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Binding of ^{3}H-Lectins from Kintoki Bean and Taro Tuber to Small Intestine of the Mouse

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Abstract

The major objective of this study carried out was to compare the binding of Kintoki bean lectin (KBL) and Taro tuber lectin (TTL) to the mouse intestinal segments using ^{3}H-labeled lectins and to assess the effect of such binding on the ability of the small intestine. Binding of ^{3}H-KBL or ^{3}H-TTL was studied under various conditions of time course, temperature, concentration, pH and additives of sugars, EDTA or unlabeled native lectin. The interaction of the lectins to intestinal tissue was stronger in KBL than in TTL, which was supposed to be the major reason for the stronger antinuritional effect of KBL. The optimal binding conditions were at 37°C for 60mins and at pH 7. The binding of both lectins were inhibited by fetuin and EDTA.

Key words : Kintoki bean lectin (KBL), Taro tuber lectin (TTL), binding amounts of lectins, ^{3}H-labeled lectin, small intestine

INTRODUCTION

Lectins have been studied principally for the interest regarding their physiological role in plants, but they may also be good sources of new plant lectins of unique carbohydrate-binding properties. From many reports, lectins are known to have severe effects on the small intestinal brush border and to induce hyperplasia in the gut[1,2]. They are also absorbed intact from the gut[3,4] and have systemic effects including the induction of cell degradation[5], a rapid depletion of body fat and glycogen and a reduction in plasma insulin[6], and an accelerated rate of protein loss leading to increased urinary urea excretion[7].

The implication of KBL and TTL in the role of naturally-occurring toxicants acting *via* the small intestine is now established, and recent works from our laboratory concerned with the nutritional toxicity and binding patterns have done much to indicate probable modes of action of these lectins in the mouse and rat. However, the majority of studies utilizing fluorescein isothiocyanate (FITC)-conjugated lectins have relied on semi-quantitative estimators of the labeling, and it is not known whether this accurately reflects quantitative differences in the expression of the binding of lectin to intestine.

In this study, an experiment has been conducted to determine quantitatively the occurrence of binding of ^{3}H-labeled KBL and TTL to normal mouse intestinal tissue. Also, we have compared their binding activities in order to explain the differences in toxicity between KBL and TTL. TTL is less toxic than KBL.

MATERIALS AND METHODS

Toxic lectins were isolated from the Kintoki bean and Taro tuber as previously described[8-10]. Lectins were labeled with ^{3}H by the method based on Hunter and Greenwood. The labeling was initiated with N-succinimidyl [2,3-^{3}H] propionate (Pavkard Co.) and halted by the removal of the reagent from the reaction mixture on gel filtration. Three hundred milligrams of each KBL or TTL was dissloved in 5ml of phosphatase buffered saline (PBS) for 5hrs at 5°C. Then the entire solution was centrifuged at 18,000 rpm for 10mins, and the supernatant was mixed with the ^{3}H-reagent for 2hrs at 5°C. The sample

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was then applied to a 2×30cm column of Sephadex G-50 gel. The radioactivity (cpm) was measured in a five-channel automatic liquid scintillation counter (Tri-curd 460 C by Pavkard, Japan) and from A_{280} determination the micromoles of lectin in the collected solution were calculated.

For the preparation of intestinal tissue, male mice of ddY strain, weighing about 30g, were killed. The small intestine was removed and cut into pieces approximately 2cm long. They were reversed, rinsed and freeze-dried as described previously.

For the calculation of the ^{3}H-lectin bound on intestine, actual experimental conditions were set, such as time course, temperature, pH and additives. The mixture of ^{3}H-lectin and reversed intestinal segments were adjusted to a final volume of 1ml. After binding time, the radioactivity of the supernatant was calculated in a Pavkard 460 counting system. The binding values were calculated as % of the working concentration of ^{3}H-lectin. The changes of binding amounts at different times (15, 30, 60, and 120mins) were read to obtain an optimal time and an optimal temperature (5° C, 37° C) for the binding of these lectins to the intestine. The differences in binding amounts between two parts of the intestine, 0~15cm and 15~30cm from pylorus, were also estimated. For the observation of binding changes at various ^{3}H-lectin concentrations, lectin solutions of 0.25, 0.5, 0.75, 1.0, 1.2, 1.6, 2.0, 3.5 and 5.0mg/ml were prepared and rinsed for the binding experiment in order to draw Scatchard plots. The most suitable pH was estimated with McIlvain's buffer solutions. In order to test the inhibition of ^{3}H-lectin binding, 100mg/ml concentration of sugars or EDTA was mixed with the same volume of ^{3}H-lectin solution, 0.5ml. Unlabeled lectins were also prepared at the concentrations of 0, 1, 10, 50 folds of each ^{3}H-lectin. Intestines were also treated with one of the unlabeled solutions before binding of ^{3}H-lectins was determined with the pretreated intestines.

RESULTS AND DISCUSSION

The KBL and TTL were purified on CM-cellulose. The recoveries of these lectins from starting materials were 1.2% for KBL and 0.014% for TTL. The amounts of the finally purified lectins of KBL and TTL required for the complete HA of 1ml of 1% mouse erythrocyte suspension were 4.15μg/ml and 15.23μg/ml respectively. Generally, the total binding patterns of ^{3}H-KBL and ^{3}H-TTL were somewhat similar to each other.

The results of the experiments involving intestinal binding amounts were presented in Fig. 1, which compared the bound amounts of ^{3}H-KBL and ^{3}H-TTL by two parts of intestinal segments up 120 mins. The time required for the complete binding was 60mins. Judging from the hemagglutinating activities of the contents in the gut, the lectins seemed to not be rapidly hydrolyzed *in vivo*[11~14]. Therefore, there must be time enough for the exhibition of their toxic functions before being inactivated in the digestive tract. It was considered that the ingested lectins must reveal their physiological the epithelial

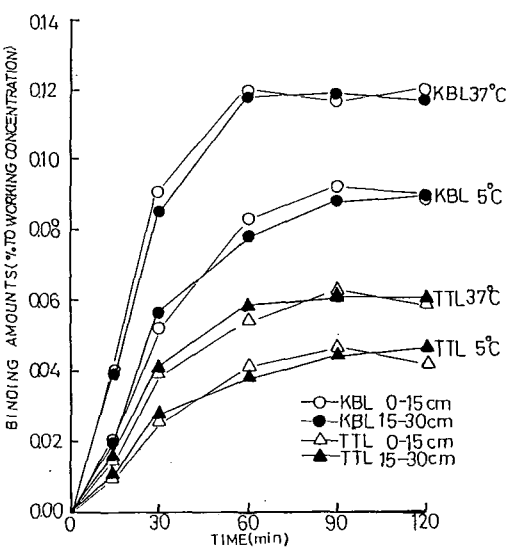

Fig. 1. Binding amounts of ^{3}H-KBL and ^{3}H-TTL to mouse intestine(time course and temperature).
The optimal time and temperature in the ^{3}H-lectin bind were calculated. It was 60min at 37° C. The binding to the tissue proceeded quickly and reached a plateau at 37° C after 60min, during which time the lectin must maintain its stability in the gut. Everted segments of the two different parts of the small intestine were estimated simultaneously. There were no differences statistically. TTL was a poor binder to intestine as compared to KBL. There were about 2 folds in the KBL and TTL ^{3}H-lectin binding.

cells before it was enzymatically degraded into the inactive forms. Fig. 2 provided the quantitative measurements of the binding amounts of the two ^{3}H-lectins to intestine at various lectin concentrations. The values provided the data for the construction of Scatchard plots. The Ka and binding amounts calculated from the graphs were shown in the footnotes of Fig. 2. When the degree of growth depression on mice fed KBL and TTL were compared, it was clear that KBL was a stronger antinutritional factor than TTL : mice given 10mg of KBL daily showed a growth retardation similar to that of mice given 30mg of TTL. It corresponded well with our previous reports[5,7~10]. Reasons for the difference in toxicity might be multiple. One was that TTL was more susceptible to digestive enzymes and hydrolyzed to inactive forms before it reached the target tissue on intestinal surface. The present experiment also presented another reason for the weaker toxicity of TTL by proving that the amount of bound TTL on mouse intestine was smaller, about one third that of KBL, than KBL. A good coincidence was observed between the two lectins in terms of their toxic intensities and their binding activities. In both categories, KBL was 3times stronger than TTL. The optimum pH in binding activity of KBL or TTL was pH 7, which was the pH of body fluid. On the other hand, their lectins agglutinated red blood cells in a highly pH-dependent manner (data not shown).

The KBL and TTL appeared to be specific toward fetuin and EDTA by hemagglutinating inhibition and fluorescein spectroscopy analysis[15, 16]. The binding of these ^{3}H-lectin was observed in the presence of inhibitors (Fig. 3). From these results, the predictive values towards the relationship between the degree of binding of lectins to intestinal tissues and the damaging effects on the intestinal epithelium could be easily interpreted. These lectins were valuable probes of carbohydrate structure that could be adopted to biochemical techniques for quantitative purposes. Lectins, due to their ability to

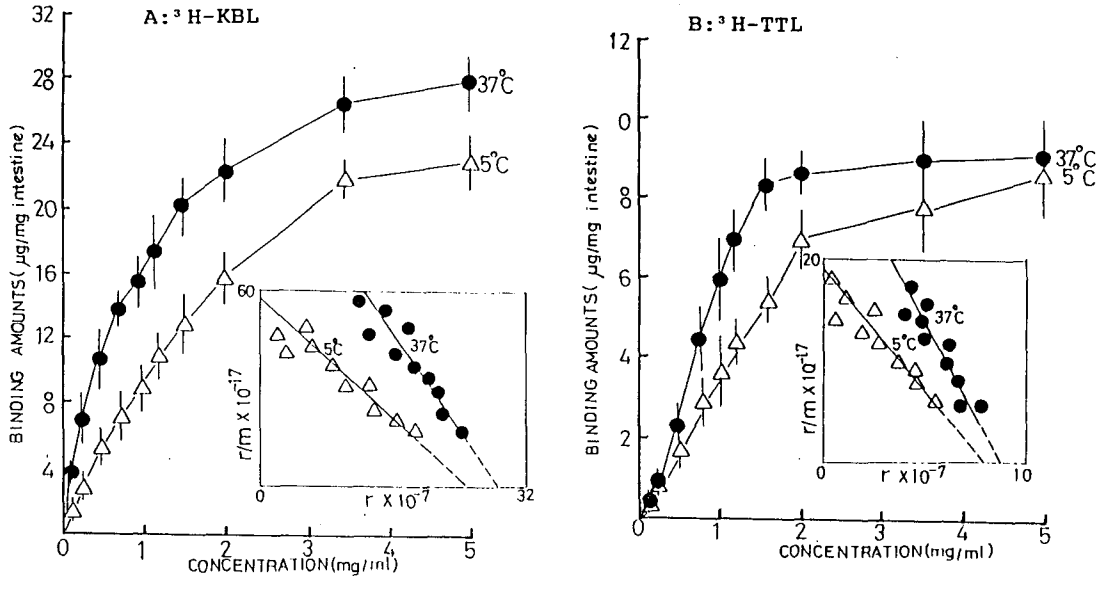

Fig. 2. Binding amounts of ^{3}H-KBL and ^{3}H-TTL to mouse intestine at various conditions and Scatchard plot for the binding amounts.

For the ^{3}H-lectin concentrations higher than 2mg/ml, the curve shows a tendency of saturation in TTL not show in KBL. It was also found that nonspecific binding increased with the increase of lectin concentrations. The plotting of the data according to Scatchard showed more clearly the cooperation of lectin binding. It shows these curves for ^{3}H-KBL and ^{3}H-TTL, at 5° C and 37° C, the shape of the plot was similar to each other. The association rate constants obtained for KBL($K_a = 2.39 \times 10^{10} M^{-1}$ at 5° C and $K_b = 4.13 \times 10^{10} M^{-1}$ at 37° C) and TTL($K_c = 2.7 \times 10^{10} M^{-1}$ at 5° C and $K_d = 4.07 \times 10^{10} M^{-1}$ at 37° C) are in close agreement, suggesting the binding of two lectins to intestinal villi by similar mechanism. The number of binding sites per mg intestine deduced from the plot are 24.7×10^{7}, 28.1×10^{7} for KBL and 7.89×10^{7}, 8.6×10^{7} for TTL, in order to 5° C and 37° C.

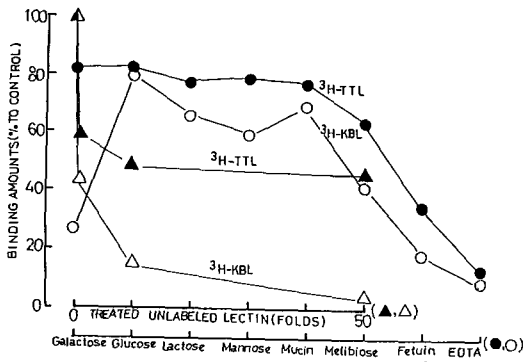

***Fig. 3.* Inhibitions of the binding amounts of ^{3}H-KBL and ^{3}H-TTL to mouse intestine by various sugars and EDTA (% to binding amounts of control).**
In addition to carbohydrates, the inhibitors exceeding 50% were galactose, melibiose and fetuin for KBL, but only fetuin for TTL. Fetuin was an inhibitor more than other sugars in both of all, leading to 81.3% inhibition for KBL and 64.5% inhibition for TTL. EDTA showed more obvious inhibition than fetuin. Almost all specially bound radioactivity could be released with EDTA. It was shown that cooperatibility is a function determined by the lectin itself and also by the characteristics of the cell membrane. In the additive native KBL or TTL, it can not be excluded in the interaction of intestinal villi during the binding procedure. The bind was more obviously interrupted in the case of KBL than TTL.

bind to cell-surface carbohydrates, have become widely used tools for exploring the structure and dynamics of cell surfaces[17]. An elucidation of the specificity and mechanism of saccharide binding to the lectins is necessary to understand the mechanism of their interaction with cell-surface receptors. Lectin-sugar interaction must be studied in more detail.

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콩과 토란에서 추출한 ^{3}H-Lectin의 마우스 소장에의 흡착량 정량

서 영 주

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요 약

렉틴의 항영양작용기구 해명의 하나로서 마우스 반전소장에의 흡착양상을, ^{3}H로 레벨한 렉틴을 가지고, 방사활성을 측정했다. 그 결과, 소장부위에의한 흡착량은 유의차가 없었고, 흡착포화시간은 60분간 37°C에서, 흡착량은 8.6×10^{7}/1mg intestine이 되고, 렉틴이 소장세포와 반응해서, 여러가지 항영양작용을 유도한다고 생각된다. ^{3}H 레벨한 렉틴 농도가 높아짐에 따라 흡착량은 크게되고, 2mg/ml에서 거의 포화에 달했다. pH 7부근에서 흡착량이 제일 크고, 생체내에서의 소장은 렉틴이 가장 흡착되기 쉬운 상태임을 알수있었으며 저해인자는 fetuin과 EDTA이었다.