			X				X	15.00 (2.01)^{ab}	13.60 (0.50)^{a}

Results are means ± standard deviation of three determinations.

[a–f]Means within a fraction with the same subscripts are not significantly different ($p < 0.05$).

decreasing the frequency of protein–protein encounters, and by ''shoring up'' the protein superstructure.

Surface hydrophobicity of the 11S fractions followed a trend similar to the isolate forms (Table 2). Sucrose alone had no significant ($p < 0.05$) effect on hydrophobicity (data not shown). However, when sucrose and heat treatment were combined, surface hydrophobicity was reduced to levels far below those of the native protein. In this case, sucrose may be causing or allowing a dramatic reorganization of the protein structure. In their seminal study, Lee and Timasheff (1981) suggested that proteins preferentially exclude sucrose molecules from their immediate surroundings. Resulting protein molecules are extremely stable, with the maximum number of hydrophobic groups buried in the interior. Antipova and Semenova (1995) documented similar 11S behaviuor in their biopolymer research. They explained this increased hydrophilicity by suggesting that sucrose molecules surround the protein, forming a hydrophilic layer. The solubility and surface hydrophobicity similarity between the 11S fractions and isolates suggests that these fractions are dominant in the overall isolate structure.

In contrast to the isolates and 11S fractions, the 7S fraction was less soluble when subjected to high sucrose levels and temperature (Table 1). The 7S fraction, a trimer with no disulfide bonds, is more sensitive to temperature changes. High temperatures cause partial unfolding of the 7S structure, exposing hydrophobic residues (Galazka, Dickinson, & Ledward, 1999). With the introduction of high sucrose concentrations, the 7S subunits condense into their most thermodynamically stable, albeit having decreased solubility, forms (Hermansson, 1978).

Surface hydrophobicity characteristics of the 7S fractions differed among the textured and non-textured forms (Table 2). Neither heat nor sucrose affected the hydrophobicity of the soy flour 7S form, whereas these treatments decreased hydrophobicity in 7S fractions derived from the textured flours. Although this appears contradictory in light of the fact that solubility also declined, low hydrophobicity does not necessarily bring about increased solubility (Wagner et al., 2000). Several factors, including processing method of the original flours, may affect solubility and hydrophobicity of the 7S fractions. Thermal degradation of the 7S trimer, and subsequent mixing of polypeptide fragments, may mask or alter the true solvation characteristics.

The solubility profile of puroindoline suggests different sensitivities to sucrose and heat, depending on pH (Table 1). At pH 5.5, neither sucrose nor heat alone affected solubility. However, in combination, they increased it. At pH 7.5, increasing sucrose significantly increased solubility, while higher temperature decreased it. In combination, solubility decreased with higher sucrose levels and temperatures, possibly indicating that the sucrose effect on puroindoline is greater than that of temperature. The solubility profile of BSA was similar to that of puroindoline with respect to its reaction to the sucrose/temperature combination (data not shown). Puroindoline and BSA are both complex lipid-transfer proteins, containing regions in their polypeptide chains that are rich in hydrophobic residues. Heat treatment of these molecules may cause partial exposure of these regions. In addition, sucrose may amplify the hydrophobic nature of these proteins by aligning and interacting with the amino acids adjacent to the hydrophobic residues through the hydrophobic regions present in the glucose units (Duan, Hall, Nikaido, & Quiocho, 2001).

Surface hydrophobicity measures for puroindoline also indicate an extreme shift in characteristics based on pH. At pH 5.5, puroindoline exhibited significant surface hydrophobicity alterations which increased with both sucrose and heat. In contrast, at pH 7.5 surface hydrophobicity declined with only minor heat and sucrose addition. This pH effect may reflect the approaching isoelectric point of the protein. The exact p*I* of this complex protein has not been documented, but estimates range from pH 10 to 11 (Le Guernevé, Seigneuret, & Marion, 1998). Theoretical calculation of the isoelectric points of several of the puroindoline A precursor sequences (i.e., mature puroindoline A minus tryptophan-rich region) places them near the pH range used in these studies. Heat and/or purification treatments may have altered the puroindoline fractions and selected for these lower p*I* units. These units may condense into more thermodynamically stable forms in the higher pH treatments, lowering surface hydrophobicity.

Gliadin and glutenin solubilities and surface hydrophobicities were similar to those reported in previous research (Chung & Pomeranz, 1979; Popineau & Pineau, 1987). Heat and sucrose increased the solubility of both proteins (Tables 1 and 2). Gliadins reached their maximum solubility at pH 5.5 and glutenins at 7.5. This is inversely correlated with the isoelectric points of gliadin and glutenin, which are 8.1 and 7.1, respectively. Surface hydrophobicity of both proteins decreased with increasing heat and sucrose while pH had no significant effect.

3.2. Binding constant

Purified puroindoline and soy protein fractions gave well-resolved peaks when applied to the Sepharose column (Fig. 1). Elution times of each fraction were similar, whether run separately or together (data not shown). SDS–PAGE of each of the collected fractions was pure for each protein. These data indicate that complex interaction between the protein fractions, if it occurred, equilibrated faster than the column separation time of $\approx$40 min. Therefore, the equilibrium chromatography technique of Hummel and Dryer (1962) was used to

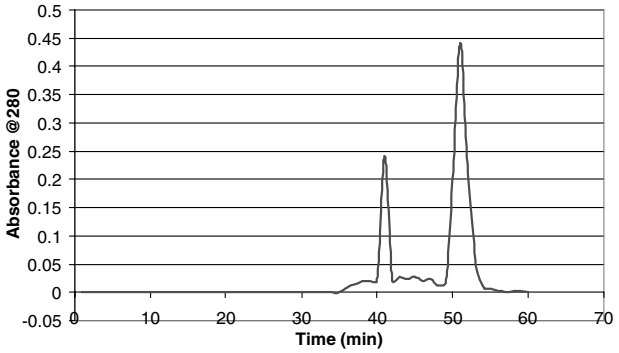

Fig. 1. Gel filtration trace of puroindoline and native glycinin.

detect the presence of reversible binding between puroindoline and the soy fractions.

Equilibrium chromatography measures dissociation constant (K_d) by detecting the decrease in ligand concentration at its normal elution time. In this experiment, the gel filtration column was equilibrated with buffer-containing 20 μM puroindoline. Samples of each of the soy fractions (S) to be evaluated for binding were dissolved in the same buffer. When binding occurred between puroindoline and a soy fraction, the concentration of free puroindoline [P_{free}] in the sample decreased by an amount equal to the puroindoline–soy fraction aggregate formed. This sample was applied to the column and further eluted with the puroindoline buffer. Eluting fractions from the column were assayed spectrophotometrically. Due to its increased molecular weight, the puroindoline–soy (P–S) aggregate exhibited a higher mobility in the column, eluting faster than free puroindoline. Because P_{free} in the sample was now less than that contained in the column buffer, a trough was observed at the normal elution time for puroindoline (Fig. 2). As the soy fraction concentration increased, the trough size increased (data not shown). Aggregates, and their corresponding troughs in the spectrophotometric traces, were formed for each of the isolates and the 11S fractions. However, no evidence of puroindoline interaction occurred with the 7S fractions.

To determine the binding constant of the P–S complex, the concentration of P_{free} was accomplished by adding additional puroindoline to the soy fraction-containing sample buffer. Puroindoline interacts with the soy fraction (S) and forms a complex, lowering the total free puroindoline present in the sample to levels less than that in the column and produces the trough observed. As more puroindoline is added to the sample, more aggregates are formed, but the level of P_{free} slowly increases as excess is not consumed through complex formation. As long as the level of P_{free} present in the sample is lower than that in the column, a trough will be formed. When [P_{free}] is greater than the column concentration, a peak will form. When no trough or peak in puroindoline is observed (the cross-point), the P_{free} in the sample equals the concentration with which the column was eluted. The concentration of puroindoline bound to the soy fraction [P–S] equals the concentration at the cross-point minus the initial column concentration. Figs. 2 and 3 illustrate this procedure using puroindoline and soy flour 11S fraction. Fig. 4 shows the cross-point plot used for dissociation constant determination. Assuming the formation of an equimolar com-

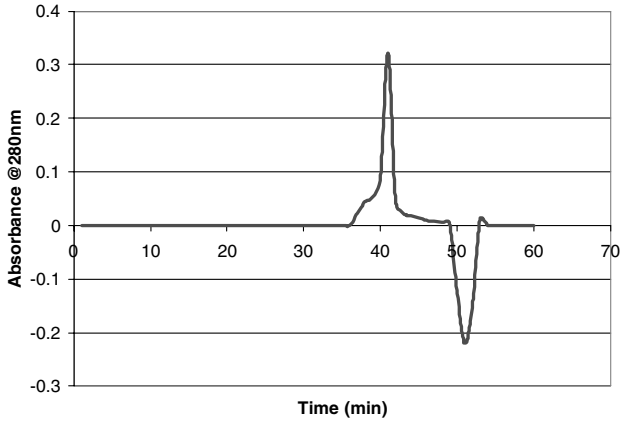

Fig. 2. Equilibrium gel filtration of puroindoline and soy flour 11S fraction: 30 μM.

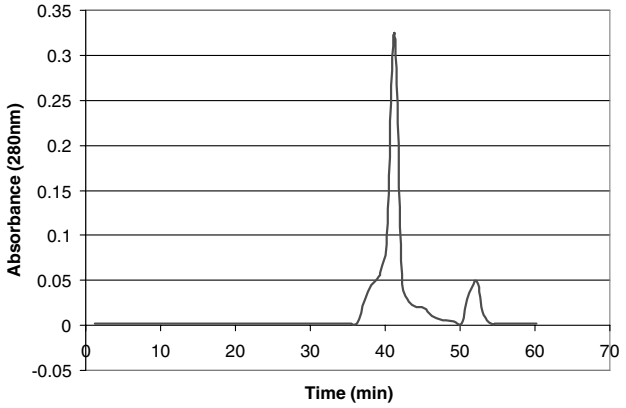

Fig. 3. Equilibrium gel filtration of puroindoline and soy flour 11S fraction: 50 μM.

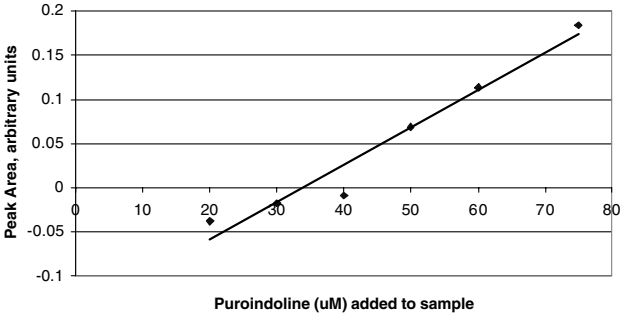

Fig. 4. Cross-point determination plot for equilibrium gel filtration of puroindoline and soy flour 11S fraction.

plex between puroindoline and the soy fractions, the dissociation constant from these data can be calculated:

$K_d = [S_{free}] \times [P_{free}]/[P–S]$.

The dissociation constants (K_d [μM]) for the isolates and their corresponding 11S fractions were: SFI, 30.6; TVP isolate, 27.9; TSP isolate, 24.1; Soy flour 11S fraction, 37.1; TVP 11S fraction, 24.8; and TSP 11S fraction, 22.1 (data not shown). These data indicate that textured soy proteins, and their corresponding 11S fractions, have a greater binding affinity for puroindoline than do non-textured soy protein.

4. Conclusions

Textured soy protein fractions displayed higher solubility and surface hydrophobicity profiles than their non-textured counterparts. Sucrose addition decreased hydrophobicity in the textured proteins, but increased it in the non-textured proteins. Characteristics of the isolate as a whole appear to be dictated by those of its 11S moiety, a role mirrored in the binding studies. Based on solvation profiles, protein–protein interaction between soy fractions and puroindoline is possible through hydrophobic interactions that are, perhaps, enhanced through heat and sucrose addition. However, the dissociation constants indicate an extremely short-lived protein–protein interaction in all cases. If puroindolines are the source of soy protein adhesion on the surface of the wheat-starch granule, the mechanism by which they adhere is not fully explained through the method elucidated in these in vitro studies. Aggregates, and their corresponding troughs in the spectrophotometric traces, were formed with each of the isolates and the 11S fractions. However, samples containing the 7S fractions contained no evidence of an interaction with puroindoline.

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