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– Minireview –

The Comparative Studies on the Lectins from Kintoki Bean and Taro Tuber

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Abstract

The comperisons of Kintoki bean lectin (KBL) and Taro tuber lectin(TTL) which have been studied in our laboratory are summerized. The recoveries of pure lectins are 0.12% and 0.014%, respectively. They seem to have slight differences in isoelectric points (pH) ; 5.19~5.67 for KBL and 6.41~7.42 for TTL. The minimum concentrations of HA are 2.8μg/ml and 21.6μg/ml. The enzymatic modification on HA, growth inhibition, inhibition of nutritional absorption and binding capacities (FITC, ^{3}H) of KBL are demonstrated to be much greater than those of TTL.

Key words : Kintoki bean lectin (KBL), Taro tuber lectin (TTL)

INTRODUCTION

Lectin is a cell-agglutinating and carbohydrate-binding protein occurring in many plants, particularly in legume seeds, and also in animals and microorganism[1~3]. Since their discovery by Stillmark in caster bean, nearly 100 years ago, a number of studies on plant lectins have been performed. Generally, lectins are neither chemically, nor structurally alike. Moreover, even those belonging to the same family, or genus, differ in their physiochemical and biological properties[4~7]. In earlier experiments from our laboratories[8~20], among some 53 different plants treated, about 27 of them have been founded to have mouse erythrocyte agglutinating activity and showed toxicity after intraperitoneal injection. And the purification steps and toxicity of lectins from Kintoki bean (*Phaseolgaris vulgaris*), Tora bean (*Phaseolus vulgaris*) and Taro tuber (*Colocasia antiquorum*) have shown. In this paper, conclusions drawn are presented with respect to the chemical characteristics and nutritional effects of the KBL and TTL, namely, purification, electrophoresis, hemagglutining activity, enzymatic modification, heat stability, growth rate, the absorption of major nutrients, intestinal enzyme activity and binding to intestine.

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ANALYTICAL ELECTROPHORESIS AND CHEMICAL CHARACTERIZATION

The Kintoki bean is a represesntative legume and Taro tuber is accepted as food in Japan. They are used for cooking, generally. Each of them is purified chromatographycally to four isolectin and also analyzed electrophoretically or by ultracentrifugation (Table 1). About 1mg of each finally purified lectins is usually obtained from 0.83g Kintoki bean or 7g Taro tuber. Their recoveries from purified amounts are 0.12% and 0.014%, respectively. Probably, there are some

Table 1. Physiochemical properties of KBL and TTL

		Isolectin			
		I	II	III	IV
Isoelectric points	KBL	5.19	5.20	5.48	5.67
	TTL	6.41	6.82	7.21	7.42
Molecular weight[1]	KBL	33,000	33,000	33,000	33,000
	TTL	11,000	11,000	11,000	11,000
Carbohydrate (%)	KBL[2]	4.30	4.16	4.25	4.25
	TTL[3]	2.0	2.0	2.0	2.0
Minimum Con. (μg/ml)[4]	KBL	3.0	1.5	1.5	0.7
	TTL	1.7	2.5	1.1	2.2

[1] Determined by SDS-polyacrylamid gel electrophoresis
[2] Detected in the gas chromatographic analysis
[3] Determined by phenol-sulfuric acid method as galactose
[4] Required for the agglutination of 1ml suspension of 1% mouse erythrocyte

losses of lectin due to purification. The purification yield of KBL is about 8.5 fold comparing to TTL. They seem to have slight differences in isoelectric points; 5.19, 5.20, 5.48, 5.67 for KBL and 6.41, 6.82, 7.21, 7.42 for TTL. Each lectin seems to be a tetramer of a 33,000 dalton subunit in KBL and to be a trimer of a 11,000 dalton subunit in TTL. The purified lectins are found to be a glycoprotein containing 4% and 2% carbohydrate in all the isolectins, in order of KBL and TTL. Major amino acids are essentially the same among each of the isolectins and all of their isolectins are abundant in aspartic acid, leucine and glycine. However, they are absent or rare in cystine and methionine, which is a distinguishable common feature of many other plant lectins (data not shown). The major plant proteins, especially bean seed, have been observed in their electrophoretic patterns to differ significantly from each other in their physiochemical properties when thay are obtained from different cultivators[21,22]. Therefore, a possible explanation will be that the properties of the lectins from different materials may also differ.

HEMAGGLUTINATION ASSAYS

Blood group specific agglutination

Agglutination of human and animal erythrocytes by KBL and TTL is shown in Table 2. Human red blood cells, regardless of their types, are agglutinated by KBL to similar intensities, but not by TTL. Mouse erythrocyte is most strongly agglutinated in both of their lectins. Agglutination titer of KBL is much higher compared to that of TTL, generally. The minimum amounts of KBL and TTL that are needed to agglutinate mouse erythrocytes are 2.8μg/ml and 21.6μg/ml, respectively.

Table 2. Agglutination of various red blood cells by KBL and TTL

Erythrocyte	Minimum concentration (μg/ml)	
	KBL	TTL
Human A	44.8	-
B	44.8	-
C	44.8	-
Rat	22.4	43.2
Rabbit	11.2	43.2
Sheep	5.6	21.6
Mouse	2.8	21.6

pH and heat stability

The heat stability of the lectin is examined by heating for 1hr at various temperatures in a phosphate-buffered saline solution. Immediately after cooling in ice, it is used for the agglutinating test. The activities of KBL and TTL are not affected by heating at 60°C for 1hr. But the activity of KBL is lowered rapidly, above 80°C, and is completely lost at 100°C after 1hr. However, in case of TTL, a large part of the activity is remained even after heating at 90°C or 100°C. Each purified lectin is stable at the pH range 2.0~10.0. At pH 10.0 the HA of the TTL decreaesed to 50% of the initial value, but not in KBL.

Inhibition of agglutination by sugars

Table 3 shows the inhibition of HA (hemagglutinating activity) of the lectin by sugars. Among the tested sugars, fetuin is found to be the best inhibitor for both of them. The sugars of galactose type are able to inhibit KBL, although a relatively high concentration is required for detecting an inhibition pattern.

Enzymatic modification on HA

Table 4 shows that KBL is relatively more resistant

Table 3. Inhibition of hemagglutinating activity (HA) by various sugars

Sugars	Minimum con. (mg/ml) for inhibiting the HA	
	KBL	TTL
Galactose	150	-
Lactose	150	-
GalNAc	75	-
Fetuin	37.5	62.5

The following sugars are not inhibitory to the both lectins glucose, mannose, fructose, fucose, xylose, gluNAc

Table 4. Effects of enzymatic modification on the HA

Enzyme treatment	Minimum concentration (μg/ml)	
	KBL	TTL
Control	3.9	15.6
Trypsin 1/100-fold	3.9	125
1/1000-fold	3.9	125
Pepsin 1/100-fold	3.9	125
Chymotrypsin 1/50-fold	3.9	-
Pronase 1/100-fold	-	62.3

to trypsin and pepsin than TTL. In particular, KBL retains its full activity even after it is mixed with pepsin at 1/100-fold the amount of lectin at pH 2.

TOXIC EFFECTS ON MICE

Mortality of mice injected with crude or pure lectin intraperitoneally

Table 5 shows that the mortality caused by the intraperitoneal injection of KBr and TTL increased as their purification steps proceeded. For the 100% death of injected mice, for the KBL and TTL, 1.5mg and 3.0mg of crude extracts, 0.5mg and 2.0mg of crude lectins and 0.2mg and 1.5mg of pure lectins are required per gram body weight. In intraperitoneal injection testing, the mortality increased 7 times when the KBL purification folds increased 6 times but the mortality by TTL in creased only twice when purification folds in creased 4 times.

Table 5. Lethal activity of KBL or TTL injected to mice intraperitoneally at various purification steps

Purification steps	mg injected per g b. wt.		Mortality (dead mice/injected)		Minimum con. (μg/ml)	
	KBL	TTL	KBL	TTL	KBL	TTL
Crude extract	0.50	2.00	0/5	1/3	25	92
	1.00	2.80	0/5	2/3		
	1.50	3.00	5/5	3/3		
Crude lectin	0.25	1.40	0/5	1/3	10	66
	0.38	1.90	2/5	2/3		
	0.50	2.00	5/5	3/3		
Pure lectin	0.15	0.50	2/5	1/3	4	21
	0.20	1.00	5/5	2/3		
	0.25	1.50	5/5	3/3		

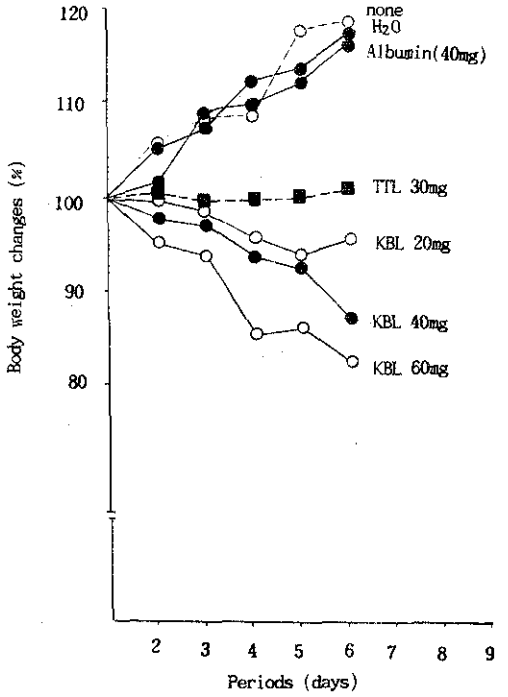

Fig. 1. Growth of mice fed various amounts of KBL and TTL by stomach-feeding.

Growth inhibition

Fig. 1 shows the results of comparative growth experiments with KBL and TTL. Experimental mice on a basal diet are fed KBL or TTL by stomach intubation. The KBL diet caused a continuous decrease in body weight and one third or two third of the mice in the 40mg and 60mg lectin feeding groups died in a few days, while the TTL diet only brought a growth retardation. The antinutritional effects of KBL is demonstrated to be much greater than those of TTL. When these lectins are autoclaved at 120°C for 1hr, body weight gains of the mice are comparable to those from the basal albumin diet. Comparing to the control mice, their body weights are reduced down to 84%, 76 % and 71% after 1 day when KBL dosaage was 20 mg, 40mg and 60mg, respectively. On the other hand, mice given TTL 30mg are reduced to 88.4%, comparing to the control groups. The PERs are −1.04, −2.73, −3.02 and +1.92 in the order of 20mg, 40 mg, 60mg, for KBL and 30mg for TTL groups, which is comparable to the control PER, 2.89 or 2.40.

Digestibility

Table 6 shows the apparent rates of intestinal ab-

Table 6. Effects of the addition of KBL or TTL to each diet on the absorption of major nutrients by rats or mice

	Control[1]	KBL	Control[2]	TTL
Body weight change (g/day)	+1.2	−1.3	+1.2	+0.68
Food intake (g/day)	7.8	4.8	5.4	4.5
N absorbed (%)	55.5	26.3	91.6	85.5
Carbohydrate absorbed (%)	99.6	94.4	97.2	94.6
Lipid absorbed (%)	92.6	83.3	–	–
PER	+0.65	−0.37	+2.21	+0.51

[1]Autoclaved Kintoki bean
[2]0% casein diet

Table 7. Effect of the KBL or TTL on the intestinal enzyme activity of mice

Diet	KBL-crude (200mg)	KBL-pure (20mg)	TTL-crude (150mg)	TTL-pure (30mg)
Body weight gain (g/day)	0.11	0.45	0.95	1.13
Alkaline phosphatase*	91.2	93.8	69.4	88.6
Sucrase*	47.2	65.6	70.7	95.7
LA-peptidase*	59.5	65.9	74.2	76.1

*expressed by % to control

sorption/digestion of main nutrients. Using Kintoki bean as the single source of protein, the rate of absorption of proteins is considerably low, 55.5%, even in the control group based on the autoclaved bean diet. Addition of the lectin, the absorption rate much lowered to 26.3%. Although to a smaller extent, the rates of carbohydrate and lipid are also lowered. On the other hand, for the mice ingested TTL, the absorption rate of protein and carbohydrate are lowered to 93.3% and 97.3%, respectively.

Effects on the intestinal enzyme

Table 7 shows the study of body weight gain with KBL or TTL added to the basal diet by stomach-intubation. This feeding is carried out for 6 days with paired feeding. The activities of alkaline phosphatase, sucrase and leucine aminopeptidase in the small intestine of the mice given KBL or TTL diet decreased compare to those of the control groups. The sucrase activity of the small intestine based on KBL diet decreased to 50~65% compared to the basal diet group. Except for the alkaline phosphatase activity, the activities of other enzymes based on KBL diet Showed less than TTL diet group.

Morphological appearance of the mouse small intestine

The micrographs of the small intestine are taken from the mice fed on a basal diet with KBL or TTL (data not shown). In the mice fed lectin, the villus were seen short, irregular and the arrangement were abnormal. When FITC-conjugated lectin intubated, it is 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 locallized. It seems that, the affinity the KBL is much stronger than TTL.

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팥콩 Lectin과 토란 Lectin의 특성 비교

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요 약

지금까지 발표된 본 연구실의 논문 중 팥콩 lectin (KBL)과 토란 lectin (TTL)에 관하여, 물리화학적인 특성과 독활성을 중심으로, 각 논문의 자료[9~20]를 토대로 비교 정리하였다.