The Role of Intestinal Bacteria in the Transformation of Sodium Picosulfate

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ABSTRACT—Sodium picosulfate, a laxative, was biotransformed to 4,4'-dihydroxydiphenyl-(2 pyridyl)methane by intestinal flora that produced a novel sulfotransferase (not sulfatase). The biotransformation was activated by adding phenolic compounds such as phenol, acetaminophen and flavonoids. The enzyme activity related to this biotransformation was the highest in the contents of the caecum region of the intestine. The enzyme activity was 3.0 μmole/hr/g wet feces in humans and 0.75 in rats (pH 8.0). The optimal pH was 9.0.

Keywords: Picosulfate, Biotransformation, Sulfotransferase, Intestinal flora, Laxative

It is clear that sodium picosulfate, which is widely used as a laxative, inhibits the absorption of water and electrolytes, and increases their secretion into the intestinal lumen. Its hydrolysate, 4,4'-dihydroxydiphenyl-(2-pyridyl)methane (DPM), is known to be an essential moiety of the laxative, but this is absorbed from the upper part of the intestine before reaching the target organ, the colon, when it is administered orally to rats (1, 2).

After the conjugation with glucuronic acid and sulfonic acid in the liver, a small amount of its conjugates is excreted via the bile duct into the intestinal lumen. However, when DPM conjugates are administered orally, little is absorbed (3, 4). Therefore, sodium picosulfate has been considered to be hydrolyzed in the distal segment of the intestine by an intestinal microbial enzyme, sulfatase.

Recently, we discovered a species of sulfotransferase-producing bacteria from human intestine when phenylsulfate esters were used as the substrate (5, 6). Furthermore, the sulfotransferase activity was higher than that of the sulfatase in rat and human feces (7). Here, we present the role of the sulfotransferase-producing intestinal bacteria in the laxative action of sodium picosulfate.

MATERIALS AND METHODS

Materials

p-Nitrophenylsulfate (PNS), p-nitrophenylacetate and bovine serum albumin were purchased from Sigma Chem. Co. (U.S.A.). Sephacryl S-300 fine, hydroxyapatite and DEAE-cellulose were from Pharmacia Fine Chem. (Sweden). Phenol was from Wako Pure Chem. Ind., Ltd. (Japan), and general anaerobic medium (GAM) was from Nissui Seiyaku Co. (Japan). Sodium picosulfate was kindly donated by Ms. S.-H. Yook of the Korean National Institutes of Health.

Preparation of feces sample for enzyme activity assay

Feces (0.5 g) were collected from 3 healthy men (Twenties, male) and pooled. Male rats (SDD Wistar, 180–220 g) were maintained on pellet food (Samyang, Korea) and tap water ad lib, and 3 rats were used per group. In the case of rats treated with antibiotics, an antibiotics mixture (175 mg chloramphenicol, 500 units nystatin, 20 mg streptomycin, 10 mg erythromycin and 200 units penicillin per rat) was administered orally once a day for 3 days before the experiments.

To assay enzyme activities of the intestinal contents, the rat was anesthetized with ether, and then the intestine was taken out and divided into seven fractions: stomach, upper small intestine (duodenum), lower
small intestine (jejunum), caecum, upper large intestine, lower large intestine and feces. The contents of each fraction were collected, suspended and made up to a known volume (10-fold) with 20 mM Tris-HCl buffer, pH 7.0.

Assay of the enzyme activity

The sulfotransferase activity was assayed in a 1.26-ml reaction mixture consisting of 60 μl of 50 mM PNS (occasionally sodium picosulfate), 0.58 ml of 20 mM tyramine (occasionally the other phenolic compounds), 0.42 ml of 0.1 M Tris-HCl buffer, pH 8.0 or 7.0, and 0.2 ml of the enzyme solution. The reaction mixture was incubated at 37°C for 15 min–2 hr with shaking. The reaction was stopped by the addition of 1 N NaOH (0.8 ml), and then the mixture was centrifuged at 3000 rpm for 10 min. The absorbance at 405 nm of the resulting supernatant solution was measured. Sulfatase activity was measured under the assay conditions for the sulfotransferase activity without the addition of the acceptor tyramine. The assay mixture of carboxylesterase activity contained 0.4 ml of 1 mM p-nitrophenylacetate, 0.55 ml of 50 mM Tris-HCl buffer, pH 8.0 or 7.0, and 50 μl of the enzyme solution in a cuvette of 1-cm light path. The reaction was started by adding the substrate, and the absorbance at 405 nm was time-scanned at 25°C.

Purification of sulfotransferase from Eubacterium A-44

The bacterium, Eubacterium A-44, was isolated from human feces in a GAM broth containing 0.15% agar and then cultured in 40 ml GAM broth under a strictly anaerobic condition. The cultured medium was inoculated in 2 l of GAM broth containing 1 mM PNS and cultured at 37°C for 16–20 hr in an anaerobic box. The cultured medium was centrifuged at 7000 rpm for 20 min at 4°C. The harvested cells were washed twice with saline. The resulting precipitate was suspended in 0.1 M acetate buffer, pH 5.5, and disrupted by a sonicator (Wakenyaku Co., Ltd., Japan). The sonicated solution was centrifuged at 13000 rpm for 30 min at 4°C. The supernatant fluid was used as a starting material for the purification. To purify the enzyme, ammonium sulfate fractionation, DEAE-cellulose, hydroxyapatite and Sephacryl S-300 fine column chromatographies were carried out according to the previous method (6). The purified enzyme had a specific activity of 61.4 μmole/min/mg protein.

Determination of protein

Protein was determined by the method of Lowry et al. (8) with bovine serum albumin as the standard.

RESULTS

Activities of sulfotransferase, sulfatase and carboxylesterase from rat intestinal contents

In preliminary experiments, enzymic hydrolysis of PNS was assayed in human and rat feces. The activity was 0.5–3.0 μmole/hr/g wet feces in humans and 0.15–0.75 in rats. Oral administration of antibiotics markedly decreased the activity of rats to 5% that of the control rats, but the enzyme activity was restored one month after stopping the administration of antibiotics.

The activities of sulfotransferase, sulfatase and carboxylesterase in intestinal contents of rat treated with antibiotics and of control rat were assayed (Table 1). Sulfotransferase and sulfatase activities of control rats were the highest in the caecum, followed by colon and feces. Both enzyme activities were 2.2–2.4-fold higher

| Table 1. The activity of enzymes (pH 7.0) in the intestine of rats treated with antibiotics and control rats |
|--------------------------------------------------|-----------|-----------|---|---|---|---|
| Enzymes (treated with antibiotics)                | Stomach   | Duodenum  | Jejunum | Caecum | Upper colon | Lower colon | Feces |
| Carboxylesterase (μmole/min/g)                    | 0.01      | 3.07      | 0.05     | 0.01   | 0.03        | 0.05        | 0.03  |
| Sulfatase (μmole/min/g)                           | 0.06      | 1.30      | 1.50     | 1.80   | 1.10        | 1.90        | 1.30  |
| Sulfotransferase (μmole/min/g)                    | 1.90      | 1.60      | 1.90     | 0.90   | 0.90        | 0.90        | 0.20  |
| Enzymes (control)                                |           |           |          |        |             |             |       |
| Carboxylesterase (μmole/min/g)                    | 0.02      | 3.39      | 0.47     | 0.14   | 0.06        | 0.05        | 0.05  |
| Sulfatase (μmole/min/g)                           | 2.60      | 0.20      | 4.10     | 5.60   | 3.40        | 3.00        | 3.80  |
| Sulfotransferase (μmole/min/g)                    | 2.80      | 7.00      | 12.80    | 25.20  | 19.40       | 16.40       | 16.90 |
at pH 8.0 than at pH 7.0. The optimal pHs of the caecum sulfotransferase and sulfatase were 9.0 (Fig. 1). These activities were decreased to one tenth by oral administration of antibiotics. However, carboxylesterase activity was not affected by antibiotics treatment and was the highest in the contents of the duodenum. The optimal pH of the duodenum carboxylesterase was 7.8. Here, the sulfatase activity of the intestinal contents may contain sulfotransferase activity, because the contents of the intestine contained phenolic compounds which originated from endogeneous and exogeneous materials, bile duct excreted products and the diet, and these can serve as acceptor substrates of the actual sulfotransferase. In addition, rat diet and feces whose sulfotransferase and sulfatase were inactivated by heating were also used as acceptor substrates for the sulfotransferase purified from Eubacterium A-44 (Table 2).

Transformation of picosulfate with rat feces

To investigate the formation of the free phenol, DPM, which is the genuine laxative in the target region, picosulfate was incubated with rat feces. Feces which contain a small amount of phenolic compounds have a phenylsulfate hydrolyzing enzyme activity towards PNS when it used as substrate, making it a better substrate than picosulfate. However, transformed products of picosulfate, DPM and 4,4'-monosulfoxydihydroxydiphenyl-(2-pyridyl) methane (MDPM), were not detected in the reaction mixture (Fig. 2a). When phenol was added in the reaction mixture, the transformed products were formed and their production was proportional to the reaction time. When 1 mM picosulfate and 1 mM phenol was incubated at 37°C with 10 mg rat feces for 10 hr, 21% of the picosulfate was hydrolyzed and 1.4% of it was transformed to the free phenol. In the present studies, phenylsulfate esters hydrolyzing bacteria were also screened from human and rat intestinal bacteria. The screened bacteria, K-35, K-36, D-1, D-2 and D-3, produced sulfotransferase(s) whose properties were unknown, but did not produce sulfatase(s) when PNS was used as the donor substrate. However, the sulfatase-producing bacteria could not be isolated.

Transformation of picosulfate with sulfotransferase of Eubacterium A-44

To investigate the role of Eubacterium A-44, which was isolated from human intestinal flora, in the formation of DPM, the partially purified sulfotransferase from Eubacterium A-44 was incubated with sodium picosulfate and phenol. The picosulfate was transformed to DPM and MDPM, and the HPLC pattern of the products was similar to that of rat feces (Fig. 2b).

Table 2. Substrate specificity of inactivated feces and diets of rats (donor substrate, PNS)

| Substrate*             | Activity (unit/mg protein) |
|------------------------|----------------------------|
| Inactivated rat feces** | 0.62                       |
| Rat diet               | 0.49                       |

*Final concentration, 2 mg/ml. **Heated at 90°C for 10 min.
DPM and MDPM were not produced when phenol was absent in the reaction mixture. Acceptor substrate specificity was investigated using PNS as the donor substrate (Table 3). The best substrate was acetaminophen, followed by tannic acid and naringin which are both contained in foods. These compounds may act as acceptor substrates of the sulfotransferase of intestinal bacteria in the laxative action of sodium picosulfate.

**Table 3. Acceptor substrate specificity (donor substrate, PNS)**

| Substrate         | Activity**(%) |
|-------------------|---------------|
| Phenol            | 100           |
| p-Acetaminophen   | 129           |
| Tannic acid       | 35            |
| Naringin          | 30            |
| Gallic acid       | 15            |

*Final concentration, 5 mM. **Specific activity in the presence of phenol + PNS was 61.4 unit/mg protein.*

**Fig. 2.** HPLC chromatogram: (A) The formation of DPM with rat feces. The reaction mixture (10 ml) of (a) contained 1 mM sodium picosulfate + 1 mM phenol; that of (b), 1 mM sodium picosulfate + 10 mg feces; and that of (c), 1 mM sodium picosulfate + 1 mM phenol + 10 mg feces; Reactions were carried out at 37°C for 10 hr with shaking. (B) The formation of DPM with the sulfotransferase of Eubacterium A-44. The reaction mixture (1 ml) contained 1 mM sodium picosulfate, 1 mM phenol, 35 mM tris-HCl buffer and the enzyme; It was incubated at 37°C for 0 min (a), 10 min (b) or 30 min (c). A 5-μl aliquot of the reaction mixture was injected after millipore filtration. HPLC (Shimadzu system) conditions used: column, Chemcosorb 7-ODS-H; solvent, 45% MeOH (0.5% TFA); Flow rate, 1 ml/min; wavelength, 254 nm. Retention time: sodium picosulfate, 1.73 min; MDPM, 1.93 min; DPM, 2.24 min; phenol, 4.73 min.
DISCUSSION

Sodium picosulfate is known to be hydrolyzed to DPM, which is an essential moiety of the laxative. It has been thought that this hydrolysis is catalyzed by the microbial sulfatase in the intestine. However, the sulfotransferase activity of the intestinal contents was higher than that of the sulfatase at pH 7, which is around the intestinal pH. Furthermore, intestinal contents contained acceptors of the sulfotransferase, phenolic compound(s), which originate from endogeneous and exogeneous materials. This suggests that the measured sulfatase activity was partially due to the sulfotransferase activity. When picosulfate was incubated with rat feces, transformed products, DPM and MDPM, were not detected. However, when phenol was added in the same reaction mixture, the transformed products were formed. These results suggest that the transformation of picosulfate to the phenol, the genuine laxative, requires phenolic compound(s) and was actually catalyzed by the sulfotransferase (not the sulfatase) of intestinal bacteria. Furthermore, this suggestion was supported by the fact that picosulfate was transformed to DPM and MDPM with the *Eubacterium A-44* sulfotransferase which is isolated from human intestinal flora. Acceptor substrates of the sulfotransferase were flavonoids, acetaminophen, tannic acid, etc.

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