Effect of *Phaseolus vulgaris* lectins on glucose absorption, transport, and metabolism in rat everted intestinal sacs

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Many legume seeds contain lectins. Some of these lectins are toxic when fed to humans or laboratory animals. Although the mechanism for such toxicity has not been clearly elucidated, lectin binding to the intestinal mucosa is an obligatory step. Therefore, the interaction of lectin and enterocytes may interfere with the digestive process. Lectins from toxic (red kidney bean) and non-toxic (mountaineer half runner) Phaseolus vulgaris *varieties were tested for their effect on the intestinal absorption, transport, and metabolism of glucose. Both lectins were purified by affinity chromatography on Con A-Sepharose 4B. Everted sacs, 5 cm long, from the first third of the small intestine were incubated for 20 min in the presence of 10 mmol/L glucose in both the luminal and serosal media. Pre-incubation (15 min) of the everted sacs with increasing concentrations of the red kidney bean lectin (50–200 μg/mL mucosal solution) before glucose addition reduced both the absorption and transport of the sugar from the luminal to the serosal side. Glucose metabolism as judged by lactate formation was not affected. At a concentration of 100 μg/mL of red kidney bean lectin, absorption, and transport were inhibited by 40% and 72%, respectively. Lactate production was decreased by only 5%. Under the same conditions (100 μg/mL) mountaineer half runner lectin inhibited glucose absorption and transport by less than 10%. Interference of lectins with the absorption and transport of nutrients could partially explain the toxic effect of lectins from some* Phaseolus vulgaris *varieties.*

Keywords: *Phaseolus vulgaris;* lectins; intestine; glucose transport

Introduction

Legume seeds are an important source of energy and protein for both animal and human consumption. However, their biological utilization is often impaired by their content of a number of antinutritional factors.[1,2] Ingestion of diets based on raw legume seeds, such as red kidney beans (RKB) *(Phaseolus vulgaris),* produces weight loss and eventual death of experimental animals.[1,3,4]

Feeding raw legume seeds or their lectins impairs the absorption of various nutrients, including amino acids and carbohydrates.[5–13] These harmful effects have been attributed primarily to the binding of the lectins to the surface of the intestinal epithelial cells,[3,14–16] causing, among other alterations, a non-specific interference with the final digestion and absorption of nutrients.[14,17–19] In addition, lectin binding produces changes in the structure and metabolism of epithelial cells and bacterial overgrowth.[20]

Nevertheless, not all legume seed lectins are toxic. The extent of growth inhibition produced by them seems to be related to the strength of their initial binding to the mucosal surface.[21]

In 1972 Jaffé et al.[22,23] separated the hemagglutinating activity of various *Phaseolus vulgaris* cultivars into four specificity types: A, B, C, and D. Those that agglutinate trypsin treated cow-erythrocytes (A and C) were toxic. In contrast, type D, which only agglutinate pronase-treated hamster or mice red blood cells, was non-toxic.

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Therefore, this simple test has been used as a predictor of bean lectin toxicity.

Jaffé and Gómez[24] tested the intraperitoneal toxicity in mice of raw extracts and partially purified lectin fractions from RKB and mountaineer half runner (MHR). Mortality was considerably higher in RKB-treated animals, in comparison with those that received MHR. An extensive account of these experiments was recently provided by Jaffé and Seidl.[25] In addition, RKB lectin produces disruption of gut mucosa when administered by stomach intubation; no histological changes were observed after MHR treatment.[26]

In the present study the effect of the lectins from a toxic (RKB; group A) and a non-toxic (MHR; group D) *Phaseolus vulgaris* varieties on the intestinal absorption, transport, and metabolism of glucose were tested. Results indicate that only the toxic lectin reduces both glucose absorption and transport from the luminal to the serosal side of rat everted intestinal sacs.

Methods and materials

Materials

Seeds. RKB were purchased from a local market. MHR seeds were a gift from Keystone Seed Co., Hollister, CA, USA.

Animals. Male Sprague-Dawley rats (180–230 g) were obtained from the colony kept at the School of Biology, Central University of Venezuela, Caracas, Venezuela. They were housed individually in wire-bottom cages and had free access to a non-purified commercial diet (Purina, Valencia, Venezuela) and water.

Chemicals. Hexokinase, glucose-6-phosphate dehydrogenase, lactate dehydrogenase (type II from rabbit muscle), and trypsin (type III) were from Sigma Chemical Co. (St. Louis, MO USA), Pronase from *Streptomyces griseus* from Calbiochem-Behring Co. (Frankfurt, Germany). All other chemicals were of analytical grade.

Methods

Lectin purification. Finely ground RKB and MHR seeds were extracted overnight in 0.85% NaCl, pH 7.5 (10% wt/vol) at 4° C. Extracts were centrifuged at 36,000*g* for 20 min. The supernatants were brought to 50% saturation with $(NH_4)_2SO_4$ and the precipitates (globulins) were discarded. The supernatants were then adjusted to 100% saturation with $(NH_4)_2SO_4$ and centrifuged at 36,000*g*, for 10 min. Precipitates were dissolved in 1 volume of distilled water, dialyzed against water for 4 days (2 changes daily), clarified by centrifugation, concentrated in a rotavapor (Buchi, Schweitz, Switzerland), and lyophilized. RKB and MHR lectins were obtained from the latter fractions by affinity chromatography in a Concanavalin A-Sepharose 4B column following the procedure of Data and Ray.[27]

Hemagglutination assays. Cow, hamster, and rabbit red blood cells were used. Those of cow were treated with trypsin and the hamster erythrocytes with pronase.[22] Hemagglutination was assessed in microtiter plates (Dynatech Laboratories, Alexandria, VA USA). Titers were expressed as the maximal geometric dilution in which visible macroscopic hemagglutination was observed after 1 hour. The hemagglutinating activity (units × mg^{-1}) was calculated from the respective titers according to Kilpatrick and Yeoman.[28]

Measurement of glucose absorption, transport and metabolism. Small intestine segments (5–10 cm long) were obtained from rats starved for 18 hours. Everted sacs were incubated in 50 mL conical flasks containing 10 mL of Krebs-Ringer phosphate buffer, pH 7.2, modified to contain 3 mEq of Ca^{2+} per liter.[29] Glucose (10 mmol/L) was present initially in both the luminal and serosal sides. When present, the lectins were added to the luminal solution.

After incubation, sacs were rinsed with cold buffer and drained to recover the serosal solution. Samples of the mucosal and serosal solutions were deproteinized with 1 M $HClO_4$. Neutralized aliquots of both solutions were analyzed for glucose and lactate, before and after the incubation. Glucose absorption and transport were calculated as the difference between the final and initial values.

Protein, glucose and lactate assays. Protein was measured using the procedure of Lowry et al.;[30] glucose as described by Barthelmai and Czok[31] and lactate according to Hohorst.[32]

Results and discussion

Specificity type of the Phaseolus vulgaris *seeds. Lectin purification*

Table 1 shows the hemagglutinating activity of RKB and MHR seeds. As expected, RKB strongly agglutinated the erythrocytes of all species tested. In contrast, MHR agglutinated pronase-treated hamster red blood cells and only slightly those of cow or rabbit. Therefore, based on previous reports from our laboratory,[22] RKB belongs to the specificity group A (toxic) and MHR to type D (non-toxic).

The lectins from RKB and MHR seeds were purified by affinity chromatography on a Concanavalin A-Sepharose 4-B column *(Figure 1)*. Lectin yield amounted to 2.7% (RKB) and 2.1% (MHR). Although seed toxicity has been related to lectin content,[3] in this case no large differences in lectin yield between the toxic and non-toxic varieties were observed.

The purified lectins exhibited the same specificity as the raw seed extracts (see *Table 1*). This specificity reflects differences in isolectin content in the seeds. The model of Yachnin and Svenson[33] provides a rational basis for explaining the hemagglutinating, leucoagglutinating, and mitogenic properties of *P. vulgaris* lectins but, as pointed out by Jaffé,[2] it does not explain varietal differences in toxicity.

Oral toxicity may well be the result of various processes including lectin binding to surface receptors,[34] disruption of the mucosal surface of the gut,[26,34,35] interference with processes of digestion and absorption of nutrients,[5,13] extent of internalization by endocytosis, and bacterial overgrowth.[21,36]

Effect of RKB and MHR lectins on glucose transactions in everted intestinal sacs

The intestinal mucosa possess the capability to perform a complex set of carbohydrate transactions, including

Table 1 Specificity and hemagglutinating activity of raw extracts and purified lectins from RKB and MHR seeds

Fraction	Protein (mg/mL)	Hemagglutinating activity		
		Cow-trypsin	Rabbit	Hamster-pronase
RKB raw extract	22.50	3,723	910	1.91×10^9
RKB lectin (RKBL)	1.00	163,840	40,960	4.19×10^7
Heated RKBL	1.00	0	0	0
MHR raw extract	17.50	9	0	2.34×10^3
MHR lectin (MHRL)	1.00	160	80	1.02×10^4

Protease-treated cow and hamster erythrocytes and untreated rabbit red blood cells (4% vol/vol in saline) were used for the agglutination tests. Hemagglutinating activity (units/mg protein) was calculated as described in the text.

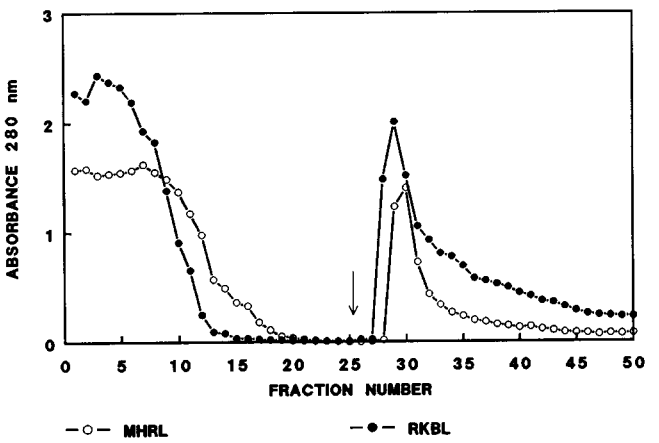

Figure 1 Purification of *Phaseolus vulgaris* lectins by affinity chromatography. RKB and MHR seeds were extracted as described in the text. The lyophilized materials (120 mg) were dissolved in 8 mL of phosphate buffer (0.1 M; pH 6.8) containing 0.15 M NaCl and 0.1 M of $MgCl_2$, $MnCl_2$, and $CaCl_2$, respectively, and seeded on top of Con A-Sepharose 4B columns (10 × 1.6 cm). The columns were washed with the same buffer (16 $ml \cdot h^{-1}$; 3 mL fractions). The arrow indicates elution with 0.2 M alpha-methyl mannoside. The hemagglutinating activity against cow-trypsin (RKBL) or hamster-pronase erythrocytes (MHRL) appeared between fractions 27–45.

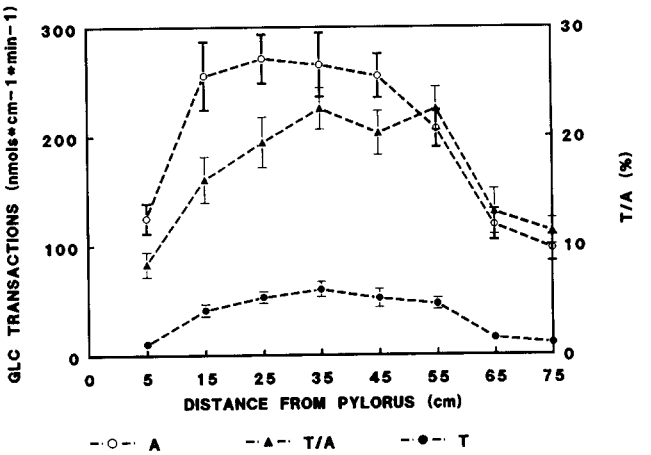

Figure 2 Longitudinal distribution of intestinal glucose transactions in everted intestinal sacs. Everted sacs (10 cm each) were prepared from everted rat small intestines, within 5–85 cm away from the pylorus, and incubated for 20 min as described in the text. Results for glucose absorption (A) (○--○), transport to the serosal side (T) (●--●), and percentage of absorbed glucose transported (T/A) (▲--▲) are the means ± S.E.M. for five experiments.

the final digestion of sugars and the absorption, transport to the serosal side, and metabolism of the resulting monosaccharides.

Jaffé and Camejo[5] reported a decrease in the glucose absorption capacity of rats fed raw black beans. Similar results were obtained more recently by Donatucci et al.,[13] who measured glucose uptake employing a sophisticated vascular intestinal perfusion system. However, to the best of our knowledge, there are few studies to evaluate the direct interference of the glucose absorption process by lectins, and none comparing the effect of lectins from a toxic and a non-toxic variety of *P. vulgaris*.

Figure 2 shows the longitudinal distribution of the glucose absorption and transport activities in rat everted intestinal sacs. As expected for the duodenal region, the first intestinal segment (0–10 cm from the pylorus) had a low sugar absorption capacity, while in the subsequent sacs (10–50 cm) glucose uptake was twice as active. No significant differences were observed among the means within this group. Thereafter this activity progressively decreased.

Glucose transport to the serosal side paralleled the longitudinal distribution of the absorption activity, reaching a maximum within the 20–50 cm region, in which 20% of the absorbed glucose appeared internally. Similar results were reported by Pritchard and Porteus[29] for rats fed a commercial non-purified diet.

In the everted sacs, glucose absorption was linear for at least 30 min, and increased linearly depending on the initial luminal glucose concentration from 2.5–20 mmol/L (results not shown). These results are in accordance with previous reports.[29,37,38] Considering that plasma glucose concentration fluctuates between 5–12 mmol/L (fasting or post-absorptive values),[39] for further studies 10 mmol/L glucose was added to both the luminal and serosal fluids.

Table 2 shows the effect of RKB and MHR lectins on intestinal glucose transactions. When the sacs were incubated simultaneously with glucose and RKB lectin (RKBL) (50–200 μg/mL mucosal medium) only minor differences were observed in comparison to control sacs (no lectin). In contrast, pre-incubation for 15 min with RKBL (100 μg/mL) prior to glucose addition, caused a remarkable inhibition of both glucose absorption (40%) and transport (75%). Apparently, the lectin-mucosa in-

Table 2 RKBL and MHRL effects on intestinal glucose transactions

Lectin μg/mL	P.I.T. min	Glucose		T/A %	Lactate production
		Absorption	Transport		
		μmoles/5 cm/20 min			μmoles/5 cm/20 min
0	0	25.86 ± 1.22	4.16 ± 0.12	16.08 ± 0.75	18.15 ± 0.41
RKB 50	0	24.28 ± 1.18	4.16 ± 0.09	17.14 ± 0.89	18.27 ± 0.33
100	0	22.94 ± 1.17[a]	3.67 ± 0.08[a]	16.01 ± 0.95	17.30 ± 0.21
200	0	21.99 ± 1.02[a]	3.56 ± 0.06[a]	16.20 ± 0.77	13.68 ± 0.10
0	15	26.42 ± 2.16	2.88 ± 0.15[a]	10.97 ± 0.96[a]	13.29 ± 0.52[a]
RKB 50	15	19.25 ± 2.21[a]	1.44 ± 0.20[a]	7.52 ± 0.54[a]	12.90 ± 0.53
100	15	16.08 ± 1.61[ab]	0.80 ± 0.21[ab]	4.95 ± 1.05[ab]	12.50 ± 0.71[b]
200	15	15.16 ± 1.50[ab]	0.65 ± 0.21[ab]	4.31 ± 1.22[ab]	12.08 ± 0.63[b]
MHR 50	15	26.22 ± 3.12[c]	2.60 ± 0.28[c]	9.91 ± 0.15[c]	13.04 ± 1.29
100	15	26.52 ± 1.66[c]	2.42 ± 0.22[c]	9.13 ± 0.64[c]	13.08 ± 1.41
200	15	25.03 ± 2.31[c]	2.35 ± 0.23[c]	9.43 ± 0.97[c]	12.86 ± 1.08

Intestinal sacs were pre-incubated or not for 15 min with the indicated lectin prior to the addition of glucose (10 mmol/L) to the luminal solution. Results are means ± S.E.M. for eight experiments for RKBL and four for MHRL. P.I.T.: pre-incubation time in min. T/A.: % of absorbed glucose that appears in the serosal solution.

[a]Significantly different ($P < 0.05$, two-way ANOVA; Duncan Test) from the respective control.

[b]Significantly different from the values obtained with 50 μg/mL RKBL.

[c]Significantly different from the respective RKBL value.

teraction is affected by a high glucose concentration. This may explain why pre-incubation of the sacs with RKBL in the absence of glucose caused a larger inhibition of absorption and transport.

It should be mentioned that pre-incubation of the sacs in the absence of both glucose and lectin caused a significant decrease in the amount of glucose transported to the serosal side. It is possible that when the tissue is kept at 37° C in the absence of luminal glucose there are changes that decrease its ability to transport glucose to the serosal side but not sugar absorption *(Table 2)*.

Addition of 200 μg/mL RKBL did not increase the inhibition of either glucose absorption or transport, *(Table 2)* suggesting that after saturation of cell receptors, further increases in lectin concentration will not cause additional effects. These results are in agreement with those of Cuperlovic et al.,[40] who found that the binding of *P. vulgaris* lectin to isolated intestinal cells reached saturation above 75 μg/mL. In addition, Urbach and Levy-Benshimol* reported that the mortality of enterocytes incubated with RKBL followed a similar saturation curve.

On the other hand, pre-incubation with MHR lectin did not produce significant changes in glucose transactions *(Table 2)*. It is likely that either the MHRL interaction with the mucosal surface is very weak, or after binding to its receptors it does not interfere with the activity of the glucose transporter. Recent observations from our laboratory* indicate that both RKBL and MHRL were cytotoxic to isolated enterocytes, although the effect of the RKBL was stronger. Nonetheless, it is not clear yet whether MHRL actually binds to receptors on the intact mucosal surface because it is possible that receptors specific to MHR lectin could be exposed as a consequence of the hyaluronidase treatment used to prepare the enterocytes.

The inhibition of glucose absorption and subsequent transport to the serosal side cannot be attributed to cell death because lactate production was not substantially affected in any case. It is likely that the serosal glucose is the major precursor for lactate production in lectin-treated sacs. Therefore, it appears that neither lectin is able to interfere with intracellular glucose metabolism, as measured by lactate production, provided there is an alternative sugar supply. Total lactate production and utilization of serosal glucose *(Table 2)* are in agreement with previous results,[37,38] indicating that tissue anoxia was not very severe. Glucose balance calculations demonstrated that we could only account for 70% of the absorbed sugar. It is likely that the unaccounted glucose could be transformed into other products, such as pyruvate, alanine, and CO_2.[29,37]

The results presented in this paper demonstrate that the lectins from toxic and non-toxic *Phaseolus vulgaris* varieties had different effects on glucose absorption and transport that parallel their effects on cell viability. Knott et al.[20] reported depression of insulin levels in rats fed RKB or purified phytohemagglutinin. The inhibition of glucose absorption by RKBL may explain why these rats did not develop hyperglycemia despite the decrease in plasma insulin levels.

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