Effects of Phenolic Environmental Estrogens on the Sulfotransferase Activity of the Mouse Intestine and a Human Colon Carcinoma Cell Line, Caco-2

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In order to explore the possible role of sulfation in the inactivation of environmental estrogens at gastrointestinal sites and their subsequent removal, we investigated the effects of phenolic environmental estrogens on sulfotransferase (ST) activity. The mouse intestine and a human colon carcinoma cell line, Caco-2, were studied. ST enzymes were found to have a high affinity for diethylstilbestrol (DES) and bisphenol A (BPA), whereas phenol ST (PST) activity was strongly inhibited by nonylphenol and genistein in both mice and humans. Kinetic analysis showed that this inhibition was competitive. These observations suggest that nonylphenol and genistein compounds might inhibit PST activity in the human intestine and that they might escape detoxification by sulfation.

Key words —— environmental estrogen, bisphenol A, diethylstilbestrol, sulfotransferase, Caco-2

INTRODUCTION

Orally administered environmental chemicals are first taken up by epithelial cells of the gastrointestinal tract and it is possible that some are metabolized and excluded outside the cells, however, little is known about the mechanism by which environmental chemicals are metabolized within the gastrointestinal tract. Several reports have suggested that intestinal sulfotransferase (ST) activity might be involved in the metabolism of orally administered drugs or xenobiotics, thereby facilitating their detoxification. Moreover, it has been suggested that dietary exposure to food constituents or environmental chemicals might modulate this activity.[1–3] Recent reports have indicated that sulfation and/or glucuronidation of bisphenol A (BPA) by cytosolic sulfotransferases (STs), and/or UDP-glucuronosyltransferases, respectively, are pathways by which compounds might be eliminated from the body.[4,5] STs catalyze the transfer of a sulfate group from 3′-phosphoadenosine 5′-phosphosulfate (PAPS) to an acceptor molecule, containing either a phenol, enol, alcohol or amine.[6–8] Previously, we characterized phenol ST (PST) activity within the mouse intestine and a human colon carcinoma cell line, Caco-2.[9] Here, we examined the ability of PST activity within each model to detoxify phenolic environmental estorgens and found that some phenolic environmental estrogens are good substrates, while others, in fact, inhibit PST activity *in vitro*.

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MATERIALS AND METHODS

Materials —— All phenolic compounds were purchased from Wako Chemicals Co. (Tokyo, Japan). [^{35}S]PAPS (82.78 Ci/mmol) was purchased from NEN Dupont (Detroit, U.S.A.). The prokaryotic expression vector, pRSET, was purchased from Invitrogen (California, U.S.A.).

Expression of Mouse Intestinal PST in *E. coli* —— Mouse intestinal cDNA was expressed using the expression vector, pRSET, within *E. coli* BL21(DE3) (Stratagene) as previously described.[10] Briefly, the transformed cells were cultivated in 30 ml LB broth at 30°C. Four hours after induction with 1 mM 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 $\times$ *g* for 60 min, the supernatant was assayed for PST 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% CO_2.

Preparation of the Cytosolic Extract of Caco-2 Cells —— Caco-2 cells ($1–2 \times 10^7$) were removed from their 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 $\times$ *g* for 15 min and the supernatant was then centrifuged at 105000 $\times$ *g* for 60 min. The clear lysate was used in the following studies.

Assay of PST Activity —— PST activity within each sample of lysate was determined using [^{35}S]PAPS as the sulfate donor, and 2-naphthol as a sulfate acceptor, according to a slight modification of the procedure of Foldes and Meek.[11] Briefly, the reaction mixture (500 μl) consisted of 10 mM phosphate buffer (pH 7.4), 50 μM 2-naphthol or environmental chemicals, 1.0 μM [^{35}S]PAPS (0.1 μCi), and the lysate sample (2–10 μg of protein). 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, unconverted [^{35}S]PAPS was precipitated by the addition of 0.1 ml of both 0.1 M $Ba(OH)_2$ and 0.1 M $ZnSO_4$. The precipitate was removed by centrifugation at 12000 $\times$ *g* for 5 min. This precipitation procedure was then repeated. After the second round of precipitation, the remaining supernatant (300 μl) was transferred to a 3 ml liquid scintillator and the amount of radioactivity was counted. Controls were obtained by omitting the acceptor substrate from the reaction mixture. The effect of phenolic environmental chemicals on PST activity was determined by assaying for PST activity in the presence of varying concentrations of these compounds.

RESULTS AND DISCUSSION

Sulfation of Phenolic Environmental Estrogens by Intestinal STs

PST catalyzes the sulfation of simple phenols and catecholamines.[6–8] Previously, we cloned copies of a mouse intestinal PST cDNA, which encodes for a PST isozyme belonging to the SULT1B subfamily. This isozyme is heavily expressed in the mouse intestine.[10] Thus, we examined whether *E. coli*-expressed mouse intestinal PST could catalyze phenolic environmental estrogens. As is shown in Fig. 1A, *E. coli*- expressed mouse intestinal PST had

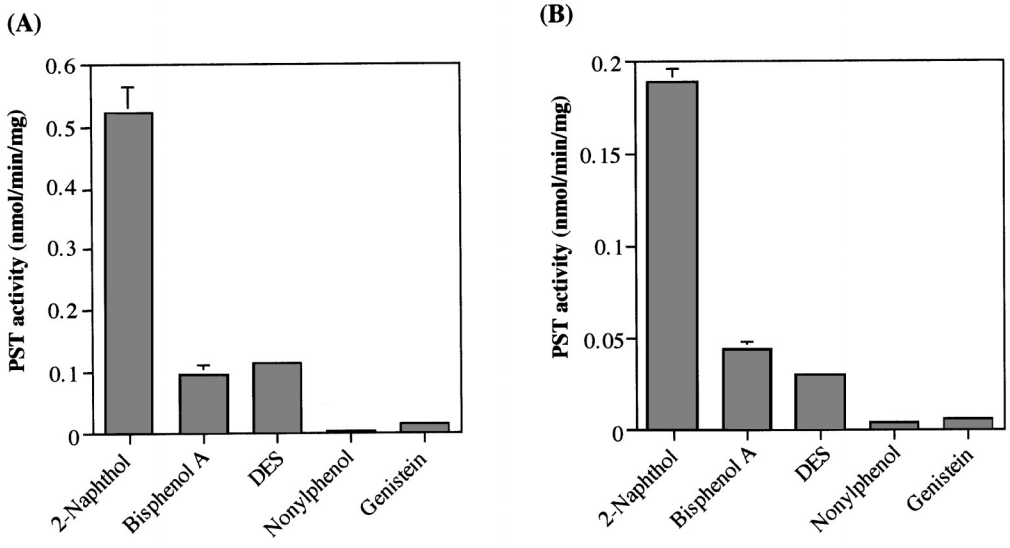

Fig. 1. ST Activity against Phenolic Environmental Estrogens of *E. coli*-Expressed Mouse Intestinal PST (A) and a Caco-2 Cell Extract (B)

Determination of ST activity is described in the Materials and Methods section. 50 μM of substrate and 10 μg of protein were used for each assay. 2-naphthol served as a control. The bars represent the average values of two assays with the deviations.

a high affinity for diethylstilbestrol (DES) and BPA. In fact, DES, BPA, and 2-naphthol were observed to undergo the same amount of catalysis, following exposure to *E. coli*-expressed mouse intestinal PST. On the other hand, nonylphenol and genistein were poor substrates for the enzyme. The Km values of DES and BPA were calculated to be 9.6 μM and 27 μM, respectively, based on Lineweaver-Burk plot analysis (n = 3) (Table 1). This illustrates that these chemicals have a high affinity for mouse intestinal PST.

Next, we examined the ability of the Caco-2 ST enzyme to sulfate DES, BPA, nonylphenol and genistein. The Caco-2 ST enzyme yielded similar, but not identical, results to that of mouse intestinal PST (Fig. 1B). Caco-2 ST had a high affinity for DES (Km = 3 μM), but a much lower affinity for BPA (Km = 190 μM) (Table 1). Previously, we demonstrated that two different forms of PST contribute to the PST activity of Caco-2 cells: simple phenol PST (P-PST), and monoamine PST (M-PST).[9] Previously, it has been found that M-PST predominates in Caco-2 cells.[9] Therefore, the preference for DES observed in the present experiment might be due to the activity of M-PST, which has a different substrate specificity than that of the mouse intestinal PST, a SULT1B subfamily.

Here, we described the ability of gastrointestinal PSTs to metabolize DES and BPA at μM concentrations. Suiko *et al*. have demonstrated that both P-PST and estrogen ST (EST) are very active against environmental estrogens.[12] We detected EST mRNA expression in Caco-2 cells (data not shown), however, we believe that EST activity made only a small contribution to the sulfation of DES and BPA in this experiment because Caco-2 cells were found to have very low EST activity (1.1 pmole/min/mg) against 17β-estradiol.

Inhibitory Effect of Phenolic Environmental Estrogens on PST Activity

We examined the effect of phenolic environmental estrogens on PST activity in the mouse intestine and in Caco-2 cells. As is shown in Fig. 2, 50 μM of

Table 1. Kinetic Parameters for Bisphenol A and DES of the PST Activities

	mouse intestinal		Caco-2	
substrates	Km (μM)	Vmax (nmol/min/mg)	Km (μM)	Vmax (nmol/min/mg)
Bisphenol A	27.3 ± 6.4	0.67 ± 0.19	190 ± 36	0.045 ± 0.006
DES	9.6 ± 1.2	1.95 ± 0.12	3.0 ± 0.4	0.026 ± 0.002

All values are the average of 3 experiments with S.D.

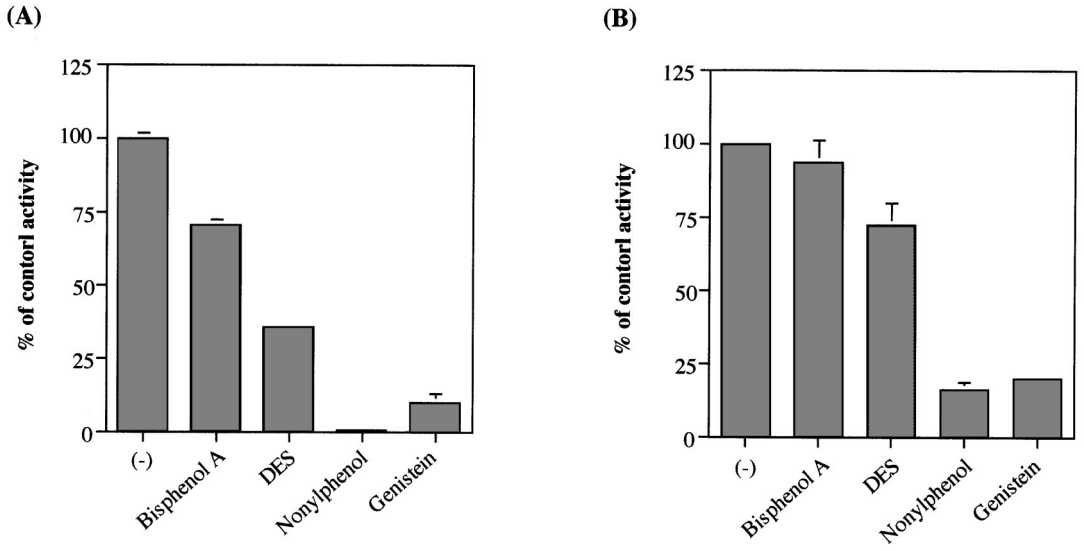

Fig. 2. Effect of Phenolic Environmental Estrogens on the PST Activity of Mouse Intestinal (A) and Caco-2 Cells (B)

PST activity *vs*. 2-naphthol (50 μM) was determined in the presence of environmental estrogens (50 μM). Residual activity is shown as a percentage of control activity (–). The bars represent the average values of two assays with the deviations.

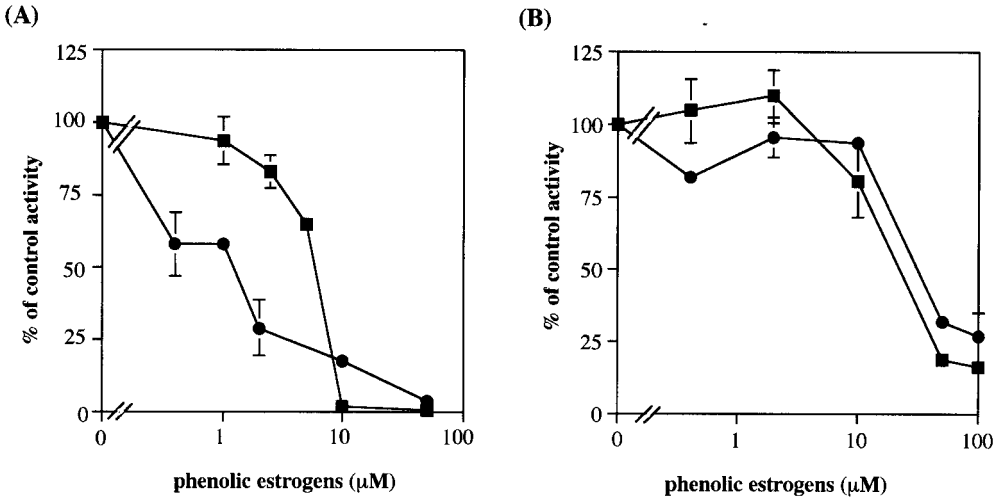

Fig. 3. Inhibitory Effect of Genistein and Nonylphenol on PST Activity

The effect of genistein (●), and nonylphenol (■), on PST activity *vs.* 50 μM 2-naphthol was determined at each indicated concentration. (A) Effect on mouse intestinal PST activity (10 μg of protein). IC_{50}s of 1.7 ± 0.6 and 4.3 ± 1.3 μM were calculated for genistein and nonylphenol, respectively (n = 3). (B) Effect on PST activity of the Caco2-cell extract (10 μg of protein). Points represent the averages of two assays with the deviations.

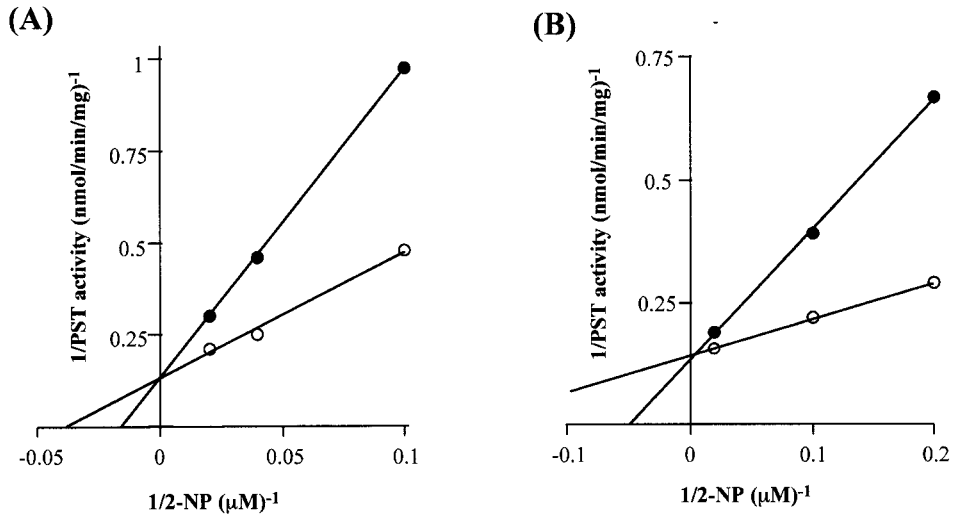

Fig. 4. Lineweaver-Burk Plots of Genistein and Nonylphenol Inhibition

Mouse intestinal PST activity *vs.* 5–50 μM of 2-naphthol was determined with (●), or without (○), 0.8 μM genistein (A) and 2 μM nonylphenol (B). All points represent an average of two assays.

nonylphenol and genistein strongly inhibited the PST activity of both cell lines against 50 μM 2-naphthol, while the PST activity of mouse intestinal and Caco-2 cells against DES and BPA was only moderately inhibited. The IC_{50}s of mouse intestinal activity were determined to be 4.3 ± 1.3 μM and 1.7 ± 0.6 μM for nonylphenol and genistein, respectively (n = 3) (Fig. 3). The inhibitory activity of these compounds was observed to be greater than that of 2,6-dichloro-4-nitrophenol (DCNP), a specific inhibitor of mammalian PST activity (IC_{50} of mouse intestinal PST activity = 30 μM).[10,13] Less inhibition of Caco-2 PST activity was observed compared to that of mouse intestinal PST activity (Fig. 3B). In addition, we detected residual Caco-2 PST insensitivity toward nonylphenol (*ca.* 25%) and genistein (*ca.* 35%), even at concentrations of 100 μM (Fig. 3B), in Caco-2 cells. This was probably due to monoamine PST activity, which is less sensitive to DCNP.[12] Kinetic analysis revealed that both nonylphenol and genistein were competitive inhibitors (Fig. 4).

The inhibitory action of these kinds of environ-

mental estrogens on gastrointestinal PST activity might influence the detoxification of toxic chemicals, the metabolism of pharmaceuticals, or the inactivation/activation of endogenous compounds such as dopamine and tri-iodothyronine that are catalyzed by PST enzymes. Further studies must be carried out to clarify the interactions between environmental estrogens and PSTs at gastrointestinal sites.

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