Effect of Ingested Winged Bean Lectin on Gastrointestinal Function in the Rat

Toshizo Kimura, Shinobu Nakata, Yoji Harada, and Akira Yoshida

1Laboratory of Food and Nutrition, Department of Home Economics, Osaka Kyoiku University, Ikeda, Osaka 563, Japan
2Laboratory of Nutritional Biochemistry, Department of Agricultural Chemistry, Nagoya University, Chikusa-ku, Nagoya 464, Japan

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Summary The present study was undertaken to provide further evidence for mechanisms proposed for the toxicity of ingested winged bean lectin in animals: to determine its effect on activities of some hydrolases localized in the brush border membrane of the small intestine. An adaptive increase in sucrase activity of rats given a high-sucrose diet (HSD) was restrained by the addition to HSD of a lectin fraction (WBLF) isolated from raw winged beans but not by that of heated WBLF or soybean trypsin inhibitor. Restraining effects of WBLF added to HSD on time-course changes in activities of sucrase, alkaline phosphatase and leucine aminopeptidase of rats after giving HSD were similar to those of concanavalin A, which had been observed in the previous study. These results substantiate that the mechanism of the toxicity of ingested winged bean lectin involves its binding to the luminal surface of the small intestine and in turn disturbing the functional formation of the brush border membrane.

Key Words winged beans, lectin, small intestine, sucrase, alkaline phosphatase, leucine aminopeptidase

The poor nutritional properties of raw beans have been ascribed to the trypsin inhibitor, lectins and other toxic substances present (1–3). Jaffe and Korte (4) reported that trypsin inhibitor, amylase inhibitor and lectins are present in raw winged beans (Psophocarpus tetragonolobus) and that diets prepared with the raw beans are toxic to rats. In our previous study (5), the lectins or similar substances present in raw winged beans were suggested to be major factors in the toxicity.

Regarding the mechanisms involved in the toxicity of winged beans, Higuchi et
al.(6) proposed that the binding of the lectin to epithelial cells of the gastrointestinal tract might be the initial step of the induction of the adverse effects by lectin. We recently demonstrated(7) that the mechanisms of adverse effects of ingested concanavalin A (Con A) involve its binding to the surface of epithelial cells of the small intestine, whereby disturbance occurs in the functional formation of the brush border membrane, and that this might apply to gastrointestinal disorders observed with ingestion of other toxic bean lectins.

Thus, the purpose of this study was to verify the above-mentioned suggestion via observation of the effects of ingested lectins isolated from raw winged beans on activities of intestinal enzymes localized in the brush border membrane of the small intestine(8). The activity of these enzymes has been adopted as criteria for typical functioning of the brush border membrane.

MATERIALS AND METHODS

Animals and diets. Male rats of the Wistar strain (Shizudokyo, Shizuoka, Japan) weighing approximately 100 g were individually housed in suspended wire-mesh cages in a room kept at 21 to 24°C with a lighting period automatically regulated to provide a 12-h light period (08:00–20:00h) and a 12-h dark period (20:00–08:00h). Rats were fasted for 48 h before the start of the experiment. The composition of the basal diet (in percent) was as follows: casein, 10; salt mixture (9), 4; vitamin mixture (10), 2; corn oil, 5; choline chloride, 0.2; L-methionine, 0.3 and sucrose, 78.5. Retinol palmitate, 350 IU, ergocalciferol, 35 IU, and all-rac-α-tocopherol, 5 mg, were added to 100 g of the diet.

Isolation of lectin fraction from raw winged beans. The lectin fraction was isolated by the procedure of Honavar et al.(11), who isolated lectin fraction from raw kidney beans. One kg of the finely ground meal of dried raw winged beans, produced in the Philippines, containing 32.2% of protein (N×6.25), was suspended in 1% NaCl solution and agitated overnight at 4°C. The material that remained insoluble was removed by centrifugation, and the supernatant was adjusted to pH 4 with concentrated HCl. The precipitate found upon acidification was centrifuged off and discarded. A bentonite-celite mixture (95 g) (1:1) was added to the supernatant which served to absorb selectively the trypsin inhibitor used in this procedure. The suspension was stirred overnight at 4°C, and then filtered. The filtrate was brought to 0.75 saturation with (NH₄)₂SO₄ and the precipitate was collected by centrifugation and redissolved in one liter of distilled water. Precipitation of the protein with 0.75 saturated (NH₄)₂SO₄ was repeated three times, and the protein obtained after the third precipitation was dissolved in the minimum amount of water, dialyzed exhaustively, and finally lyophilized. The yield of the isolated winged bean lectin (WBLF) was 20.1 g. Hemagglutinating activity and antitryptic activity were determined by the method of Jaffé and Lette(12) and that of Lakowsky(13), respectively. Hemagglutinating activity per mg of WBLF was ten times as high as that per mg of protein in the crude protein fraction extracted with 1% NaCl. No
antitryptic activity in WBLF was detected.

Experiment 1. Rats were divided into 5 groups. Rats in group 1 were killed before refeeding. Those in groups 2 and 4 were given 5 g of the basal diet mixed with 100 mg of casein, the control diet, or with 100 mg of WBLF, the WBLF diet, respectively, and were killed 24 h later. Rats in groups 3 and 5 were given ad libitum the control diet and WBLF diet, respectively, and were killed 24 h later. The small intestine was rapidly removed, and a jejunal segment representing the second 15 cm segment from the pylorus was excised. The segment was slit longitudinally after rinsing with cold saline and then homogenized by an Ultra Turrax homogenizer (Ika Werk, West Germany). The resulting homogenate was used for determination of sucrase activity.

Experiment 2. Rats were divided into 3 groups. Rats in groups 1 and 2 were given 5 g of the control diet, and were killed 25 h later. The animals in group 1 received no additional food before being killed, but those in group 2 were allowed free access to the WBLF diet for 5 h before being killed. Rats in group 3 were given 5 g of the WBLF diet and allowed free access to the control diet for 5 h before being killed, though they were killed 25 h after being given the WBLF diet. The small intestine was treated as described in preceding experiment for determination of sucrase activity.

Experiment 3. Rats were divided into 5 groups. The animals in group 1 were killed before refeeding. Rats in the other groups were given 5 g of the control diet, WBLF diet or the basal diet mixed with 100 mg of WBLF heated at 120°C for 60 min, the heated WBLF diet, or 100 mg of soybean trypsin inhibitor (type II-2, Sigma Chemical, St. Louis, USA), the TI diet, respectively, and were killed 24 h later. The small intestine was prepared in the manner described in preceding experiments for determination of sucrase activity.

Experiment 4. Rats were divided into 7 groups. The animals in group 1 were killed before refeeding. Rats in groups 2 to 4 were given 5 g of the control diet, and those in groups 5 to 7 were given 5 g of the WBLF diet. One groups each was selected from groups 2 to 4 and from 5 to 7 and rats were killed 8, 16 or 24 h after being given the respective diets. The small intestine was treated as described in experiment 1 for determination of enzyme activities.

Analytical procedure. Sucrase activity was determined by the method of Dahlqvist (14), alkaline phosphatase activity was measured by the method of Kind and King (15), and leucine aminopeptidase activity was assayed by the method of Goldberg and Rutenburg (16). The protein content of the small intestine was determined by the method of Lowry et al. (17) using bovine serum albumin as a standard.

Statistical analysis. Statistical analysis was done by Student’s “t” test and the least significant difference calculated according to the method of Snedecor and Cochran (18).
RESULTS AND DISCUSSION

Intestinal sucrase, alkaline phosphatase and leucine aminopeptidase are localized in the brush border membrane of the mucosa in the small intestine (8). Many investigators have reported that rats manifest adaptive responses of these enzyme activities to dietary nutrients (19–26). Thus, the measurement of these enzyme activities has been adopted as criteria for functional disturbances in the brush border membrane. The length of the small intestine was little altered by the diet, whereas its weight altered significantly owing to changes in mucosal weight (27,28). In the present study, enzyme activity is expressed in terms of micromoles of substrate hydrolyzed per unit length of the small intestine (designated as the segmental activity), which is considered to be more suitable than the conventional method of expressing micromoles of substrate hydrolyzed per mg protein in the small intestine, the specific activity.

As shown in Fig. 1 (experiment 1), intestinal sucrase activity in rats 24 h after being given restricted or unrestricted amounts of the control diet increased to a level about two times as high as that in those before refeeding, although there was definite difference in the amount of food consumed between restricted feeding, 5 g, and ad libitum feeding, 9.1 ± 0.1 g. These results confirmed the data reported earlier from our laboratory that a significant increase in sucrase activity owing to feeding a high sucrose diet was unaffected by the amount of food consumed by animals (21,25). On the other hand, ingestion of the WBLF diet regardless of the

![Fig. 1](image-url)
Table 1. Effect of winged bean lectin fraction (WBLF) added to a basal diet on intestinal sucrase activity in rat\(^1\) (experiment 2).

<table>
<thead>
<tr>
<th>1st fed diet</th>
<th>Basal +2% casein</th>
<th>Basal +2% casein</th>
<th>Basal +2% WBLF</th>
</tr>
</thead>
<tbody>
<tr>
<td>2nd fed diet (^2)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body weight</td>
<td>g</td>
<td>82.4 ± 1.6(^a)</td>
<td>82.2 ± 0.4(^a)</td>
</tr>
<tr>
<td>Food intake</td>
<td>1st</td>
<td>g</td>
<td>5.0</td>
</tr>
<tr>
<td></td>
<td>2nd</td>
<td>g</td>
<td>—</td>
</tr>
<tr>
<td>Small intestine (SI)</td>
<td>mg/g SI</td>
<td>500 ± 15(^a)</td>
<td>523 ± 5(^ab)</td>
</tr>
<tr>
<td>Intestinal protein</td>
<td>mg/g SI</td>
<td>147 ± 6.6(^a)</td>
<td>152 ± 4.4(^a)</td>
</tr>
<tr>
<td></td>
<td>mg/segment (^4)</td>
<td>73.8 ± 4.7(^a)</td>
<td>79.2 ± 1.7(^a)</td>
</tr>
<tr>
<td>Sucrase activity</td>
<td>U(^5)/g SI</td>
<td>960 ± 102(^a)</td>
<td>896 ± 114(^a)</td>
</tr>
<tr>
<td></td>
<td>U/mg protein</td>
<td>6.53 ± 0.56(^a)</td>
<td>5.90 ± 0.78(^a)</td>
</tr>
<tr>
<td></td>
<td>U/segment</td>
<td>480 ± 54(^a)</td>
<td>464 ± 59(^a)</td>
</tr>
</tbody>
</table>

\(^1\) Rats after a 48 h fast were given 5 g of the 1st diet, and were killed 25 h later. \(^2\) The 2nd diets were administered for 5 h before killing. \(^3\) Mean ± SE (n=4); means in the same row not sharing a common superscript letter are significantly different (p<0.05). \(^4\) Second 15 cm segment from the pylorus. \(^5\) Micromoles of sucrose hydrolyzed per hour.

amount of food consumed restrained the adaptive increase in sucrase activity owing to dietary sucrose. This restraining effect of WBLF added to a high-sucrose diet on intestinal sucrase activity is common to that of Con A added to the same diet, which has been previously reported by Nakata and Kimura (7).

The results of experiment 2 are shown in Table 1. Sucrase activity in rats 25 h after being given 5 g of the control diet was unaffected by the delayed feeding of the WBLF diet, which was resumed 20 h later, whereas the restraining effect of WBLF diet feeding on sucrase activity was not ameliorated by delayed feeding of the control diet. We previously reported that the adaptive changes in sucrase activity of rats given a high sucrose diet were prevented by the addition of Con A to the diet, but were unaffected by the delayed feeding of the diet containing Con A (7). These findings have excluded the possibility that ingested Con A might bring about the luminal loss of the brush border membrane owing to ingested Con A (29). The results of the present experiment strongly suggest that the effect of ingested WBLF on intestinal sucrase activity might be compatible with that of ingested Con A (7).

Table 2 shows the results of experiment 3. As observed in experiment 1, sucrase activity in rats 24 h after being given the control diet was significantly increased as compared with that in rats before refeeding, the increase being prevented by replacing supplemented casein with WBLF in the diet. However, the heated WBLF diet or TI diet refeeding in rats after a 48 h fast had no effect on the adaptive increase in sucrase activity owing to dietary sucrose. These results reproduce the adaptive changes observed in sucrase activity of rats given a high-sucrose diet,
Table 2. Effect of winged bean lectin fraction (WBLF) or soybean trypsin inhibitor (TI) added to a basal diet on intestinal sucrase activity in rats (experiment 3).

<table>
<thead>
<tr>
<th>Diet</th>
<th>Before refeeding</th>
<th>Basal + 2% casein</th>
<th>Basal + 2% WBLF</th>
<th>Basal + 2% heated WBLF</th>
<th>Basal + 2% TI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body weight</td>
<td>g</td>
<td>91.5 ± 0.6</td>
<td>92.5 ± 2.3</td>
<td>92.3 ± 1.2</td>
<td>92.3 ± 1.4</td>
</tr>
<tr>
<td>Small intestine (SI)</td>
<td>mg/segment</td>
<td>406 ± 24</td>
<td>478 ± 14</td>
<td>466 ± 13</td>
<td>470 ± 15</td>
</tr>
<tr>
<td>Intestinal protein</td>
<td>mg/g SI</td>
<td>165 ± 2.4</td>
<td>166 ± 3.7</td>
<td>169 ± 5.9</td>
<td>177 ± 4.6</td>
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<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>mg/segment</td>
<td>66.9 ± 2.6</td>
<td>79.2 ± 3.5</td>
<td>78.3 ± 0.6</td>
<td>83.0 ± 3.1</td>
</tr>
<tr>
<td>Sucrase activity</td>
<td>U/g SI</td>
<td>753 ± 63</td>
<td>1,102 ± 52</td>
<td>718 ± 27</td>
<td>949 ± 56</td>
</tr>
<tr>
<td></td>
<td>U/mg protein</td>
<td>4.59 ± 0.44</td>
<td>6.6 ± 0.43</td>
<td>4.28 ± 0.25</td>
<td>5.39 ± 0.29</td>
</tr>
<tr>
<td></td>
<td>U/segment</td>
<td>308 ± 37</td>
<td>525 ± 19</td>
<td>338 ± 18</td>
<td>437 ± 27</td>
</tr>
</tbody>
</table>

1 Rats after a 48 h fast were killed before refeeding. 2 Rats after a 48 h fast were given 5 g of the diet, and were killed 24 h later. 3 Heated at 120°C for 60 min. 4 Mean ± SE (n = 4); means in the same row not sharing a common superscript letter are significantly different (p < 0.05). 5 Second 15 cm segment from the pylorus. 6 Micromoles of sucrose hydrolyzed per hour.
Effect of winged bean lectin fraction (WBLF) added to a high-sucrose diet on time-course changes in intestinal enzyme activities of rats (experiment 4). Rats were given 5g of a high-sucrose diet mixed with 100 mg of casein (○) or 100 mg of WBLF (●), respectively, after a 48-h fast. These enzyme activities are expressed as a percentage of the activities in rats before refeeding. In rats before refeeding, these enzyme activities (micromoles of substrate hydrolyzed per hour in the second 15 cm segment second from the pylorus) were as follows; sucrase activity, 220 ± 35; alkaline phosphatase activity, 1,968 ± 101; leucine aminopeptidase activity, 198 ± 12. Vertical bars represent the SE of the mean for five rats. Values without common letters are significantly different at p < 0.05.

As demonstrated in the preceding experiments, and support the suggestion that the lectins present in raw winged beans are major factors in toxicity owing to ingestion of the raw beans (5).

As shown in Fig. 2 (experiment 4), intestinal sucrase activity of rats given the control diet remained constant for 16h, and increased considerably by 24h. In contrast to time-course changes in sucrase activity, alkaline phosphatase and leucine aminopeptidase activities markedly increased within several hours. However, adaptive changes observed in these enzyme activities of rats given a high-sucrose diet were prevented by the addition of WBLF to the diet. The time-course
changes in the hydrolase activities in rats given the high-sucrose diet were similar to those observed in the previous study (7), this restraining effect of WBLF diet feeding on enzyme activities being consistent with that of the Con A diet feeding (7).

Higuchi et al. (6) have reported that hemagglutinating activity was detected in the intestinal mucosa and feces of rats ingesting winged bean lectin, which is resistant to digestive enzymes. Our previous study (7) demonstrated that: 1) Ingested Con A is unaltered during its passage through the gastrointestinal tract and is rapidly excreted. 2) The mechanisms of the adverse effect of ingested Con A involve its binding to the surface of epithelial cells of the small intestine, as has been shown by Padolsky and Weiser (30), whereby disturbance occurs in the functional formation of the brush border membrane. 3) The toxicity of ingested Con A might apply to gastrointestinal disorders observed with ingestion of other toxic bean lectins.

The effects of WBLF, observed in the present study, added to the high sucrose diet on the hydrolase activities of enzymes localized in the brush border membrane of the small intestine were similar to those of Con A observed in the previous study (7).

Accordingly, the following conclusion may be drawn: the mechanisms of the adverse effect of ingested WBLF involve its binding to the surface of epithelial cells in the small intestine, as has been demonstrated by Higuchi et al. (6), whereby disturbance occurs in the functional formation of the brush border membrane.

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REFERENCES


