JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Effects of Phenolic Acids on Human Phenolsulfotransferases in Relation to Their Antioxidant Activity

CHI-TAI YEH AND GOW-CHIN YEN*

Department of Food Science, National Chung Hsing University, 250 Kuokuang Road, Taichung 40227, Taiwan, Republic of China

Sulfate conjugation by phenolsulfotransferase (PST) enzyme is an important process in the detoxification of xenobiotics and endogenous compounds. There are two forms of PST that are specific for the sulfation of small phenols (PST-P) and monoamines (PST-M). Phenoilc acids have been reported to have important biological and pharmacological properties and may have benefits to human health. In the present study, human platelets were used as a model to investigate the influence of 13 phenolic acids on human PST activity and to evaluate the relationship to their antioxidant activity. The results showed that chlorogenic acid, syringic acid, protocatechuic acid, vanillic acid, sinapic acid, and caffeic acid significantly ($p < 0.05$) inhibited the activities of both forms of PST by 21−30% at a concentration of 6.7 μM. The activity of PST-P was enhanced ($p < 0.05$) by *p*-hydroxybenzoic acid, gallic acid, gentisic acid, *o*-coumaric acid, *p*-coumaric acid, and *m*-coumaric acid at a concentration of 6.7 μM, whereas the activity of PST-M was enhanced by gentisic acid, gallic acid, *p*-hydroxybenzoic acid, and ferulic acid. The phenolic acids exhibited antioxidant activity as determined by the oxygen radical absorbance capacity (ORAC) assay and Trolox equivalent antioxidant capacity (TEAC) assay, especially gallic acid, *p*-hydroxybenzoic acid, gentisic acid, and coumaric acid, which had strong activity. The overall effect of phenolic acids tested on the activity of PST-P and PST-M was well correlated to their antioxidant activity of ORAC value ($r = 0.71$, $p < 0.01$; and $r = 0.66$, $p < 0.01$). These observations suggest that antioxidant phenolic acids might alter sulfate conjugation.

KEYWORDS: Platelet; human phenolsulfotransferases; phenolic acids; antioxidant activity

INTRODUCTION

Sulfation (sulfonation) is one of the major phase II conjugative reactions involved in the biotransformation of various endogenous compounds, drugs, and xenobiotics as well as in steroid biosynthesis, catecholamine metabolism, and thyroid hormone homeostasis (*1*, *2*). This reaction is controlled by a family of cytosolic sulfotransferases, which catalyze the transfer of a sulfate group from 3′-phosphoadenosine-5′-phosphosulfate (PAPS) onto the target molecules to increase the excretion of harmful substrates (*3*). In humans, there are two major subfamilies of cytosolic sulfotransferases involved in the metabolism of xenobiotics: phenolsulfotransferase (PST) and hydroxysteroid sulfotransferase (HST) (*4*). HST is responsible for the sulfation of steroids. Two functionally distinct forms of PST have been identified in human tissue. One form, PST-P, is relatively specific for the sulfation of small phenols and structurally related neutral compounds, whereas PST-M is primarily responsible for the sulfation of monoamines, such as dopamine. These cytosolic sulfotransferases are particularly active in platelets and are generally present in the intestinal wall, adrenal gland, and brain (*5*). However, the PST in human platelets, liver, and gut show great variation in their activities.

PST-P levels in platelets are particularly interesting because they are highly correlated with the corresponding PST-P levels in the human liver, cerebral cortex, and small intestinal mucosa (*6*). Thus, it is feasible to use the PST activity in blood platelets to reflect any drug metabolizing activity in other tissues of interest. There is evidence that harmful substrates might accumulate in the body when the PST activity is inhibited (*7*, *8*).

In addition to the well-known vitamins C and E, or carotenoids, research has shown that fruits and vegetables contain many phytonutrients which possess antioxidant properties (*9*). Phenolic acids such as hydroxybenzoic acids and hydroxycinnamic acids are antioxidant compounds in fruits and vegetables (*10*). Research on phenolic acids is of current interest due to the important biological and pharmacological properties attributed to their antioxidant properties (*11*). Nevertheless, in the literature data regarding the effects of phenolic acids on phenolsulfotransferase activities is limited.

On this basis, this work uses human platelets as a model to assess the influence of phenolic acids on the activity of human phenolsulfotransferases (PST-P or PST-M). In addition, the antioxidant capacity of phenolic acids was investigated by using

* Author to whom correspondence should be addressed (telephone +886-4-2287-9755; fax +886-4-2285-4378; e-mail gcyen@mail.nchu.edu.tw).

10.1021/jf0208132 CCC: $25.00
Published on Web 01/28/2003

the oxygen radical absorbance capacity (ORAC) assay (*12*) and Trolox equivalent antioxidant capacity (TEAC) assay (*13*). The effect of phenolic acids on the activities of both forms of phenosulfotransferases in relation to their antioxidant capacities was determined.

MATERIALS AND METHODS

Materials and Reagents. Gentisic acid, chlorogenic acid, syringic acid, protocatechuic acid, *p*-hydroxybenzoic acid, vanillic acid, ferulic acid, gallic acid, caffeic acid, sinapic acid, *o*-coumaric acid, *p*-coumaric acid, *m*-coumaric acid, *p*-nitrophenol, dopamine, sucrose, Na_2EDTA, β-phycoerythnin (β-PE) from *Porphydium cruentum*, and 2,2′-azinobis-(3-ethylbenzthiazoline-6-sulfonate) (ABT^+) were all obtained from Sigma Chemical Co. (St. Louis, MO). [^{35}S]-3′-Phosphoadenosine-5′-phosphosulfate ($PAPS^{35}$) (1.0−1.5 Ci/mmol) was obtained from Du Pont-New England Nuclear (Boston, MA). 2,2′-Azobis(2-amidinopropane) hydrochloride (AAPH) was purchased from Wako Chemicals (Osaka, Japan). 6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) was obtained from Aldrich (Milwaukee, WI).

Platelet Preparation. Blood (50 mL) was collected from healthy adult volunteers by venepuncture and added into 1 mL of 5% Na_2-EDTA with gentle mixing. Samples were kept at room temperature and processed within 2 h. The samples were centrifuged at 300*g* for 10 min, and platelet-rich plasma was carefully aspirated and recentrifuged at 8000*g* for 5 min. The platelet pellet was washed twice with 0.9% sodium chloride and resuspended in 10 mM potassium phosphate buffer, pH 7.0, prior to homogenization. The homogenate was then stored at −20 °C until analyzed.

Platelet Protein Estimation. The protein content of platelet cytosols was estimated by the method of Bradford (*14*) with bovine serum albumin as a standard.

Assay of PST Activity. PST activity was measured according to the method of Folds and Meek (*15*) with a modification. *p*-Nitrophenol (final concentration = 3 μM) and dopamine (final concentration = 10 μM) were used as substrate for measuring PST-P and PST-M, respectively. Platelet homogenates (20 μL) were incubated with substrates and 6.7 μM ^{35}S-labeled PAPS in a final volume of 180 μL of potassium phosphate buffer, pH 7.0, for 20 min at 37 °C. The reaction was terminated by adding 0.1 M barium acetate. Protein, unreacted PAPS, and free sulfate were removed by precipitation with 0.1 M barium hydroxide and 0.1 M zinc sulfate. After centrifugation, 500 μL of the supernatant was thoroughly mixed with 4 mL of scintillant, and radioactivity was measured by liquid scintillation spectrometry. Incubation was performed in triplicates with a blank (no homogenate) being subtracted.

Effect of Phenolic Acids on PST Activity. The influence of phenolic acids on PST activity was determined according to the method of Fold and Meek (*15*) and Bamforth et al. (*16*) with a slight modification. The incubation mixture contained 100 μL of 0.1 M potassium phosphate buffer (pH 7.0), 20 μL of the platelet homogenates, 20 μL of the substrate, and 20 μL of phenolic acids (final concentrations = 6.7 μM for individual compound); 20 μL of ^{35}S-labeled PAPS (final concentration = 6.7 μM) was added at successive intervals to tubes at 37 °C in a water bath, and the reaction was terminated after 20 min by the addition of 0.1 M barium acetate (200 μL). Any unreacted PAPS, free sulfate, or protein was precipitated by two additions of 0.1 M barium hydroxide (200 μL) followed by 0.1 M zinc sulfate (200 μL). After centrifugation (11 500*g* for 3 min), 500 μL of the supernatant was thoroughly mixed with 4 mL of scintillant, and radioactivity was measured by liquid scintillation spectrometry. All assays were performed in triplicate.

Trolox Equivalent Antioxidant Capacity (TEAC) Assay. The antioxidant activities of various phenolic acids were measured according to the method of Miller et al. (*13*) using the TEAC assay. Briefly, ABTS radical cation ($ABTS^+$) solution was diluted with PBS (5 mM) to obtain a solution of 0.8 unit in absorbance at 734 nm. The solution was transferred into a plastic cuvette to which 50 μL of the phenolic acids was added (final concentration = 6.7 μM for individual compound), and the absorbance was read after exactly 1 min. A dose−response curve was plotted for Trolox, and the TEAC value is expressed as millimolar to a concentration of Trolox solution having the antioxidant equivalent to a 1000 ppm solution of the sample under investigation. A higher TEAC value of the sample indicates a stronger antioxidant activity.

Table 1. Effect of Phenolic Acids on Human Platelet Sulfotransferase Activity[a]

phenolic acid	PST activity (pmol/min/mg of protein)	
	p-nitrophenol	dopamine
gentisic acid	0.50 ± 0.00(–25)[b]	3.28 ± 0.18 (–21)
chlorogenic acid	0.35 ± 0.05 (4.4)	2.59 ± 0.09 (4.8)
syringic acid	0.15 ± 0.05 (22.0)	2.32 ± 0.25 (15.0)
protocatechuic acid	0.17 ± 0.05 (21.0)	2.31 ± 0.08 (15.0)
p-hydroxybenzoic acid	0.44 ± 0.01 (–1.6)	2.81 ± 0.19 (–3.4)
vanillic acid	0.07 ± 0.04 (29.0)	2.22 ± 0.07 (18.0)
ferulic acid	0.30 ± 0.04 (9.2)	2.88 ± 0.06 (–6.1)
gallic acid	0.56 ± 0.00 (–14.0)	2.82 ± 0.06 (–3.9)
caffeic acid	0.36 ± 0.05 (8.4)	2.64 ± 0.10a[c] (2.9)
sinapic acid	0.30 ± 0.01 (9.3)	2.06 ± 0.04 (24.0)
o-coumaric acid	0.56 ± 0.04 (–13.0)	2.11 ± 0.02 (23.0)
p-coumaric acid	0.67 ± 0.06 (–23.0)	2.00 ± 0.23 (26.0)
m-coumaric acid	0.47 ± 0.05 (–5.4)	2.66 ± 0.06a (2.1)

[a] PST-P and PST-M activities of control (without phenolic acid) were 0.40 ± 0.07 and 2.71 ± 0.03 pmol/min/mg of protein, respectively. Data are expressed as mean specific activity ± SD from three experiments. The phenolic acid concentration used in sulfotransferase assay was 6.7 μM. Blood was collected from three healthy males. [b] Values in parentheses represent the inhibition percentage of phenolic acids on human platelet sulfotransferase. [c] Values within a column without a letter are significantly different from controls at $p < 0.05$.

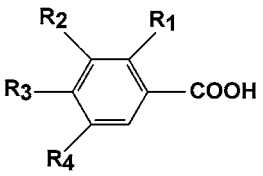
Oxygen Radical Absorbance Capacity (ORAC) Assay. The automated ORAC assay was carried out on a Fluostar Galaxy plate reader (BMG LabTechologies, GmbH, Offenburg, Germany) with a fluorescent filter (ex 540 nm; em 565 nm). The procedure was based on the previous report of Cao et al. (*17*) with a slight modification. Briefly, in the final assay mixture, β-PE (16.7 nM) was used as a target of free radical (or oxidant) attack with AAPH (40 mM) as a peroxyl radical generator. Trolox (1 μM) was used as a standard and prepared fresh daily. The analyzer was programmed to record the fluorensence of β-PE every 5 min after AAPH was added. All fluorescence measurements were expressed relative to the initial reading. Final results were calculated using the differences of area under the β-PE decay curves between the blank and a sample and expressed as micromoles of Trolox equivalents per micromoles of sample.

Statistical Analysis. Correlation and regression analyses and principal component analysis (PCA) were performed using the SigmaPlot scientific graph system. The component loading included ORAC and TEAC activities as well as activities of P-form and M-form phenolsulfotransferases. All data were analyzed by one-way analysis of variance (ANOVA). Significant differences ($p < 0.05$) between means were determined using Duncan's multiple-range tests.

RESULTS

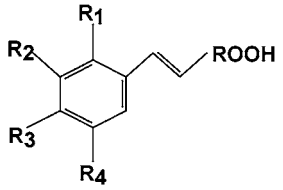
Influence of Phenolic Acids on Human Platelet Sulfotransferase Activity. Phenolic acids are widely distributed in nature and can be derived from two nonphenolic molecules: benzoic and cinnamic acids, respectively. Gallic, vanillic, syringic, gentisic, and *p*-hydroxybenzoic acids are hydroxy derivatives of benzoic acid, whereas caffeic, ferulic, sinapic, and *p*-coumaric acids are hydroxy derivatives of cinnamic acid. In this study, the effects of 13 phenolic acids on the sulfation of *p*-nitrophenol and dopamine were determined. The chemical structures of these phenolic acids are shown in **Figure 1**. The results shown in **Table 1** indicate that the PST-P and PST-M activities of the control (without phenolic acids) were 0.40 and 2.71 pmol/min/mg of protein, respectively. Moreover, the addition of phenolic acids could influence the activities of both forms of PST. The inhibition percentage of phenolic acids on

Hydroxybenzoic acids

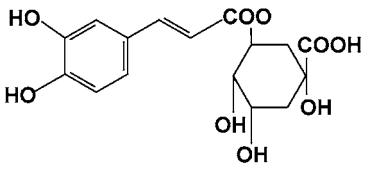


R_1=R_2=R_4=H, R_3=OH	*p*-Hydroxybenzoic acid
R_1=R_4=H, R_2=R_3=OH	Protocatechuic acid
R_1=R_4=H, R_2=OCH_3, R_3=OH	Vanillic acid
R_1=H, R_2=R_3=R_4=OH	Gallic acid
R_1=H, R_2=R_4=OCH_3, R_3=OH	Syringic acid
R_1=R_4=OH, R_2=R_3=H	Gentistic acid

Hydroxycinnamic acids



R_1=OH, R_2=R_3=R_4=H	*o*-Coumaric acid
R_1=R_3=R_4=H, R_2=OH	*m*-Coumaric acid
R_1=R_2=R_4=H, R_3=OH	*p*-Coumaric acid
R_1=R_4=H, R_2=R_3=OH	Caffeic acid
R_1=R_4=H, R_2=OCH_3, R_3=OH	Ferulic acid
R_1=H, R_2=R_4=OCH_3, R_3=OH	Sinapic acid



Chlorogenic acid

Figure 1. Chemical structures of phenolic acids.

human platelet sulfotransferase is also presented in **Table 1**. It was found that syringic acid, protocatechuic acid, and vanillic acid showed strong inhibitory effects (21−30%) on platelet PST-P activity at a concentration of 6.7 μM. Although vanillic acid showed the strongest inhibition on PST-P, it had a weaker inhibition (18%) on PST-M activity ($p < 0.05$). Protocatechuic acid and syringic acid could moderately inhibit the PST-M and PST-P activities by 21−22%. Chlorogenic acid, ferulic acid, caffeic acid, and sinapic acid showed lower inhibitory effects on PST-P activity, whereas *o*-coumaric acid, *p*-coumaric acid, and sinapic acid had marked inhibition on PST-M activity. Moreover, other phenolic acids (gentisic acid, ferulic acid, *p*-hydroxybenzoic acid, and gallic acid) showed no influence on PST-M activity.

Some compounds, including *p*-hydroxybenzoic acid, gentisic acid, gallic acid, *o*-coumaric acid, *p*-coumaric acid, and *m*-coumaric acid, were found to increase ($p < 0.05$) the PST-P activity. PST-P activity was elevated by these phenolic acids in the order of gentisic acid > *p*-coumaric acid > gallic acid > *o*-coumaric acid > *p*-hydroxybenzoic acid ≒ *m*-coumaric acid. Gentisic acid was the most active compound to increase ($p < 0.05$) the activity of PST-M, followed by gallic acid, *p*-hydroxybenzoic acid, and ferulic acid. As shown in **Table 1**, the activity of PST-P increased in the presence of *o*-coumaric acid, *p*-coumaric acid, and *m*-coumaric acid. In contrast, PST-M

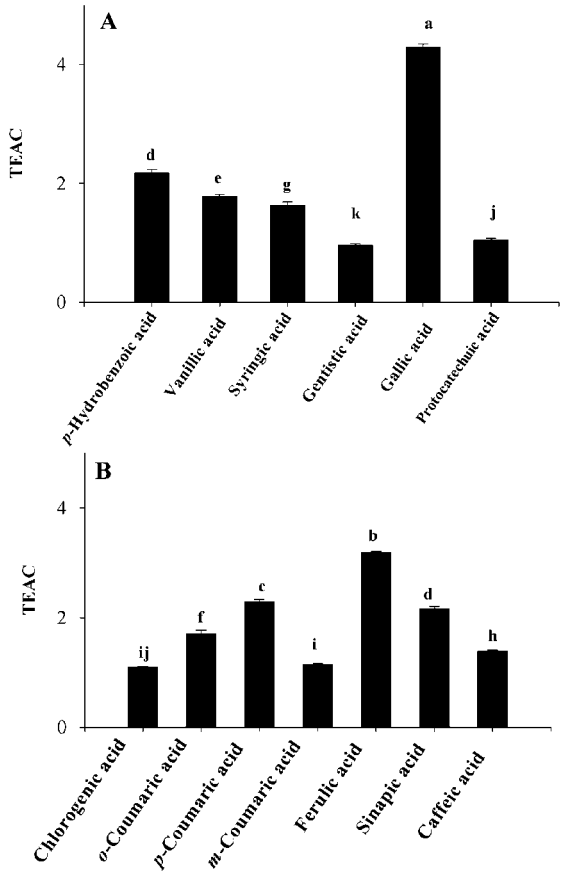


Figure 2. TEAC values of phenolic acids: (A) hydroxy derivatives of benzoic acids; (B) hydroxy derivatives of cinnamic acids. The TEAC value is the millimolar concentration of a Trolox solution having the antioxidant capacity equivalent to a 1000 ppm solution of the sample. Data are expressed as mean ± SD from three experiments. Bars topped by different letters are significantly different at $p < 0.05$.

activity was significantly inhibited by *o*-coumaric acid, *p*-coumaric acid, and *m*-coumaric acid. Gallic acid, a trihydroxybenzoic acid, caused an increase in the activities of both forms of PST.

Antioxidant Activity of Phenolic Acids Evaluated by TEAC and ORAC Assays. Figure 2 shows the antioxidant activity of phenolic acids evaluated by the TEAC assay. All phenolic acids had higher molar equivalent antioxidant capacity than that of Trolox ($p < 0.05$). The TEAC values of gallic acid and *p*-hydroxybenzoic acid were 4.3 and 2.2 mM Trolox equiv, respectively, at a concentration of 6.7 μM ($p < 0.05$). Both gallic acid and *p*-hydroxybenzoic acid were effective scavengers for an $ABTS^+$ radical. The antioxidant activity of hydroxybenzoic acids was in the order of gallic acid > *p*-hydroxybenzoic acid > vanillic acid > syringic acid > protocatechuic acid > gentisic acid. The results also indicated that the antioxidant activity of hydroxycinammic acids was found to be in the order of ferulic acid > *p*-coumaric acid > sinapic acid > *o*-coumaric acid > *m*-coumaric acid > caffeic acid > chlorogenic acid. Ferulic acid and *p*-coumaric acid had the highest TEAC values, 3.2 and 2.3 mM Trolox equiv, respectively.

Figure 3 shows the antioxidant activity of various phenolic acids against peroxyl radical ($ORAC_{ROO\cdot}$ activity). The data indicate that gallic acid had the highest $ORAC_{ROO\cdot}$ value (Trolox equiv, 14 μM) among the 13 phenolic acids tested. *p*-Coumaric acid, *m*-coumaric acid, gallic acid, gentisic acid, and *p*-hydroxybenzoic acid had $ORAC_{ROO\cdot}$ values within the range of 8.3−12.8 μM Trolox equiv. Caffeic acid, sinapic acid, vanillic

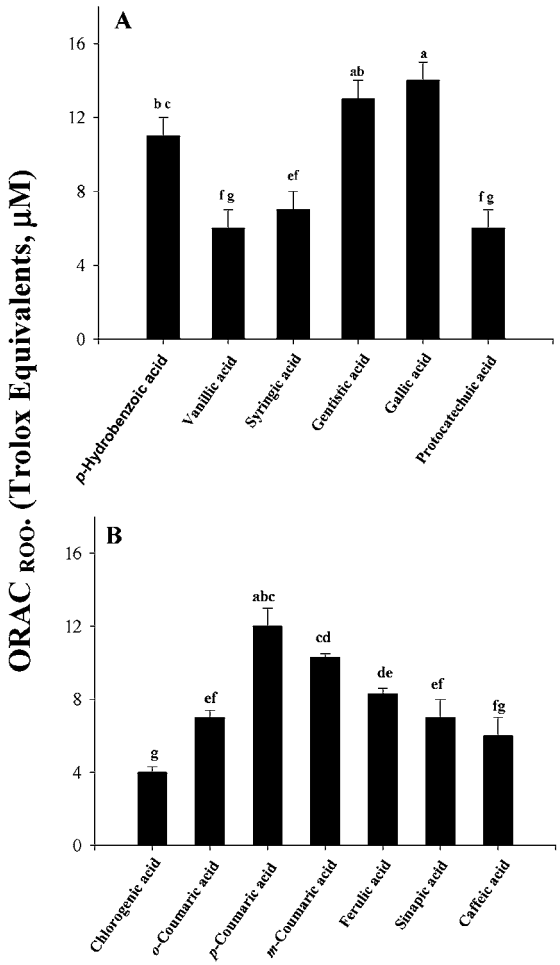


Figure 3. $ORAC_{ROO\cdot}$ activity (Trolox equiv, μM) of phenolic acids: (A) hydroxy derivatives of benzoic acids; (B) hydroxy derivatives of cinnamic acids. Data are expressed as mean ± SD from three experiments. Bars topped by different letters are significantly different at $p < 0.05$.

acid, syringic acid, and chlorogenic acid were in the lower $ORAC_{ROO\cdot}$ values (Trolox equiv, 4−6 μM). Among the hydroxycinnamic acids, both the *p*-coumaric acid and *m*-coumaric acid had higher $ORAC_{ROO\cdot}$ activity than any of the others.

Of the tested phenolic acids including gallic acid, *p*-hydroxybenzoic acid, gentisic acid, and coumaric acid, all acted as antioxidants against peroxyl radical ($ROO^{\bullet}$) in a dose-dependent manner (data not shown). In addition, the results obtained from $ORAC_{ROO\cdot}$ assay were similar to those of the TEAC assay. In general, phenolic acids that inhibited PST activity were found to have lower antioxidant capacities.

Relationship between the Influence of Phenolic Acids on PST Activity and Their Antioxidant Activity. The influence of phenolic acids on PST-P activity in relation to their antioxidant activity is presented in **Figure 4**. It was found that there is a significant linear correlation between the influence of phenolic acids on PST-P activity and their antioxidant activity. A correlation coefficient ($r = 0.71$, $p < 0.01$) was observed between the influence of phenolic acids on PST-P activity and their ORAC values (**Figure 4A**). In addition, the influence of phenolic acids on PST-P activity was linearly related to the TEAC measurement ($r = 0.71$, $p < 0.01$) (**Figure 4B**). A similar result can be seen in **Figure 5**, where there is a significant linear correlation between the influence of phenolic acids on PST-M activity and their ORAC values ($r = 0.66$, $p < 0.01$) (**Figure 5A**) or TEAC values ($r = 0.66$, $p < 0.01$) (**Figure 5B**). These results suggest that phenolic acids tested on the activity of PST-P and PST-M were well correlated to their antioxidant activity.

DISCUSSION

This paper describes the influence of phenolic acids on the activity of both forms of PST in relation to their antioxidant activity. The results demonstrate that syringic acid, protocatechuic acid, vanillic acid, chlorogenic acid, sinapic acid, and caffeic acid were moderate inhibitors of both forms of PST at a concentration of 6.7 μM (**Table 1**). Vanillic acid, the most abundant compound in vanillin, showed 28 and 13% inhibition on PST-P and PST M activity, respectively. Vanillin was reported to inhibit 50% of liver 17α-ethinylestradiol sulfotransferases (EE_2ST) activity (IC_{50}) at a concentration of ∼1.3 μM (*16*). Caffeic acid from coffee and tea was found to be a potent inhibitor of PST-P (*2*). Quercetin was found to be a potent inhibitor of PST-P, with an IC_{50} value of 0.10 μM (*18*). Our results agreed with those reports and also found that vanillic acid, caffeic acid, and quercetin (data not shown) were potent inhibitors for both forms of PST. In contrast, the results presented in the present study comprise the first report to show that gallic acid, gentisic acid, and *p*-hydroxybenzoic acid could enhance the activity of both PST-P and PST-M. There was a significant correlation between the influence of phenolic acids on PST activity and their antioxidant activity (**Figures 4** and **5**).

Phenolic acids, especially hydroxycinnamic acids and hydroxybenzoic acids, are secondary plant products and are commonly found in plant-derived foodstuffs. Ferulic acid and *p*-coumaric acid have been reported to act as scavengers of thiol free radicals (*19*). In addition, ferulic acid has a strong antioxidant activity by preventing oxidative DNA damage induced by the Fenton reaction (*20*). Gentisic acid was reported to have an inhibitory action in the myeloperoxidase system and was able to impair the tyrosyl radical catalyzed low-density lipoprotein peroxidantion (*21*). Recently, gallic acid has been shown to induce cell death in cancer cells (*22*).

The ORAC assay is one of the methods used to evaluate the antioxidant capacity of various biological substrates, ranging from pure compounds such as melatonin (*23*) and flavonoids (*24*) to complex matrices such as vegetables (*9*) and animal tissues (*25*). Both the TEAC and ORAC assays were used in this study to evaluate the antioxidant activities of phenolic acids that increased the activity of PST. In the present study, it was found that there was a significant correlation between the influence of phenolic acids on both forms of PST activities and their ORAC or TEAC values ($r = 0.71$, $p < 0.01$; and $r = 0.66$, $p < 0.01$). According to the results of recent studies conducted in our laboratory (unpublished data), it was found that there was a significant correlation between the platelet content and the activities of both forms of PST in different patients with hypertension, migraine, or hypothyroidism ($r = 0.68$, $p < 0.01$; $r = 0.63$, $p < 0.01$). These data indicate that the activity of platelet PST varied among the patients and had a lower level of PST activity. Our data in this study indicate that varied disorders may affect the activity of platelet PST. Therefore, the activities of both forms of PST in the platelet of these patients were significantly increased by gallic acid, ferulic acid, gentisic acid, and coumaric acid (data not shown).

These findings have clinical significance as the cytosolic phenolsulfotransferases inactivated phenols and amines by catalyzing their conjugations with sulfate donated from PAPS. The M-form enzymes catalyzed the sulfate conjugation of micromolar concentrations of dopamine and other phenolic

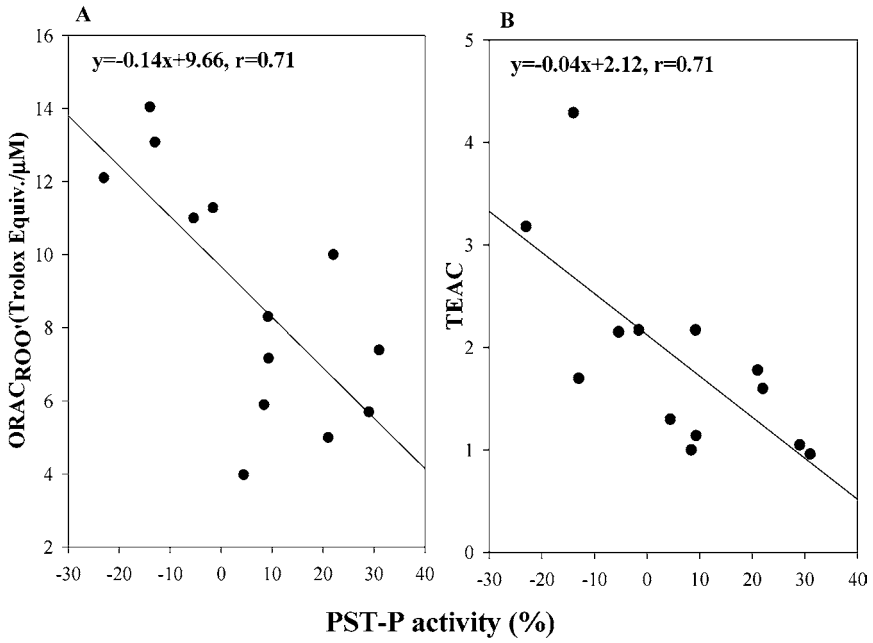


Figure 4. Effects of phenolic acids on P-form phenosulfotransferase activity$_{(X)(\text{pmol/min/mg of protein})}$ in relation to their (A) ORAC$_{ROO^{\bullet}(Y)(\text{Trolox equiv}/\mu M)}$ and (B) TEAC$_{(Y)(\text{TEAC, mM})}$. Each value is the mean ± SD of three experiments.

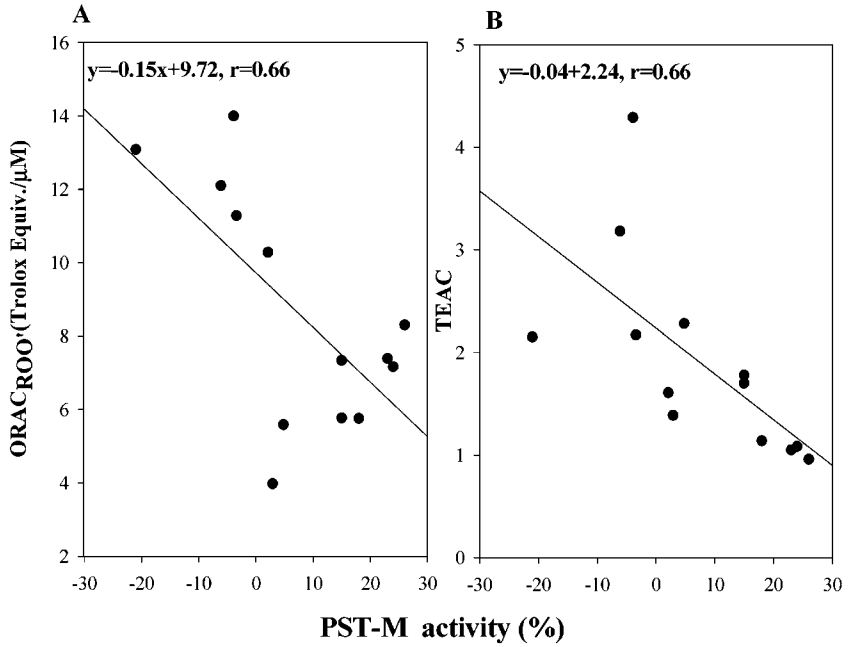


Figure 5. Effects of phenolic acids on M-form phenosulfotransferase activity$_{(X)(\text{pmol/min/mg of protein})}$ in relation to their (A) ORAC$_{ROO^{\bullet}(Y)(\text{Trolox equiv}/\mu M)}$ and (B) TEAC$_{S(Y)(\text{TEAC, mM})}$. Each value is the mean ± SD of three experiments.

monoamines. Patients with migraine appear to have significantly lower levels of platelet M-form PST activity, leading to raised central nervous system levels of catecholamines, which are believed to be a major factor in headaches (*26*). Friberg et al. (*27*) demonstrated that reduced metabolism of bioactive compounds such as dopamine or other monoamines following inhibition of PST-M might result in an increase in the bioavailability of dopamine and the consequent altered pharmacological action such as migraine. Alternatively, changes in PST activity might be linked to a pharmacological response, a lower sulfating process that has been related to depressive patients (*28*). However, the capacity of these phenolic acids to modulate conjugation reactions may be a factor in the interindividual variation found in xenobiotic metabolism and could make it necessary for volunteers in drug metabolism studies to adhere to a common dietary regime.

In conclusion, our results demonstrate that chlorogenic acid, syringic acid, protocatechuic acid, vanillic acid, sinapic acid, and caffeic acid all inhibited both forms of PST activity. On the contrary, *p*-hydroxybenzoic acid, gentisic acid, gallic acid, *o*-coumaric acid, *p*-coumaric acid, and *m*-coumaric acid were found to increase the PST-P activity. However, gentisic acid, gallic acid, *p*-hydroxybenzoic acid, and ferulic acid could increase PST-M activity. In addition, gallic acid, ferulic acid, gentisic acid, and coumaric acid had antioxidant capacity in both the ORAC and TEAC assay system. There was a significant correlation between the influence of phenolic acids on the activity of both forms of PST and their antioxidant capacity of

ORAC value. Because PST is a key enzyme in the catalysis of xenobiotic metabolism, increased activity of PST will therefore promote the efficiency of detoxification as well as steroid biosynthesis and thyroid hormone homeostasis. Our results provide better understanding of the effects of phenolic acids on human PST activities, as well as information regarding the intake of phenolic antioxidants for human health. Further studies are planned to explore the systematic combination of two and three compounds to evaluate the interaction of phenolic acids on promoting PST activities.

LITERATURE CITED

(1) Brix, L. A.; Nicoll, R.; Zhu, X.; McManus, M. E. Structural and functional characterization of human sulfotransferases. *Chem. Biol. Interact.* **1998**, *109*, 123−127.

(2) Coughtrie, M. C. H.; Sharp, S.; Maxwell, K.; Innes, N. P. Biology and function of the reversible sulfation pathway catalysed by human sulfotransferase and sulfatases. *Chem. Biol. Interact.* **1998**, *109*, 3−27.

(3) Weinshilboum, R.; Aksoy, I. Sulfation pharmacogenetics in humans. *Chem. Biol. Interact*. **1994**, *92*, 233−237.

(4) Brix, L. A.; Barnett, A. C.; Duggleby, R. G.; Leggett, B.; McManus, M. E. Analysis of the substrate specificity of human sulfotransferases SULT1A1 and SULT1A3:site-directed mutagenesis and kinetic studies. *Biochemistry* **1999**, *38*, 10474−10479.

(5) Littlewood, J. T.; Glover, V.; Sandler, M. Red wine contains a potent inhibitor of phenolsulphotransferase. *Br. J. Clin. Pharmacol*. **1985**, *19*, 275−278.

(6) Anderson, R. J.; Kudlacek, P. E.; Clemens, D. L. Sulfation of minoxidil by multiple human cytosolic sulfotransferases. *Chem. Biol. Interact.* **1998**, *109*, 53−67.

(7) Weinshiboum, R. M. Phenol sulfotransferase in humans: properties, regulation and function. *Fed. Proc*. **1986**, *45*, 2223−2228.

(8) Falany, C. N. Molecular enzymology of human liver cytosolic sulfotransferase. *Trends Pharmacol. Sci*. **1991**, *12*, 255−259.

(9) Cao, G.; Sofic, E.; Prior, R. L. Antioxidant capacity of tea and common vegetables. *J. Agric. Food Chem.* **1996**, *44*, 3426−3431.

(10) Bagchi, D.; Hassoum, E.; Bagchi, M.; Stohs, S. Protective effects of antioxidants against Endrin-induced hepatic lipid/peroxidantion, DNA damage and excretion of urinary lipid metabolites. *Free Radical Biol. Med*. **1993**, *15*, 217−222.

(11) Tanaka, T.; Kojima, T.; Kawamori, T.; Wang, A.; Suzui, M.; Okamoto, K.; Mori, H. Chemoprevention of diethynitro-samine-induced hepatocarcinogenesis by a simple phenolic acid protocatechuic acid in rats. *Cancer Res.* **1993**, *53*, 2775−2779.

(12) Cao, G.; Alessio, H. M.; Culter, R. G. Oxygen-radical absorbance capacity assay for antioxidants. *Free Radical Biol. Med*. **1995**, *14*, 303−311.

(13) Miller, N. J.; Rice-Evans, C. A.; Davies, M. J.; Gopinathan, V.; Milner, A. A novel method for measuring antioxidant capacity and its application to monitoring the antioxidant status in premature neonates. *Clin. Sci.* **1993**, *84*, 407−412.

(14) Bradford, M. M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein- dye binding. *Anal. Biochem.* **1976**, *72*, 248−254.

(15) Foldes, A.; Meek, J. L. Rat brain phenolsulfotransferase—partial purification and some properties. *Biochim. Biophys. Acta* **1973**, *327*, 365−374.

(16) Bamforth, K. J.; Jones, A. L.; Roberts, R. C.; Coughtrie, M. W. H. Common food additives are potent inhibitors of human liver 17α-ethinyloestradiol and dopamine sulphotransferases. *Biochem. Pharmacol.* **1993**, *46*, 1713−1720.

(17) Cao, G.; Sofic, E.; Prior, R. L. Antioxidant and pro-oxidant behavior of flavonoids: structure−activity relationships. *Free Radical Biol. Med.* **1993**, *22*, 749−760.

(18) Walle, T.; Eaton, E. A.; Walle, U. K. Quercetin, a potent and specific inhibitor of the human P-form phenolsulphotransferase. *Biochem. Pharmacol.* **1995**, *50*, 731−734.

(19) D'Aquino, M.; Buillion, C.; Chopra, M.; Devi, D.; Dunster, C.; James, G.; Niki, E.; Willson, R. Sulphydryl (thiyl) free radical formation, biochemically, by sonolysis, by radiolysis, and thermally: vitamin A, curcumin, muconic acid and related conjugated olefins as reference activity models. *Methods Enzymol*. **1994**, *233*, 34−36.

(20) Aruoma, O. I. Antioxidant action of plant foods: use of oxidative DNA damage as a tool for studying antioxidant efficacy. *Free Radical Res.* **1999**, *30*, 419−427.

(21) Hermann, M.; Kapiotis, S.; Hofbauer, R.; Seelos, Ch.; Held, I.; Gmeiner, B. Salicylate promotes myeloperoxidase-initiated LDL oxidation: Antagonization by its metabolite gentistic acid. *Free Radical Biol. Med.* **1999**, *26*, 1253−1260.

(22) Serrano, A.; Palacios, C.; Roy, G.; Cespon, C.; Villar, M. L.; Nocito, M.; Porque, P. G. Derivates of gallic acid induce apoptosis in tumoral cell lines and inhibit lymphocyte proliferation. *Arch. Biochem. Biophys.* **1998**, *350*, 49−54.

(23) Aejmelaeus, R.; Holm, P.; Kaukinen, U.; Metsä-Ketelä,T. J. A.; Laippala, P.; Hervonen, A. J. L.; Alho, H. R. O. Age-related changes in the peroxyl radical scavenging capacity of human plama. *Free Radical Biol. Med.* **1997**, *23*, 69−75.

(24) Cao, G.; Verdon, C. P.; Wu, A. H. B.; Wang, H.; Prior, R. L. Automated oxygen radical absorbance capacity assay using the COBAS FARA II. *Clin. Chem*. **1997**, *41*, 1738−1744.

(25) Kohen, R.; Beit-Yannai, E.; Berry, E. M.; Tirosh, O. Overall low molecular weight antioxidant activity of biological fluids and tissues by cyclic voltammetry. *Methods Enzymol.* **1999**, *300*, 285−296.

(26) Humphrey, P. P. A.; Fenuik, W.; Perren, M. J.; Beresford, I. J. M.; Skingle, M.; Whalley, E. T. Serotonin and migraine. *Ann. N.Y. Acad. Sci*. **1990**, *600*, 587−600.

(27) Friberg, L.; Olesen, J.; Iversen, H. K.; Sperling, B. Migraine pain associated with middle cerbral artery dilation: reversal by sumatriptan. *Lancet* **1991**, *338*, 13−17.

(28) Mine, K.; Okada, M.; Mishima, N.; Fujiwara, M.; Nagakawa, T. Plasma-free and sulfoconjugated MHPG in major depressive disorders: Differences between responders to treatment and non-responders. *Biol. Psychiatry* **1993**, *34*, 654−660.

Received for review July 23, 2002. Revised manuscript received November 25, 2002. Accepted November 25, 2002. This research work was partially supported by the National Science Council, the Republic of China, under Grant NSC90-2313-B005-154.

JF0208132