Inhibitory Effects of Green Tea and Grape Juice on the Phenol Sulfotransferase Activity of Mouse Intestines and Human Colon Carcinoma Cell Line, Caco-2

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Tea and fruit juices are beverages consumed daily all over the world. The present study reports the inhibitory effects of these beverages on the activity of mammalian intestinal phenol sulfotransferases (P-STS). Green tea strongly inhibited the E. coli-expressed mouse intestinal P-STS activity in vitro. (−)-Epigallocatechin gallate (EGCG) was found to be the most potent inhibitor among the catechins tested (IC₅₀ = 0.93 μM). (−)EGCG also inhibited the P-STS activity of the human colon carcinoma cell line, Caco-2. Kinetic analysis showed that the inhibition was competitive. Among fruit juices examined (apple, grape, grapefruit and orange), grape juice exhibited the most potent inhibitory action on the P-STS activity of mouse intestines and human colon carcinoma cells. The inhibitory activity of grape juice was located mainly in the skin and seeds. Flavonols, such as quercetin and kaempferol, inhibited the P-STS activity at low concentrations. These observations suggest the possible inhibition of P-STS activity in human intestines by green tea or grape juice.

Key words  Caco-2; catechin; green tea; flavonol; inhibition; sulfotransferase

Cytosolic sulfotransferases (STs) catalyze the transfer of the sulfate group from 3'-phosphoadenosine 5'-phosphosulfate (PAPS) to acceptor molecules possessing phenols, enols, alcohols or amines.1−5 Sulfo-conjugation confers greater polarity and water solubility on the parent molecules, thereby, facilitating biliary or urinary excretion and detoxification.1−5

Although most studies on STs have been performed using liver, enzymatic activity and immunological signals of certain STs have been found in other tissues including brain, kidney, lung, intestine, platelets and placenta.2−6 Recently, we reported the isolation and characterization of a mouse intestinal phenol ST (P-STS).7 This P-STS belongs to the ST1B1 subfamily of P-STSs based on its sequence homology and substrate specificity, and its expression was high in the intestine as well as in the liver.7 Several reports have suggested that intestinal ST activity may be involved in the metabolism of orally administered drugs or xenobiotics, facilitating their detoxification8−10 and dietary exposure to food constituents or environmental chemicals might modulate this activity.11 In order to elucidate the potent effect of food constituents on intestinal ST activity, we investigated the effects of green tea and fruit juices on the P-STS activity of mouse intestines and human colon carcinoma cells.

MATERIALS AND METHODS

Materials  Catechins and other flavonoids were purchased from Funakoshi Co. (Tokyo, Japan). Green tea leaves (Sencha) were produced in Uji, Kyoto, Japan and extracted by the standard Japanese method as described below. Natural fruit juices (Kirin Tropicana 100%) were obtained from Kirin Beverage Co. (Tokyo, Japan). [³⁵S]PAPS (82.78 Ci/mmol) was purchased from NEN Dupont (Detroit, U.S.A.). Prokaryotic expression vector, pRSET, was purchased from Invitrogen (California, U.S.A.).

Expression of Mouse Intestinal P-STS in E. coli  The mouse intestinal cDNA was expressed using an expression vector, pRSET, and E. coli BL21(DE3) (Stratagene) as described previously.7 Briefly, the transformed cells were cultivated in 30 ml LB broth at 30 °C. Four hours after induction with 1 mM isopropyl-β-D-thiogalactopyranoside, the cells were harvested, washed with 0.9% NaCl and resuspended in 2 ml buffer A (50 mM Tris–HCl, pH 7.5, 250 mM sucrose, 0.1 mM EDTA, 3 mM 2-mercaptoethanol, 0.1 mM phenylmethylsulfonyl fluoride, 5 μg/ml antipain and 5 μg/ml pepstatin). The cells were then disrupted by sonication (Branson sonifier), and, after centrifugation at 105000×g for 60 min, the supernatant was used to assay for P-STS activity.

Cell Culture  Caco-2 cells were obtained at passage 40 from Riken Cell Bank, Japan. Cells were grown in minimum essential medium (MEM) with 10% fetal bovine serum, 2 mM glutamine, 10 U/ml penicillin, 10 U/ml streptomycin and non-essential amino acids at 37 °C in a humidified atmosphere containing 5% CO₂.

Preparation of Cytosolic Extract of Mouse Intestines and Caco-2 Cells  Small intestines were excised from male ddY mice (10 week-old) and washed with 0.9% NaCl. Tissues were sliced with scissors and homogenized in 5 ml buffer A. Caco-2 cells (1−2×10⁴) were removed from culture flasks using 0.05% trypsin/0.53 mM EDTA, washed with phosphate buffered saline, and then homogenized in 1 ml buffer A. The debris was removed by centrifugation at 3000×g for 15 min and the supernatant was then centrifuged at 105000×g for 60 min. The clear lysates were used for the following studies.

Assay of P-STS Activity  P-STS activity in the lysates was determined using [³⁵S]PAPS as the sulfate donor and 2-naphthol or dopamine as a sulfate acceptor, according to the procedure of Foddef and Meek12 with a slight modification.13,14 Briefly, the reaction mixture (500 μl) consisted of 10 mM phosphate buffer, pH 7.4, 50 μM 2-naphthol or dopamine, 1.0 μM [³⁵S]PAPS (0.1 μCi) and lysates (2−10 μg of proteins). The mixture was incubated at 37 °C for 15 min and the reaction was stopped by the addition of 0.1 ml cold 0.1 M barium acetate. Then the unconverted [³⁵S]PAPS was precipitated by...
the addition of 0.1 ml of both 0.1 M Ba(OH)\textsubscript{2} and 0.1 M ZnSO\textsubscript{4}. The precipitate was removed by centrifugation at 12000×g for 5 min. This precipitation procedure was then repeated. The supernatant (300 μl) after the second precipitation was transferred to 3 ml liquid scintillator and counted. Blanks were monitored by omitting acceptor substrates. The effects of green tea, fruit juices and flavonoids on the P-ST activity were assayed in the presence of these compounds at various concentrations.

**Preparation of Green Tea Extract** The leaves of green tea (2 g) were extracted with 100 ml hot water (75°C) for 2 min. The extract was then centrifuged at 3000×g for 5 min and the supernatant was divided into small aliquots and stored at -80°C. The dried weight of 1 ml extract was 6.1 mg.

**RESULTS**

**Effect of Green Tea and Catechins on P-ST Activity** Previously, we have cloned a mouse intestinal P-ST cDNA which encodes an isoyme belonging to the STIB subfamily of P-STS. P-ST catalyzes the sulfation of simple phenols as well as catecholamines.\textsuperscript{7} The effects of green tea on the *E. coli*-expressed mouse intestinal P-ST activity were analyzed. As shown in Fig. 1A, less than 0.1% (v/v) green tea extract was enough to inhibit half of the P-ST activity toward 50 μM 2-naphthol. The IC\textsubscript{50} was estimated as 0.08% (v/v). This value is equivalent to 4.9 μg dried-green tea extract per milliliter reaction mixture. Since catechins are the major phenolic compounds found in green tea extract, among green tea polyphenols,\textsuperscript{15,16} we examined the effect of catechins on the P-ST activity. As shown in Fig. 1B, of the 6 catechins examined, (−)-epigallocatechin gallate (EGCG) and (−)-epicatechin gallate (ECG) strongly inhibited P-ST activity, whereas the other catechins only exhibited moderate inhibitory activity. The IC\textsubscript{50}s were determined as 0.93±0.37 μM for EGCG, 0.78±0.32 μM for ECG and 13.4±5.3 μM for (−)-catechin, respectively (n=3). The inhibitory activity of these catechins was higher than that of 2,6-dichloro-4-nitrophenol (DCNP), a specific inhibitor of P-ST activity (IC\textsubscript{50}=30 μM).\textsuperscript{7,17} A similar inhibition by catechin was observed on the P-ST activity in mouse intestinal extract (data not shown). However, we did not investigate this further since our preliminary data suggested that there might be several P-ST isozymes in the extract.

Caco-2 is a human colon carcinoma cell line which has been used as a model for human intestinal drug metabolism.\textsuperscript{18,19} Caco-2 cells showed significant P-ST activity on 2-naphthol and dopamine. As a first step to elucidate the possible effect of green tea on human intestinal P-ST activity, we investigated the effects of catechins on the P-ST activity of Caco-2 cells. As shown in Fig. 2A, (−)EGCG exhibited the most potent inhibition of Caco-2 P-ST activity for 2-naphthol. The IC\textsubscript{50} of (−)EGCG inhibition was calculated to be 14.5±1.5 μM (n=3) (Fig. 2B). Similar inhibition was observed when dopamine was used as a substrate (data not shown). Unlike the data obtained with the mouse intestinal P-ST, (−)ECG exhibited only slight inhibition. Kinetic analysis of (−)EGCG inhibition indicated that the mode of (−)EGCG inhibition was competitive (Fig. 2C).

**Effect of Grape Juice on P-ST Activity** As fruit juices are daily consumed like green tea, we investigated the effects of fruit juices on *E. coli*-expressed mouse intestinal and Caco-2 P-ST activities. Of the 4 fruit juices tested (apple, grape, grapefruit and orange), grape juice exhibited the most potent inhibitory activity (Fig. 3A and B). The IC\textsubscript{50} of grape juice was calculated as 0.2% (v/v) for mouse intestinal P-ST activity and 0.8% (v/v) for Caco-2 P-ST activity, respectively (Fig. 3C). Biphasic inhibition was observed for the Caco-2 P-ST activity. To localize the inhibitory activity in the grape, grapes were divided into three parts (skin, seeds and flesh) and each part was extracted with an equal volume of water, because our preliminary analysis suggested that the inhibitory activity was water-soluble. The P-ST activity was measured in the presence of each extract and the inhibitory activity was mainly found in the skin and seed extracts (data not shown). Since grape skin and seeds contain a lot of polyphenols and flavonoids are a major component of polyphenols,\textsuperscript{20} the effects of flavonoids (quercetin, naringenin and kaempferol) on mouse intestinal and Caco-2 P-ST activities were measured. As shown in Fig. 4A, quercetin and kaempferol exhibited strong inhibitory activity while naringenin showed only weak inhibition. Kinetic analyses indicated that the mode of inhibition by quercetin was competi-

![Fig. 1. Effects of Green Tea and Catechins on *E. coli*-Expressed Mouse Intestinal P-ST Activity](image-url)
Fig. 2. Effects of Catechins on Caco-2 P-ST Activity

(A) P-ST activity on 2-naphthol (50 μM) was determined in the presence of catechins (50 μM). (B) Inhibitory effects of (-)-EGCG were measured over the range 0.8—200 μM. The IC₅₀ was calculated as 14.5 ± 1.5 μM (n=3). (C) Lineweaver-Burk plots of (-)-EGCG inhibition. P-ST activity on 2-naphthol in the range of 1—20 μM was determined with (squares) or without (circles) (-)-EGCG (5 μM). The Kᵣ was estimated as 5.83 ± 0.72 μM (n=3).

Fig. 3. Effects of Grape Juice on P-ST Activity

P-ST activity on 50 μM 2-naphthol was measured in the presence of 10% (v/v) fruit juices. (A) E. coli-expressed mouse intestinal and (B) Caco-2 cells. (C) Effects of grape juice on the P-ST activity of mouse intestine (closed circles) or Caco-2 cells (open circles).

Fig. 4. Effects of Flavonols on P-ST Activity

(A) P-ST activity on 50 μM 2-naphthol was assayed in the presence of flavonols (50 μM); dashed bars, E. coli-expressed mouse intestinal P-ST; closed bars, Caco-2 P-ST. Control activity (without flavonols) was referred to as 100%. (B) Dose-dependent inhibition by quercetin of the E. coli-expressed mouse intestinal PST activity. The IC₅₀ was calculated to be 2.62 μM (n=3). (C) Lineweaver-Burk plots of quercetin inhibition. P-ST activity on 2-naphthol over the range 1—50 μM was determined in the presence (squares) or absence (circles) of quercetin (2.6 μM). The Kᵣ was estimated as 1.65 μM (n=3).
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