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Differential Roles of Human Monoamine (M)-Form and Simple Phenol (P)-Form Phenol Sulfotransferases in Drug Metabolism

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Cytosolic sulfotransferases (STs) are traditionally known as Phase II drug-metabolizing or detoxifying enzymes that facilitate the removal of drugs and other xenobiotic compounds. In this study, we carried out a systematic investigation on the sulfation of drug compounds by two major human phenol STs (PSTs), the monoamine (M)-form and simple phenol (P)-form PSTs. Activity data obtained showed the differential substrate specificity of the two enzymes for the thirteen drug compounds tested. Kinetic studies revealed that the M-form PST displayed stereoselectivity for the chiral drug, isoproterenol. The effects of divalent metal cations on the activity of the M-form and P-form PSTs toward representative drug compounds were quantitatively evaluated. Results obtained indicated that the drug-sulfating activities of the two human PSTs were partially or completely inhibited or stimulated by the ten divalent metal cations tested at a 5 mM concentration. The two enzymes appeared to be less sensitive to the effects of physiologically more abundant metal cations such as Mg^{2+} and Ca^{2+}, but more sensitive to the detrimental effects of other metal cations that may enter the body as environmental contaminants.

Key words: drug metabolism, phenol sulfotransferase, stereoselectivity, substrate specificity, sulfation.

Abbreviations: ST, sulfotransferase; PST, phenol sulfotransferase; PAPS, 3′-phosphoadenosine 5′ phosphosulfate.

Sulfate conjugation is a major pathway *in vivo* for the biotransformation and excretion of drugs and xenobiotics, as well as endogenous compounds such as steroid and thyroid hormones, catecholamines, cholesterol, and bile acids (*1–3*). The responsible enzymes, called the "cytosolic sulfotransferases (STs)", catalyze the transfer of a sulfonate group from the active sulfate, 3′-phosphoadenosine 5′-phosphosulfate (PAPS), to an acceptor substrate compound containing either a hydroxyl or an amine group (*4*). Sulfate conjugation may result in the inactivation or activation of the substrate compounds or increase their water-solubility, thereby facilitating their removal from the body (*1–3*).

In searching for the cytosolic ST enzymes involved in the sulfation of drugs, earlier studies revealed two distinct forms of the "phenol ST" (PST) in human platelets (*5*, *6*). The monoamine (M) (thermolabile)-form PST catalyzes more effectively the sulfation of monoamines such as dopamine and epinephrine, and the simple phenol (P) (thermostable)-form PST preferentially sulfates neutral phenols such as p-nitrophenol and α-naphthol (*7*). It is now clear that, in addition to their presence in platelets, these two PSTs have a somewhat widespread tissue distribution in the human body. In particular, the M-form PST has been found in the upper gastro-intestinal tract and brain (*8*), and the P-form PST in the adrenal gland, lung, and liver (*9*). Both forms are thought to be constitutive enzymes though little is known about the regulation of their enzymatic activity (*1*). In the past several years, however, studies performed in our laboratory have revealed that some divalent metal cations may exert stimulatory or inhibitory effects on these enzymes (*10*, *11*). For example, the addition of Mn^{2+} to the reaction mixture resulted in a dramatic increase in the Dopa/tyrosine-sulfating activity of the M-form PST, while Cd^{2+} inhibited this activity. These findings indicate that divalent metal cations may play a significant role in regulating the activity of the M-form PST and perhaps cytosolic STs in general. It may therefore be important to investigate whether divalent metal cations that enter the body in food or as environmental contaminants exert significant stimulatory or inhibitory effects on the sulfation of drugs. Another interesting aspect of the metabolism of drugs through sulfation is the stereoselective sulfate conjugation of some of these compounds. A study using rat hydroxysteroid ST (STa) has revealed the stereoselective sulfation of chiral secondary alcohols (*12*). Studies using homogenates of, or intact, human liver, intestine, or platelets have also demonstrated the stereoselective sulfation of some chiral drugs including isoproterenol and albuterol (*13*, *14*). In view of our previous studies showing that the M-form PST displayed stereoselectivity for the D-enantiomers of Dopa and tyrosine (*11*), it is interesting to investigate whether the M-form PST also exhibits stereoselectivity toward chiral drugs.

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We report here a systematic investigation on the differential activities of the M-form and P-form PSTs toward a variety of drug compounds. The kinetics of sulfation of the two enantiomeric forms of the chiral drug isoproterenol by the M-form PST was examined. Moreover, the effects of divalent metal cations on the drug-sulfating activities of these two enzymes were studied.

MATERIALS AND METHODS

Materials—Acebutolol, acetaminophen, adenosine 3′-phosphate 5′-phosphosulfate (PAPS), bupivacaine, dexamethasone, dobutamine, lidocaine, (±)isoproterenol, (+)isoproterenol, (–)isoproterenol, 4-methylumbelliferone, (±)metoprolol, minoxidil, DL-propanolol, (±)salbutamol, (±)sotalol, dopamine, *p*-nitrophenol, and adenosine 5′-triphosphate (ATP) were products of Sigma. Carrier-free sodium [^{35}S]sulfate was from ICN Biomedicals. Cellulose thin-layer chromatography (TLC) plates were from EM Science. All other chemicals were of the highest grade commercially available.

Preparation of Purified Human M-Form and P-Form PSTs—Human M-form (SULT1A3) and P-form (SULT1A1) PSTs were cloned, expressed, and purified using the pGEX-2TK Glutathione S-transferase Gene Fusion System based on the procedure previously established (*15*, *16*).

Enzymatic Assay—Sulfotransferase activities of purified M-form and P-form PSTs were assayed using [^{35}S]PAPS as the sulfonate group donor. The standard assay mixture, in a final volume of 25 μl, contained 50 mM potassium phosphate buffer (pH 7.0), 15 μM [^{35}S]PAPS, and 50 μM of the drug compound tested. The enzyme dilutions were prepared in 50 mM potassium phosphate buffer (pH 7.0) containing 10% glycerol and 8 mM dithiothreitol. The reaction was started by the addition of 5 μl of the enzyme preparation, allowed to proceed for 3 min (so that the reaction reached no more than 5–10% of completion) at 37°C, and terminated by heating at 100°C for 2 min. The precipitates were cleared by centrifugation for 1 min, and the supernatant was subjected to the analysis of [^{35}S]sulfated product based on the TLC procedure previously established (*17*). Each experiment was performed in triplicate, together with a control without enzyme. The results obtained were calculated and expressed in nanomoles sulfated product formed/min/mg protein.

Determination of the Stimulatory/Inhibitory Effects of Divalent Metal Cations on the Sulfation of Drug Compounds by Human M-Form or P-Form PST—To determine the patterns of stimulation/inhibition of purified human M-form or P-form PST by divalent metal cations, enzymatic assays were performed in the presence or absence of such cations. Standard assay mixture, in a final volume of 25 μl, contained the test divalent cation (5 mM), 14 μM PAP[^{35}S], 50 mM potassium phosphate buffer (pH 7.0), the enzyme being assayed, and a specified concentration of the drug compound as substrate. Controls containing all the reagents, but without divalent metal cation or with 5 mM EDTA, were assayed in parallel. The reaction was started by the addition of the enzyme and allowed to proceed for 3 min at 37°C, followed by heat inactivation of the enzyme at 100°C for 2 min. The precipitates were cleared by centrifugation for 1 min, and the supernatant was subjected to the analysis of [^{35}S]sulfated product based on the TLC procedure previously established (*17*).

Table 1. **Specific activity of human M-form and P-form PSTs.**[a]

Drug compounds (50 μM)	Specific activity (nmol/min/mg protein)	
	M-PST	P-PST
Acebutolol	N.D.[b]	2.9 ± 0.2
DL-Propanolol	N.D.	2.4 ± 0.1
Bupivacaine	N.D.	2.0 ± 0.1
(±) Metoprolol	N.D.	2.0 ± 0.1
(±) Sotalol	N.D.	2.3 ± 0.1
Dexamethasone	N.D.	2.7 ± 0.1
Dobutamine	68.8 ± 4.4	106.0 ± 2.0
4-Methylumbelliferone	9.4 ± 0.6	95.1 ± 2.9
Lidocaine	0.05 ± 0.02	0.3 ± 0.1
(±)Isoproterenol	147.0 ± 1.1	22.1 ± 1.1
(±)Salbutamol	25.5 ± 0.2	2.9 ± 0.5
Acetaminophen	2.8 ± 0.1	11.7 ± 0.8
Minoxidil	0.5 ± 0.1	4.8 ± 0.1

[a]Data shown represent the mean ± SD derived from three determinations. [b]Activity not detected.

Miscellaneous Methods—[^{35}S]PAPS was synthesized from ATP and carrier-free [^{35}S]sulfate using the bifunctional human ATP sulfurylase/APS kinase and its purity determined as previously described (*18*). The [^{35}S]PAPS synthesized was then adjusted to the required concentration and specific activity by the addition of cold PAPS. SDS–polyacrylamide gel electrophoresis was performed on 12% polyacrylamide gels using the method of Laemmli (*19*). Protein determination was based on the method of Bradford with bovine serum albumin as standard (*20*).

RESULTS AND DISCUSSION

Biological sulfation was first discovered when phenyl sulfate was isolated from the urine of a patient who had been treated with phenol (*21*). This finding had largely positioned the research on sulfation and the responsible sulfotransferase enzymes within the general area of pharmacology/toxicology for over a century. Indeed, a great many studies using experimental animals or volunteer human subjects have demonstrated the metabolism of drugs through sulfation (*22*, *23*). The current study aimed to examine the differential roles of the M-form and P-form PSTs in the sulfation of drug compounds and the regulatory effects of divalent metal cations.

Differential Activities of Human M-Form and P-Form PSTs toward Drug Compounds—Purified M-form and P-form PSTs were assayed using a variety of drug compounds as substrates. Activity data compiled in Table 1 showed that all drug compounds tested (at a concentration of 50 μM in the assay mixture) could be used as substrates by the P-form PST, with dobutamine and 4-methylumbelliferone giving the highest specific activities. In contrast, the M-form PST displayed a more distinct substrate preference, with high activities toward dobutamine, salbutamol, and (±)isoproterenol (all of which are dopamine analogs) and very low or undetectable activities with the other substrates (the concentration of the substrates used was 50 μM). These results showed clearly the differential substrate specificity of the M-form

and P-form PSTs toward the drug compounds tested. It is interesting to note that, in line with its general role as a detoxifying enzyme (*2*, *3*), the P-form PST displays a broader substrate specificity than the M-form PST, which is believed to play a more specific role in the homeostasis of dopamine in the brain and perhaps the detoxification of this and other deleterious monoamines and dopamine analogs in the upper gastrointestinal tract (*8*). Table 2 shows the kinetic constants determined for the sulfation of acetaminophen (one of the most extensively used drugs, which is believed to be detoxified in the liver) and minoxidil (which is activated by conversion to its sulfate-conjugated form) by the P-form PST and the sulfation of dobutamine by the M-form PST. Based on these data, the catalytic efficiency of the M-form PST for the sulfation of dobutamine appeared to be considerably higher than that of the P-form PST for the sulfation of acetaminophen or minoxidil. Previous studies using liver homogenates demonstrated the stereoselective sulfation of (±)isoproterenol (*13*). In view of our previous finding that the M-form PST displayed stereoselectivity for the D-enantiomers of Dopa and tyrosine (*11*), we decided to test the kinetics of sulfation of the two enantiomeric forms of isoproterenol by this enzyme. Results shown in Fig. 1 indicated that the apparent K_m values determined for the two enantiomers of isoproterenol are nearly identical, whereas the V_{max} for the (+)enantiomer is almost two times that for the (–)enantiomer. The preferential sulfation of one enantiomeric form over the other may have important pharmacological implications for drugs that are administered in the racemic form.

Effects of Divalent Metal Cations on the Sulfation of Drug Compounds by Human M-Form and P-Form PSTs—Divalent metal cations are known to play important roles in the function of biological molecules (*24*). Some of them, including manganese, zinc, copper, iron, and cobalt, are essential components of different enzymes, while others such as magnesium and calcium are required for the actions of certain enzymes or proteins. In contrast to these biologically useful metal cations, some metal cations (*e.g.*, lead and mercury) that enter the body primarily as environmental contaminants have been shown to exert deleterious effects (*25*, *26*). Our previous studies have revealed that some divalent metal cations may exert stimulatory or inhibitory effects on the cytosolic STs (*10*, *11*). We were interested in investigating the effects of these divalent metal cations on the sulfation of drug compounds. Enzymatic assays, using

Table 2. **Kinetic constants of human M-form and P-form PSTs with dobutamine, acetaminophen, and minoxidil as substrates.**[a]

	K_m (μM)	V_{max} (nmol/min/mg)	V_{max}/K_m
M-form PST			
Dobutamine	14.8	384.6	26.0
P-form PST			
Acetaminophen	430.4	22.1	0.05
Minoxidil	200.6	6.2	0.03

[a]Data shown represent mean ± SD derived from three determinations.

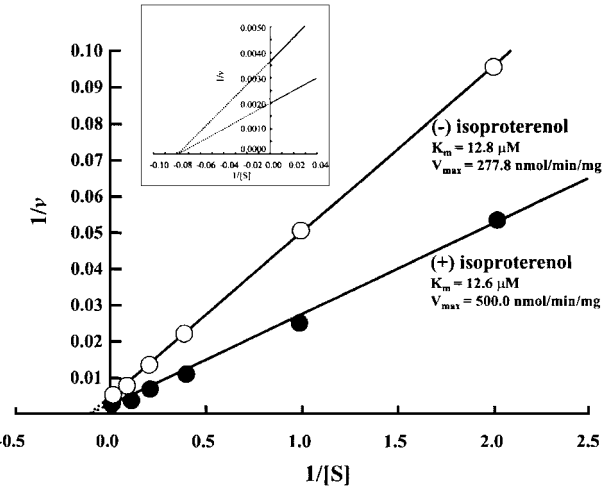

Fig. 1. **Lineweaver-Burk double-reciprocal plot of the human M-form PST with (+)Isoproterenol or (–)Isoproterenol as substrate.** Concentrations of (+)isoproterenol or (–)isoproterenol are expressed in μM, and velocities are expressed in nmol of product formed/min/mg enzyme. Each data point represents the mean value derived from three determinations.

Table 3. **Inhibitory/stimulatory effects of divalent metal cations on the human M-form and P-form PSTs.**[a]

	M-form PST		P-form PST	
	Dobutamine (5.0 μM)	(±) Isoproterenol (10 μM)	Acetaminophen (500 μM)	Minoxidil (250 μM)
	(nmol/min/mg protein)			
Control	112 ± 3	126 ± 1	9.5 ± 0.5	3.1 ± 0.3
$FeCl_2$	73.9 ± 1.1	90.7 ± 2.1	13.1 ± 0.3	3.3 ± 0.2
$HgCl_2$	3.8 ± 0.2	ND[b]	1.4 ± 0.1	0.06 ± 0.02
$CoCl_2$	103 ± 0.2	119 ± 2	5.3 ± 0.2	1.2 ± 0.1
$ZnCl_2$	99.6 ± 0.4	65.5 ± 0.4	3.6 ± 0.1	0.6 ± 0.1
$Pb(CH_3COO)_2$	107 ± 3	120 ± 1	10.5 ± 0.9	3.0 ± 0.1
$CdCl_2$	111 ± 2	122 ± 2	8.7 ± 0.1	2.0 ± 0.2
$MnCl_2$	99.5 ± 1.3	124 ± 3	12.6 ± 0.1	3.7 ± 0.2
$CaCl_2$	114 ± 3	115 ± 3	10.3 ± 0.6	2.8 ± 0.1
$MgCl_2$	113 ± 1	135 ± 2	10.8 ± 0.8	3.5 ± 0.2
$CuCl_2$	0.8 ± 0.1	2.4 ± 1.2	0.05 ± 0.02	0.04 ± 0.02
NaCl	112 ± 3	118 ± 0.3	9.5 ± 0.8	3.0 ± 0.2

[a]Data shown represent mean ± SD derived from three determinations. [b]Activity not detected.

acetaminophen and minoxidil (for the P-form PST) or dobutamine and (±)isoproterenol (for the M-form PST) as substrates (at the concentrations specified), were carried out in the absence or presence of various divalent metal cations at a concentration of 5 mM. As a control for the counter ion, Cl^-, parallel assays in the presence 10 mM NaCl were also performed. Results obtained are compiled in Table 3. The degrees of inhibition or stimulation were evaluated by comparing the activities determined in the presence of metal cations with the activities determined in the absence of metal cations. Both the M-form and P-form PSTs were found to be partially or completely inhibited or stimulated by the majority of the divalent metal cations tested, and both were less sensitive to the effects of physiologically more abundant metal cations such as Mg^{2+} and Ca^{2+}. In contrast, they were more sensitive to the detrimental effects of metal cations that may enter the body as environmental contaminants. At a concentration of 5 mM, Cu^{2+} and Hg^{2+} exerted nearly complete inhibition of the activity of the M-form and P-form PSTs toward the four drug compounds tested. These dramatic inhibitory effects imply that the presence of these metal cations may interfere with the metabolism of drugs through sulfation. To what extent the inhibitory effects of these metal cations on the M-form and P-form PSTs may help prolong the presence of drug compounds *in vivo*, however, remains to be clarified.

Finally, it should be pointed out that, in addition to the differential substrate specificity of the human M-form and P-form PSTs toward drug compounds, recent studies have revealed the genetic polymorphism of the P-form PST (*27*, *28*). It may be important, therefore, to examine the drug-sulfating phenotypes of different P-form PST allozymes. Information of this kind will likely help in understanding the individual differences in drug metabolism and may in the future aid in developing tailored drug regimens that suit individual needs.

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REFERENCES

1. Mulder, G.J. and Jakoby, W.B. (1990) Sulfation in *Conjugation Reactions in Drug Metabolism* (Mulder, G.J. and Jakoby, W., eds.) pp. 107–161, Taylor and Francis, London
2. Falany, C. and Roth, J.A. (1993) Properties of human cytosolic sulfotransferases involved in drug metabolism in *Human Drug Metabolism; From Molecular Biology to Man* (Jeffery, E.H., ed.) pp. 101–115, CRC Press, Boca Raton, FL
3. Weinshilboum, R. and Otterness, D. (1994) Sulfotransferase enzymes in *Conjugation-Deconjugation Reactions in Drug Metabolism and Toxicity* (Kaufmann, F.C., ed.) pp. 45–78, Springer-Verlag, Berlin
4. Lipmann, F. (1958) Biological sulfate activation and transfer. *Science* **128**, 575–580
5. Rein, G., Glover, V., and Sandler, M. (1982) Multiple forms of phenolsulphotransferase in human tissues: selective inhibition by dichloronitrophenol. *Biochem Pharmacol.* **31**, 1893–1897
6. Mwaluko, G. and Weinshilboum, R. (1982) alpha-Methyldopa, alpha-methyldopamine an alpha-methylnoradrenaline: substrates for the thermolabile form of human platelet phenol sulphotransferase. *Br. J. Clin. Pharmacol.* **14**, 231–239
7. Weinshilboum, R.M. (1986) Phenol sulfotransferase in humans: properties, regulation, and function. *Fed. Proc.* **45**, 2223–2228
8. Rubin, G.L., Sharp, S., Jones, A.L., Glatt, H., Mills, J.A., and Coughtrie, M.W.H. (1996) Design, production and characterization of antibodies discriminating between the phenol- and monoamine-sulphating forms of human phenol sulphotransferase. *Xenobiotica* **26**, 1113–1119
9. Heroux, J.A., Falany, C.N., and Roth, J.A. (1989) Immunological characterization of human phenol sulfotransferase. *Mol. Pharmacol.* **36**, 29–33
10. Sakakibara, Y., Suiko, M. and Liu, M.-C. (1994) *De novo* sulfation of L-tyrosine in HepG2 human hepatoma cells and its possible functional implication. *Eur. J. Biochem.* **226**, 293–301
11. Suiko, M., Sakakibara, Y., Nakajima, H., Sakaida, H., and Liu, M.-C. (1996) Enzymic sulphation of dopa and tyrosine isomers by HepG2 human hepatoma cells: stereoselectivity and stimulation by Mn^{2+}. *Biochem. J.* **314**, 151–158
12. Banoglu, E. and Duffel, M.W. (1997) Studies on the interactions of chiral secondary alcohols with rat hydroxysteroid sulfotransferase STa. *Drug Metab. Dispos.* **25**, 1304–1310
13. Pesola, G.R. and Walle, T. (1993) Stereoselective sulfate conjugation of isoproterenol in humans: comparison of hepatic, intestinal, and platelet activity. *Chirality* **5**, 602–609
14. Walle, T., Walle, U.K., Thornburg, K.R., and Schey, K.L. (1993) Stereoselective sulfation of albuterol in humans. Biosynthesis of the sulfate conjugate by HEP G2 cells. *Drug Metab. Dispos.* **21**, 76–80
15. Sakakibara, Y., Takami, Y., Nakayama, T., Suiko, M., and Liu, M.-C. (1998) Localization and functional analysis of the substrate specificity/catalytic domains of human M-form and P-form phenol sulfotransferases. *J. Biol. Chem.* **273**, 6242–6247
16. Liu, M.-C., Suiko, M., and Sakakibara, Y. (2000) Mutational analysis of the substrate binding/catalytic domains of human M form and P form phenol sulfotransferases. *J. Biol. Chem.* **275**, 13460–13464
17. Liu, M. -C. and Lipmann. F (1984) Decrease of tyrosine-O-sulfate-containing proteins found in rat fibroblasts infected with Rous sarcoma virus or Fujinami sarcoma virus. *Proc. Natl Acad. Sci. USA* **81**, 3695–3698
18. Yanagisawa, K., Sakakibara, Y., Suiko, M., Takami, Y., Nakayama, T., Nakajima, H., Takayanagi, K., Natori, Y., and Liu, M. -C. (1998) cDNA cloning, expression, and characterization of the human bifunctional ATP sulfurylase/adenosine 5′-phosphosulfate kinase enzyme. *Biosci. Biotechnol. Biochem.* **62**, 1037–1040
19. Laemmli, U.K. (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **227**, 680–685
20. Bradford, M.M. (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **72**, 248–254
21. Baumann, E. (1876) In: *Ber Dtsch. Chem. Ges.*, pp.54–58
22. Nelson, S.D. and Gordon, W.P. (1983) Mammalian drug metabolism. *J. Nat. Prod.* **46**, 71–87
23. Chen, X., Zhong, D., and Blume, H. (2000) Stereoselective pharmacokinetics of propafenone and its major metabolites in healthy Chinese volunteers. *Eur. J. Pharm. Sci.* **10**, 11–16
24. Frausto da Silva, J.J.R. and Williams, R.J.P. (1991) *The Biological Chemistry of the Elements. The Inorganic Chemistry of Life*, Clarendon Press, Oxford.
25. Bressler, J., Kim, K.A., Chakraborti, T., and Goldstein, G. (1999) Molecular mechanisms of lead neurotoxicity. *Neurochem. Res.* **24**, 595–600
26. Cranmer, M., Gilbert, S., and Cranmer, J. (1996) Neurotoxicity of mercury–indicators and effects of low-level exposure: overview. *Neurotoxicology* **17**, 9–14
27. Carlini, E.J., Raftogianis, R.B., Wood, T.C., Jin, F., Zheng, W., Rebbeck, T.R., and Weinshilboum, R.M. (2001) Sulfation pharmacogenetics: SULT1A1 and SULT1A2 allele frequencies in Caucasian, Chinese and African-American subjects. *Pharmacogenetics* **11**, 57–68
28. Iida, A., Sekine, A., Saito, S., Kitamura, Y., Kitamoto, T., Osawa, S., Mishima, C. and Nakamura, Y. (2001) Catalog of 320 single nucleotide polymorphisms (SNPs) in 20 quinone oxidoreductase and sulfotransferase genes. *J. Hum. Genet.* **46**, 225–240