J. Korean Soc. Food Nutr.
22(4), 494 ~ 499(1993)

한국영양식량학회지

Light and Electron Microscopical Observation of the Binding of Lectin to Mouse Intestine

Young-Ju Seo[§]

Human Life and Environmental Sciences Graduate Division of Human Culture, Nara Women's University, Nara 630, Japan

Abstract

The morphological and histochemical observation of the lectin binding to intestine *in vivo* or *in vitro* was investigated. Our finding demonstrates the validity of semi-quantitative estimates of lectin binding to mouse intestine. The fluorescence patterns obtained after treatment of intestine sections with FITC-conjugated lectin revealed that Kintoki bean lectin (KBL) and Taro tuber lectin (TTL) were localized on the cell membrane, especially the top and upper sites of the villi and showed that KBL was more strongly located than TTL under various conditions. In the reverted intestine of mice fed lectin, the villi were considerably disordered and conspicuously disrupted.

Key words : Kintoki bean lectin (KBL), Taro tuber lectin (TTL), FITC-lectin, small intestine, microscopical observation

INTRODUCTION

Many plant lectins possess a strong affinity to the intestinal epithelium. Histochemical studies revealed that the *Phaseolus vulgaris* lectin binds to the glycocalyx or surface coat of the small intestine and also to some of the subepithelial structure[1]. It has been shown that some lectins are also bound to the intestinal epithelium when introduced into isolated intestinal segments or fed intact to animals either in purified forms or as raw beans[2-6]. The precise mechanism responsible for the impairment in the absorptive capacity of the intestines is no doubt associated with the profound changes in the morphology and ultrastructure of the intestine that accompany the ingestion of various lectins[7-12]. The aim of this paper is to study by means of light microscopy and scanning electron microscopy the morphological alterations of mice small intestine which will be correlated to functional and biochemical reactions[13,14]. Similar associations have been described in studies on kidney bean toxicity in rats and pigs[7,8,15]. The present study describes the binding states of FITC-lectins and the changes of mouse intestine in mice offered a diet containing lectins. These observations were made comparatively with KBL and TTL.

MATERIALS AND METHODS

Preparations of two lectins (KBL and TTL) were purified according to the methods described previously[16,17]. Hemagglutinating activity was assayed with a micro titer plate by the serial double dilution method using 2% mouse blood cells.

For the light microscopic observation of binding to the intestinal sections, the FITC-lectins (fluorescein isothiocyanate-conjugated lectins) were used for the investigation of the binding parameters to intestinal tissues. Normal mice were taken from the Shizodokyo, Japan (ddY strain, males weighing about 30g). The small intestines were extracted and divided into several sections. After repeatedly rinsing with PBS, the intestines were fixed using 4% (v/v) glutaraldehyde in PBS for overnight and dehydrated in graded series of ethanol for about 12hours in each step. And then 5~8μm thick sec-

[§] Present address : 103-1304 Lotte Apt, Gwan-Um Dong, Puk-Gu, Daegu 702-260, Korea

tions were prepared. After washing, the tissue sections were incubated with the FITC-KBL or FITC-TTL under various conditions. 50μl of FITC-conjugated KBL or TTL was applied to the dehydrated intestinal tissue sections and incubated at 5°C for 60mins or at 37°C for 0, 15, 30 and 60mins. The various concentrations of the FITC-lectins were also mixed with intestine and incubated at 37°C for 60mins. The working solutions of the FITC-lectins were of 10, 50, 100 and 200 μg/ml concentrations. The binding of FITC-lectins was observed in the presence of inhibitors (galactose, fetuin and EDTA) and under various pH conditions (pH 2.02, 5.0, 7.3, and 9.16). The binding pattern on the intestine of mice fed the lectins was also observed at 37°C for 60mins.

The microscopic observations of reverted intestine, mice were anesthetized after a lectin feeding periods of 6 days. Segments of 3cm length were prepared, reverted, fixed in 4% glutaraldehyde buffered and freeze-dried. The reverted small intestinal sections were splattered with gold and observed in a scanning electron microscope (Jeol JFC 1100) at 1100V, 9mA for 7mins without tilting. For the light microscopic observation, the intestines of mice fed 10% protein diet with or without TTL for 6 days were segmented. Each intestinal segment was excised, fixed and embedded in paraffin. Serial 5~ 8μm sections were cut and stained with hematoxylin, eosin and perodicacid-Schiff. These sections were observed under a light microscope (Olympus BH2) and photographed at ASA 100.

RESULTS AND DISCUSSION

Hemagglutinating activities (HA) of KBL and TTL, either in intact form or in FITC-conjugated form, were shown in the Table 1 when FITC-KBL and FITC-TTL were applied to the column, with Sephadex they were eluted as a single low molecular weight peak (data not shown). It indicated that the conjugated

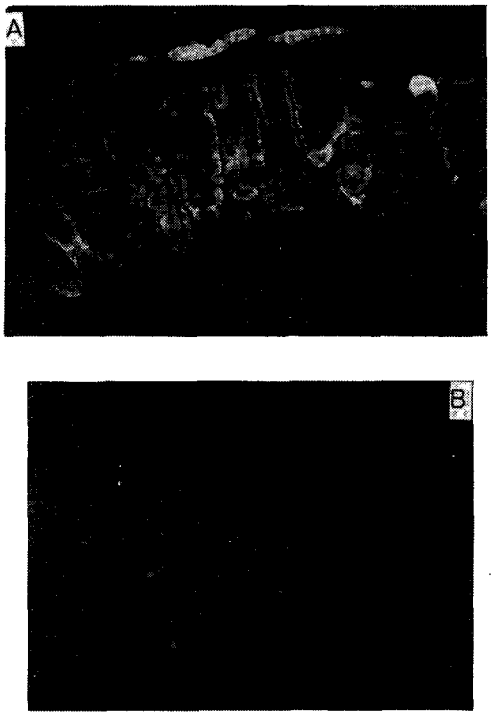

Fig. 1. Fluorescein light microscope of the part of an intestinal section.
A shows immunofluorescence in brush border region and within apical cytoplasm of erythrocytes, but not at the crypt. It is the part of the jejunum of a mouse fed FITC-KBL *in vitro* (×100).
B shows like normal, the binding of FITC-KBL to intestinal section could be nearly completely abolished by the pretreatment with fetuin. It is the part of the jejunum of a mouse previously incubated with fetuin before fed FITC-KBL *in vitro* (×100).

Table 1. Effects of FITC conjugation on the hemagglutinating activity of Kintoki bean lectin(KBL) and Taro tuber lectin(TTL)**

Concentration of 2^n (μg/ml)	280	2^0	2^1	2^2	2^3	2^4	2^5	2^6	2^7	2^8	2^9	Minimum* conc.(μg/ml)
KBL	280	+	+	+	+	+	+	+	−	−	−	4.4
FITC-KBL	150	+	+	+	+	+	+	−	−	−	−	4.7
TTL	250	+	+	+	+	+	−	−	−	−	−	15.6
FITC-TTL	260	+	+	+	+	−	−	−	−	−	−	32.5

* Required for the agglutination of 1ml suspension of 1% mouse erythrocyte **Mouse red blood cells were agglutinated by KBL or TTL and FITC-KBL or FITC-TTL. Mouse cells strongly agglutinated by the KBL or FITC-KBL but less TTL or FITC-TTL. KBL was not affected by the FITC conjugating, but TTL was slightly decreased in HA from their minimum concentrations, the HA of KBL or FITC-KBL was about 2^2, 2^3 folds of TTL or FITC-TTL, respectively

lectins were not denatured and could be used in the binding experiments.

Tissues fixed in 4% glutaraldehyde and embedded in paraffin were actually suitable for the identification of lectin in light microscopy, with the use of FITC-conjugated lectins, and the paraffin was removed from the tissue sections. FITC-lectin was allowed to react with intestinal sections on a slide glass. The binding patterns of FITC-KBL and FITC-TTL in the intestinal sections were illustrated in Fig. 1. The apical membrane and the junctional complex were outlined rather clearly, which disappeared in the presence of fetuin. All the results of the observations, including various experimental conditions, were depicted in Table 2 to compare to binding patterns of FITC-conjugated KBL and TTL. Intensity of binding was regarded from absence (-) to weak (±) and strong (+). In general, the binding of FITC-KBL was more strongly localized in each intestinal sections, than FITC-TTL.

From Fig. 2 and Fig. 3, effects of lectin binding to mouse intestinal villi were observed both at the light microscopic level and electron microscopic level. The use of intestinal segments for electron microscope resulted in a more precise recognition of the differences between two competitive intestines fed lectin or not, than that for light microscope. The gut lesion was characterized by the disturbance of the normal palisade arrangement of the apex microvilli of the enterocyte in the proximal small intestine, with the severe effects confined to the villus tips and possibly correlating with the distribution of lectin-receptor activity. The small intestinal tissue of experimental mice was histologically and ultrastructually distinguishable from that of mice which had been fed a control diet. Thus, the gross changes in morphology appeared to be a consequence of the binding of active KBL or TTL supplied in the diet, although the precise mechanism leading to the observed changes remained to be elucidated. It appeared that intensive staining of the intestinal membrane was related not only to the glycocalyx but also to the carbohydrate-rich materials which were an integral part of the membrane structure. No adverse effect could be attributed to either glutaraldehyde fixation or paraffin-embedding. The binding of lectin might be then followed by further events, such as diarrheal disease[18,19] or penetration of macromolecu-

Table 2. The binding patterns of FITC-conjugated lectins on various conditions

Experimental conditions		FITC-KBL	FITC-TTL
Time	0 (37° C)	-	-
(mins)	15 (37° C)	±	±
	30 (37° C)	+	±
	60 (37° C)	++	±
	60 (5° C)	+	±
pH	2.02	±	
	5.0	-	
	7.3	++	
	9.16	±	
Additives	Fetuin	-	±
	EDTA	-	-
Intestine fed lectin		±	±

* The intensity of binding to intestinal cross section if regarded from absence (-), to weak (±), to strong (+) and to heavily strong (++)

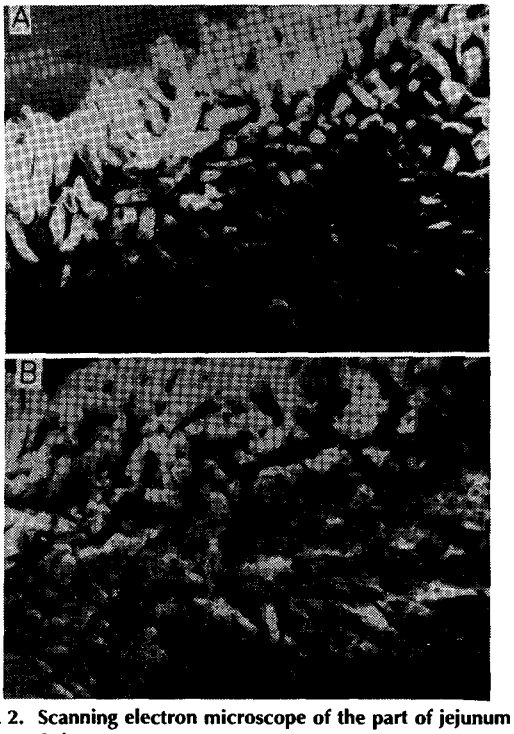

Fig. 2. Scanning electron microscope of the part of jejunum fed KBL.
A shows the control mouse fed on a basal diet (×16). B shows the experimental mouse fed 20mg KBL per daily (×16). Compared to the control intestine, the normal mucosa is weak after lectin digestion and limited to the luminal borders of the epithelium in the everted intestine fed lectin.

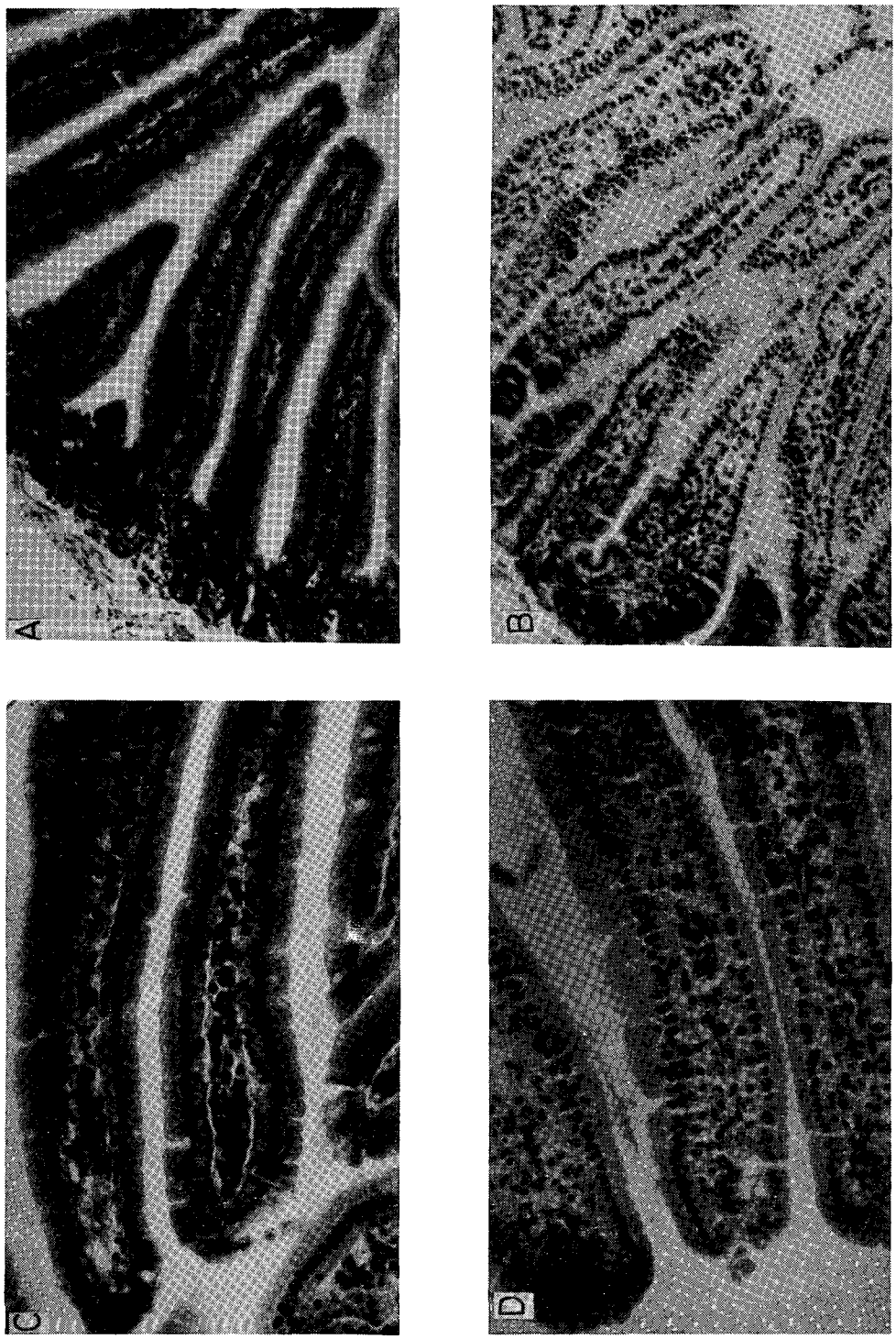

Fig. 3. Light microscope of the part of jejunum of a mouse intestinal cross section fed TTL of not *in vivo* (HE-stain).
In mouse intestine fed TTL, the goblet cells were faintly stained. Moreover, the apical membrane of the epithelial cells displayed less staining than in the control mouse. A is the control intestinal section (×200). B is the experimental section (×200). C is the control intestinal section (×400). D is the experimental section (×400).

les into the intestinal mucosa[20]. Our finding also confirmed that the affinity of cellular structures for lectins was not significantly affected by its fixation and embedding as in other reports[19-22]. Although quantitative correlation between the staining intensity and the binding amount could not be deduced, it was noted.

REFERENCES

1. Jaffe, W. G. : Hemagglutinins (Lectins). In "*Toxic constituents of plant foodstuffs*" Liener, I. E. (ed.), Academic Press, New York and London, p.73 (1980)
2. Haynes, R. and Feeny, R. E. : Fractionation and properties of trypsin and chymotrypsin inhibitors from lima beans. *J. Biol. Chem.*, **242**, 5378 (1967)
3. Liener, I. E. : Legumes toxins in relation to protein digestibility. *J. Food Sci.*, **41**, 1076 (1976)
4. Brady, P. G., Vanniner, A. M. and Banwell, J. G. : Identification of the dietary lectin, wheat germ hemagglutinin, in human intestinal contents. *Gastroenterology*, **75**, 236 (1978)
5. His, H. and Sharon, N. : The biochemistry of plant lectins (phytohemagglutinins). *Annu. Rev. Biochem.*, **42**, 541 (1973)
6. Simpson, D. L., Thorne, D. R. and Loh, H. H. : Endogenous carbohydrate-binding proteins from vertebrate tissues : Functional role in recognition process. *Life Sci.*, **22**, 727 (1978)
7. Etzler, N. E. and Branstrator, M. L. : Differential localization of cell surface and secretory components in rat intestinal epitherium by use of lectins. *J. Cell Biol.*, **62**, 329 (1974)
8. Miyoshi, M., Nakabayashi, J., Hara, T., Yawata, T., Tsukamoto, I. and Hamaguchi, Y. : The lethal protein from Kintoki beans (*Phaseolus vulgaris*) identified as a lectin. *J. Nutr. Sci. Vitaminol.*, **28**, 255 (1982)
9. Jaffe, W. G. : Über phutotoxins aus Bohnene (*Phaseolus vulgaris*), *Arzneim. Frosch*, **12**, 1012 (1960)
10. Kimura, T., Satanchote, C. and Yoshida, A. : Effect of feeding of raw winged bean seeds on gastrointestinal function in rats. *J. Nutr. Sci. Vitaminol.*, **28**, 27 (1982)
11. Santidrian, S., Sobrini, F. J., Billo, J. and Larralde, J. : Guanidinoacetate methyltransferase activity in growing male rat fed on a raw field bean (*Vicia faba* L.) diet. *Enzyme*, **26**, 103 (1981)
12. Boedeker, E. C. and Boldt, D. H. : Effect of plant lectins on the binding of human intrinsic factor-vitamin B_{12} complex to isolated guinea pig brush border membranes. *Gastroenterology*, **68**, 866 (1975)
13. Putztai, A., Clarke, E. M. W., Grant, G. and Kin, T. P. : The toxicity of *Phaseolus vulgaris* lectins. Nitrogen balance and immunochemical studies. *J. Sci. Food Agric.*, **32**, 1037 (1981)
14. Pusztai, A., Vlarke, E. M. W. and King, T. P. : The nutritional toxicity of *Phaseolus vulgaris* lectins. *Proc. Nutr. Soc.*, **38**, 115 (1979)
15. Takiko, Y., Tomoko, H, Ikuyo, T. and Masamitsu, M. : Toxic effects of Kintoki bean lectin on cultured animal cells. *J. Nutr. Sci. Vitaminol.*, **29**, 65 (1983)
16. Seo, Y. J., Kamitani, S. and Miyoshi, M. : Purification and some properties of Taro tuber lectin. *Nippon Kasei Gakkaishi* (*J. Home Econ. Jpn*), **40**, 805 (1989)
17. Hamaguchi, Y., Yagi, N., Nishino, A., Mochizuki, T., Mizukami, T. and Miyoshi, M. : The isolation and characterization of a lethal protein from Kintoki bean (*Phaseolus vulgaris*). *J. Nutr. Sci. Vitaminol.*, **23**, 525 (1977)
18. Osawa, T. : Lectin. In "*Lectin*" (in Japanese) Osawa, T. and Mori, R. (eds.), Kodansha Press, Tokyo, p.1 (1976)
19. Moon, H. W. : Mechanisms in the pathogenesis of diarrhea. *J. Am. Veter. Med. Assoc.*, **172**, 443 (1978)
20. Ugolev, A. M. and Lay, P. D. : Membrane digestion. A concept of enzymic hydrolysis on cell membranes. *Biochim. Biophys. Acta.*, **300**, 105 (1973)
21. Lorenzsonn, V. and Olsen, W. A. : *In vivo* responses of rat intestinal epithelium to intestinal dietary lectins. *Gastroenterology*, **82**, 838 (1982)
22. Higuchi, M., Suga, M. and Iwai, K. : Participation of lectin in biological effects of winged bean. *Agric. Biol. Chem.*, **47**, 1879 (1983)

(Received June 28, 1993)

콩과 토란에서 추출한 FITC-Lectin의 마우스 소장조직에 대한 현미경 관찰

서 영 주
일본 나라여자대학교 인간문화연구과

요 약

렉틴투여 마우스의 소장을 고정절편으로 해서, HE 염색한 후 광학현미경관찰 및 조직을 반전고정해서 주사형전자현미경관찰을 하여, 소장점막의 미융모막의 변화를 대조군과 비교했다. 그 결과 소장융모의 팽윤, 단평화, 소장벽의 박약화, 상피세포의 밀도화 및 흐트러짐 등이 관찰되었다. 즉 렉틴이 정상적인 생체기능을 방해한다는 의미에서의 독활성이 있다는 것은, 소장조직에의 영양소흡수부전이 하나의 요인이 됨을 알수 있다.