pH-INDUCIBLE β-GLUCOSIDASE AND β-GLUCURONIDASE OF INTESTINAL BACTERIA

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β-Glucosidase and β-glucuronidase of human and rat fecal bacteria were induced by cultivation in alkaline media although their growths were not affected. When a bacterium isolated from human feces producing each enzyme was cultured in a medium at pH 5 for 12-15 h and then adjusted to pH 8, β-glucosidase and β-glucuronidase were induced 9.2-fold and 11.5-fold, respectively.

KEYWORDS β-glucosidase; β-glucuronidase; intestinal bacteria; enzyme induction

Geographic variations in the incidence of colon cancer indicate that environmental factors such as diet are important to the etiology and pathogenesis of colon cancer.[1] The incidence of intestinal cancer is apparently related to economic development. It is noteworthy that these tumors are still comparatively rare in developing countries. Some theories have implicated high dietary intake of meat in the development of colon cancer, although other influences have not been excluded.[2] The meat diet induced some enzymes, such as β-glucuronidase, azoreductase, nitroreductase and steroid 7α-dehydroxylase, of several different bacteria.[3,4] These enzymes are associated with conversion of procarcinogens to carcinogens; β-glucosidase catalyzes the hydrolysis of glycosides of natural products, such as cycasin and amygdalin,[5] and β-glucuronidase catalyzes the hydrolysis of glucuronic acid conjugates of xenobiotics, such as benzo(α)pyrene.[6] Kinoshida and Gelboin confirmed that β-glucuronidase of intestinal bacteria catalyzes DNA-binding with metabolites of benzo(α)pyrene excreted via the bilary duct.[7] Feeding of viable culture of *Lactobacillus acidophilus* also influenced these fecal enzyme activities.[8] Such cultures fed as supplements to rats and humans significantly lowered the activities of fecal bacterial β-glucuronidase, nitroreductase and azoreductase in rats and humans consuming meat diets. Thornton and Samelson *et al.* reported that high colonic pH promotes colorectal cancer[9] and that populations with alkaline fecal pH are at greater risk for colon cancer than those with acid fecal pH.[10] Here we investigated the induction of β-glucuronidase or β-glucosidase of intestinal bacteria by pH of media.

MATERIALS AND METHODS

Materials *p*-Nitrophenyl-β-D-glucopyranoside and *p*-nitrophenyl-β-D-glucuronide were purchased from Sigma Chem. Co. (U.S.A.). General anaerobic medium (GAM) and Tryptic soy agar (TS) medium were from Nissui Seiyaku Co., Ltd.(Japan). The other chemicals were of analytical reagent grade.

Bacteria *Escherichia coli* HB 101 was kindly donated from Korean Type Culture Collection. Bacteria (HGO-7 and HGU-3) producing β-glucosidase or β-glucuronidase were isolated from fecal microflora of a healthy man in his 20s according to the method of Mitsuoka.[11]

Enzyme Assay Feces of rats or humans and their cultivated bacterial broth were assayed for the enzyme activity. About 1 g of feces was suspended in 10 ml of 25 mM phosphate buffer, pH 7.0. The suspensions were cultured in GAM broth, centrifuged at 7,000 rpm for 20 min and resuspended in 10 ml of 25 mM phosphate buffer at pH 7.0.

β-Glucosidase was assayed in a 1 ml reaction mixture consisting of 40 ml of 10 mM *p*-nitrophenyl-β-D-glucopyranoside, 0.76 ml of 0.1 M phosphate buffer, pH 7.0, and 0.2 ml of the sample. β-Glucuronidase was assayed in a 1 ml reaction mixture consisting of 0.4 ml of 2 mM *p*-nitrophenyl-β-D-glucuronide, 0.6 ml of 0.1 M phosphate buffer, pH 7.0, and 0.2 ml of the sample. The reaction was stopped by the addition of 0.25 N NaOH, and then the mixture was centrifuged at 3,000 rpm for 10 min. The absorbance at 405 nm was measured within 10 min.

RESULTS AND DISCUSSION

Using *p*-nitrophenyl-β-D-glucopyranoside as substrate, the β-glucosidase activities of rat and human feces were 1.69 and 3.94 μmol/min/g wet feces, respectively. The optimal pH of β-glucosidase of these feces was 6-8. However, β-glucosidase produced by

bacteria isolated from these specimens showed two optimal pHs, 6-8 and 8-10. Using *p*-nitrophenyl-β-D-glucuronide, β-glucuronidase activities of rat and human feces were 1.54 and 2.92 μmol/min/g wet feces, respectively. Their optimal pH was 6-7. After 50 μl of the 100-fold diluted stool samples of healthy men and rats were inoculated in GAM broths which were adjusted to pH 5, 6, 7, 8, 9 and 10, followed by incubation for 24 h under an anaerobic condition, the β-glucosidase and β-glucuronidase activities were measured (Fig. 1). When the samples were cultured in alkaline media, β-glucosidase activities were induced: those of humans and rats were the highest in media of pH 10 and 9, respectively. In particular, the activity of humans increased in proportion to pH increase. β-Glucuronidase activities were also increased in the alkaline media. The pH of media for the highest enzyme activities of humans and rats were 8 and 9, respectively.

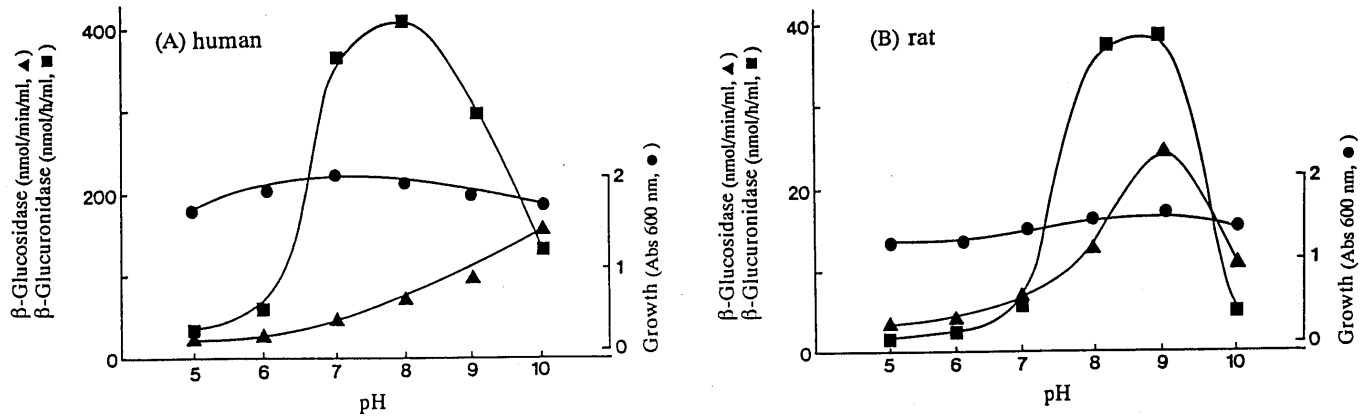

Fig. 1. Effect of pH of Media on Induction of β-Glucosidase and β-Glucuronidase of Intestinal Bacteria

(A) Fresh feces of healthy men were inoculated into the media of various pHs and cultured for 24 h under anaerobic conditions.

(B) Rat (Wistar male, 200 g) intestinal bacteria were cultured according to the method of (A).

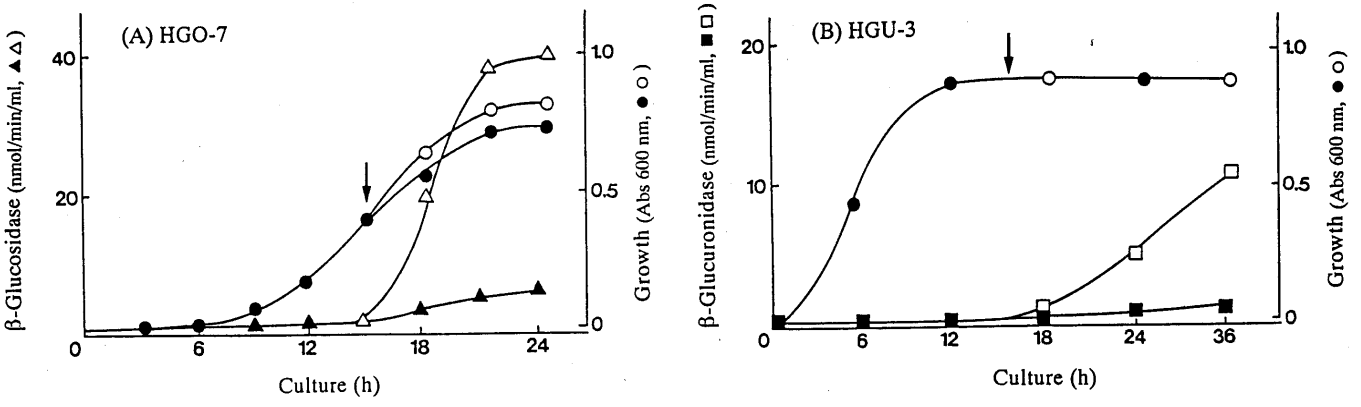

Fig. 2. Induction of β-Glucosidase or β-Glucuronidase of a Human Intestinal Bacterium, HGO-7 or HGU-3

(A) The bacterium, HGO-7, was inoculated in GAM broth of pH 5 and then cultured. Thereafter, the medium was adjusted to pH 8. Symbols indicate growth [●] and β-glucosidase activities [▲] of bacteria cultured in pH 5-medium, and growth [○] and the enzyme activity [△] of bacteria cultured in medium whose pH was changed from 5 to 8 at 16 h-cultivation. Arrows indicate the point at which pH of the medium was changed to 8. (B) The method was performed according to (A), using β-glucuronidase producing bacterium, HGU-3. Symbols indicate growth [●] and β-glucuronidase activity [■] in pH 5-medium, growth [○] and the enzyme activity [□] in pH 8-medium.

Several bacteria producing β-glucosidase and β-glucuronidase were isolated from human intestinal bacteria by using alkaline GAM agar plates; these bacteria were inoculated in media of pH 5 and cultured to reach the stationary phase, and then these cultured media were adjusted to pH 8. By changing pH of the media to alkaline, β-glucosidase and β-glucuronidase activities of the cultured bacteria were dramatically induced although their bacterial growths were not affected (Fig. 2). These results suggest that these enzymes may be inducible by the pH of intestine contents: it has been known that intestinal pH is affected by diet and lowered by dietary fiber and increase of acid-producing bacteria such as *Bifidobacterium* and *Lactobacillus* in lower parts of the intestine.

We suggest that the enzymes may be affected by colonic pH: high colonic pH induces the enzymes, β-glucosidase and β-glucuronidase, and promotes colon cancer.

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