Mechanisms of Adverse Effect of Amaranth Feeding in the Rat

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Summary In order to clarify the mechanism of the adverse effects of dietary amaranth, trisodium 1-(4-sulfo-l-naphthylazo)-2-naphthyl-3,6 disulfinic acid, the effects of amaranth in vitro and in a jejunum perfusion in vivo on intestinal sucrase were investigated in rats. The inhibitory effect of amaranth in vitro on the sucrase activity was not detected even at the concentration of 1%, whereas the remarkable release of intestinal sucrase from intestine was observed with the jejunum perfusion of Ringer bicarbonate solution (RBS) containing amaranth at the 1% level. On the other hand, the perfusion of RBS containing tris(hydroxymethyl)aminomethane, a strong inhibitor of intestinal disaccharidase activities, did not produce the release of intestinal alkaline phosphatase. These findings suggest that the toxicity of dietary amaranth is due to the exfoliating or solubilizing effects of amaranth on the brush border membrane of the small intestine.

Key Words small intestine, sucrase, perfusion, amaranth, tris(hydroxymethyl)aminomethane

Ershoff (1) and Ershoff and Marshall (2) reported that toxicities, significant growth retardation and severe diarrhea of non-ionic surface-active agents added to a highly purified diet were counteracted by the concurrent feeding of dietary fiber. In the previous studies (3–6) we reported that the mechanisms were the exfoliating or solubilizing effects of these detergents in the brush border membrane of the small intestine, and that the dietary fiber prevented these effects. The toxicities of amaranth feeding as well as those of non-ionic surface-active agent feeding were prevented by the concurrent feeding of dietary fiber (7, 8). However, the mechanism of the toxicities caused by amaranth feeding remains unclear, although we suggested

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that it was mainly due to the exfoliating or solubilizing effects of amaranth on the brush border membrane, not being due to the inhibition of enzyme activities involved in the membrane digestion (3).

The present study was undertaken to elucidate the mechanism of the toxicity caused by amaranth feeding from the experimental observations as follows; 1) the effect of amaranth in vitro on intestinal sucrase activity, which was localized in the brush border membrane (9), being considered to have important nutritional significance (10), and 2) the releasing effect of amaranth and tris(hydroxymethyl)aminomethane (Tris), a strong inhibitor of intestinal disaccharidase activities (11, 12), on the brush border membrane in a jejunum perfusion in vivo.

MATERIALS AND METHODS

Animals. Male rats of the Wistar strain (Shizudokyo, Shizuoka, Japan) weighing approximately 120 g were used.

Effects of amaranth on intestinal sucrase activity in vitro. The enzyme preparation was the homogenate of rat jejunum, which was made by the same method used in the previous studies (4, 5). The maximum concentration, 1%, of amaranth in the incubation medium for the determination of sucrase activity corresponded with that in the lumen of the small intestine in rats fed on the diet containing a toxic dose of amaranth (3). Sucrase activity was assayed according to the method of Dahlqvist (13). In order to avoid color interference due to amaranth in the incubation solution, Sanfix 555 (Sanyo Chemical Industry Co., Ltd., Kyoto, Japan), a cationic surface-active agent, was used. Prior to the determination of glucose by the glucose oxidase method, Sanfix 555 was added into the solution to form a precipitable Sanfix 555-amaranth complex, which was removed by centrifugation.

Effects of amaranth and Tris on release of intestinal sucrase and alkaline phosphatase in jejunum perfusion in vivo. The animals fasted with access to water overnight were anesthetized with pentobarbital sodium (5 mg/100 g body weight). A proximal jejunal segment (about 10 cm length) was perfused with Ringer bicarbonate solution (RBS) (14) at a constant rate (0.5 ml/min). Perfusion continued for 150 min, allowing 30 min for equilibration and 120 min for collection of the perfusate in 30 min aliquots. The control group was perfused with RBS, and the experimental group was perfused with RBS containing 1% amaranth or 0.25% Tris. The concentrations of amaranth or Tris added into RBS were one-fifth of the toxic levels of these chemicals added to a high sucrose diet (3, 10), since these chemicals in the diet were diluted one-half to one-tenth in the lumen of the small intestine with drinking water and secreting water (4). Intestinal sucrase or alkaline phosphatase activities in the resulting perfusate were adopted as the criteria for exfoliation or solubilization of the brush border membrane. Sucrase activity was assayed by the method mentioned above, and alkaline phosphatase activity was determined by the method of Kind and King (15).

Statistical analysis. Statistical analysis was done by Student's "t" used to
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determine significant differences between treatment means (16).

RESULTS AND DISCUSSION

Effects of amaranth on intestinal sucrase activity in vitro

Figure 1 shows changes in optical density at 420 nm caused by removing of amaranth with the addition of Sanfix 555 into the solution. The minimum optical density was so sufficiently low and thus, Sanfix 555 was available for the determination of glucose with the glucose oxidase method. In addition, it was confirmed that Sanfix 555 did not affect the determination of glucose formed by the enzyme reaction in a preliminary experiment. Figure 2 represents the relationship between the sucrase activity and the concentration of amaranth in the incubation solution, indicating that the sucrase activity was not affected by amaranth in the

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Fig. 1. Changes in optical density at 420 nm with removal of amaranth by Sanfix 555.

Fig. 2. Effect of amaranth on activity of intestinal sucrase in vitro.

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incubation solution. The effects of amaranth on intestinal alkaline phosphatase and leucine aminopeptidase activities in vitro could not be determined. However, it has been reported that there were no relationships between the inhibitory effects of several kinds of chemicals in vitro on these enzyme activities and the toxic effects of these chemicals in the diet on gastrointestinal function (4, 5). From these findings, the toxicity of dietary amaranth was demonstrated to be produced with other effects than the inhibitory effect on intestinal enzyme activities.

**Effects of amaranth and Tris on release of intestinal sucrase and alkaline phosphatase in jejunum perfusion in vivo**

Figure 3 shows changes in the release of sucrase from jejunum into the perfusate. The release of sucrase in the control group lasted at a constant rate for 120 min after the first 30 min RBS perfusion. The constant liberation rate of sucrase activity was corresponding to the physiological cell desquamation (6, 17). The releasing patterns of maltase and alkaline phosphatase as well as protein in the control group were exactly similar to that of sucrase (6). The sucrase activity in the second 30 min RBS-perfusate was adopted as a standard for the relative activities of sucrase released by RBS and amaranth-RBS perfusion for the last 90 min. The presence of amaranth at the 1% level in RBS gradually increased the release of sucrase during all periods. The increased release of sucrase in amaranth-RBS group

![Graph](image)

**Fig. 3.** Time course of release of sucrase from rat jejunum by perfusion of amaranth. The jejunum was perfused with Ringer bicarbonate solution (RBS) for 60 min, and then followed with RBS containing 1% amaranth for 90 min. The relative activity of sucrase in perfusate is shown as the ratio to the activity released with RBS perfusion in the second 30 min, as 100. ○, RBS (control). ●, RBS+1% amaranth. These symbols located at a half period in 30 min aliquots. Vertical bars represent SE of the mean for 6 rats.
Fig. 4. Time course of release of alkaline phosphatase from rat jejunum by perfusion of tris(hydroxymethyl)aminomethane (Tris). The jejunum was perfused with Ringer bicarbonate solution (RBS) for 60 min, and then followed with RBS containing 0.25% Tris for 90 min. The relative activity of alkaline phosphatase in perfusate is shown as the ratio to the activity released with RBS perfusion in the second 30 min, as 100. ○, RBS (control). ●, RBS + 0.25% Tris. These symbols located at a half period in 30 min aliquots. Vertical bars represent SE of the mean for 6 rats.

was 2 to 5 times as compared with the release of sucrase in the control group. The release of sucrase with amaranth-RBS perfusion was not so significant as compared with that of sucrase with sodium taurocholate- and sodium deoxycholate-RBS perfusion (6), but was more remarkable than that of sucrase with Tween 20- and Tween 60-RBS perfusion (6). Figure 4 shows the release of alkaline phosphatase activity with Tris-RBS perfusion at the 0.25% level, at which the sucrase activity in vitro was completely inhibited by Tris (10). The release of alkaline phosphatase as well as sucrase with RBS perfusion shown in Fig. 3 stayed at a constant level during 30 to 150 min after the beginning of RBS perfusion. There was no difference in the releasing pattern of alkaline phosphatase activity between RBS perfusion and Tris-RBS perfusion.

These results demonstrated that the primary effect of the toxicity of amaranth feeding was not the inhibition of intestinal sucrase activity caused by dietary amaranth, but the exfoliation or solubilization of the brush border membrane due to dietary amaranth. However, we could not technically find a way to prevent the release of intestinal sucrase by the presence of dietary fiber in amaranth-RBS perfusion. As reported in the previous studies (4, 5), the toxicity of Tween 20 or Tween 60 feeding and the significant release of intestinal enzyme activities with Tween 20-RBS perfusion were counteracted by the addition or the presence of a small amount of dietary fiber to the diet or in the perfusion. However, in spite of the fact that sodium taurocholate and sodium deoxycholate had no inhibitory effect on intestinal sucrase activity in vitro, the toxicity of feeding of these chemicals could not be prevented by the concurrent feeding of a large amount of dietary fiber. It indicated that differences between the adverse effects of non-ionic and anionic surface-active agents depended on the degree of exfoliation or solubilization of the brush border membrane of the epithelial cells of the small intestine. The adverse effects of amaranth feeding could be counteracted by the concurrent feeding of a
large amount of dietary fiber (3, 7, 8), and the inhibitory effect of amaranth on intestinal sucrase activity was not found in the present study. In consideration of these findings, the primary cause of the toxicity of amaranth feeding is suggested to be a moderate degree of exfoliating or solubilizing effects of dietary amaranth on the brush border membrane. On the other hand, inhibition of intestinal sucrase activity caused by Tris feeding, which was not accompanied with a significant exfoliation or solubilization of the brush border membrane, brought about severe gastrointestinal disturbance (10). These findings lent further support to the suggestion that intestinal sucrase played an important role in nutritional significance.

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