Immunochemical Studies on Gastric and Intestinal Digestion of Soybean Glycinin and β-Conglycinin in Vivo

Jean-Paul Lallès,*,† Hussaini M. Tukur,†,‡ Paulo Salgado,† E. N. Clare Mills,§ Michael R. A. Morgan,§ Laurence Quillien,|| Didier Levieux,⊥ and René Toullec†

Laboratoire du Jeune Ruminant, INRA, 65 rue de Saint-Brieuc, 35042 Rennes Cedex, France; Department of Food Biochemistry, Institute of Food Research, Norwich Laboratory, Norwich Research Park, Colney, Norwich NR4 7UA, United Kingdom; Laboratoire de Biochimie et de Technologie des Protéines, INRA, B.P. 71 627, 44316 Nantes Cedex 03, France; and Station de Recherches sur la Viande, Unité d'Immunochimie, INRA, Centre de Clermont-Theix, 63122 Saint-Genès-Champanelle, France

Two experiments were conducted to study gastric and small intestinal digestion of soybean glycinin and β-conglycinin in preruminant calves fed milk replacers containing a mixture of skim milk powder and antigenic heated soybean flour. In experiment 1, duodenal passage of immunoreactive β-conglycinin lasted for a much longer time after the morning meal than that of glycinin. Western blotting revealed the early abomasal outflow of glycinin subunits that associated nearly intact basic polypeptides to partially degraded acidic polypeptides. Intact β-conglycinin was evidenced at most sampling times. In experiment 2, intact basic glycinin ($M_r = 21000$) associated with partially digested acidic glycinin ($7000 < M_r < 25000$) was demonstrated in ileal digesta up to 8−10 h after the meal. β-Conglycinin immunoreactivity could not be evidenced by Western blotting in ileal digesta.

Keywords: *Calf nutrition; ELISA; protein digestion; soy globulin; Western blotting*

INTRODUCTION

Soybeans are widely used by the food industry as a cheap source of protein or as an additive for improving the functional properties of foods. However, optimizing its in vivo digestion requires adequate processing to inactivate antinutritional factors such as protease inhibitors and lectins and to reduce its allergenic potential [review by Lallès et al. (1993)].

Soybean flours, concentrates, and isolates, contain approximately 50, 70, and 90% protein, respectively, of which the storage globulins glycinin and β-conglycinin are the major constitutents (Murphy and Resurreccion, 1984; Plumb et al., 1994). Glycinin ($M_r \approx 360000$), also called the 11S globulin due to its sedimentation coefficient, is made up of six subunits ($M_r \approx 60000$). Each subunit comprises one acidic, A ($M_r \approx 40000$), and one basic, B ($M_r \approx 20000$), polypeptide, which are disulfide-bonded together [review by Nielsen (1985)]. These polypeptides are highly heterogeneous; up to 13 A and 11 B forms have been identified using two-dimensional electrophoresis (Lei et al., 1983). Moreover, pairings between A and B polypeptides are nonrandom, leading to five major A-covalent associations of highly variable methionine content (Staswick et al., 1981). Finally, soybean cultivars appear to differ in actual numbers of A (6 to 7) and B (3 to 8) polypeptides constituting glycinin superstructures (Mori et al., 1981). β-Conglycinin which is glycosylated comprises three subunits, α, α′, and β, with M_r of ≈76000, 72000, and 53000, respectively, that associate randomly into trimers of $M_r \approx 150000-220000$ (Nielsen, 1985).

Soy globulins are known to resist heat processing and digestion by mammalian enzymes (Nielsen et al., 1988; Sissons and Thurston, 1984). The A polypeptides of glycinin are hydrolyzed more rapidly than B polypeptides in vitro, and pepsinolysis is more extensive than trypsin digestion (Lynch et al., 1977; Kella et al., 1986). Depending on experimental conditions, A polypeptides can be degraded by pepsin into peptides of M_r below 16000 (Kella et al., 1986), 12000 (Lynch et al., 1977), or even 7000 (Richardson and Catsimpoolas, 1979). Peptic treatment of the B polypeptides generates one major fragment of $M_r = 16000$, whereas pancreatin digestion is virtually complete (Kella et al., 1986). Finally, glycinin is more susceptible to trypsin in vitro than legumin, the 11S globulin of pea (Plumb and Lambert, 1990). Data pertaining to β-conglycinin digestion are scarce, but Astwood et al. (1996) mentioned that the basic (β) subunit was more resistant than the acidic (α, α′) subunits to gastric digestion in vitro. This was consistent with the sequential degradation of β-conglycinin subunits during germination [review by Bau et al. (1997)] and fermentation in the rumen of sheep (Romagnolo et al., 1990; Aufrère et al., 1994).

The proteolysis of soy globulins in vivo has not been studied extensively. The presence of soluble forms of immunoreactive glycinin and β-conglycinin in ileal digesta of calves sensitive to heated soybean flour was first reported by Sissons and Thurston (1984). More recently, ileal flow of glycinin and β-conglycinin were estimated by ELISA to be 10.3 and 0.9% of respective amounts of immunoreactive globulins ingested by pre-

* Author to whom correspondence should be addressed [fax (+33) (0) 2-99-28-53-70; e-mail lalles@roazhon.inra.fr].
† Laboratoire du Jeune Ruminant.
‡ Present address: Department of Animal Sciences, Faculty of Agriculture, Usmanu Danfodiyo University, PMB 2346, Sokoto, Nigeria.
§ Department of Food Biochemistry.
|| Laboatoire de Biochimie et de Technologie des Protéines.
⊥ Station de Recherches sur la Viande.

10.1021/jf980882+ CCC: $18.00
Published on Web 06/16/1999

Table 1. Properties of Antibodies Used To Characterize Immunoreactive Glycinin and β-Conglycinin in Soybean Products and Intestinal Digesta of Calves

antigen injected	working dilution	produced in	code name[a]	polypeptide or subunit recognized	reference
			Anti-glycinin Antibodies		
native glycinin	1:500	rabbit	LJR J4 (Pab)	acidic (A)	Tukur et al. (1993)
SDS-denatured and reduced glycinin	1:500	rabbit	LJR 9205 (Pab)	acidic (A) and basic (B)	present work
pea legumin B	1:1000	rabbit	BTP 02 (Pab)	basic (B)	L. Quillien et al. (unpublished data)
subtilisin-treated glycinin	1:10 000	rat	IFRN 0025 (Mab)	acidic (A)	Carter et al. (1992)
			Anti-β-conglycinin Antibodies		
native β-conglycinin	1:400	mouse	IFRN 0089 (Mab)	acidic (α, α′)	Plumb et al. (1995)
native β-conglycinin	1:200	rabbit	LJR J2 (Pab)	acidic (α, α′) and basic (β)	Tukur et al. (1993)

[a] Pab, polyclonal antibody; Mab, monoclonal antibody.

ruminant calves (Tukur et al., 1993). However, these components were not characterized biochemically.

The aim of the present work was to provide further information on the gastric and small intestinal digestion of glycinin and β-conglycinin in the calf, as assessed by ELISA and Western blotting techniques using appropriate antibodies.

MATERIALS AND METHODS

Materials. All of the reagents including horseradish peroxidase conjugates of anti-rabbit IgG, anti-mouse IgG, and anti-rat IgG and substrates used for ELISA [2,2′-azinobis(3-ethylbenzthiazoline-6-sulfonic acid)] and Western blotting assays (diaminobenzidine) were purchased from Sigma Chemical Co., St. Quentin, France. Microtiter plates F16 Maxisorp were from Nunc, Roskilde, Denmark. The Argus 300 ELISA plate reader was from Packard Instruments Co., Meriden, CT. Molecular weight standards kit (ref 17-0446-01) was from Pharmacia, Uppsala, Sweden. It included phosphorylase *b* (94000), bovine serum albumin (BSA) (67000), ovalbumin (43000), carbonic anhydrase (30000), soybean trypsin inhibitor (21100), and α-lactalbumin (14400). Nitrocellulose membranes (Hybon-C super) were from Amersham International, U.K. Electrophoresis system model 6450 was from Atto-Touzard (Touzard & Matignon, France). Proteins were transferred from gels to nitrocellulose membranes using a transblot apparatus (Pharmacia).

Purification of Glycinin and β-Conglycinin and Antibody Preparation. Glycinin and β-conglycinin were prepared from raw defatted soy flour (Thanh and Shibasaki, 1976).

Rabbit polyclonal antisera (Pab) were produced against native glycinin (LJR J4; Tukur et al., 1993) and SDS-denatured and reduced glycinin (LJR 9205; H. M. Tukur and J. P. Lallès, unpublished data). A rabbit Pab (BTP 02) was raised to the B polypeptide of pea legumin and found to cross-react with the corresponding polypeptide of glycinin by ELISA (L. Quillien et al., unpublished data). Monoclonal antibodies (Mabs) specific for glycinin A (IFRN 0025; Carter et al., 1992) and specific for an epitope that is common to α and α′ subunits, but not present in the β subunit, of β-conglycinin (IFRN 0089; Plumb et al., 1995) were added to this panel of Pabs. The properties of these antibody preparations are summarized in Table 1.

Digestion Studies in Vivo. *(1) Experiment 1—Duodenal Sampling.* This trial was designed to study changes in immunoreactive glycinin and β-conglycinin concentrations in duodenal digesta over time after the morning meal. Five Holstein heifer calves were fitted with a soft T-piece cannula in the duodenum at the age of 3 months. After recovery, they were randomly adapted for 2 weeks to milk replacers containing either skim milk powder only (SMP1; Sofivo, Condé-sur-Vire, France) or mixtures of SMP and soybean products [1:1, on a digestible crude protein (CP) basis)]. The soy products were a heated soybean flour (HSF1; Protisoya from Société Industrielle des Oléagineux, Bougival, France) and an ethanol-treated soybean protein concentrate (SPC1; Danproveal from Central Soya, Aarhus, Denmark). HSF1 was considered as antigenic because it contained 132 and 30 mg of immunoreactive glycinin and β-conglycinin per gram of CP, respectively, as determined by ELISA (Tukur et al., 1993, 1996). Neither of these immunoreactivities were detected in SPC1. Therefore, it was considered to be nonantigenic. The soy-based diets were formulated to contain similar levels of digestible protein and energy (Table 2). The milk replacers were mixed with lukewarm water (180 g of powder/kg) prior to being fed by bucket twice daily at a level of 55 g of dry matter/kg of body weight$^{0.75}$/day. Duodenal digesta were collected 0.5, 2, 4, 6, and 8 h after the morning meal in five, three, and four calves receiving the HSF1, SPC1, and SMP1 diets, respectively. Digesta pH was measured immediately, and a mixture of preservatives [ovomucoid type II 335 mg/kg of digesta, 15 mM disodium ethylenediamine tetraacetate (EDTA), 1 mM sodium azide, 2 mM phenylmethanesulfonyl fluoride (PMSF)] was added to limit digesta proteolysis by enzymes. The samples were vigorously shaken and frozen at −20 °C prior to freeze-drying.

(2) Experiment 2—Ileal Sampling. Conditions of this trial have been published elsewhere (Tukur et al., 1993). Briefly, six preruminant Holstein heifer calves were fitted with an abomasal catheter and a reentrant ileo-cecal cannula at the age of 2 months. They were fed three milk replacer diets for 2 weeks each, according to a double Latin square design. Protein in diets was provided by skim milk powder only (SMP2) or by a mixture (1:1; CP basis) of skim milk powder and heated soybean flour (HSF2; Arkasoy 50 from Société Industrielle des Oléagineux) or ethanol-treated soy protein concentrate (SPC2;

Table 2. Composition of Diets in Experiment 1

	SMP1[a]	HSF1	SPC1
diet ingredients (g/kg)			
skim milk powder		270.0	270.0
whey powder		74.4	74.4
HSF1 product		320.0	
SPC1 product			164.2
tallow		225.6	180.0
sucrose			23.8
lactose		58.5	223.4
dicalcium phosphate		8.7	16.1
sodium chloride		5.3	5.6
potassium chloride			7.0
calcium chloride		3.7	
magnesium sulfate			1.7
others[b]		33.8	33.8
chemical composition (g/kg DM)			
OM	926.0	929.0	926.0
ash	74.3	71.0	74.4
CP (N × 6.25)	226.0	278.0	230.0
digestible CP[d]	212.0	202.0	201.0
digestible energy[cd]	4964	4674	4583

[a] Ingredient composition not disclosed by the manufacturer. [b] L-Lysine HCl, 3.9; DL-methionine, 2.0; pregelatinized starch, 20.0; sorbitol, 5.0; vitamin and mineral premix, 2.9. [c] kcal/kg of DM for digestible energy. [d] Calculated from previous experiments with similar products in this laboratory.

Table 3. Postprandial Changes in Duodenal Concentrations of Immunoreactive Soybean Globulins and Milk Proteins in Calves Fed a Milk Replacer Containing a Mixture of Skim Milk Powder and Antigenic Heated Soybean Flour (HSF1) in Experiment 1 (Data Expressed on a CP Basis as Percentages of Corresponding Concentrations in Diets) (Means ± SEM)[a]

	time after the morning meal				
protein	0.5 h	2 h	4 h	6 h	8 h
soybean globulins					
glycinin	209 ± 17	46 ± 7	18 ± 3	6 ± 4	0 ± 0
β-conglycinin	185 ± 38	111 ± 14	147 ± 16	56 ± 17	25 ± 11
milk proteins					
β-lactoglobulin	11 ± 3	50 ± 4	23 ± 4	11 ± 2	7 ± 1
α-lactalbumin	5 ± 3	30 ± 8	5 ± 1	1 ± 0.1	0.4 ± 0.02
BSA	440 ± 60	298 ± 22	134 ± 43	27 ± 4	87 ± 57
IgG	71 ± 7	57 ± 7	29 ± 6	4 ± 1	10 ± 4

[a] No specific soybean globulin immunoreactivity was found in duodenal digesta of calves fed the SMP1 or SPC1 diet in experiment 1.

Danpro from Aarhus Olie, Denmark). HSF2 contained 173 and 29 mg of immunoreactive glycinin and β-conglycinin per gram of CP, respectively, whereas SPC2 was devoid of them. Total digesta were collected from ileal cannulas over 96 h during the second week of each experimental period in flasks containing sodium benzoate (final concentration 69 mM). Representative average samples of the 96-h digestibility period were prepared, frozen at −20 °C, and freeze-dried. Digesta were also collected hourly for 10 h after the morning meal of the fourth day in flasks containing sodium benzoate and PMSF (69 and 1.4 mM, respectively) and treated the same as the average samples.

Protein Extraction and Assay. Protein from dietary sources and digesta was extracted in borate buffer for 1.5 h at room temperature (Bush et al., 1992; Tukur et al., 1993). Concentration of protein in solutions was determined according to the method of Lowry et al. (1951) using bovine serum albumin (BSA) as the standard. Total nitrogen was also determined by the Kjeldahl method, and CP calculated as total N × 6.25.

Immunoassays for Soy and Milk Proteins. *(1) ELISAs for Soybean Globulins.* The concentrations of immunoreactive glycinin and β-conglycinin in soy products and duodenal digesta were carried out by inhibition ELISAs using specific hyperimmune sera (LJR J4 and LJR J2 of Table 1, respectively) as described previously by Tukur et al. (1993, 1996). Concentrations in digesta were expressed on a CP basis as a percentage of respective concentrations of soybean globulins in the diet.

(2) Radial Immunodiffusion Assays for Whey Proteins. The duodenal passage of some whey proteins including β-lactoglobulin, α-lactalbumin, BSA, and IgG was studied comparatively to that of soybean globulins, with the HSF1 diet in experiment 1. The concentrations of these proteins were measured by specific radial immunodiffusion assays as described elsewhere (Yvon et al., 1993), and data were expressed as above for ELISAs.

Duodenal Passage of Liquid Phase and Apparent Mean Retention Times of Immunoreactive Proteins in the Abomasum. An indigestible marker of the liquid phase of digesta, chromium (Cr)-EDTA (1.5 mg of Cr/kg of body weight), was added to the liquid substitute diets just before feeding in experiment 1. The liquid substitute diets and duodenal digesta were freeze-dried and ashed, and Cr was assayed from ash by atomic absorption spectrometry as described previously (Lallès and Poncet, 1990). The apparent mean retention times (MRTs) of Cr-EDTA and proteins of interest in the proximal part of the gut (i.e., mostly abomasum) were calculated algebraically from duodenal concentrations according to the method of Thielmans et al. (1978).

SDS−PAGE Electrophoresis and Immunoblotting. *(1) Electrophoresis.* This was carried out using minigels (80 × 90 mm) as described by Laemmli (1970) in the presence of Tris−glycine buffer (25 mM Tris, 192 mM glycine, pH 8.3) with a 12.5% acrylamide separating and a 4% acrylamide stacking gel. Loadings were 5 μg of protein per track for pure glycinin or β-conglycinin, 40 μg for duodenal digesta from experiment 1, and 65 μg for ileal digesta from experiment 2. Molecular weight standards were also loaded in a separate well. Electrophoresis was performed for 1.5 h at 40 mA. Gels were stained for protein using Coomassie blue R250.

(2) Protein Transfer. After electrophoresis, the gels were equilibrated for 15 min in the transfer buffer and proteins were electrotransferred (1 h, 100 mA) to nitrocellulose membranes in Tris−glycine buffer (25 mM Tris, 192 mM glycine, pH 8.3) containing 0.1% SDS (w/v) and 20% methanol (v/v). Protein transfer was monitored by staining membranes with Ponceau red.

(3) Immunoblotting. Membranes were saturated using 5% (w/v) skim milk powder in Tris buffer (20 mM Tris, 37 mM NaCl, pH 7.6) for 1 h at room temperature. They were incubated overnight at room temparature with optimal dilutions of specific antibodies (Table 1). After washing, membranes were incubated with appropriate horseradish peroxidase conjugated antibodies diluted at 1:1000 (v/v) for 2 h at room temperature. Finally, immunolabeling was revealed by incubating membranes in Tris buffer containing diaminobenzidine (0.7 mg/mL) for 5−10 min.

RESULTS

Duodenal Passage of Soybean Globulins and Milk Proteins in Experiment 1. *(1) Soybean Globulins.* The levels of immunoreactive glycinin and β-conglycinin in duodenal digesta collected 0.5 h after the morning meal were 209% ± (SEM) 17 and 185% ± 38 of those in the HSF1 milk replacer (Table 3), suggesting a faster abomasal outflow of immunoreactive soy globulins compared to milk proteins. At 2, 4, 6, and 8 h postfeeding, the levels of glycinin in digesta were 22, 8.6, 2.9, and 0% of the value at 0.5 h. Abomasal emptying of β-conglycinin was quite different from that of glycinin. Its concentration in duodenal digesta was transiently lower at 2 h, higher at 4 h, and then decreased to levels still measurable at 8 h. Immunoreactive glycinin and β-conglycinin were detected in neither protein source nor duodenal digesta of calves fed SPC1 and SMP1 diets.

(2) Whey proteins. The levels of immunoreactive β-lactoglobulin and α-lactalbumin in duodenal digesta of calves fed the HSF1 diet progressively increased at 0.5 h from 11 and 5% of the concentrations in the diet, respectively, to reach maxima of 50 and 30% at 2 h and then decreased to 7 and 0.4% at 8 h after feeding. In contrast, levels of immunoreactive BSA and IgG were initially high (440 and 71%, respectively), progressively decreased to 27 and 4% at 6 h, and then increased to 87 and 10% at 8 h. This increase in protein concentration observed at 8 h, that is, before the distribution of the evening meal, probably reflected the clearance of BSA and IgG from plasma to lumen of the intestine.

Table 4. Summary of Western Blotting for Soybean Globulins in Pure Form, in HSF1 Product, and in Duodenal Digesta Collected after the Morning Meal in a Calf Fed a Milk Replacer Containing a Mixture of Skim Milk Powder and Antigenic Heated Soybean Flour (HSF1)in Experiment 1 ($M_r \times 1000$)

antibody/condition[a]	pure antigen	HSF1 extract	digesta collected at 0.5 h	2 h	4 h	6 h	8 h
glycinin							
IFRN 025/with reduction	**36**[c]	**36**	36, **23**, **15**	**36**, 33	**36**, 33	**36**, 33	nd[c]
IFRN 025/without reduction	**67**	**64**	**42**, **32**	**62**, (41), **34**	**64**, (34)	**64**, (34)	nd
BTP 02/with reduction	**36, 20**	**36, 20**	**20**	36, 20	(20)	(20)	nd
BTP 02/without reduction	**67**	**67**	**43**, **33**, (29)	**64**, (41, 32)	67	65	nd
β-conglycinin							
IFRN 089	**89**, **84**	**89**, **84**	(94, 89)	**94**, **89**	(94, 89)	**94**, **89**	nd
LJR J2[b]	**58**	**57**	**60**, 25	**57**−**93**	**57**−**93**	**57**−**93**	57−93

[a] SDS−PAGE carried out under reducing or nonreducing conditions. [b] Bands corresponding to the basic subunit only are indicated. [c] Intensity of staining: strong staining in bold; light staining in parentheses; nd, not detected.

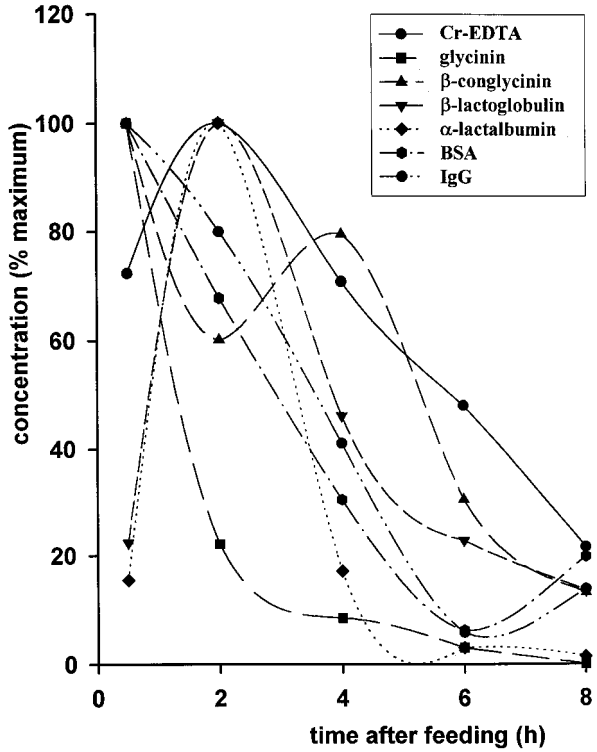

Figure 1. Postprandial changes in the concentration of soybean globulins and milk proteins in duodenal digesta of calves of experiment 1 fed a milk replacer containing heated soybean flour (data expressed as a percentage of maximal concentrations).

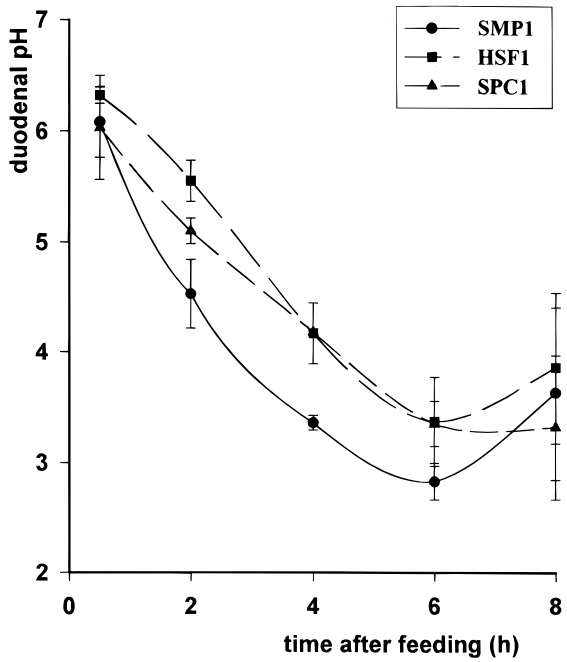

Figure 2. Postprandial changes in the duodenal pH of calves of experiment 1 fed milk replacers containing skim milk powder (SMP1) or a mixture of skim milk powder and either heated soybean flour (HSF1) or ethanol-treated soy protein concentrate (SPC1) (means ± SEM).

Whey protein assays were not carried out in protein sources and digesta of calves fed SPC1 and SMP1 diets because immunoreactive soy globulins were absent in these samples.

Apparent Mean Retention Time of Cr-EDTA and Dietary Proteins in the Abomasum and pH of Duodenal Digesta. The kinetics of Cr-EDTA, soy globulins, and milk proteins in duodenal digesta were standardized by expressing concentrations as a percentage of respective maximal concentrations (Figure 1). This clearly illustrated that most of the proteins studied had high initial (i.e., at 0.5 h) concentrations, suggesting that they were not trapped by abomasal clotting. However, initial concentrations for β-lactoglobulin and α-lactalbumin were low, suggesting a delayed abomasal mixing. Kinetics of passage were even more variable among the different proteins, probably reflecting their individual susceptibilities to abomasal digestion and subsequent loss of immunoreactivity. In that respect, glycinin appeared to be the most sensitive, and β-conglycinin and β-lactoglobulin the least, although the latter two still had contrasting kinetics. Apparent MRTs in the abomasum of calves fed the HSF1 diet were calculated to be 208 ± 6, 87 ± 10, 205 ± 11, 208 ± 9, 161 ± 18, 163 ± 15, and 156 ± 8 min for Cr-EDTA, immunoreactive glycinin, β-conglycinin, β-lactoglobulin, α-lactalbumin, BSA, and IgG, respectively. Besides, the MRT of Cr-EDTA in the abomasum was found to be similar among diets (208 ± 16, 208 ± 6 and 225 ± 14 min with SMP1, HSF1, and SPC1 diets, respectively).

Duodenal pH decreased from 6.0 to 6.3 at 0.5 h after the meal to values between 2.8 and 3.4 at 6 h and then tended to increase toward 3.3−3.9 (Figure 2). Duodenal pH was usually higher with the HSF1 diet than with the SMP1 diet. The calves fed the SPC1 diet presented pH values that were intermediate between those observed with HSF1 and SMP1 diets at 0.5, 2, and 8 h but close to that with the HSF1 diet at 4 and 6 h.

Western Blotting of Duodenal Digesta of Calves Fed a Diet Containing Treated Soy. *(1) Glycinin.* Immunoblots of reduced (Figure 3b,d) and nonreduced (Figure 3a,c) samples were developed with A polypeptide specific Mab IFRN 0025 (Figure 3a,b) and a polyclonal

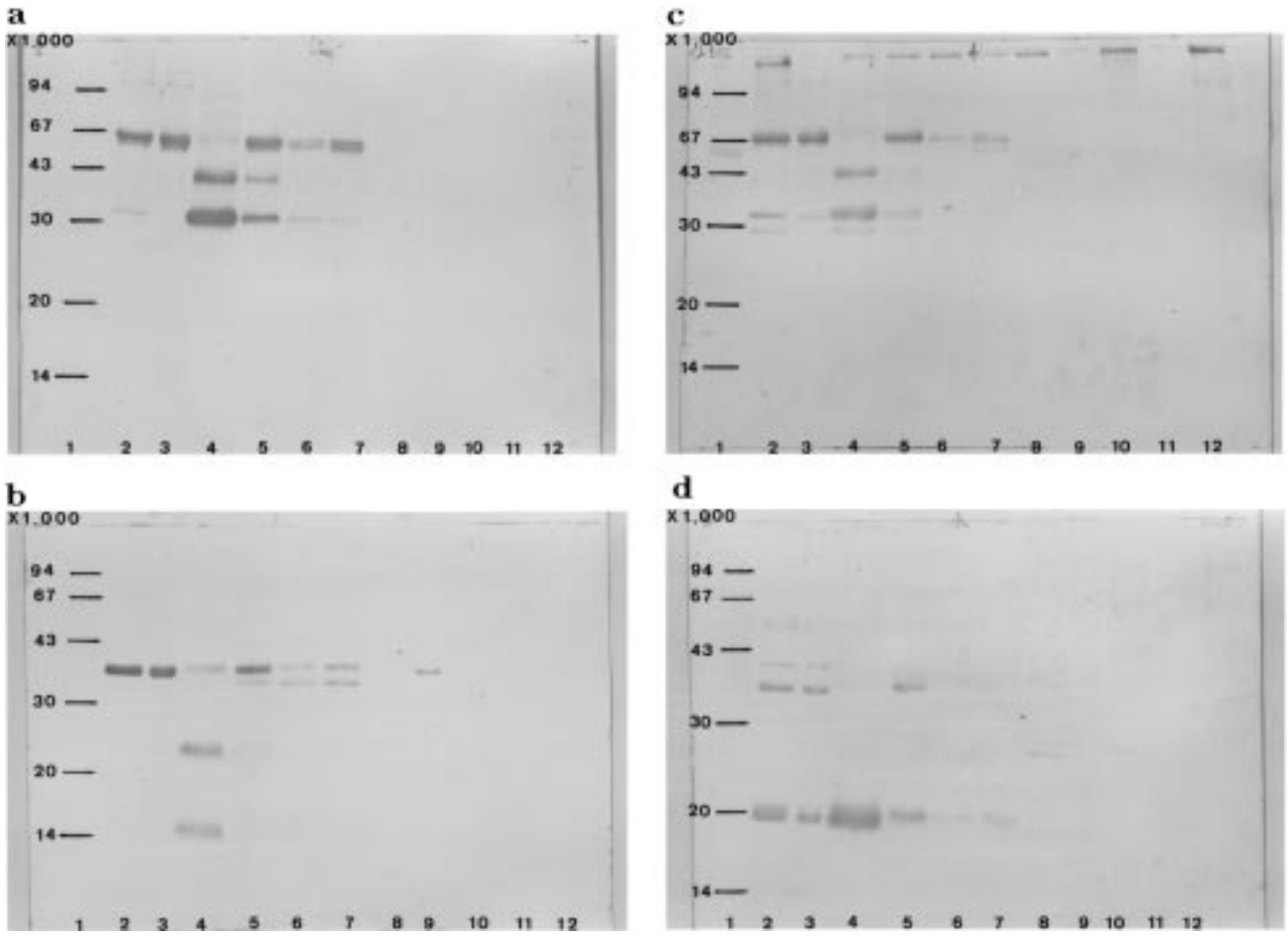

Figure 3. Immunoblots of duodenal digesta developed with anti-glycinin antibodies: pure glycinin (lane 2), heated soybean flour (HSF1, lane 3), and duodenal digesta collected at 0.5 (lane 4), 2 (lane 5), 4 (lane 6), 6 (lane 7), and 8 (lane 8) h after the morning meal in a calf of experiment 1 fed the HSF1 diet; molecular weight markers (lane 1), soy protein concentrate SPC1 (lane 9), duodenal digesta collected at 0.5 h in a calf fed the SPC1 diet (lane 10), skim milk powder SMP1 (lane 11), and duodenal digesta collected at 0.5 h in a calf fed the SMP1 diet (lane 12). SDS−PAGE was carried out under nonreducing (a, c) and reducing (b, d) conditions. Immunoblots were developed using antibodies against A- (Mab IFRN 0025; a, b) and B-polypeptides (Pab BTP 02; c, d) of glycinin. Molecular weight markers: phosphorylase *b*, 94000; bovine serum albumin, 67000; ovalbumin, 43000; carbonic anhydrase, 30000; soybean trypsin inhibitor, 20100; α-lactalbumin, 14400.

antiserum that recognizes B polypeptides (Figure 3b,d). In the absence of reducing agent both antibody preparations revealed the presence of intact (A + B) subunits in pure glycinin and soybean flour (lanes 2 and 3, respectively) with $M_r \approx 64000-67000$. A fainter band was also present at $M_r \approx 60000$. Analysis of digesta 0.5 h after feeding showed the presence of two fragments of $M_r \approx 42000$ and 33000 recognized by both antibody preparations (Figure 3a,c, lane 4). These were much fainter in digesta 2 h after feeding, although a major polypeptide of $M_r \approx 63000$ was detected in this sample (lane 5) and in those digesta collected 4 and 6 h after feeding (lanes 6 and 7). Fainter bands below 63000 were also seen, which may correspond to the band at $M_r \approx 60000$ seen in lanes 2 and 3. No reaction was seen with SPC1 and SMP1 (lanes 9 and 11) or the digesta of animals fed the SPC1 or SMP1 diets (lanes 10 and 12). Immunoblots of the same samples analyzed after reduction developed with the A-specific Mab showed the $M_r \approx 35000$ A polypeptide in glycinin and the soybean flour (Figure 3b, lanes 2 and 3). This was only faintly present in digesta 0.5 h after feeding, although two smaller fragments of $M_r \approx 23000$ and 15000 were recognized by this Mab (lane 4). The intact A polypeptide was more abundant in digesta 2 h after feeding (lane 5) than in the 4 and 6 h digesta (lanes 6 and 7). A fainter polypeptide of $M_r \approx 33000$ was also detected in these samples. A faint staining of the A polypeptide was observed in the SPC1 product (lane 9). Pab BTP02 was raised to the B polypeptide of pea legumin and recognized the B polypeptide of glycinin at $M_r \approx 20000$ (Figure 3d, lanes 2 and 3). However, for unknown reasons in this part of the study, this antiserum also labeled the A polypeptide glycinin at $M_r \approx 36000$ and a faint band at $M_r \approx 40000$ in the pure preparation and in HSF1 (Figure 3d, lanes 2 and 3). In the digesta 0.5 h after feeding, only the $M_r \approx 20000$ polypeptide could be detected, but binding to the 35000 polypeptide reappeared 2 h after feeding (lanes 4 and 5). Binding to the B polypeptide could only be faintly observed in the 4 and 6 h samples, and not at all in the 8 h sample (lanes 6−8).

(2) β-Conglycinin. IFRN 0089 recognized the α/α′ subunits in the purified β-conglycinin and soybean flour (Figure 4a, lanes 2 and 3), which gave anomalously high apparent molecular weights of $M_r \approx 90000$. There was evidence of extensive aggregation in both samples with a range of higher M_r polypeptides being detected. The polyclonal anti-β-conglycinin recognized the α/α′ and β subunits in these samples in addition to aggregated material (Figure 4b, lanes 2 and 3). The Mab recognized only intact material in the digesta 2 and 6 h after

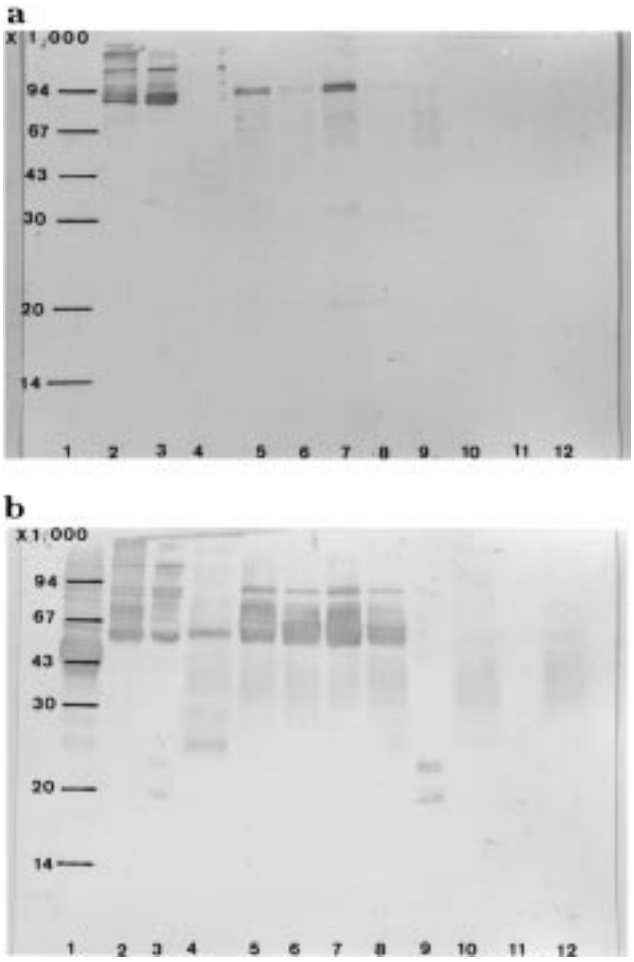

Figure 4. Immunoblots of duodenal digesta developed with anti-β-conglycinin antibodies: pure β-conglycinin (lane 2), heated soybean flour (HSF1, lane 3), and duodenal digesta collected at 0.5 (lane 4), 2 (lane 5), 4 (lane 6), 6 (lane 7), and 8 (lane 8) h after the morning meal in a calf of experiment 1 fed the HSF1 diet; molecular weight markers (lane 1), soy protein concentrate SPC1 (lane 9), duodenal digesta collected at 0.5 h in a calf fed the SPC1 diet (lane 10), skim milk powder SMP1 (lane 11), and duodenal digesta collected at 0.5 h in a calf fed the SMP1 diet (lane 12). SDS−PAGE was carried out under nonreducing conditions. Immunoblots were developed using antibodies against acidic (α, α′) (Mab IFRN 0089; a) and acidic (α, α′) and basic (β) (Pab LJR J2; b) subunits of β-conglycinin. Molecular weight markers: phosphorylase *b*, 94000; bovine serum albumin, 67000; ovalbumin, 43000; carbonic anhydrase 30000; soybean trypsin inhibitor, 20100; α-lactalbumin, 14400.

feeding (Figure 4a, lanes 5 and 7). A more complex pattern of polypeptides was detected with the Pab. Thus, 0.5 h after feeding digesta contained $M_r \approx 60000$ and 25000 polypeptides (Figure 4b, lane 4), whereas the digesta 2−8 h after feeding contained a complex mixture of β-conglycinin-derived polypeptides with $M_r \approx 60000-93000$ (Figure 4b, lanes 5−8). The Pab recognized two low M_r polypeptides of ≈25000 and ≈21000 Da in size present in the SPC1, but nothing was detected in SPC1 digesta, SMP1, or SMP1 digesta (Figure 4b, lanes 9−12).

Western Blotting of Ileal Digesta of Calves Fed Treated Soy. Glycinin presence was detected by anti-glycinin and anti-A and anti-B polypeptide antibody preparations. The anti-glycinin recognized the $M_r \approx 35000$ A and $M_r \approx 20000$ B polypeptide present in reduced glycinin (Figure 5b, lane 2). No intact A polypeptide was detected in the individual ileal digesta of the six calves fed HSF2 (lanes 3−8), although the M_r 20000 B polypeptide was clearly visible, together with a faster mobility M_r 17000 polypeptide. Analysis under nonreducing conditions (Figure 5a) showed the presence of the A + B subunit at $M_r \approx 67000$. The digesta of calves fed HSF2 were recognized only poorly (lanes 3−8) with broad bands of $M_r \approx 43000$ and 35000 being evident. The anti-B-polypeptide Pab recognized only the B polypeptide of glycinin (Figure 5d, lane 2). This was usually poorly recognized in the ileal digesta of calves fed HSF2 (lanes 3−8). High M_r material of ≈40000 Da was detected in the digesta of two animals fed HSF2 (lanes 5 and 8) and faintly in one animal fed SMP2 (lane 10). The A-specific Mab again bound to the nonreduced A + B glycinin subunit of M_r 68000 (Figure 5d, lane 2) and recognized the M_r 35000 subunit in the ileal digesta of calves fed HSF2 (lanes 3−7), except in one calf (lane 8). No staining was observed for SPC2 or SMP2 digesta (lanes 9 and 10).

Time Course of Glycinin in Ileal Digesta in Individual Animals. The average ileal digesta appeared to contain only the $M_r \approx 20000$ B polypeptide (Figure 6a, lane 3). This could not be detected in ileal digesta until 4 h after feeding (lane 6), which disappeared ∼9 h after feeding (lane 10). Essentially the same pattern of binding was observed for the second animal (Figure 6b). However, in this calf, a labeling at $M_r \approx 40000$ corresponding to the A polypeptide of glycinin was observed in the average ileal digesta (lane 3) and in the digesta collected at 4 and 5 h after the meal (samples H4 and H5).

DISCUSSION

The present work clearly demonstrates in vivo the large differences existing in the gastric (abomasal) and intestinal digestion of soybean globulins glycinin and β-conglycinin. It also supports the view that protein structure is crucial to determining the extent and kinetics of protein digestion. This in turn may modulate the role of plant proteins, their degradation products, and undigested residues on gut secretion and absorption (Santoro et al., 1997) and immune-mediated hypersensitivity reactions [review by Lallès and Peltre (1996)].

On the basis of immunological investigations, β-conglycinin appeared to be more resistant to digestion in the abomasum, but less so than glycinin in the small intestine of calves. This apparent paradox may reflect a number of interacting factors such as protein structure, enzymatic systems, and gut physiology. On the one hand, both native and denatured pure β-conglycinins have been found to be poorly digested in vitro by pepsin (Astwood et al., 1996; Nielsen et al., 1988). This is in agreement with the present observations in vivo. The B polypeptide of glycinin, compared to its A polypeptide, also resists pepsinolysis in vitro (Kella et al., 1986). On the other hand, the soybean proteins appear to follow a sequential breakdown pattern fairly similar during germination [review by Bau et al. (1997)], ruminal fermentation (Romagnolo et al., 1990; Aufrère et al., 1994), or feed supplementation with enzyme mixtures (Marsman et al., 1997): β-conglycinin is digested more quickly than glycinin, the acidic chains of both globulins are more susceptible than basic chains, and the B chain of glycinin shows the highest resistance to proteolysis. Our observations on the digestion of soy globulins at the end of the small intestine are in agreement with these observations. Two major features of soy globulins

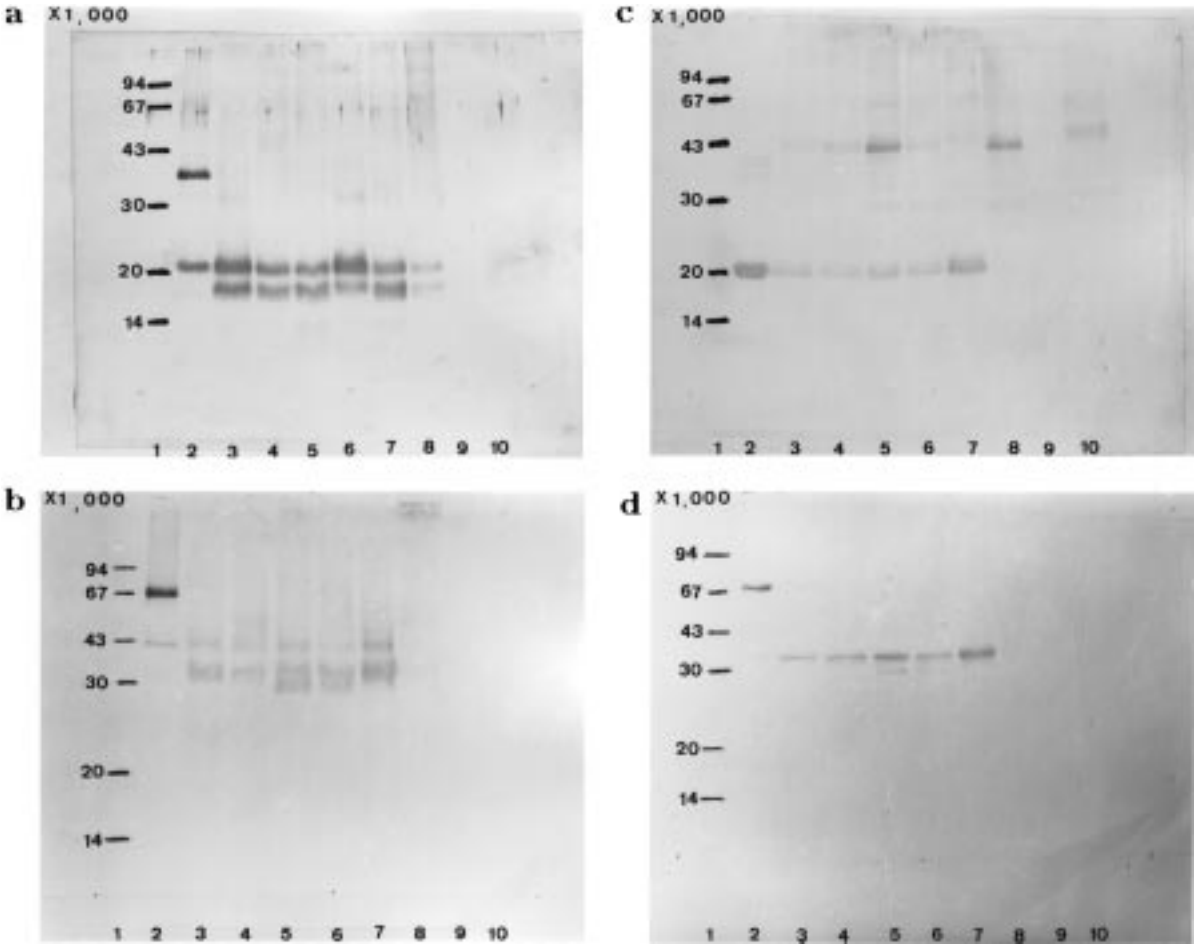

Figure 5. Immunoblots of ileal digesta developed with anti-glycinin antibodies: pure glycinin (lane 2) and average individual samples of ileal digesta collected in the six calves of experiment 2 fed a milk replacer containing heated soybean flour (HSF2) (lane 3−8), ethanol-treated soy protein concentrate SPC2 (lane 9) or skim milk powder SMP2 (lane 10); molecular weight markers (lane 1). SDS−PAGE was carried out under nonreducing (a, c) and reducing (b, d) conditions. Immunoblots were developed using antibodies Pab JR 9205 directed against SDS-denatured and reduced glycinin (a, b), Mab IFRN 0025 against A polypeptide (c), and Pab BTP 02 against B polypeptide (d) of glycinin. Molecular weight markers: phosphorylase *b*, 94000; bovine serum albumin, 67000; ovalbumin, 43000; carbonic anhydrase, 30000; soybean trypsin inhibitor, 20100; α-lactalbumin, 14400.

probably determine this susceptibility to proteolysis: (1) the richness of glycinin in intra- and intermolecular disulfide bonds, which contribute to the compact structure of this protein, and (2) the hydrophobicity of basic polypeptides, which determines their propensity to aggregate into large insoluble complexes that limit access of proteolytic enzymes (Fukushima, 1991; Yamauchi et al., 1991).

However, other explanations must be sought to account for the divergent effects of abomasal digestion. In the abomasum, the formation of a curd is followed by hydrolysis of κ-casein by chymosin and pepsin. Curd firmness is reduced and the rate of abomasal emptying is increased when substantial amounts of skim milk powder are replaced by plant protein [review by Toullec and Lallès (1995)]. We hypothesize that changes in duodenal (and therefore abomasal) pH over time are partly responsible for the differential behavior of soybean globulins in this digestive compartment. Indeed, duodenal pH was always below the isoelectric point of glycinin (pI = 6.4), whereas it reached that of β-conglycinin (pI = 4.9) at ~3 h after the meal (that is probably earlier in the abomasum). This may have favored transient insolubilization of β-conglycinin, thus explaining its biphasic passage in the duodenum. None of the milk proteins assayed behaved like glycinin or β-conglycinin. From the data presented, it would appear that glycinin did not aggregate with the milk proteins in the clot, whereas β-conglycinin may have partially aggregated. The apparent MRT of β-conglycinin in the abomasum was 2.4 times longer than that of glycinin, which may have favored its intestinal digestion as a faster abomasal outflow is known to decrease ileal digestibility of protein [review by Toullec and Lallès (1995)]. We found that the concentration of immunoreactive β-conglycinin in processed soybean products was a better predictor of apparent fecal digestibility of soybean nitrogen in calves than that of glycinin (Lallès et al., 1996b; Tukur et al., 1996), and it is possible that this is linked with the poor gastric digestion of β-conglycinin, although this remains unproven.

Our data indicate that a proportion of intact B polypeptides of glycinin can survive small intestinal digestion in the calf, whereas the A polypeptides appear to be more readily digested. This agrees with in vitro data which indicate that A polypeptides are more susceptible to pepsinolysis and trypsinolysis than B polypeptides (Lynch et al., 1977; Kamata and Shibasaki, 1978; Kella et al., 1986; Plumb and Lambert, 1990). A polypeptide residues would have M_r below 16000 (Kamata and Shibasaki, 1978; Kella et al., 1986), 12000 (Lynch et al., 1977), or even 7000 (Richardson and

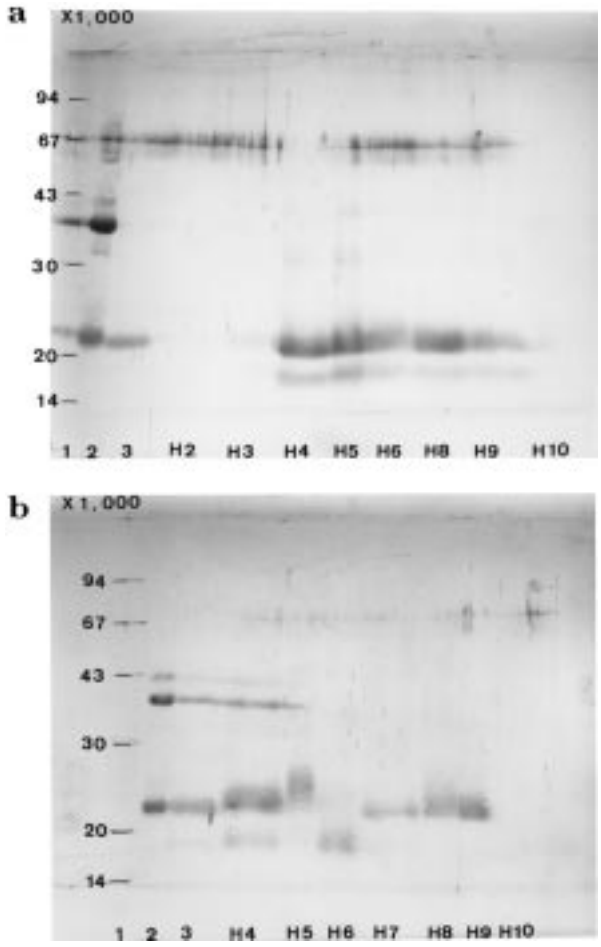

Figure 6. Immunoblots of ileal digesta collected kinetically after the morning meal: pure glycinin (lane 2), of average ileal digesta sample (lane 3), and of ileal digesta collected hourly (lane 4−10) after the morning meal in two calves (a, b) of experiment 2 fed a milk replacer containing heated soybean flour (HSF2). Lanes 4−10 correspond to ileal samples collected during hours H2, H3, H4, H5, H6, H8, and H9 for calf a and hours H4, H5, H6, H7, H8, H9, and H10 for calf b. Molecular weight markers are shown in lane 1. SDS−PAGE was carried out under reducing conditions. Immunoblots were developed using antibody Pab LJR 9205 directed against SDS-denatured and reduced glycinin. (An artifact was observed at M_r = 67000.) Molecular weight markers: phosphorylase *b*, 94000; bovine serum albumin, 67000; ovalbumin, 43000; carbonic anhydrase, 30000; soybean trypsin inhibitor, 20100; α-lactalbumin, 14400.

Catsimpoolas, 1979). SDS−PAGE by Kella et al. (1986) clearly indicated that B polypeptide pepsinolysis generated one band of $M_r \sim 16000$ with some B molecules remaining unmodified, whereas two peptides of M_r = 16000 and 14000 were obtained by peptic digestion of A polypeptides. Thus, the glycinin-derived band of M_r 17000 could originate from either A or B polypeptides. Trypsinolysis of native glycinin in vitro up to 30 min led to SDS−PAGE patterns with polypeptides of M_r estimated to be around 31000 and 27000 from the A polypeptide and 21000 polypeptide, which may represent the intact B polypeptide. Other fragments of 17000, 15500, 14000, and 11000 may originate from either the A or B chain of glycinin (Kella et al., 1986). These data resemble the present observations and suggest that the products generated by proteolysis in vitro and in vivo of glycinin are fairly similar. The glycinin component identified at M_r = 35000 may represent an AB-type structure but does not preclude the presence of A fragments at the same M_r position. Indeed, an intermediate degradation product of M_r = 33000 was identified after in vitro trypsinolysis of pea and soy 11S globulins (Plumb et al., 1989; Plumb and Lambert, 1990). In addition, Caugant et al. (1993) using amino acid profile techniques to analyze ileal digesta from calves fed antigenic soy, concluded that A polypeptides of glycinin, probably partially digested, might constitute a part of the soy protein escaping small intestinal digestion. Whether these undigested fragments contribute to increase ileal losses of endogenous nitrogen as shown with phaseolin in rats (Santoro et al., 1997) is not known.

Little is known about the digestion behavior of legume globulins in vivo. Bush et al. (1992) reported that immunoreactive pea legumin fragments detected at the ileum of calves had M_r of 200000, 70000, and 40000, as determined by gel filtration under nondenaturing and nonreducing condition. They comprised nearly intact light A polypeptide (M_r = 25000) and B polypeptides (M_r = 22000, together with heavy A polypeptides and related fragments (M_r 43000, 40000, 25000, and 20000) (Lallès et al., 1998). Crévieu et al. (1997) found that the A polypeptide (M_r = 39000) of pea legumin was present in the gizzard but had disappeared in the jejunum of chickens. In contrast, fragments of M_r in the range 19500−25000 (probably from A polypeptide of legumin) persisted to the end of the digestive tract. Collectively, these data are consistent with an external location of A polypeptides of 11S globulins, which are accessible to proteases, and a more internal position of B polypeptides of the 11S proteins that are therefore less digestible (Plumb et al., 1989; Plumb and Lambert, 1990; Carter et al., 1992), as is suggested by the three-dimensional structure (Lawrence et al., 1994; Utsimi et al., 1996).

Immunoreactive β-conglycinin determined by ELISA in the ileum of calves represented ∼1% of the amount ingested (Tukur et al., 1993), but we were unable to characterize immunoreactive fragments further by Western blotting in the present study. Bush et al. (1992) could not measure by ELISA immunoreactive vicilin, the 7S globulin of *Pisum sativum*, in ileal digesta of calves. Crévieu et al. (1997) indicated no native pea vicilin was detectable in the upper jejunum, but a vicilin peptide of M_r ∼18000 was present in the terminal ileum. Begbie and Ross (1993) showed the presence of large fragments (M_r = 22000−26000) of phaseolin, the 7S globulin of *Phaseolus vulgaris*, in ileal digesta of pigs. Unfortunately, none of these studies identified the molecular origin (i.e., from the α, α′, or β subunit) of these immunoreactive components of the 7S globulins.

Most legume proteins are allergenic in humans and young farm animal species [review by Lallès and Peltre (1996)]. In the case of soybean, Astwood et al. (1996) observed intact basic (β) subunit of β-conglycinin, with few detectable proteolytic fragments, after 60 min of incubation in vitro with pepsin. Intact glycinin A polypeptide and acidic (α) subunit of β-conglycinin were observed during 0.5−60 and 2 min, respectively, with intermediate fragments present for variable periods of time. Astwood et al. (1996) concluded that important food allergens in humans, such as β-conglycinin, were stable to gastric digestion in vitro. The present results support this view. Indeed, in calves suffering from gut hypersensitivity reactions to heated soybean flour, it was found that β-conglycinin elicited stronger responses than glycinin, in direct skin tests and in vitro lympho-

proliferation assays (Lallès et al., 1996a). Thus, such immunological activity of β-conglycinin may be linked with its resistance to gastric digestion and consequent sensitization of the small intestine.

CONCLUSION

This study demonstrates that β-conglycinin was more resistant than glycinin to gastric digestion in vivo. Immunoreactive glycinin escaped digestion in the small intestine, mainly in the form of intact basic polypeptides disulfide-bonded to partially digested acidic polypeptides. These observations are in broad agreement with soybean protein susceptibility to in vitro degradation with gastric or pancreatic enzymes. The present data help to explain the usually lower apparent digestibility of soybean products in vivo, due to increased ileal flow of dietary protein. Whether such undigested fragments stimulate ileal losses of endogenous protein as shown with phaseolin in rats awaits further investigation.

ACKNOWLEDGMENT

We thank all of the staff for animal care, Mrs. A. Huet and R. Cabral for technical assistance, and Mrs. J. Quillet for collecting the literature.

LITERATURE CITED

Astwood, J. D.; Leach, J. N.; Fuchs, R. L. Stability of food allergens to digestion *in vitro*. *Nat. Biotechnol.* **1996**, *14*, 1269−1273.

Aufrère, J.; Graviou, D.; Michalet-Doreau, B. Degradation in the rumen of protein of two legumes: soybean meal and field pea. *Reprod. Nutr. Dev.* **1994**, *34*, 483−490.

Bau, A. M.; Villaume, C.; Nicolas, J. P.; Mejean, L. Effect of germination on chemical composition, biochemical constituents and antinutritional factors of soya bean (*Glycine max*) seeds. *J. Sci. Food Agric.* **1997**, *73*, 1−9.

Begbie, R.; Ross, A. W. Resistance of the kidney bean reserve protein, phaseolin, to proteolysis in the porcine digestive tract. *J. Sci. Food Agric.* **1993**, *61*, 301−307.

Bush, R. S.; Toullec, R.; Caugant, I.; Guilloteau, P. Effects of raw pea flour on nutrient digestibility and immune responses in the preruminant calf. *J. Dairy Sci.* **1992**, *75*, 3539−3552.

Carter, J. M.; Lee, H. A.; Mills, E. N. C.; Lambert, N.; Chan, H. W.-S.; Morgan, M. R. A. Characterisation of polyclonal and monoclonal antibodies against glycinin (11S storage protein) from soya (*Glycine max*). *J. Sci. Food Agric.* **1992**, *58*, 75−82.

Caugant, I.; Toullec, R.; Formal, M.; Guilloteau, P.; Savoie, L. Digestibility and amino acid composition of digesta at the end of the ileum in preruminant calves fed soyabean protein. *Reprod. Nutr. Dev.* **1993**, *33*, 335−347.

Crévieu, I.; Carré, B.; Chagneau, A. M.; Quillien, L.; Guéguen, J.; Bérot, S. Identification of resistant pea (*Pisum sativum* L.) proteins in the digestive tract of chickens. *J. Agric. Food Chem.* **1997**, *45*, 1295−1300.

Fukushima, D. Recent progress of soybean protein foods: chemistry, technology and nutrition. *Food Rev. Int.* **1991**, *7*, 323−351.

Kamata, Y.; Shibasaki, K. Formation of digestion intermediate of glycinin. *Agric. Biol. Chem.* **1978**, *42*, 2323−2329.

Kella, N. K. D.; Barbeau, W. E.; Kinsella, J. E. Effect of oxidative sulfitolysis of disulfide bonds of glycinin on solubility, surface hydrophobicity, and *in vitro* digestibility. *J. Agric. Food Sci.* **1986**, *34*, 251−256.

Laemmli, U. K. Cleavage of structural proteins during the assembly of bacteriophage T4. *Nature (London)* **1970**, *227*, 680−685.

Lallès, J. P.; Peltre, G. Biochemical features of grain legume allergens in humans and animals. *Nutr. Rev.* **1996**, *54*, 101−107.

Lallès, J. P.; Poncet, C. Ruminal and intestinal digestion of pea and soyabean meal by dairy calves during and after weaning. 1. Digestion of organic matter and nitrogen. *Livest. Prod. Sci.* **1990**, *24*, 129−142.

Lallès, J. P.; Salmon, H.; Bakker, N. P. M.; Tolman, G. H. Effects of dietary antigens on health, performance and immune system of calves and piglets. In *Recent Advances of Research in Antinutritional Factors in Legume Seeds*; van der Poel, A. F. B., Huisman, J., Saini, H. S., Eds.; Wageningen Pers: Wageningen, The Netherlands, 1993; pp 253−270.

Lallès, J. P.; Dréau, D.; Salmon, H.; Toullec, R. Identification of soya bean allergens and immune mechanisms of dietary sensitivities in preruminant calves. *Res. Vet. Sci.* **1996a**, *60*, 111−116.

Lallès, J. P.; Tukur, H. M.; Toullec, R.; Miller, B. G. Analytical criteria for predicting apparent digestibility of soy protein in preruminant calves. *J. Dairy Sci.* **1996b**, *79*, 475−484.

Lallès, J. P.; Quillien, L.; Toullec, R. Immunoblotting of ileal digesta of calves fed pea. In *Proceedings of the International Conference on Plant Proteins from European Crops*; Guéguen, J., Popineau, Y., Eds.; Springer-Verlag: Berlin, Germany, 1998; pp 193−197.

Lawrence, M. C.; Izard, T.; Beuchat, M.; Blagrove, R. T.; Colman, P. M. Structure of phaseolin at 2.2. Å resolution. Implication for a common vicilin/legumin structure and the genetic engineering of seed storage proteins. *J. Mol. Biol.* **1994**, *238*, 748−776.

Lei, M. G.; Tyrell, D.; Bassette, R.; Reeck, G. R. Two-dimensional electrophoretic analysis of soybean proteins. *J. Agric. Food Chem.* **1983**, *31*, 963−968.

Lowry, O. H.; Rosebrough, N. J.; Farr, A. L.; Randall, R. J. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **1951**, *193*, 265−275.

Lynch, C. J.; Rha, C. K.; Catsimpoolas, N. Note on the rapid proteolysis of glycinin by pepsin and trypsin. *Cereal Chem.* **1977**, *54*, 1282−1285.

Marsman, G. J. P.; Gruppen, H.; Mul, J. A.; Voragen, A. G. J. *In vitro* accessibility of untreated, toasted and extruded soybean meals for proteases and carbohydrases. *J. Agric. Food Chem.* **1997**, *45*, 4088−4095.

Mori, T.; Utsumi, S.; Inaba, H.; Kitamura, K.; Harada, K. Differences in subunit composition of glycinin among soybean cultivars. *J. Agric. Food Chem.* **1981**, *29*, 20−23.

Murphy, P. A.; Resurreccion, A. P. Varietal and environmental differences in soybean glycinin and β-conglycinin content. *J. Agric. Food Sci.* **1984**, *32*, 911−915.

Nielsen, N. C. The structure and complexity of the 11S polypeptides in soybeans. *J. Assoc. Off. Chem. Soc.* **1985**, *62*, 1680−1686.

Nielsen, S. S.; Deshpande, S. S.; Hermodson, M. A.; Scott, M. P. Comparative digestibility of legume storage proteins. *J. Agric. Food Chem.* **1988**, *36*, 896−902.

Plumb, G. W.; Lambert, N. A comparison of the trypsinolysis products of nine 11S globulin species. *Food Hydrocolloids* **1990**, *3*, 465−473.

Plumb, G. W.; Carr, H. J.; Newby, V. K.; Lambert, N. A study on the trypsinolysis of pea 11S globulin. *Biochim. Biophys. Acta* **1989**, *999*, 281−288.

Plumb, G. W.; Mills, E. N. C.; Tatton, M. J.; D'Ursel, C. C. M.; Lambert, N.; Morgan, M. R. A. Effect of thermal and proteolytic processing on glycinin, the 11S globulin of soy (*Glycine max*): a study utilizing monoclonal and polyclonal antibodies. *J. Agric. Food Chem.* **1994**, *42*, 834−840.

Plumb, G. W.; Lambert, N.; Mills, E. N. C.; Tatton, M. J.; D'Ursel, C. C. M.; Bogracheva, T.; Morgan, M. R. A. Characterisation of monoclonal antibodies against β-conglycinin from soya bean (*Glycine max*) and their use as probes for thermal denaturation. *J. Sci. Food Agric.* **1995**, *67*, 511−520.

Richardson, D. P.; Catsimpoolas, N. The effect of thermal denaturation on the tryptic hydrolysis of glycinin. *J. Sci. Food Agric.* **1979**, *30*, 463−468.

Romagnolo, D.; Polan, C. E.; Barbeau, W. E. Degradability of soybean meal protein fractions as determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. *J. Dairy Sci.* **1990**, *73*, 2379−2385.

Santoro, L. G.; Grant, G.; Pusztai, A. Effects of short-term feeding of rats with a highly purified phaseolin preparation. *Plant Foods Hum. Nutr.* **1997**, *51*, 61−70.

Sissons, J. W.; Thurston, S. M. Survival of dietary antigens in the digestive tract of calves intolerant to soyabean products. *Res. Vet. Sci.* **1984**, *37*, 242−246.

Staswick, P. E.; Hermodson, M. A.; Nielsen, N. C. Identification of the acidic and basic subunit complexes of glycinin. *J. Biol. Chem.* **1981**, *256*, 8752−8755.

Thanh, V. H.; Shibasaki, K. Major protein of soybean seeds. A Straightforward fractionation and their characterization. *J. Agric. Food Chem.* **1976**, *24*, 1117−1121.

Thielmans, R. F.; François, E.; Bodart, C.; Thewis, A. Mesure du transit gastrointestinal chez le porc à l'aide de radiolanthanides. Comparaison avec le mouton. *Ann. Biol. Anim. Biochim. Biophys.* **1978**, *18*, 237−247.

Toullec, R.; Lallès, J. P. Digestion dans la caillette et dans l'intestin grêle. In *Nutrition des Ruminants Domestiques: Ingestion et Digestion*; Jarrige, R., Ruckebusch, Y., Demarquilly, C., Farce, M. H., Journet, M., Eds.; INRA Editions: Paris, France, 1995; pp 527−581.

Tukur, H. M.; Lallès, J. P.; Mathis, C.; Caugant, I.; Toullec, R. Digestion of soya-bean globulins, glycinin, α-conglycinin and β-conglycinin in the preruminant and the ruminant calf. *Can. J. Anim. Sci.* **1993**, *73*, 891−905.

Tukur, H. M.; Lallès, J. P.; Plumb, J. W.; Mills, E. N. C.; Morgan, M. R. A.; Toullec, R. Investigation of the relationship between *in vitro* ELISA measures of immunoreactive soy globulins and *in vivo* effects of soy products. *J. Agric. Food Chem.* **1996**, *44*, 2155−2161.

Utsumi, S.; Gidamis, A. B.; Takenaka, Y.; Maruyama, N.; Adachi, M.; Mikami, B. Crystallization and X-ray analysis of normal and modified recombinant soybean proglycinins. In *Macromolecular Interactions in Food Technology*; Parris, N., Kato, A., Creamer, L. K., Pierce, J., Eds.; American Chemical Society: Washington, DC, 1996; pp 257−270.

Yamauchi, F.; Yamagishi, T.; Iwabichi, S. Molecular understanding of heat-induced phenomena of soybean protein. *Food Rev. Int.* **1991**, *7*, 283−322.

Yvon, M.; Levieux, D.; Valluy, M. C.; Pélissier, J. P.; Patureau-Mirand, P. Colostrum protein digestion in newborn lambs. *J. Nutr.* **1993**, *123*, 586−596.

Received for review August 10, 1998. Revised manuscript received April 23, 1999. Accepted April 28, 1999. Part of the grant for this work came from the French Ministère des Affaires Etrangères, the Ministère de l'Agriculture et de la Pêche (DGER 91-131), the Région de Bretagne, The Département d'Ille-et-Vilaine, and the District de Rennes, to whom we are grateful.

JF980882+