0026-895X/87/060813-07$02.00/0

MOLECULAR PHARMACOLOGY, 32:813–819

Human and Rat Liver Phenol Sulfotransferase: Structure-Activity Relationships for Phenolic Substrates

NORMAN R. C. CAMPBELL, JON A. VAN LOON, RAJAH S. SUNDARAM, MATTHEW M. AMES, CORWIN HANSCH, and RICHARD WEINSHILBOUM

Clinical Pharmacology Unit, Department of Pharmacology (N.R.C.C., J.A.V.L., R.S.S., R.W.) and Division of Developmental Oncology Research, Department of Oncology (M.M.A.), Mayo Clinic/Mayo Foundation, Rochester, Minnesota 55905 and Department of Chemistry, Pomona College, Claremont, California 91711 (C.H.)

Received May 5, 1987; Accepted September 24, 1987

SUMMARY

Phenol sulfotransferase (PST) catalyzes the sulfate conjugation of many phenolic drugs. Human liver contains thermostable (TS) and thermolabile forms of PST. Ion exchange chromatography shows that two isozymes of TS PST (peaks I and II) are present in human liver preparations. Rat liver contains four forms of PST that can be separated by ion exchange chromatography. Quantitative structure-activity relationship (QSAR) analysis was used to study phenolic substrates for both human and rat liver PST. Thirty-six substituted phenols were tested as substrates for partially purified human liver TS PST peak I. QSAR analysis resulted in derivation of the following equation: log $1/K_m$ = 0.92 (±0.18)log P − 1.48 (±0.38)MR'_4 − 0.64 (±0.41)MR_3 + 1.04 (±0.63)MR_2 + 0.67(±0.44) σ^- + 4.03 (±0.42). In this equation K_m is the Michaelis constant, P is the octanol-water partition coefficient, MR is the molar refractivity of substituents at the 2-, 3-, and 4-positions, and σ^- is the Hammett constant. Values of log $1/K_m$ calculated with this equation were highly correlated with log $1/K_m$ values (r = 0.950) that were observed experimentally. Nine phenols were also tested as substrates for partially purified human liver TS PST peak II. Log $1/K_m$ values for these compounds were significantly correlated for the two isozymes of TS PST (r = 0.992, $p < 0.001$). QSAR analysis was also used to derive equations that described the behavior of phenolic substrates for rat liver PST forms I and II. These equations differed substantially from the equation derived for compounds tested with human liver TS PST peak I. Therefore, the characteristics of the active sites of human liver TS PST peak I and rat liver PST forms I and II appear to differ. Application of these equations may make it possible to predict K_m values of phenolic substrates for human liver TS PST and for rat liver PST forms I and II.

Phenol sulfotransferase (PST) (EC 2.8.2.1) catalyzes the sulfate conjugation of many phenolic and catechol drugs, xenobiotic compounds, and neurotransmitters (1–3). All human tissues that have been studied contain at least two forms of PST (4–7). These forms differ in their substrate specificities, sensitivity to inhibitors, physical properties, and regulation among individuals. One form is thermostable (TS) and catalyzes the sulfate conjugation of μM concentrations of 4-nitrophenol, phenol, and other "simple" phenols (4, 8–10). The other form is thermolabile (TL) and catalyzes the sulfate conjugation of μM concentrations of dopamine and other catechol and phenolic monoamines (4, 8–10). However, at high concentrations phenol and 4-nitrophenol are substrates for TL PST (9, 10), and at high concentrations dopamine can serve as a substrate for the TS form of the enzyme (6). It has been suggested that these two enzymes be named the "P," or phenol-metabolizing, and the "M," or monoamine-metabolizing forms of PST (4, 8). However, because of significant overlap in their substrate specificities, we will refer to them subsequently as TS or TL PST on the basis of the unequivocal differences in their thermal stabilities. In addition to the TS and TL forms of the enzyme, two isozymes of TS PST are present in human liver and brain on the basis of the elution of two peaks of TS PST activity during DEAE ion exchange chromatography (6, 11). TL PST elutes after the two isozymes of TS PST during ion exchange chromatography (6, 10).

Multiple forms of PST are also present in rat liver. There are at least four forms of rat liver PST based on their sequential elution during DEAE ion exchange chromatography (12–14). Only three of the four forms of rat liver PST have been well characterized. Forms I and II have molecular weights of approximately 64,000 and similar substrate specificities. They catalyze the sulfate conjugation of many simple phenols and, at high pH values, of catechols. Rat liver PST form IV has a slightly lower apparent molecular weight than do forms I and II, and a high affinity for catechol substrates (13). Form III is

This work was supported in part by National Institutes of Health Grants GM 28157 (R. W.), GM 35720 (R. W.), CMC-30302 (C. H.), and Research Career Development Award CA00755 (M. M. A.), and by National Institutes of Health Contract ES 55110 (R. W.).

ABBREVIATIONS: PST, phenol sulfotransferase; TS, thermostable; TL, thermolabile; QSAR, quantitative structure-activity relationship; PAPS, 3′-phosphoadenosine 5′-phosphosulfate; BSA, bovine serum albumin.

so rapidly inactivated after purification that it has not been well characterized (14).

In the experiments to be described here we used QSAR analysis to study both human and rat liver PST. In the first set of experiments, the ability of a series of phenols to serve as substrates for human liver TS PST peak I was determined so that QSAR analysis could be performed. A smaller number of phenols was also tested as substrates for human liver TS PST peak II to determine whether relative K_m values for that isozyme were similar to and correlated with those found when the same compounds were used as substrates for human liver TS PST peak I. Finally, QSAR analyses were also performed with previously published data for rat liver PST peaks I, II, and IV.

Materials and Methods

PST assay. PST activity was assayed by the method of Foldes and Meek (15) as modified by Anderson and Weinshilboum (16) and adapted by Campbell *et al.* (6) for the measurement of the human liver enzyme activity. Samples of partially purified human liver TS PST peaks I and II were incubated at 37° with sulfate acceptor substrates and ^{35}S-PAPS, the sulfate donor for the reaction. Samples that contained no sulfate acceptor were used as blanks. After incubation for 15 min, reactions were terminated by the precipitation of PAPS and protein with barium hydroxide, barium acetate, and zinc sulfate. Each sample was then centrifuged, the supernatant fluid was aspirated and mixed with 3a70 liquid scintillation counting fluid (Research Products International Corp., Mt. Prospect, IL), and radioactivity was measured in a Beckman LS 7500 liquid scintillation counter. Details of the PST assay have been described elsewhere (6, 16).

Protein assay. Protein concentrations were measured by the dye binding method of Bradford (17) with BSA as a standard.

PST substrate studies. A series of phenols was tested as substrates for PST. The purity of each compound was determined by high performance liquid chromatography performed with a 10-μm C18 reverse phase IBM column with methanol and water in varying proportions as the mobile phase and with detection by UV absorption at 254 nm. All of the compounds eluted as single peaks.

Initial substrate kinetic experiments were performed with a series of concentrations of each compound that differed by several orders of magnitude. Apparent K_m values were estimated on the basis of data from a second series of experiments performed with at least five different concentrations close to the K_m for that compound. The effect of every concentration studied was measured in triplicate, and each apparent K_m value was measured at least twice. The K_m values reported are averages of all measurements.

Purification of PST. Human liver TS PST peaks I and II were partially purified as described elsewhere (6). Specifically, human hepatic tissue was obtained from patients who underwent clinically indicated partial hepatectomies for the removal of either primary or metastatic tumors. The hepatic tissue used was not affected by tumor. Tissue was obtained under guidelines approved by the Mayo Clinic Institutional Review Board. The hepatic tissue was homogenized in 9 volumes of 5 mM potassium phosphate buffer, pH 7.5, with a Polytron homogenizer. After initial centrifugation at 16,200 × g for 15 min at 4°, supernatant fractions were centrifuged at 100,000 × g for 1 hr at 4°. Supernatant fractions from that step were subjected to DEAE-Sepharose CL-6B ion exchange chromatography. TS PST peaks I and II were separated from each other during ion exchange chromatography. Separation of the two peaks was verified by repeat chromatography on an ion exchange column. Each peak was then subjected to gel filtration chromatography through Sephadex G-100 superfine. The enzyme used in the studies was purified at least 80-fold as compared with the activity in the supernatant after centrifugation at 100,000 × g. The two isozymes of human liver TS PST were not purified further because of rapid loss of enzyme activity in these partially purified preparations (6). The partially purified enzyme was stored in the presence of 0.75 mg/ml BSA to prevent rapid inactivation (6). Characteristics of partially purified TS PST peaks I and II have been described elsewhere (6).

Kinetic analyses. Apparent Michaelis (K_m) constants were estimated by the method of Wilkinson (18) with a computer program written by Cleland (19). An IBM PC computer was used to perform these calculations.

QSAR analysis. QSAR analyses were performed by the method of Hansch (20, 21).

Materials. ^{35}S-PAPS (2.1–2.4 Ci/mmol) was purchased from New England Nuclear Corp. (Boston, MA). Dithiothreitol (Cleland's reagent), 4-nitrophenol, and BSA were obtained from Sigma Chemical Co. (St. Louis, MO). 2-Methylphenol, 4-methylphenol, 2-bromophenol, 3-cyanophenol, 3,5-dihydroxyphenol, α,α,α-trifluoro-4-methylphenol, 4-hydroxybenzamide, 2-chlorophenol, 2-methoxyphenol, 3,4-dichlorophenol, 3-chlorophenol, 3,5-dimethylphenol, 3,5-dichlorophenol, 4-ethylphenol, 2-fluorophenol, 2-hydroxybenzyl alcohol, phenol, 4-acetamidophenol, 4-methoxyphenol, α,α,α-trifluoro-2-methylphenol, 4-n-propylphenol, 3-nitrophenol, 3-hydroxybenzyl alcohol, 3-t-butylphenol, 4-aminophenol, 3-aminophenol, 4-t-butylphenol, 2-cyanophenol, 4-chlorophenol, and 3-acetamidophenol were purchased from Aldrich Chemical Co. (Milwaukee, WI). 3-Iodophenol was obtained from Alfa Products (Danvers, MA). 1,2-Dihydroxybenzene (catechol) was purchased from Fisher Scientific Co. (Pittsburgh, PA). 4-Cyanophenol was obtained from Tridom/Fluka (Hauppauge, NY). 2-Iodophenol was purchased from Eastman Kodak (Rochester, NY). 4-Phenylphenol was obtained from Aldrich Chemical Co. and was purified by thin layer chromatography performed by Dr. G. Powis, Mayo Foundation.

Results

Three series of studies were performed. The first set of experiments involved the measurement of apparent K_m values for 36 phenolic substrates with human liver TS PST peak I. The results were used to perform QSAR analyses. K_m values for nine of these compounds were also measured with human liver TS PST peak II. Finally, previously published data were used to perform QSAR analyses with rat liver PST forms I, II, and IV.

Structure-activity analyses with human liver TS PST peak I. Apparent K_m values of partially purified human liver TS PST peak I were determined with 36 phenols (Table 1). These apparent K_m values varied by more than 5 orders of magnitude. The data in Table 1 were then used to perform regression analyses to characterize structure-activity relationships for these substrates with TS PST peak I. The hydrophobic effects of substituents at the 2-, 3-, 4-, and 5-positions were very similar when equations were developed using π values for individual substituents at each position. Therefore, log P values determined with the octanol-water system (22) were used rather than π values to characterize hydrophobic interactions.

Structure-activity relationships were studied first with 2-substituted phenols. Derivatives with substituents at the 2-position are often difficult to analyze because of interactions of substituents at this position with the adjacent moiety on the substrate molecule and/or because of intermolecular interactions with the enzyme. Structure-activity relationships for the parent compound (phenol) and for 2-substituted derivatives of phenol were described best by Eq. 1:

$$\log 1/K_m = 0.67(\pm 0.58)\log P + 2.42(\pm 1.92)F_2 + 1.10(\pm 0.94)MR_2 + 3.81(\pm 0.92) \quad (1)$$
$$n = 11,\ r = 0.941,\ s = 4.27$$

In this expression F_2 is the inductive parameter (22) and MR_2 is the molar refractivity, scaled by 0.1, of the substituent at the

TABLE 1

Log $1/K_m$ values and parameters used in the derivation of Eqs. 1–4 for human liver TS PST peak I with X-C_6H_4OH substrates

Log $1/K_m$ values were calculated with Eq. 4. Experimental values for log P were obtained from the Pomona College data bank. MR_4' values of less than 0.74 were assigned values of 0.

X	Observed log $1/K_m$	Calculated log $1/K_m$	$\lvert\Delta\log 1/K_m\rvert$	log P	σ^-	MR_4'	MR_3	MR_2
1. 2-Cl	7.41	6.75	0.66	2.15	0.27	0	0.10	0.60
2. 3,4-Cl_2	7.05	7.23	0.19	3.33	0.64	0	0.60	0.10
3. 3-I	6.92	6.18	0.74	2.93	0.35	0	1.39	0.10
4. 4-CF_3	6.89	7.21	0.32	2.95	0.65	0	0.10	0.10
5. 2-CF_3	6.88	7.49	0.61	2.80	0.65	0	0.10	0.50
6. 4-Cl	6.64	6.45	0.19	2.39	0.27	0	0.10	0.10
7. 3-Cl	6.62	6.30	0.32	2.50	0.37	0	0.60	0.10
8. 3-NO_2	6.55	5.98	0.57	2.00	0.71	0	0.74	0.10
9. 3,5-Cl_2	6.44	7.19	0.75	3.62	0.74	0	1.20	0.10
10. 2-F	6.24	5.69	0.56	1.71	0.05	0	0.10	0.10
11. 3-$C(CH_3)_3$	6.16	5.86	0.30	3.31	−0.10	0	1.96	0.10
12. 4-CN	6.12	6.21	0.09	1.60	1.00	0	0.10	0.10
13. 4-NO_2	6.08	5.56	0.52	1.91	1.24	0.74	0.10	0.10
14. 2-CH_3	5.90	6.24	0.34	1.95	−0.15	0	0.10	0.56
15. 3-CN	5.90	5.67	0.23	1.70	0.56	0	0.63	0.10
16. 4-CH_3	5.66	5.75	0.09	1.94	−0.15	0	0.10	0.10
17. 2-OH	5.40	4.97	0.43	0.88	−0.16	0	0.10	0.29
18. 3,5-$(CH_3)_2$	5.14	5.49	0.35	2.35	−0.14	0	1.12	0.10
19. 4-C_2H_5	5.08	4.81	0.27	2.58	−0.15	1.03	0.10	0.10
20. H	4.78	5.41	0.63	1.46	0.00	0	0.10	0.10
21. 4-OCH_3	4.44	4.03	0.41	1.34	−0.16	0.79	0.10	0.10
22. 4-C_3H_7	4.39	4.69	0.30	3.20	−0.15	1.50	0.10	0.10
23. 4-NH_2	3.86	4.01	0.15	0.04	−0.15	0	0.10	0.10
24. 4-$C(CH_3)_3$	3.75	4.12	0.37	3.31	−0.13	1.96	0.10	0.10
25. 4-$CONH_2$	3.51	3.35	0.16	0.33	0.63	0.98	0.10	0.10
26. 3-NH_2	3.51	3.84	0.33	0.17	−0.16	0	0.54	0.10
27. 4-$NHCOCH_3$	2.35	2.33	0.02	0.51	0.00	1.49	0.10	0.10
28. 2-OCH_3	6.77	5.89	0.88	1.32	−0.16	0	0.10	0.79
29. 2-CN	6.40	6.77	0.37	1.61	1.00	0	0.10	0.63
30. 2-Br	7.62	7.24	0.38	2.35	0.28	0	0.10	0.89
31. 3,5$(OH)_2$	3.48	4.01	0.53	0.16	0.12	0	0.58	0.10
32. 2-I	7.76	8.04	0.28	2.65	0.30	0	0.10	1.39
33. 2-CH_2OH	4.81	5.39	0.58	0.73	0.00	0	0.10	0.72
34. 3-CH_2OH	4.11	4.13	0.02	0.49	0.00	0	0.72	0.10
35. 3-$NHCOCH_3$	3.65	4.00	0.35	0.73	0.21	0	1.49	0.10
36. 4-C_6H_5	5.61*	3.21	0.40	3.20	0.10	2.54	0.10	0.10

* This value was not used in the derivations of Eqs. 2 and 4.

2-position. Values in parentheses are used to construct 95% confidence limits; n represents the number of data points; r is the correlation coefficient; and s is the standard deviation. There was very low colinearity among the variables included in Eq. 1. Correlation coefficients for pairs of these variables were: $r^2(F_2, \log P) = 0.22$; $r^2(MR_2, \log P) = 0.16$; $r^2(F_2, MR_2) = 0.03$.

The most important variable in Eq. 1 was log P, followed sequentially by F_2 and MR_2. Eq. 1 highlighted the major features of the structure-activity relationships, although too few data points were used in the derivation of the equation for great confidence to be placed in all three variables. The positive coefficient for F_2 indicates that formation of the enzyme-substrate complex is enhanced by electron withdrawal by 2-substituents. The positive coefficient for the steric parameter MR_2, primarily a measure of bulk (21), indicates that a favorable change occurs in the region of the active site of the enzyme in the presence of "bulky" 2-substituents. The Taft steric parameter (E_s) for intramolecular steric effects yielded a less significant correlation for 2-substituents than did MR_2 (data not shown).

Structure-activity relationships for phenol and for 3-, 4-, and 5-substituted derivatives of phenol were studied next. Eq. 2 was derived by regression analysis performed with all appropriate derivatives listed in Table 1 except for the 4-C_6H_5 analog:

$$\log 1/K_m = 0.91(\pm 0.17)\log P - 1.37(\pm 0.36)MR_4' + 0.92(\pm 0.45)\,\sigma^- - 0.52(\pm 0.39)MR_3 + 3.97(\pm 0.39) \quad (2)$$
$$n = 25,\ r = 0.962,\ s = 0.413$$

In this equation MR_4' is the molar refractivity of substituents at the 4-position. Substituents with MR_4' values less than that of 4-NO_2 (0.74) were assumed to have no steric effect and were assigned a value of 0. This assumption was based on a study of the residuals from the regression equations. The log $1/K_m$ value for the 4-C_6H_5 derivative was not predicted well by Eq. 2. That compound had a much smaller log $1/K_m$ value than expected without the MR_4' term, but a much larger value than expected when the MR_4' term was used. Therefore, as noted previously, the K_m value for the 4-C_6H_5 derivative was not used to derive Eq. 2. The use of σ^- gave better results than did the use of σ, as expected for phenols. The positive coefficient for σ^- in the equation indicates that electron-withdrawing groups promote the formation of the enzyme-substrate complex. The MR_3 term in Eq. 2 was the sum of MR values for both 3- and 5-meta-substituents when both were present in a molecule. More significant correlations were obtained when the sum of MR was

used than when equations were derived that used MR for only one of the meta-substituents.

When regression analysis was performed for all compounds with substituents at the 3-, 4-, and 5-positions, including the parent compound and the 4-C_6H_5 derivative, Eq. 3 was derived:

$$\log 1/K_m = 0.92(\pm0.22)\log P - 0.98(\pm0.39)MR_4' + 0.99(\pm0.59)\,\sigma^- - 0.42(\pm0.51)MR_3 + 3.78(\pm0.50) \quad (3)$$
$$n = 26,\ r = 0.929,\ s = 0.545$$

Except for MR_4', coefficients for all of the terms in Eq. 3 were very similar to those shown in Eq. 2. The MR_4' coefficient differed primarily because of the effect of the 4-C_6H_5 derivative. However, the correlation obtained with Eq. 3 was not as high as that obtained with Eq. 2, $r = 0.929$ versus 0.962, respectively.

After the preceding analyses were performed, a single equation, Eq. 4, was derived with all of the data except for that obtained with the 4-C_6H_5 derivative:

$$\log 1/K_m = 0.92(\pm0.18)\log P - 1.48(\pm0.38)MR_4' - 0.64(\pm0.41)MR_3 + 1.04(\pm0.63)MR_2 + 0.67(\pm0.44)\,\sigma^- + 4.03(\pm0.42) \quad (4)$$
$$n = 35,\ r = 0.950,\ s = 0.477$$

The stepwise development of Eq. 4 is shown in Table 2. Addition of an F_2 term did not improve the correlation obtained with the equation. When the sterimol steric parameter (22) was used instead of MR_2 and MR_4', the correlation was not as significant as that found with Eq. 4. A plot of log $1/K_m$ values observed experimentally versus those calculated with Eq. 4 is shown in Fig. 1.

Phenolic substrates for human liver TS PST peak II. Human liver contains two isozymes of TS PST (6). Preliminary substrate kinetic studies had failed to reveal striking differences in K_m values for 4-nitrophenol measured with the two isozymes of human liver TS PST (6). To compare the substrate kinetics of the two isozymes of TS PST, K_m values were measured for TS PST peak II with several of the phenolic substrates listed in Table 1. The substrates studied were chosen on the basis of a wide range of apparent K_m values for TS PST peak I. Log $1/K_m$ values of these compounds for peaks I and II are listed in Table 3, and the correlation between values obtained with the two isozymes is shown graphically in Fig. 2. K_m values for these substrates, obtained with each of the two isozymes, were similar in magnitude, and log $1/K_m$ values were very highly correlated ($r = 0.992$, $p < 0.001$). These results are compatible with the conclusion that the active sites of the two isozymes of human liver TS PST are quite similar.

Structure-activity analyses with rat liver PST. Previously published K_m data for rat liver PST (12–14) were also subjected to QSAR analyses. Log $1/K_m$ values for rat liver PST that were used in these analyses are listed in Table 4. The following equation was derived for rat liver PST form I:

$$\log 1/K_m = 1.37(\pm0.53)\,\pi_3 + 0.29(\pm0.25)MR_4' + 0.24(\pm0.25)\,\pi_4 + 2.56(\pm0.26) \quad (5)$$
$$n = 12,\ r = 0.925,\ s = 0.261$$

The most important term in Eq. 5 was π_3, followed sequentially by MR_4' and π_4. The effect of substituents at the 2-position could not be evaluated since only a single 2-congener was included in the published data (12–14). Although there was little colinearity between the variables used in the derivation of Eq. 5 (data not shown), the limited number of data points that were available made it possible to state only that hydro-

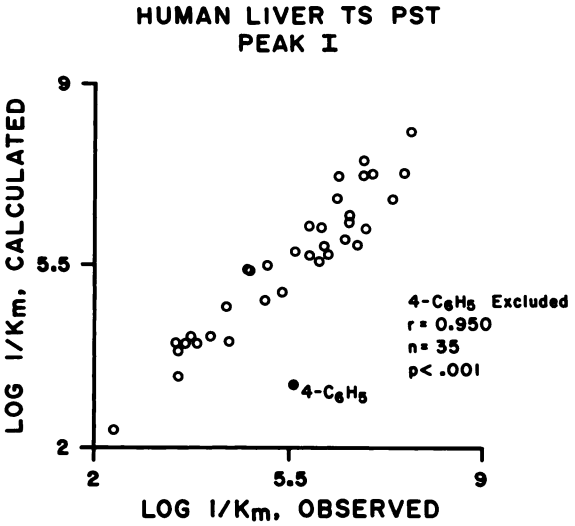

Fig. 1. Correlation between log $1/K_m$ values observed experimentally with human liver TS PST peak I and log $1/K_m$ values calculated by the use of Eq. 4. The ● (4-C_6H_5, compound 36 in Table 1) value was not used in the derivation of Eq. 4. See the text for details.

TABLE 3

Log $1/K_m$ values for human liver TS PST peaks I and II with X-C_6H_4OH substrates

X	Log $1/K_m$	
	Peak I	Peak II
1. 2-I	7.76	7.83
2. 2-Cl	7.41	7.41
3. 3-Cl	6.62	6.94
4. 4-NO_2	6.08	6.12
5. 4-CH_3	5.66	6.15
6. 3,5-$(CH_3)_2$	5.14	5.27
7. 4-OCH_3	4.44	5.07
8. 4-NH_2	3.51	4.04
9. 4-$NHCOCH_3$	2.35	2.53

TABLE 2

Stepwise development of Eq. 4

Coefficients are listed for each term in Eq. 4 at each step in the derivation of the equation. Fisher statistics (F values) at each step in the derivation are also listed.

log P	MR_4'	MR_3	MR_2	σ^-	r	s	F value
0.85					0.631	1.11	$F_{1,33} = 21.8$
0.91	−1.55				0.856	0.750	$F_{1,32} = 39.9$
1.04	−1.84	1.00			0.916	0.592	$F_{1,31} = 20.3$
0.99	−1.62	−0.73	0.92		0.932	0.542	$F_{1,30} = 7.03$
0.92	−1.48	−0.64	1.04	0.67	0.950	0.477	$F_{1,29} = 9.61$

phobic interactions at the indicated positions were the most important interactions for the analyses. Unlike the results found with human TS PST, there were no negative steric effects for 3- or 4-substituents. Significant differences in the characteristics of the hydrophobic space encountered by 3- and 4-substituents precluded the use of an overall log P term like that used in the analysis performed for human liver TS PST peak I.

Eq. 6 was derived for rat liver PST form II by using the data shown in Table 4:

$$\log 1/K_m = 1.38(\pm 0.60)\ \pi_3 + 0.28(\pm 0.28)MR_4' + 0.28(\pm 0.30)\ \pi_4 + 2.46(\pm 0.32) \quad (6)$$
$$n = 11,\ r = 0.914,\ s = 0.282$$

Eqs. 5 and 6 were very similar, although Eq. 5 provided a slightly better correlation with the experimental data than did Eq. 6, $r = 0.925$ versus 0.914, respectively. The K_m value for the 4-hydroxy compound was not used in the derivation of Eq. 6 because that derivative was 10 times more active than was predicted by the equation. Log $1/K_m$ values for the 4-C_6H_5 derivative, the behavior of which was predicted so poorly by the equations for human liver TS PST peak I, were predicted well by both Eqs. 5 and 6. Correlations between published K_m values for rat liver PST forms I and II and values calculated by the use of Eqs. 5 and 6 are shown graphically in Fig. 3. K_m values have also been published for nine compounds that were tested with rat liver PST form IV (13, 14). Unfortunately, it was not possible to derive an equation that adequately described the behavior of these compounds as substrates for rat liver PST form IV.

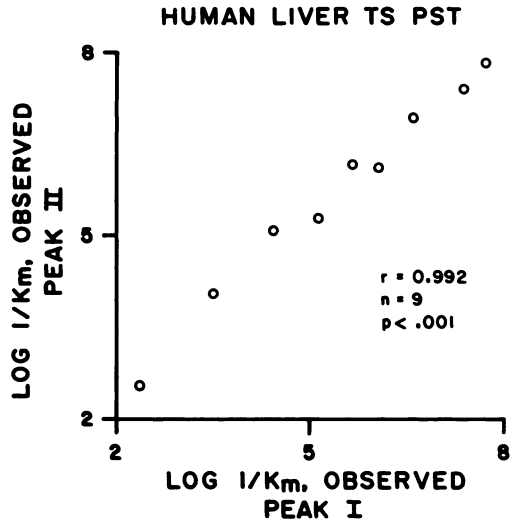

Fig. 2. Correlation between log $1/K_m$ values observed experimentally for nine phenolic substrates studied with both human liver TS PST peaks I and II.

Comparisons between the kinetic behavior of substrates for rat and human liver PST are shown graphically in Fig. 4. In that figure log $1/K_m$ values for all compounds that were tested with both human liver TS PST peak I and rat liver PST forms I and II are plotted against each other. The correlations between log $1/K_m$ values for compounds studied with PST from both species were not significant ($r = 0.469$, $p > 0.05$ and $r = 0.458$, $p > 0.05$ for rat liver forms I and II, respectively). Absolute K_m values for many of the compounds differed by up to 3 orders of magnitude. These observations, when coupled with the differences in the equations derived by structure-activity analysis, are compatible with the conclusion that the active sites of human liver TS PST peaks I and II differ substantially from those of rat liver PST forms I and II.

Discussion

Sulfate conjugation is an important pathway in the metabolism of phenolic and catechol drugs, xenobiotics and endogenous compounds (1–3). PST catalyzes the sulfation of many phenols, and multiple forms of PST are present in both human and rat liver (4–7, 12, 14). We have performed QSAR analyses of substrate kinetic data obtained with human liver TS PST peak I and of previously published data for rat liver PST forms I, II, and IV. Structure-activity relationship analyses for human liver TS PST peak I were performed sequentially with data for 2-substituted, and then for 3-, 4-, and 5-substituted, and finally, for 35 of 36 substituted phenols. The relationship between K_m values for substrates studied with human liver TS PST peaks

TABLE 4

Log $1/K_m$ values and parameters used in the derivations of Eqs. 5 and 6 for rat PST forms I and II with X-C_6H_4OH substrates

Log $1/K_m$ values for form I were calculated with Eq. 5, and log $1/K_m$ values for form II were calculated with Eq. 6. "Reported" K_m values were originally published elsewhere (12, 14). *MR_4' values of less than 0.74 were assigned values of 0. The π values are for the phenol system.*

	Log 1 K_m values for:				π_3	π_4	MR_4'
	Form I		Form II				
	Reported	Calculated	Reported	Calculated			
1. 3,4-$(CH)_4$[a]	4.22	3.87	4.05	3.80	0.66	0.66	0.87
2. H	2.74	2.56	2.59	2.46	0.00	0.00	0
3. 3-Cl	3.92	3.98	3.80	3.90	1.04	0.00	0
4. 4-Cl	2.82	2.78	2.92	2.72	0.00	0.93	0
5. 3-CH_3	3.00	3.24	2.85	3.15	0.50	0.00	0
6. 4-CH_3	2.54	2.67	2.66	2.59	0.00	0.48	0
7. 3-NO_2	3.22	3.30	3.36	3.20	0.54	0.00	0
8. 4-NO_2	2.80	2.88	2.60	2.78	0.00	0.45	0.74
9. 4-OCH_3	2.38	2.76	2.19	2.64	0.00	−0.12	0.79
10. 4-OH	2.74	2.35	3.22[b]	2.11	0.00	−0.87	0
11. 4-C_6H_5	3.74	3.70	3.60	3.64	0.00	1.74	2.54
12. 4-$NHCOCH_3$	2.68	2.72	2.80	2.55	0.00	−1.14	1.49

[a] A naphthol derivative.
[b] This value was not used in the derivation of Eq. 6.

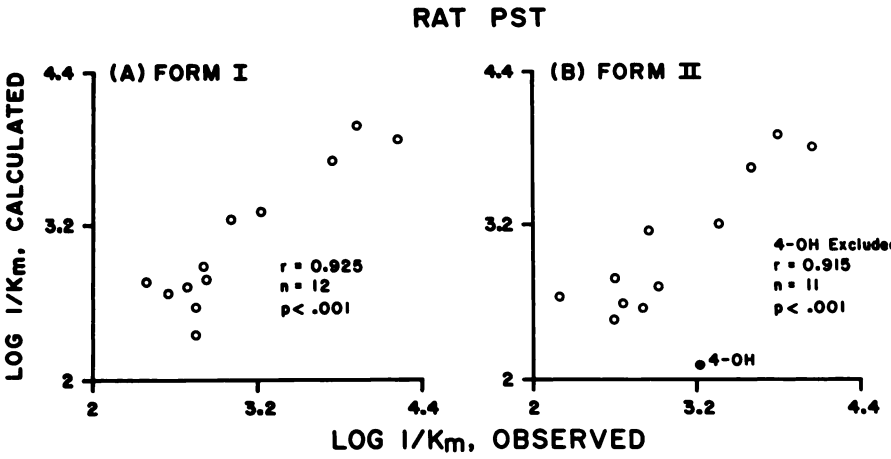

Fig. 3. Correlation between log $1/K_m$ values observed experimentally and values calculated by the use of (A) Eq. 5 for rat liver PST form I, and (B) Eq. 6 for rat liver PST form II. The ● (4-OH, compound 10 in Table 4) value was not used in the derivation of Eq. 6. See the text for details.

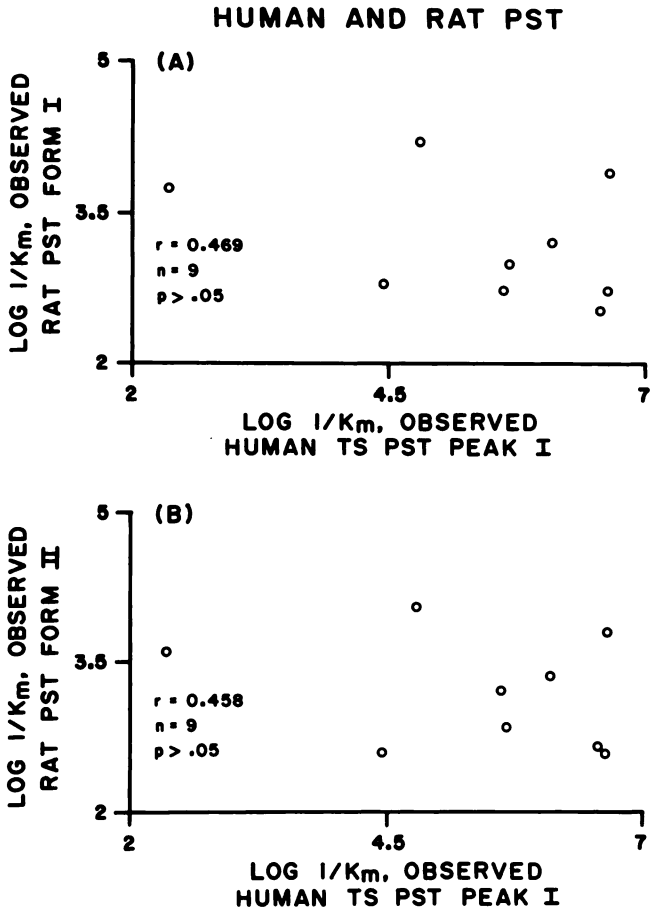

Fig. 4. Correlation between log $1/K_m$ values observed experimentally for phenolic substrates studied with human liver TS PST peak I and (A) rat liver PST form I or (B) rat liver PST form II.

I and II was also determined. Finally, structure-activity relationship analyses were also performed for rat liver PST forms I, II, and IV by using previously published substrate kinetic data (12–14).

Structure-activity analysis of data for 2-substituted phenolic substrates for human liver TS PST peak I resulted in the derivation of Eq. 1. In that equation the terms log P, F_2, and MR_2 described the major features of the structure-activity relationships. The fact that F_2 gave a better correlation than σ suggested that inductive rather than resonance electronic effects were important in the 2-position, as might be expected for substituents adjacent to the functional moiety. The positive coefficient for F_2 indicated that electron withdrawal by 2-substituents enhanced the formation of the enzyme-substrate complex. The positive coefficient for MR_2 was compatible with a favorable change in the active site region of the enzyme in the presence of bulky 2-substituents. Finally, the fact that the correlation was less significant when the Taft parameters (E_s) were used than when MR was used suggested that the steric effect was not an intramolecular one. Obviously, this conclusion is valid only for 2-substituents of the sizes of those listed in Table 1.

Eq. 2 described structure-activity relationships for human liver TS PST peak I with phenols that had 3-, 4-, and 5-substituents. The fact that a higher correlation was obtained with a summed MR_3 term for both meta-substituents than with an MR value for only one meta-substituent suggested that the site with which meta-substituents interact was of limited size. The negative coefficient for MR_4' also indicated that the binding site was limited in the direction of 4-substituents. Only 4-substituents of the size of NO_2 or larger caused the steric effect. The fact that the 4-C_6H_5 congener had greater activity than was predicted by Eq. 2 suggested that this compound might somehow "brush aside" a steric "barrier." A similar phenomenon was observed when benzamidine inhibitors of trypsin were subjected to QSAR analysis (23). The positive correlation for σ^- in Eq. 2 suggested once again that electron-withdrawing groups promoted the formation of the enzyme-substrate complex.

Eq. 4 provided an analysis for all of the phenols studied, with the exception of the 4-C_6H_5 congener. The coefficient of almost 1 for log P in Eqs. 2, 3, and 4 indicated that desolvation of the substrate during interaction with the enzyme was similar to the desolvation that occurs when the molecule partitions into octanol from water, i.e., it was essentially complete. Therefore, the active site might be pictured as a hydrophobic "hole." There was also an obstruction to 4-substituents. However, when the 4-substituent was as large as 4-C_6H_5, the obstruction could "give way" to allow access to the active site. The most active substrates in the series of compounds studied were the 2-halo compounds. Those analogs possessed the desired electron-attracting, hydrophobic, and bulk properties required for optimal activity.

Structure-activity analyses of data obtained with substrates studied previously with rat liver PST forms I and II were very similar to each other (Eqs. 5 and 6), but the equations for the rat liver enzymes were quite different from those for human liver TS PST peak I. For example, there was a small positive steric effect at the 4-position rather than the negative effect observed with the human enzyme. Behavior of the 4-C_6H_5 congener with the human enzyme was poorly predicted by Eq. 4, but the behavior of this congener was predicted very well by Eqs. 5 and 6 for the rat liver enzymes. These observations suggested that large 4-substituents might produce a conformational change in rat liver PST forms I and II. Finally, neither the absolute magnitudes nor the rank orders of K_m values for substrates studied with rat liver PST forms I and II were correlated well with those of the same compounds tested with human liver TS PST (Fig. 4).

The results of these studies may make it possible to predict K_m values for phenolic substrates of human liver TS PST peak I and for rat liver PST forms I and II, at least within the range of the data explored. Our observations have also made it possible to predict certain characteristics of the active sites of human and rat liver PST. It will be of interest to see how closely these characteristics match those found during future X-ray crystallographic studies of highly purified PST. Finally, our results suggest that there may be substantial differences between the multiple forms of human and rat liver PST. The magnitudes of K_m values for rat liver PST forms I and II and for human liver TS PST peaks I and II differ by up to 3 orders of magnitude, and the K_m values are not significantly correlated between species. In addition, characteristics of the active sites of the enzyme as predicted by structure-activity analysis differ. Finally, TS PST peaks I and II appear to be allozymes (6, 24); that is, individual human subjects have one, the other, or both peaks—probably on a genetic basis (6, 24). However, rat liver PST forms I and II appear to be isozymes; i.e., the livers of all rats contain both forms I and II (12–14). Ultimately, application of the techniques of molecular biology may be required to define the relationship between the multiple forms of PST present in human and rat liver.

Acknowledgments

We thank Luanne Wussow for her assistance with the preparation of the manuscript.

References

1. Dodgson, K. S. Conjugation with sulfate, in *Drug Metabolism from Microbe to Man* (D. V. Parke and R. L. Smith, eds.). Taylor & Francis Ltd., London, 91–104 (1977).
2. Roy, A. B. Sulfotransferases, in *Sulfation of Drugs and Related Compounds* (G. J. Mulder, ed.). CRC Press, Boca Raton, FL, 83-130 (1981).
3. Weinshilboum, R. M. Phenol sulfotransferase in humans: properties, regulation and function. *Fed. Proc.* **45**:2223–2228 (1986).
4. Rein, G., V. Glover, and M. Sandler. Phenolsulphotransferase in human tissue: evidence for multiple forms, in *Phenolsulfotransferase in Mental Health Research* (M. Sandler and E. Usdin, eds.). MacMillan Publishers Ltd., London, 98–126 (1981).
5. Young, W. F., Jr., H. Okazaki, E. R. Laws Jr., and R. M. Weinshilboum. Human brain phenol sulfotransferase: biochemical properties and regional localization. *J. Neurochem.* **43**:706–715 (1984).
6. Campbell, N. R. C., J. A. Van Loon, and R. M. Weinshilboum. Human liver phenol sulfotransferase: assay conditions, biochemical properties and partial purification of isozymes of the thermostable form. *Biochem. Pharmacol.* **36**:1435–1446 (1987).
7. Sundaram, R., and R. Weinshilboum. Human jejunal phenol sulfotransferase (PST): partial purification of the "TL" form. *Fed. Proc.* **44**:1821 (1985).
8. Rein, G., V. Glover, and M. Sandler. Multiple forms of phenolsulfotransferase in human tissue: selective inhibition by dichloronitrophenol. *Biochem. Pharmacol.* **31**:1893–1897 (1982).
9. Reiter, C., and R. Weinshilboum. Acetaminophen and phenol: substrates for both a thermostable and a thermolabile form of human platelet phenol sulfotransferase. *J. Pharmacol. Exp. Ther.* **221**:43–51 (1982).
10. Reiter, C., G. Mwaluko, J. Dunnette, J. Van Loon, and R. Weinshilboum. Thermolabile and thermostable human platelet phenol sulfotransferase: substrate specificity and physical separation. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **324**:140–147 (1983).
11. Whittemore, R. M., L. B. Pearce, and J. A. Roth. Purification and kinetic characterization of a phenol sulfating form of phenol sulfotransferase from human brain. *Arch. Biochem. Biophys.* **249**:464–471 (1986).
12. Sekura, R. D., and W. B. Jakoby. Phenol sulfotransferases. *J. Biol. Chem.* **254**:5658–5663 (1979).
13. Sekura, R. D., and W. B. Jakoby. Aryl sulfotransferase IV from rat liver. *Arch. Biochem. Biophys.* **211**:352–359 (1981).
14. Sekura, R. D., M. W. Duffel, and W. B. Jakoby. Aryl sulfotransferases. *Methods Enzymol.* **77**:197–206 (1981).
15. Foldes, A., and J. L. Meek. Rat brain phenolsulfotransferase—partial purification and some properties. *Biochim. Biophys. Acta* **327**:365–374 (1973).
16. Anderson, R. J., and R. M. Weinshilboum. Phenolsulphotransferase: enzyme activity and endogenous inhibitors in human erythrocyte. *J. Lab. Clin. Med.* **94**:158–171 (1979).
17. 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.* **72**:248–254 (1976).
18. Wilkinson, G. N. Statistical estimations in enzyme kinetics. *Biochem. J.* **80**:324–332 (1961).
19. Cleland, W. W. Computer programmes for processing enzyme kinetic data. *Nature (Lond.)* **198**:463–465 (1963).
20. Hansch, C. A. A quantitative approach to biochemical structure-activity relationships. *Acc. Chem. Res.* **2**:232–239 (1969).
21. Hansch, C. A. A computerized approach to quantitative biochemical structure-activity relationships. *Adv. Chem.* **114**:20–40 (1972).
22. Hansch, C. A., and A. Leo. *Substituent Constants for Correlation Analysis in Chemistry and Biology*. Wiley Interscience, New York (1979).
23. Recanatini, M., T. Klein, C. Z. Yang, J. McClarin, R. Langridge, and C. Hansch. Quantitative-structure-activity relationships and molecular graphics in ligand receptor interactions: amidine inhibition of trypsin. *Mol. Pharmacol.* **29**:436–446 (1986).
24. Maidak, B., N. Campbell, and R. Weinshilboum. Phenol sulfotransferase: correlation of hepatic isozymes with familial thermal stability variants, in *7th International Congress on Human Genetics*, p. 400 (1986).

Send reprint requests to: Dr. Richard Weinshilboum, Clinical Pharmacology Unit, Department of Pharmacology, Mayo Clinic/Mayo Foundation, Rochester, MN 55905.