Susceptibility of Kintoki Bean Lectin to Digestive Enzymes *in vitro* and Its Behavior in the Digestive Organs of Mouse *in vivo*

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Summary The enzymatic digestion of a Kintoki bean lectin *in vitro* resulted in neither the extensive hydrolysis nor complete inactivation of the lectin. The majority of [³H]lectin administered to mice by stomach-intubation was found in the digestive tract at levels of 88.7%, 99.4%, 99.5% and 78.6%, after 0.5, 2, 5 and 24 h of intubation, respectively. Twenty to forty percent of the administered radioactivity was found combined with the mucosa of the small intestine. After 24 h of intubation, part of the radioactivity was found in the feces. The recovery test of hemagglutinating activity and the molecular weight determination of the lectin administered to and recovered from mice revealed that more than 70% of the radioactivity detected in the digestive tract represented intact lectin.

Therefore, it was concluded that most of the Kintoki bean lectin survived its passage through the gastrointestinal tract. The lectin-binding sites in the intestinal mucosa were traced with fluorescence microscopy using the lectin conjugated with fluorescein isothiocyanate. The fluorescent sites were found located at the top and upper sites of the villi, but not at the crypt.

Key Words Kintoki bean, toxic lectin, digestion of lectin, behavior of lectin in digestive tract, binding of lectin to intestine, lectin labeled with ³H and FITC

Beans are regarded as an important source of vegetable proteins and have been consumed more and more widely. However, it has long been known that diets containing raw beans cause a significant impairment of animal growth and other noteworthy symptoms of toxicity (1-6). The anti-nutritive factors identified in beans

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include protease inhibitors, lectins (hemagglutinins), anti-niacin factor, polyphenolic compounds *etc.* (7).

We have directed our attention to a lectin contained in Kintoki beans (*Phaseolus vulgaris*) and confirmed that the purified Kintoki bean lectin impairs the growth of mice to an extent directly proportional to its dose (8). On the other hand, soybean lectin was reported to be rapidly cleaved by pepsin and thereby to be least toxic (6, 9). A storage protein of *Phaseolus* beans was reported to be less susceptible to hydrolysis by proteases *in vitro* than bovine serum albumin (10). Brady *et al.* reported that intact wheat germ lectin was detected in human intestinal contents (11). These facts indicate that lectins hardly attacked by enzymes have the opportunity of exhibiting such a toxicity in the digestive tract as the disruption of the intestinal villi of rats (8, 12).

In order to investigate whether such toxicity is directly due to some biological function of intact lectins, it should be necessary to look into the detailed behavior and action of pure lectins in the digestive tract. In recent years, a rapid increase of our knowledge of lectins has been brought about in the biological, biochemical and clinical fields (13). In the nutritional studies of lectins, however, crude preparations of lectins have been commonly used and therefore some ambiguity cannot be avoided in the interpretation of experimental results. Our previous observation on the physiological functions of the lectin purified from Kintoki bean indicated that the lectin is toxic to cultured cells and animals, that the first target of its oral toxicity is the small intestine and that this lectin must be stable enough to display its toxic activities in the digestive tract (14, 15). In this study we examined the resistance *in vitro* of the Kintoki bean lectin to several digestive enzymes and its behavior *in vivo* in the digestive tract of mice.

METHODS

Hemagglutination assay. A toxic lectin was isolated from Kintoki beans (*Phaseolus vulgaris*) as previously described (16). Hemagglutination tests were carried out on microtiter plates with 2-fold serial dilution of samples and 2% suspension of mouse erythrocytes. The amount of samples per ml in the last dilution of visually detectable agglutination was defined as one unit of hemagglutinating activity.

High-performance liquid chromatography. Gel filtration was performed on a Protein Column I-250 (exclusive MW=250,000, Waters Co., 30.5 cm × 1 cm) connected to an instrument for HPLC (Shimadzu Co.). Thirty microliters of samples were injected into the column and eluted at a room temperature with 0.28 M potassium phosphate buffer, pH 6.0, at a flow rate of 0.8 ml per min. The eluates were monitored at 280 nm and the peak areas were calculated with a computer-laid recorder (Chromatopac, C-R1A, Shimadzu). The area of a peak occurring at approximately 11 min after injection was used for the quantification of the intact lectin and hemagglutinating activity of which did not change after labeling.

Lectin labeling. 1) Labeling with ^3H : *N*-Succinimidyl [2,3- ^3H]propionate (500 μCi) and lectin (5 mg) were mixed in 1 ml of 20 mM sodium phosphate buffer, pH 7.4, and left standing at 25°C for 3 h (17). The solution was then applied on a Sephadex G-50 column to obtain [^3H]lectin eluted prior to unreacted propionate.

2) Labeling with FITC: Lectin (12.5 mg/ml) and fluorescein isothiocyanate (0.62 mg/ml, Isomer I, Sigma) were mixed in 0.1 M borate buffer, pH 8.3, and allowed to react at 5°C for 15 h in the dark (18). Free dye was removed by gel filtration on a Sephadex G-50 column. The binding ratio of FITC to lectin, F/P , was calculated from the absorbances at 280 nm and 494 nm using the formula;

$$\frac{F}{P} = \frac{A_{494}}{A_{280} - (2/7)A_{494}}$$

FITC-conjugated lectins having F/P values smaller than 0.5 were used in the present experiment because the agglutinating activity of the lectin decreased as the F/P value became higher than 1.

Digestion in vitro. Lectin (2 mg/ml) and pepsin (from hog stomach mucosa, SIGMA A, 0.04 mg/ml) were incubated at 37°C in 0.01 M HCl solution. After 2 or 4 h of incubation, 0.1 ml and 0.5 ml portions of the incubation mixture were withdrawn for HPLC and hemagglutination assay, the results of which were compared with those for the control lectin not treated with pepsin. Trypsin (from bovine pancreas, SIGMA type I) and α -chymotrypsin (from bovine pancreas, SIGMA type II) were dissolved in 1/15 M sodium phosphate buffer, pH 7.6, and cathepsin D (from bovine spleen, SIGMA) in 0.01 M sodium citrate buffer, pH 2.8. The digestion procedures with these enzymes were the same as in the case of pepsin except for the buffers used. In addition, lectin was exposed to two types of enzymes successively, pepsin plus trypsin or pepsin plus chymotrypsin, in the following way. At first, lectin (2 mg/ml) and pepsin (0.04 mg/ml) were incubated in 0.01 M HCl solution for 0.5 or 2 h. Then the pH of the incubation mixture was adjusted to 7.6 with 0.2 N NaOH, and trypsin or chymotrypsin (0.02 mg/ml) in 1/15 M sodium phosphate buffer, pH 7.6, was added to the mixture. The second digestion was carried out for 0.5 or 2 h. Assay of hemagglutinating activity and HPLC were performed in the same way as described above.

Behavior in vivo. 1) Distribution of radioactivity in mouse after stomach-intubation of [^3H]lectin: Mice (ddY, male, 4 weeks old) were fasted in advance for about 20 h. [^3H]Lectin and 10 mg of cold lectin were dissolved in 1 ml of distilled water (2×10^6 cpm/ml). This sample (1 ml) was given to each mouse by stomach-intubation and two mice each were sacrificed by decapitation after 0.5, 2, 5 and 24 h of intubation, respectively. Liver, kidney, pancreas, brain, lung, thymus, spleen, stomach, small intestine, caecum and large intestine were excised. The small intestine was divided into three sections of 12 cm length which were named small intestine 1, 2 and 3 from the pylorus to distal intestine. The contents were rinsed out with cold phosphate buffered saline (PBS) from each segment of the gastrointestinal tissues. The rinsing procedure was repeated twice. The washing fluids and the

intestinal contents were pooled as for the samples containing free lectins. The residual gastrointestinal tissues and the other excised organs were homogenized in ice-cold PBS in a Potter's homogenizer and made up to a fixed volume. Blood was collected at decapitation. Urine and feces were collected during chasing periods. A one-tenth milliliter portion of each sample was treated with hydrogen peroxide and completely dissolved in Soluen-350 (Packard Co.). An aliquot of the resultant solution was mixed with a toluene-based liquid scintillator. The radioactivity was measured in a Packard Tricarb 3375 liquid scintillation spectrometer. A part of the homogenized specimens was dissolved in 1% sodium dodecyl sulfate (SDS) followed by treatment with mercaptoethanol, and the resultant mixture was subjected to discontinuous SDS 10% polyacrylamide gel electrophoresis. Gels were cut into pieces of 5 mm length. To each piece, 0.5 ml of hydroperoxide was added, followed by incubation in a bial at 50°C overnight to dissolve the gel completely for the determination of radioactivity. As the mobility of the intact Kintoki bean lectin was 0.4 subunit MW = 35,000, it was calculated from the recovery of the radioactivity from the gel pieces at 0.35–0.45 mobility how much radioactivity of each sample represented intact lectin.

2) Hemagglutinating activity of the lectins recovered from digestive tract: Mice were fasted for 20 h. To each mouse was administered 10 mg of lectin in 0.8 ml water by stomach-intubation and two mice each were sacrificed by decapitation after 2, 5 and 24 h of intubation, respectively. Stomach, small intestine, caecum and large intestine were excised and washed with cold PBS. The contents of the digestive tract and the rinsed fluids were homogenized in a cold PBS as described above and centrifuged to collect the extracts containing free lectin. Lectin bound to the mucosa of the small intestine could not be subjected to hemagglutination assay because of the lack of reagents for releasing it from the mucosa. Urine and feces were collected for the assay of hemagglutinating activity. The recovery in activity was calculated from the total units recovered in the samples against the total units ingested by each mouse.

Binding in vitro to intestinal tissues. 1) Time course: Mice were sacrificed by decapitation after fasting for 20 h. Segments of 2 cm length were prepared from the small intestine 10–15 cm distal to the pylorus and placed in Krebs glucose medium (KGM) equilibrated with 95% O₂ and 5% CO₂. The intestinal segments were everted, blotted and weighed. They were exposed at 37°C to the [³H]lectin solutions having the concentrations of either 10, 227 or 500 µg/ml in KGM (60,000–70,000 cpm/ml). After 15, 30, 45 and 60 min of exposure, they were removed from the media, rinsed in 2 ml KGM and centrifuged. This washing procedure was repeated twice. The washed segments were dissolved in 1 ml of Soluen-350 for the measurement of radioactivity.

2) Binding to different parts of small intestine: [³H]Lectin and cold lectin were dissolved in KGM so as to make the concentrations of 1, 2, 4 and 8 mg per ml (1.8 × 10⁵ cpm/ml). The small intestine was excised from mice fasted for 20 h and divided into three parts of equal length which were named 1, 2 and 3, starting from

the one adjacent to the pylorus. Two everted segments of 1 cm length were prepared from each part, blotted, and weighed as quickly as possible. Each segment was exposed to one of the above lectin solutions at 25°C for 30 min under an atmosphere of 95% O₂ and 5% CO₂. The amounts of lectin bound to the intestinal tissues were determined as described above.

Microscopic observation of the lectin binding to intestinal mucosa. 1) *Binding in vivo*: Ten milligrams of FITC-conjugated lectin in 1 ml of PBS was ingested, *via* a stomach-tube, by a mouse fasted for 20 h. Two hours later, the mouse was anesthetized and an intestinal segment 5 cm distal to the pylorus was excised. The segment was rinsed in PBS and frozen to prepare 10 μ m sections for microscopic observation using a thermoelectric freezing unit for microtome (Komatsu Electronics Inst.). The fluorescence due to FITC in the sections was observed under a light microscope (Olympus BH2) using an FITC interference filter.

2) *Binding in vitro*: Ten micrometer sections of the small intestine of a mouse not administered with FITC-lectin were prepared in the same way as described above. About 200 μ l of an FITC-lectin solution (mg/ml) were mounted on the sections on slide glasses and kept for 20 min at room temperature. The lectin solution was blotted off and the sections were rinsed three to four times in PBS before microscopic observation.

RESULTS AND DISCUSSION

Digestion in vitro

The hemagglutinating activity of Kintoki bean lectin was not affected after 2 h of digestion by any of the four enzymes. After 4 h of incubation, the activity was least reduced by pepsin and cathepsin, but lowered to the extents of one to two well difference on a titer plate by trypsin and chymotrypsin. Table 1 shows the results obtained with an enzyme *versus* lectin ratio of 1 : 50, by weight.

Results obtained with other ratios of 1 to 20 or 1 to 100 were similar to those for the 1 to 50 ratio (data not shown). In the right-hand column in Table 1 are shown the recoveries of intact lectin as measured by gel filtration on HPLC. The recovery was 100% or very nearly, as far as the hemagglutinating activity remained unchanged, but resulted in a detectable decrease to about 87% in the 4-h digestion with trypsin or chymotrypsin where the hemagglutinating activity also decreased to a noticeable extent. However, the decrease by titer 1 in the hemagglutinating activity should be accompanied theoretically by an approximate 50% change in the recovery of intact lectin on gel filtration. The absence of quantitative correlation between the present two measurements is probably due to the semiquantitative data on hemagglutinating activity obtained by the naked eye. Successive digestion with pepsin and trypsin or with pepsin and chymotrypsin gave results similar to those obtained by the digestion with the individual enzyme. Consequently, the susceptibility of Kintoki bean lectin to trypsin or chymotrypsin does not appear to change after treatment with pepsin, indicating that the lectin is highly resistant to pepsin. It

Table 1. Hemagglutinating activity (1% mouse blood cells) and peak recovery on HPLC after enzymatic digestion of Kintoki bean lectin (enzyme/lectin (w/w)=1/50).

Enzyme	Time (h)	Titer										Recovery on HPLC (%) ^a
		2 ⁰	2 ¹	2 ²	2 ³	2 ⁴	2 ⁵	2 ⁶	2 ⁷	2 ⁸	2 ⁹	
Control	—	++	++	++	++	+	+	±	—	—	—	100.0
Pepsin	2	++	++	++	+	+	+	±	—	—	—	96.0
	4	++	++	++	+	+	+	±	—	—	—	100.0
Trypsin	2	++	++	++	+	+	+	±	—	—	—	98.3
	4	++	++	++	+	+	±	—	—	—	—	86.8
Chymotrypsin	2	++	++	++	+	+	+	±	—	—	—	100.0
	4	++	++	++	+	+	+	±	—	—	—	87.4
Cathepsin	2	++	++	++	+	+	+	±	—	—	—	100.0
	4	++	++	++	+	+	+	±	—	—	—	100.0
Pepsin	0.5	++	++	++	+	+	+	±	—	—	—	99.5
trypsin	0.5	++	++	++	+	+	+	±	—	—	—	
Pepsin	2	++	++	++	+	+	+	±	—	—	—	97.5
trypsin	2	++	++	++	+	+	+	±	—	—	—	
Pepsin	0.5	++	++	++	+	+	+	±	—	—	—	98.8
chymotrypsin	0.5	++	++	++	+	+	+	±	—	—	—	
Pepsin	2	++	++	++	+	+	+	±	—	—	—	93.1
chymotrypsin	2	++	++	++	+	+	+	±	—	—	—	

^a The recovery was calculated from the area of peak corresponding to intact lectin.

was reported that soybean lectin was rapidly hydrolyzed by pepsin, but that *Phaseolus vulgaris* lectin was not completely inactivated even after 6 days of incubation with pepsin (9). Thus the lower toxicity of soybean lectin can be explained by its higher vulnerability to digestive enzymes. In view of the relatively high resistance to digestive enzymes, Kintoki bean lectin seems to have a chance of exhibiting its toxicity in the digestive tract before being hydrolyzed *in vivo*.

Behavior in vivo

1) *Distribution of radioactivity in mouse after stomach-intubation of [³H]lectin.* Conditions of enzymatic digestion *in vitro* apparently differ from those *in vivo*, particularly in the varieties and quantities of enzymes involved. Hence, it is not certain whether the high resistance of Kintoki bean lectin against digestive enzymes found *in vitro* in the present study is applicable to the *in vivo* cases. Therefore, we administered [³H]lectin to mice by stomach-intubation in order to investigate the behavior of the lectin in the gut. At 0.5, 2, 5 and 24 h after the ingestion of [³H]lectin (10 mg, 2 × 10⁶ cpm), the radioactivities in various organs, blood, urine, and feces were counted (Table 2). The count recoveries from whole body were 88.7%, 99.4%, 99.5%, and 78.6% after 0.5, 2, 5 and 24 h of intubation, respectively. The majority of the ingested lectin was found in the digestive tract within the experimental periods;

Table 2. Distribution of radioactivity in mouse after stomach-intubation of [^3H]lectin^b (%).

Organ	Time (h)			
	0.5	2	5	24
Main organs ^a	1.3 $\pm$ 0.2	2.5 $\pm$ 0.9	2.8 $\pm$ 0.3	3.2 $\pm$ 0.1
Blood	0.1 $\pm$ 0.0	0.4 $\pm$ 0.4	0.8 $\pm$ 0.6	2.0 $\pm$ 0.0
Urine	—	—	8.8 $\pm$ 1.8	3.0 $\pm$ 0.1
Feces	—	—	—	12.0 $\pm$ 2.5
Digestive tract free	65.8 $\pm$ 12.2	57.1 $\pm$ 0.5	60.0 $\pm$ 10.2	40.3 $\pm$ 1.0
bound	21.7 $\pm$ 3.7	39.4 $\pm$ 2.5	27.2 $\pm$ 12.3	18.2 $\pm$ 0.4
Total recovery	88.7 $\pm$ 16.0	99.4 $\pm$ 0.7	99.5 $\pm$ 0.7	78.6 $\pm$ 1.4

^a Main organs: kidney, liver, spleen, pancreas, brain, lung, heart and thymus.^b [^3H]Lectin (10 mg, 2×10^6 cpm) was intubated to each mouse.Table 3. Percentages of intact lectin^a in each part of digestive tract determined by SDS polyacrylamide gel electrophoresis (%).

Organ	Time (h)							
	0.5		2		5		24	
	Free	Bound	Free	Bound	Free	Bound	Free	Bound
Stomach	99.5	—	98.2	97.7	98.8	—	—	—
Small intestine 1	99.3	99.0	99.2	98.6	96.5	94.6	95.0	93.0
2	98.7	96.9	97.5	99.4	97.7	92.7	96.2	98.0
3	99.8	93.6	99.6	99.7	99.5	90.3	96.2	98.0
Caecum	93.4	97.0	88.7	96.1	83.7	94.5	69.6	96.1
Large intestine	—	—	96.3	93.1	83.9	89.5	70.8	90.0
Feces	—	—	—	—	—	—	65.3	—

^a The ratio was calculated from the counts from the gel segments corresponding to 0.35–0.45 mobility.

87.5%, 96.5%, 97.2% and 58.4% after 0.5, 2, 5 and 24 h of intubation, respectively. High counts indicated considerable binding to the intestine. The binding ratio reached a maximum of 39.4% of the total lectin administered at 2 h and gradually decreased to 18.2% at 24 h. Counts became detectable in feces at 24 h. However, these radioactivities might be due to some hydrolyzed lectins.

To make this point clear, aliquots of all the samples were subjected to SDS 10% polyacrylamide gel electrophoresis. In the samples other than those from the

Fig. 1. Distribution of radioactivity in the digestive tract of mice given [^3H]lectin. Total radioactivity in the digestive tract was taken as 100%. The values were corrected for the recovery on SDS polyacrylamide gel electrophoresis to represent the ratios due only to intact lectin.

digestive tract and feces, few counts were found at the mobility of 0.4 where intact Kintoki bean lectin was to be located, but significant counts at the mobilities higher than 0.4. Therefore, we assume, as regards our detectability, that most of the radioactive materials absorbed by mice may not represent intact lectin. This is in contrast to the findings of Putzai *et al.* that an immune-precipitate from the serum of rat fed kidney bean contained the subunit of lectin (19). Results obtained with the samples from the digestive tract and feces are summarized in Table 3. Most of the values in Table 3 are over 90% indicating that the ingested lectin is fairly stable *in vivo*. But in the contents of the caecum, the radioactivity at around a mobility of 0.4 representing 88.7% for 2-h fragments, was detected at mobilities of 0.6–0.8 in these samples. Similar results were obtained with samples from the large intestine. In the caecum, the large intestine and the feces, part of the lectin was apparently decomposed by enzymatic and probably bacterial actions. Figure 1 shows the distribution patterns of intact lectin (free and bound) in the digestive tract of mice at four different time intervals. The percentages were calculated on the basis of the

total radioactivities present in the digestive tract at each time as 100% and corrected for the recoveries from SDS-PAGE to represent specifically the amounts of intact lectin. Free lectins in stomach, small intestine 1 and 2 amount to 10–20% after 0.5 to 2 h of ingestion, and afterward most of them moved down to the lower small intestine, the caecum and the large intestine where the lectins were found to be largely in the free state. Little free lectin was found in stomach and small intestine 1 and 2, after 24 h of ingestion. On the other hand, the distribution patterns of bound lectin indicate that a considerable part of the ingested Kintoki bean lectin quickly binds to the small intestine, and remains bound especially to small intestine 2 and 3 for at least 24 h. The total amounts of the bound lectin were the highest, approximately 40% of the total present in the digestive tract, at 2 h after stomach-intubation and about 20% were still bound after 24 h. The bound lectin seems to be gradually liberated from the tissues with time. Little binding occurred in the stomach, the caecum and the large intestine.

2) *Hemagglutinating activities of the lectins recovered from digestive tract after their stomach-intubation.* The behavior of Kintoki bean lectin in the digestive tract was examined using [^3H]lectin by identifying the lectin on SDS-PAGE. The main conclusion drawn from this experiment is that the lectin ingested orally is not easily degraded, and moves in the gut with some being bound to the tissues. We have already proven that Kintoki bean lectin is toxic to cultured animal cells (15). If the lectin retains its activity during its passage through the gut, it will be possible for the active lectin to exhibit its toxicity on the epithelial cells in the small intestine. In an attempt to examine whether the lectin is still active in the gut after ingestion into the mouse, we assayed the hemagglutinating activities of the contents recovered from the gut mice administered with Kintoki bean lectin (Table 4). As lectin bound to the intestines could not be intentionally liberated for the assay, the

Table 4. Hemagglutinating activity recovered from contents of digestive tract (%).

Organs	Time (h)			
	0.5	2	5	24
Stomach	25.0	3.1	0.8	0.4
Small intestine 1	12.5	3.1	3.1	0.8
2	12.5	25.0	3.1	1.0
3	6.3	3.1	12.5	6.3
Caecum	1.6	2.2	12.5	25.0
Large intestine	0.8	0.8	8.8	6.3
Faeces	—	—	—	5.3
Urine	—	—	—	—
Total recovery	58.7	37.3	40.8	45.1

10 mg lectin (25,200 units) was ingested by each mouse. Lectin bound to intestine was not taken into account.

recovery was as low as 30% of the total activity ingested after 2 h of intubation when the binding was supposed to reach a maximum degree, while it increased gradually to 59% and 83% after 5 and 24 h of intubation, respectively, as the lectin once bound was released *in vivo* into the digestive contents with time as shown in Fig. 1.

If the bound lectins are taken into account on the basis of the results shown in Table 2, the recoveries in the hemagglutinating activity will be over 70% in total at all the incubation periods. Consequently, it is concluded, as similarly as in the experiment on the movement of [^3H]lectin in the gut, that the majority of the ingested lectin passes through the gut, retaining a large part of its hemagglutinating activity. Higuchi *et al.* reported similar results for winged bean lectin (6). It is noteworthy that the feces collected after 24 h of intubation contained some active lectin. About 30% of the ingested [^3H]lectin was found in the feces as shown in Table 2, but the amounts calculated from the hemagglutinating activity represented only 7% (Table 4). Slow but steady progress of lectin digestion is suggested by the findings that only 65 to 70% of radioactivity represents the intact lectin in the caecum, large intestine and the feces after 24 h of intubation (Table 2).

Binding to intestine in vitro

Judging from the hemagglutinating activities of the contents of the gut, Kintoki bean lectin seemed not to be rapidly hydrolyzed *in vivo*. Therefore, there must be time enough for the lectin to exhibit its toxic functions before being inactivated in

Fig. 2. Time course of the lectin binding to small intestine *in vitro*. Lectin concentration was 10 µg/ml (△), 227 µg/ml (○) and 500 µg/ml (●).

Fig. 3. Amounts of lectin bound to small intestine *in vitro*. Everted sacs of small intestine 1, 2 and 3 were placed in lectin solutions of various concentrations for 30 min; ○, small intestine 1; △, small intestine 2; ●, small intestine 3.

the digestive tract. When Kintoki bean lectin was orally ingested by mice, several physiological effects such as decreases in body weight and in the digestibility of major nutrients, and severe disruption of the epithelial cells in the small intestine were observed (8). In the present study, we monitored the time-course of the binding reaction using everted segments of small intestine and lectin solutions of 10, 227 and 500 µg/ml. The binding to the tissue proceeded quickly and reached a plateau after 30 min, during which time the lectin must maintain its stability in the gut according to the above results (Fig. 2). Therefore, it is considered that the ingested Kintoki bean lectin must reveal its physiological activity on the epithelial cells before it is enzymatically degraded into the inactive forms. Everted segments of the 3 different parts of the small intestine were exposed to several lectin solutions of higher concentrations to see how much lectin could bind to each part of the intestine (Fig. 3). Little difference was found in the binding capacity of the various parts of the small intestine used. The amounts of bound lectin increased in proportion to the lectin concentration, saturation not occurring within the tested concentration ranges. These amounts represent about 15% of the lectin present in the solutions. This result is in good agreement with the above one in that about 10 to 20% of the ingested lectin became bound to the intestine after 0.5 and 2 h of incubation in an experiment with [^3H]lectin *in vitro*.

Histological observation of the lectin binding to intestine

1) *Treatment in vivo*. To ascertain the binding of the lectin to small intestine visually, mice were allowed to ingest FITC-lectin by stomach-intubation, and 2 h

Fig. 4. A: A section of intestine 10 cm from pylorus of mouse fed FITC-lectin *in vivo* ($\times 40$).
B: A section of intestine 2 cm from pylorus of mouse fed FITC-lectin *in vitro* ($\times 100$).

later the small intestine was excised to prepare frozen sections of $10\text{ }\mu\text{m}$ thickness. The sections were observed under a microscope through a FITC interference filter (Fig. 4 A). Fluorescence was observed at the surfaces of the villi, indicating that the lectin binds at the top and upper sides of the villi where dipeptidase, sucrase and other membrane digestive enzymes are localized (20). Hence, the lectin binding to these sites will surely result in such inhibition of the digestion and absorption of nutrients as previously reported (8).

2) *Treatment in vitro*. FITC-lectin was allowed to react with intestinal sections on a slide glass. Fluorescence was not observed inside the villi, but at the top and upper sides thereof as observed *in vivo*. Lectin appeared not to rapidly bind to the crypt.

In conclusion, Kintoki bean lectin is neither hydrolyzed nor inactivated in the stomach but moves into the small intestine in an active form. In the small intestine, little is hydrolyzed and considerable amounts of the lectin bind to the intestinal mucosa. The bound lectin remains there for a relatively long period with little being absorbed or degraded. And a large portion of free lectins passes through the digestive tract, maintaining hemagglutinating activity. The binding of the lectin to intestinal epithelia must result in the retardation of the intestinal digestion and absorption of nutrients (8). In addition, Kintoki bean lectin depresses the rate of cell division as well as other general cell activities on the villi (15). Thereby, the oral toxicities of the lectin are considered to be brought about by the reduction of enzyme secretion (21) and finally by the inhibition of membrane digestion-absorption (19). A possibility of direct enzyme inhibition by this lectin has not yet been excluded. In our preliminary experiment, we observed a significant decrease in the transport of amino acids and sugars in the small intestine of mice having ingested Kintoki bean lectin. The mechanisms of the inhibition of nutrient absorption and other changes occurring following the binding of lectin to intestinal surfaces are under investigation in our laboratory.

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