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Induction and Inhibition of Indole Production of Intestinal Bacteria

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The fecal tryptophanase activities were 0.267±0.10 for rats and 0.185±0.01 μmole/min/g wet feces for humans. The activities of indole pyruvate degradation to indole, indole pyruvate lyase, of these feces were 0.051±0.02 and 0.046±0.01 μmole/min/g wet feces, respectively. The optimal pH values of tryptophanase and indole pyruvate lyase were 5.5-7.5 and 5.5-6.5, respectively. When the intestinal flora or *E. coli* HGU-3 was cultured in GAM broth having six different pH values (5 to 10), the activities of tryptophanase and indole pyruvate lyase in the medium adjusted at pH 6 were dramatically induced by elevating the pH to 9. However, when intestinal microflora were inoculated in the medium containing lactulose, the productions of these enzymes were dramatically inhibited and the pH of the medium was lower than that of the control.

Key words : Indole, Tryptophanase, Indole pyruvate lyase, *E. coli*, Intestinal bacteria

INTRODUCTION

Several metabolites of the essential amino acid tryptophan, such as indole, L-kynurenine, acetylkynurenine, 3-hyroxyanthranilic acid and quinalic acid, have been reported to be carcinogenic in the bladder of rats (Drasar and Hill, 1974: Dunning *et al*, 1950). Formation of indole and other tryptophan metabolites in the gastrointestinal tract could be important in the etiology of cancer (Chung, 1975: DeMoss and Moser, 1969: Finegold and Flora, 1975). Epidemiological studies suggest that dietary factors, such as high animal fat and protein, are prime factors in etiology of colon cancer (Goldin and Gorbach, 1976: Reddy and Wynder, 1977). Intestinal bacteria may play an important role in liberating active key intermediates, indole and kynurenine, with chemical carcinogens inducing colon tumors in experimental animals (DeMoss and Moser, 1969: Hoch and DeMoss, 1965: Botsford and MeMoss, 1972). Indole was produced by tryptophanase which catalyzes the degradation of tryptophan. Tryptophanase was produced by normal intestinal flora and tryptophanase of many bacteria was induced by tryptophan or meat diet (Hoch and DeMoss, 1965).

Recently, we discovered that β-glucuronidase and β-glucosidase of human intestinal microflora were pH-inducible: these enzymes were induced by high pH (Kim *et al.*, 1992: Kim *et al.*, 1994). Also, a high stool pH may induce these enzymes of human intestinal bacteria and be related to the incidence of colon cancer. This result could support that the population with alkaline fecal pH may have higher occurence of colon cancer than those with acidic fecal pH (Thornton, 1982: Samelson *et al.*, 1985).

We extended our study to elucidate what could induce the indole production and tryptophanase activity in the intestine.

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MATERIALS AND METHODS

Materials

Indole, dimethylaminobenzaldehyde, pyridoxal 5-phosphate and indole 3-pyruvate were purchased from Wako Pure Chemical Co., Ltd., Japan. Tryptophan was from Sigma Chemical Co., U.S.A. General anaerobic medium (GAM) was from Nissui Pharmaceutical Co., Ltd., Japan.

Fecal sample preparation for the assay of enzyme activity

Fresh feces were collected from three healthy men (twenties, 60-70 kg) and pooled. Fresh feces from male rats (SDD Wister, 180-200 g) were collected and pooled. Each feces was immediately suspended in 10ml of 25 mM phosphate buffer at pH 7.0. The suspended samples were centrifuged at 3000×g for 5

min. and the supernatants were used for the enzyme solution. Human and rat intestinal microflora and the tryptophanase-positive bacteria, *E. coli* HGU-3, were cultured in GAM (for the experiment involving the enzyme induction by tryptophan, its final concentrations were 0, 0.3 mg/ml and 1.0 mg/ml), centrifuged at 6000×g for 20 min. and suspended in 10 ml of 25 mM phosphate buffer at pH 7.0. These were used as the enzyme solution. Sample suspension and subsquent manipulation were performed at 4°C.

Enzyme assay

Tryptophanase activity was determined as follows (Chung *et al.*, 1975): 0.4 ml of reaction mixture (2.75 mg pyrophosphate, 19.6 mg disodium EDTA dihydrate and 10 mg bovine serum albumin in 100 ml of 0.05 M potassium phosphate, pH 7.5), 0.4 ml of 0.02 M L-tryptophan and 0.1ml of the enzyme solution were incubated at 37°C for 30 min. The reaction was stopped by the addition of 0.1 ml of 60% trichloroacetic acid and the precipitated protein was removed by centrifugation at 5000×g for 10 min. The supernatant was assayed for indole.

The activity of indole pyruvate degradation to indole, indole pyruvate lyase, was determined as follows: 0.4 of reaction mixture, 0.24 ml of 0.02 M indole pyruvate and 0.2 ml of the enzyme solution were incubated at 37°C for 30 min. The reaction was stopped by the addition of 0.5 ml of 60% trichloroacetic acid and the precipitated protein was removed by centrifugation at 5000×g for 10 min. The supernatant was assayed for indole.

Indole was determined as follows: 0.5 ml of the supernatant was assayed colorimetrically for indole with 2.0 ml of the color reagent (14.7g p-dimethylaminobenzaldehyde, 52 ml H_2SO_4 and 948 ml of 95% ethanol).

RESULTS

Fecal tryptophanase and indole pyruvate lyase

The fecal tryptophanase activities were 0.267±0.10 for rats and 0.185±0.01 μmole/min/g wet feces for humans. The activity of indole pyruvate degradation to indole, indole pyruvate lyase, of these feces was 0.051±0.02 and 0.046±0.01 μmole/min/g wet feces, respectively. These enzyme activities were reduced in rats by oral administration of antibiotics, but after stopping the administration of antibiotics, the enzyme activities were restored within two weeks. These results indicated that the majority of these enzymes were originated from intestinal bacteria, not from intestinal membrane.

The pH-profiles of these enzyme activities are shown in Fig. 1. The optimal pH of tryptophanase and indole pyruvate lyase was 5.5-7.5 and 5.5-6.5, respectively.

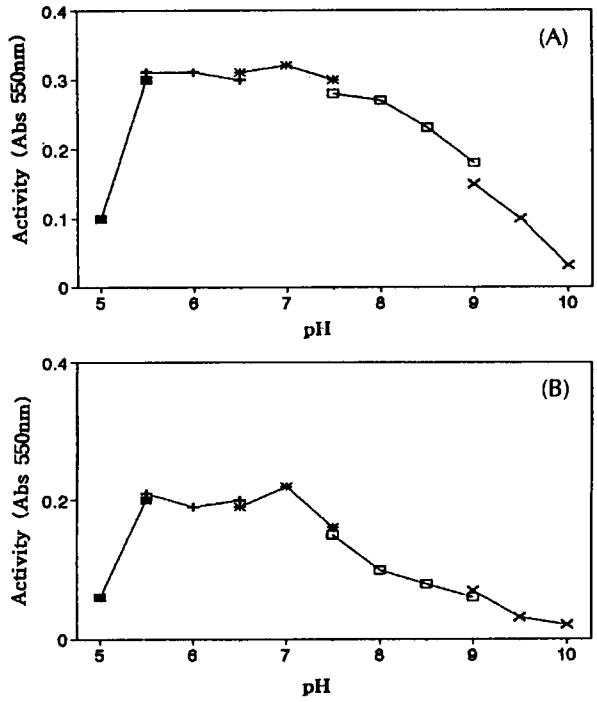

Fig. 1. pH profiles of tryptophanase (A) and indole pyruvate lyase (B) activities of human feces. Buffer used : pH 5-5.5, 0.1 M acetate buffer; pH 5.5-6.5, 0.1 M phosphate buffer; pH 6.5-7.5, 0.1 M phosphate borate buffer; pH 7.5-9, 0.1 M tris-HCl buffer; pH 9-10, 0.1 M NaOH-glycine buffer.

Induction of tryptophanase and indole pyruvate lyase by alkaline pH

When the intestinal microflora of human were cultured in GAM broth having six different pH values (5 to 10), their growth was unchanged at the different pH (Fig. 2). However, the activities of tryptophanase and indole pyruvate lyase were increased by elevating pH of the medium to 8-9 and the activities of these enzymes in the medium adjusted at pH 6 were dramatically increased 21-fold and 7.5-fold by the elevating the pH of the medium to pH 9, respectively.

When the rat intestinal flora were cultured in GAM broth having six different pH values (5 to 10), their growth were also unchanged at the different pH. However, the activities of tryptophanase and indole pyruvate lyase in the medium adjusted at pH 6 were increased to 22-fold and 6.1-fold by elevating the pH to 9, respectively.

Tryptophanase and indole pyruvate lyase of *E. coli*

When human tryptophanase-positive bacterium, *E. coli* HGU-3, was cultured in GAM broth having six different pH values (5 to 10), their growth were unchanged at the different pH (Fig. 3). However, the activities of tryptophanase and indole pyruvate lyase

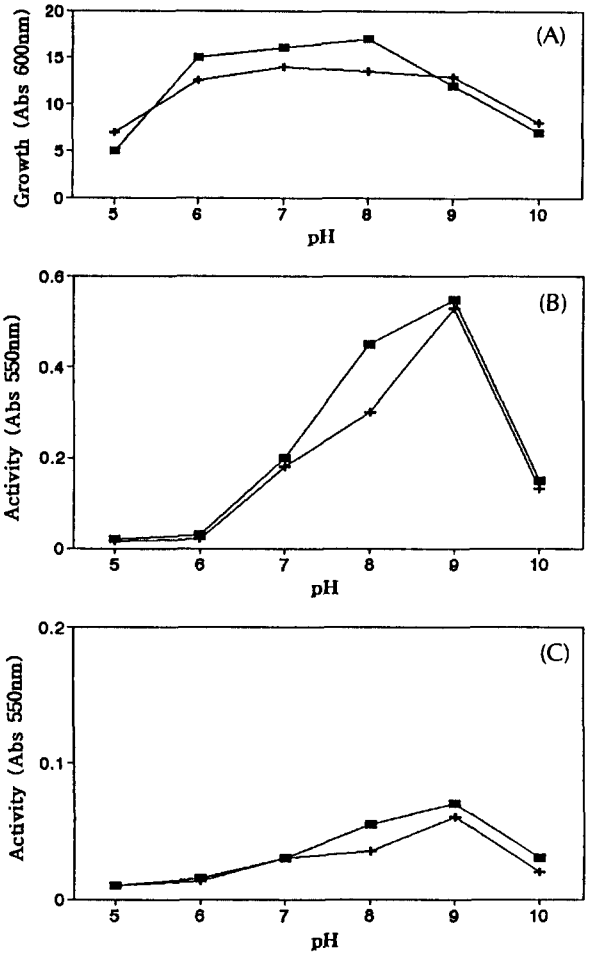

Fig. 2. Effect of pH of media on tryptophanase and indole pyruvate lyase of intestinal bacteria of human (■) and rats (+): Fresh feces of healthy men were inoculated into the media adjusted at various pHs and cultured for 24h under anaerobic conditons. (A), Growth; (B), tryptophanase activities ; (C), indole pyruvate lyase activities.

were increased by elevating pH of the medium to 9 and the activities of these enzymes in the medium adjusted at pH 6 were dramatically increased 4.8-fold and 2.6-fold by the elevating the pH values of the medium to pH 9, respectively.

The pH-profiles of these enzyme activity are shown in Fig. 4. The optimal pH values of tryptophanase and indole pyruvate lyase were 6-7 and 6, respectively.

Whether the enzyme of the intestinal bacteria or *E. coli* HGU-3 was induced by tryptophan or not was investigated. Tryptophanase of the intestinal bacteria of humans and rats was significantly induced to 3.2-fold and 2.8-fold by adding 0.5 M of the tryptophan to the medium, respectively. By the induction of tryptophanase, indole production was increased.

Inhibition of tryptophanase and indole pyruvate lyase by lactulose

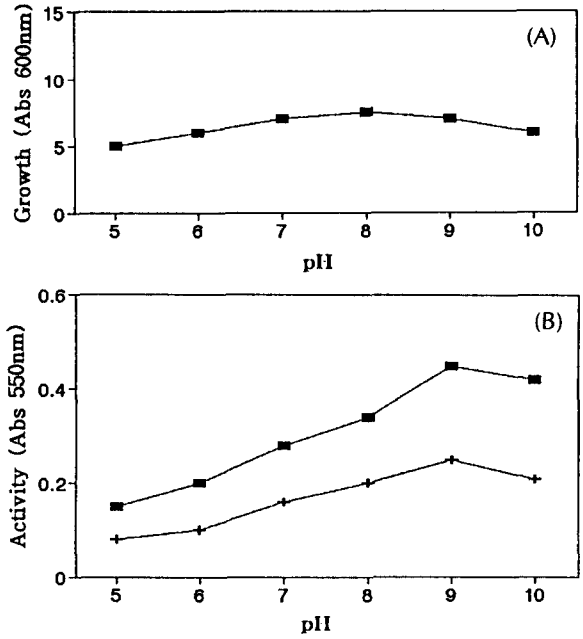

Fig. 3. Effect of pH of media on tryptophanase and indole pyruvate lyase of *E. coli* HGU-3: *E. coli* HGU-3 was inoculated into the media adjusted at various pHs and cultured for 24h under anaerobic conditons. (A), Growth; (B)-■, tryptophanase activities; (B)-+, indole pyruvate lyase activities.

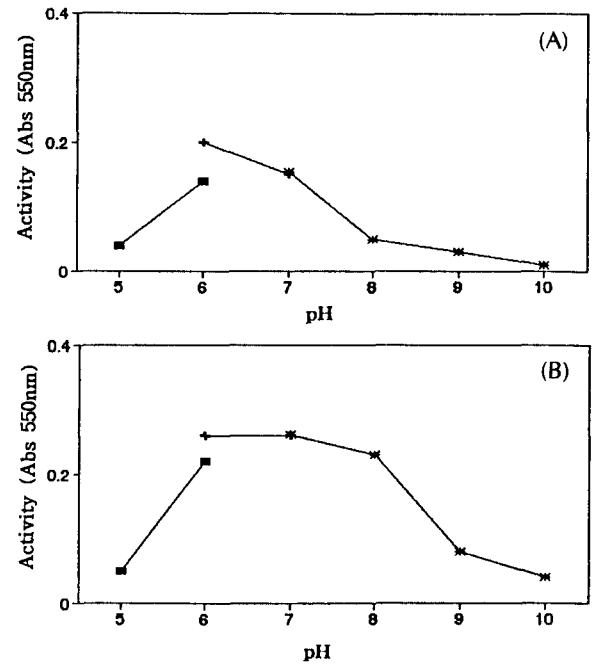

Fig. 4. pH profiles of tryptophanase (A) and indole pyruvate lyase (B) activities of E. coli HGU-3. Buffer used : pH 5-5.5, .0.1 M acetate buffer; pH 5.5-6.5, 0.1 M phosphate buffer; pH 6.5-7.5, 0.1 M phosphate borate buffer; pH 7.5-9, 0.1 M tris-HCl buffer; pH 9-10, 0.1 M NaOH-glycine buffer.

The inhibitory effect of lactulose on the enzymes, tryptophanase and indole pyruvate lyase, was in-

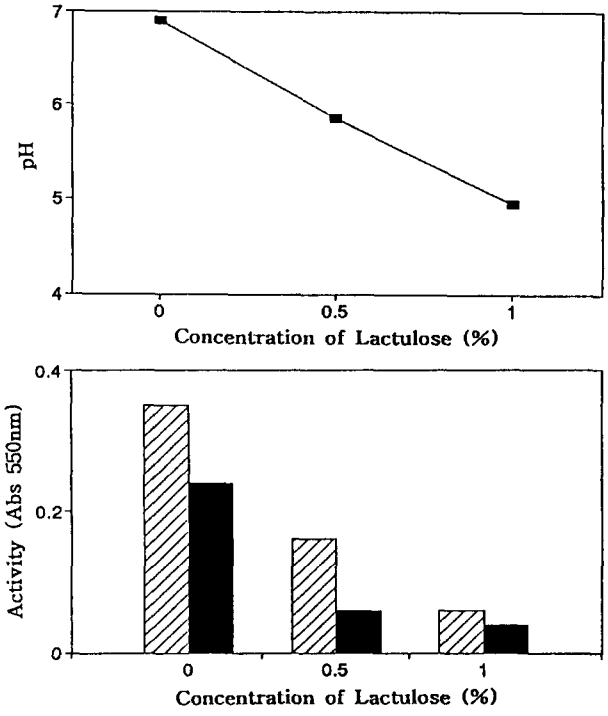

Fig. 5. Effect of lactulose of medium on tryptophanase (▨) and indole pyruvate lyase (■) activities of human intestinal bacteria. The intestinal microflora were inoculated in GAM broth (-glucose) without or with 0.5% or 1% and then cultured 24h under an anaerobic condition.

vestigated (Fig. 5). When these two enzymes were incubated with lactulose, they were not inhibited by lactulose. However, when the lactulose was added to the medium and then intestinal microflora were inoculated in this medium, the productions of these enzymes were dramatically decreased. When human intestinal microflora were cultured in the medium containing 0.5% lactulose, the activities of tryptophanase and indole pyruvate lyase were decreased to 48% and 26%, respectively. In 1% lactulose-containing medium, the activities of these enzymes were decreased to 25% and 18%, respectively. By decreasing these enzyme activities, indole production was also decreased to 26% and 24% at 1% lactulose containing medium, respectively. When intestinal microflora were cultured in the medium containing 0.5 and 1% lactulose, the pH of this medium was lower 1.1 and 1.9 than that of the control, respectively. When rat intestinal bacteria were cultured in the medium containing lactulose, the effect lowering pH of the medium and inhibiting the activity of tryptophanase and indole pyruvate lyase was also similar to those of human intestinal bacteria (Data not shown).

DISCUSSION

Tryptophanase is associated with conversion of tryptophan to indole. Formation of indole in the gastrointestinal tract could be important in the etiology of colon cancer. Thus, the induction of tryptophanase is related to the incidence of colon cancer. Also, the high pH of stool is related to the incidence of colon cancer: the population with alkaline fecal pH is greater risk for colon cancer than those with acidic fecal pH.

Thornton insisted that high pH of colon promotes colorectal cancer (Thornton, 1982). Practically, some diets are degraded by bacteria and colonic pH may become alkaline. The alkaline colonic pH induced the tryptophanase. The formation of indole responsible for the development of colorectal cancer was induced in high pH. Particularly, bacterial tryptophanase activity cultured in pH 7 was 10-fold higher than that of the cultured in pH 6. The present study supported that high pH induces the enzyme activity and promotes colon cancer. This kind of situation can occur anytime in the intestine. Lactobacillus-like beverages may also change pH of the intestine and then decrease the incidence of colon cancer (Kim and Han, 1995: Goldin *et al.*, 1980). In addition, the induction pattern of an indole pyruvate lyase activity, which was not reported until now, also was similar to that of tryptophanase. We have shown that β-glucuronidase produced by bacteria can be induced dramatically by high pH. It is also interesting that the enzyme was also related to the incidence of colon cancer. The prodcutivity of these enzymes, tryptophanase and indole pyruvate lyase, were inhibited by culturing intestinal microflora in the medium containing lactulose (lactic acid bacteria growth factor) but lactulose did not directly inhibit the enzyme. When intestinal bacteria inoculated in the lactulose-containing medium, some bacteria, such as bifidobacteria and lactobacilli utilized lactulose and grew well and then pH of the medium was decreased. By decreasing the pH of the medium which resulted in the growth of lactic acid bacteria, the productivity of these enzymes were, we thought, decreased. These results support Thornton's hypothesis that high pH of colon promotes colorectal cancer (Thornton, 1982).

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