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HUMAN LIVER PHENOL SULFOTRANSFERASE: ASSAY CONDITIONS, BIOCHEMICAL PROPERTIES AND PARTIAL PURIFICATION OF ISOZYMES OF THE THERMOSTABLE FORM*

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Abstract—Phenol sulfotransferase (PST) catalyzes the sulfate conjugation of phenolic and catechol drugs and neurotransmitters. Human platelets and brain contain at least two forms of PST. One form is relatively thermolabile (TL) and catalyzes the sulfate conjugation of monoamines such as dopamine. The other is thermostable (TS) and catalyzes the sulfation of "simple" phenols such as phenol and *p*-nitrophenol. We found that homogenates of human liver also contain two forms of PST that are similar to brain and platelet TL and TS PST with regard to substrate specificities, thermal stabilities and sensitivities to inhibitors. Optimal conditions were determined for the assay of these two activities in human liver homogenates. The apparent K_m of liver homogenate TL PST for dopamine was 27 μM. The apparent K_m of the TS form of the enzyme for *p*-nitrophenol was 0.94 μM. Human liver TS PST also catalyzed the sulfate conjugation of dopamine, but with an apparent K_m of 5 mM, over two orders of magnitude higher than that of TL PST. Two different peaks of TS PST activity were separated from the TL activity by ion exchange chromatography of human liver preparations. Both peaks of TS PST activity were partially purified and characterized. Both had similar substrate specificities and inhibitor sensitivities. K_m values of TS PST peak I for *p*-nitrophenol and for 3′-phosphoadenosine-5′-phosphosulfate were 0.91 and 0.86 μM, respectively, while the K_m values of TS PST peak II for these two cosubstrates for the reaction were 0.43 and 0.64 μM, respectively. However, the TS PST activity in peak II was significantly more thermolabile than was the activity in peak I. These results are compatible with the conclusion that human liver homogenates contain at least two forms of PST, forms with properties similar to those of TS and TL PST in homogenates of human cerebral cortex and platelets. In addition, human liver contains two isozymes of TS PST.

Sulfate conjugation catalyzed by phenol sulfotransferase (EC 2.8.2.1, PST) is an important metabolic pathway for many phenolic and catechol drugs and neurotransmitters [1–3]. Human platelets and brain contain at least two forms of PST, forms that differ in their substrate specificities, thermal stabilities, inhibitor sensitivities and regulation [4–10]. One form is relatively thermolabile (TL) and catalyzes the sulfate conjugation of micromolar concentrations of dopamine and other catechol and phenol monoamines. The other form is thermostable (TS) and catalyzes the sulfate conjugation of micromolar concentrations of phenol, *p*-nitrophenol and other "simple" phenols [6, 7]. However, at higher concentrations phenol and *p*-nitrophenol can also serve as substrates for TL PST [6, 7]. The TS form is more sensitive to inhibition by 2,6-dichloro-4-nitrophenol (DCNP) than is TL PST [5, 6, 8]. There are also individual familial variations in the thermal stability of TS PST in the human platelet [11], variations that are correlated with individual differences in the thermal stability of TS PST in human cerebral cortex [12]. Human platelet PST has been studied extensively because of the possibility that its biochemical properties and regulation might reflect those of the enzyme in less accessible drug and neurotransmitter metabolizing tissues [3]. This hypothesis has already been shown to be valid in the case of TS PST activity in the human platelet and cerebral cortex [12]. The liver plays an important role in the metabolism of both exogenous and endogenous phenolic substances. Our experiments were performed to determine whether the human liver contains PST activities with properties similar to those of the enzyme in the human platelet and brain.

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MATERIALS AND METHODS

Liver tissue acquisition and preparation

Liver tissue was obtained from eighteen male and eight female patients undergoing clinically indicated partial hepatectomies for the removal of either pri-

mary or metastatic tumors and/or for intrahepatic cholelithiasis. These tissue samples were obtained under guidelines approved by the Mayo Clinic Institutional Review Board. Grossly normal tissue, removed as far as possible from tumor, was stored at −80°. Frozen liver tissue was homogenized for 30 sec in 9 vol. of 5 mM potassium phosphate buffer, pH 7.5, with a Polytron homogenizer. Homogenates were centrifuged at 16,200 *g* for 15 min at 4°. The supernatant fractions were then centrifuged at 100,000 *g* for 1 hr at 4°, and supernatant fractions from that step were stored at −80°. For those experiments in which optimal assay conditions were determined and for experiments in which the biochemical properties of PST activity in liver homogenates were studied, equal volumes of 100,000 *g* supernatant fractions from six liver samples were pooled prior to storage at −80°. TS PST was partially purified from a pooled sample composed of equal volume aliquots of 100,000 *g* supernatant fractions from fourteen samples of hepatic tissue. For studies of PST in individual liver samples, aliquots of 100,000 *g* supernatant fractions were stored at −80° prior to assay. Three of these individual samples were used in studies of possible isozymes of TS PST. Sample "A" was from a 68-year-old woman with carcinoma of the colon metastatic to the liver; sample "B" was from a 25-year-old women with intrahepatic cholelithiasis; and sample "C" was from a 51-year-old man with carcinoma of the colon metastatic to the liver.

PST assay

PST activity was measured by the method of Foldes and Meek [13] as modified by Anderson and Weinshilboum [14]. The sulfate acceptor substrates used were dopamine and *p*-nitrophenol, model substrates in other tissues for TL and TS PST respectively [6, 8]. Samples of 100,000 *g* supernatant fractions of liver homogenates were diluted in 5 mM potassium phosphate buffer that contained dithiothreitol (DTT) and bovine serum albumin (BSA). The diluted samples were incubated at 37° with a sulfate acceptor substrate and [^{35}S]3′phosphoadenosine-5′-phosphosulfate (PAPS), the sulfate donor for the reaction. Either potassium phosphate or Tris–HCl buffers at final concentrations of 8 mM were also present. "Blanks" were samples that contained no sulfate acceptor. Pargyline, a monoamine oxidase inhibitor [15], at a final concentration of 1 mM was added to the reaction mixture when dopamine was used as a substrate. After 15-min incubations, reactions were terminated by precipitation of PAPS and protein by barium hydroxide, barium acetate and zinc sulfate. Each sample was then centrifuged. The supernatant fraction was aspirated and was mixed with 3a70 liquid scintillation counting fluid (RPI Corp.). Radioactivity was measured in a Beckman LS 7500 scintillation counter. Details of the PST assay have been described elsewhere [6–8, 14]. One unit of enzyme activity represented the formation of one nmole of product per hr of incubation at 37°.

Protein assay

Protein concentrations were measured by the dye binding method of Bradford [16] with BSA as a standard.

Ion exchange chromatography

Pooled supernatant fractions after the centrifugation of human liver homogenates at 100,000 *g* for 1 hr were applied to a 2.6 × 10 cm column of DEAE-Sepharose CL-6B that had been equilibrated with 5 mM potassium phosphate buffer, pH 7.5. The buffer contained 3 mM DTT and 100 mM NaCl. In those experiments in which large quantities of the enzyme were to be purified, a 55-ml aliquot of pooled supernatant fractions was thawed and was concentrated to 7 ml with an Amicon pressure concentrator and a PM 10 membrane. Elution was performed with a 400-ml linear NaCl gradient that varied from 100 to 225 mM in 5 mM potassium phosphate buffer, pH 7.5, that contained 3 mM DTT. The flow rate was approximately 15 ml/hr, and 6.5-ml fractions were collected. These fractions were diluted 100-fold to assay TL and 1000-fold to assay TS PST activity. Fractions that contained the two peaks of TS PST activity were pooled separately and were concentrated.

In some experiments supernatant fractions from individual liver samples were studied rather than pooled preparations. In those experiments, the DEAE Sepharose CL-6B column was 2.6 × 6 cm. Six milliliters of unconcentrated 100,000 *g* supernatant fraction was applied to the column and was eluted with a linear NaCl gradient from 100 to 200 mM in the same buffer that was used to elute the column for the purification of PST. The flow rate was approximately 10 ml/hr, and 6.5-ml fractions were collected. The fractions that contained peaks of TS PST activity were pooled, concentrated, and stored at −80° after the addition of 1 mg/ml BSA to preserve enzyme activity during storage. In some experiments, mixtures of supernatant fractions from two livers were applied to the column. In those cases, a 6-ml volume was applied, but the mixture was adjusted so that it contained approximately equal activities from each of the individual samples.

Gel filtration chromatography

A 1.5 × 70 cm column of Sephadex G-100 superfine was equilibrated with 100 mM potassium phosphate buffer, pH 7.5, that contained 3 mM DTT. The flow rate was 7 ml/hr. The column was calibrated with Dextran blue 2000, aldolase, BSA, ovalbumin, and chymotrypsinogen A so that it could be used for molecular size estimates. Four milliliters of concentrated TS PST from the ion exchange chromatography step was applied to the Sephadex column, and 3-ml fractions were collected. TS PST activity was measured using 200-fold dilutions of these fractions. Fractions that contained maximal TS PST activities were pooled and diluted with an equal volume of 5 mM potassium phosphate buffer, pH 7.5, that contained 3 mM DTT and 1.5 mg/ml BSA. These pooled enzyme preparations were stored at −80°. TS PST activity was stable for at least 3 months when stored in this fashion.

Kinetic analysis

"Apparent" Michaelis (K_m) constants were esti-

mated by the method of Wilkinson [17] with a computer program written by Cleland [18]. "True" K_m values were calculated by the method of Florini and Vestling [19]. Dixon plots [20] were used to calculate values of inhibitor (K_i) constants.

Materials

[^{35}S]PAPS (2.1 to 2.4 Ci/mmole) was purchased from the New England Nuclear Corp., Boston, MA. DTT (Cleland's reagent), *p*-nitrophenol, *p*-nitrophenol sulfate, and BSA were obtained from the Sigma Chemical Co., St. Louis, MO. Dopamine HCl was purchased from Calbiochem, San Diego, CA. Pargyline HCl was obtained from Saber Laboratories, Inc., Plainview, NY. HPLC grade phosphoric acid, HPLC grade acetonitrile, disodium EDTA, 1-heptane sulfonic acid, barium acetate, barium hydroxide and zinc sulfate were purchased from Fisher Scientific Products, Fairlawn, NJ. Molecular weight markers, DEAE Sepharose CL-6B and Sephadex G-100 superfine were purchased from Pharmacia Fine Chemicals Inc., Piscataway, NJ. Dopamine-3-*O*-sulfate and dopamine-4-*O*-sulfate were provided by Dr. A. A. Mannian, Chemical Synthesis Program, National Institute for Mental Health, Rockville, MD.

RESULTS

Two series of experiments were performed. The first involved studies of the properties of PST in human liver homogenates. Optimal conditions were determined for the assay of PST activity in the homogenates. The second series of experiments involved the partial purification of human liver TS PST, followed by studies of its biochemical properties. Preliminary experiments showed that human liver homogenates catalyzed the sulfate conjugation of both *p*-nitrophenol and dopamine. Peak enzyme activity with *p*-nitrophenol as substrate was found at a concentration of 4 μM (Fig. 1). With dopamine as a substrate, two peaks of activity were present at concentrations of 60 μM and 20 mM (Fig. 2). Therefore, 4 μM *p*-nitrophenol and two concentrations of dopamine, 60 μM and 20 mM, were used in most of the subsequent experiments.

Properties of PST in human liver homogenates

Effect of tissue concentration and incubation time. PST activities increased in a linear fashion at tissue dilutions of greater than 50,000-fold with 4 μM *p*-nitrophenol, greater than 500-fold with 60 μM dopamine, and greater than 5,000-fold with 20 mM dopamine as substrates. The use of more concentrated tissue preparations resulted in a loss of the linear relationship between tissue quantity and enzyme activity. In all subsequent experiments liver homogenates were diluted 100,000-fold with 4 μM *p*-nitrophenol as substrate, 1,000-fold with 60 μM dopamine as substrate, and 15,000-fold with 20 mM dopamine as substrate. Under those conditions, enzyme activities measured with all three substrates increased in a linear fashion for at least 20 min. An incubation time of 15 min was used in all subsequent assays.

Effect of pH. The effect of pH on PST activity in liver homogenates was determined with 4 μM *p*-nitrophenol and with both 60 μM and 20 mM dopamine as substrates. With 4 μM *p*-nitrophenol and with 60 μM dopamine, optimal activities were found with a potassium phosphate buffer that resulted in a final reaction pH of 6.6. A Tris–HCl buffer that gave a final reaction pH of 7.15 resulted in optimal activity when 20 mM dopamine was the substrate.

Effect of substrate concentration. The effect of *p*-nitrophenol concentration on PST activity in human liver homogenates is shown in Fig. 1A. A double-reciprocal plot of the data is shown in Fig. 1B. The peak of PST activity was present at a concentration of 4 μM. Eight data points from 0.1 to 4 μM *p*-nitrophenol were used to calculate an apparent K_m of 0.94 μM. A second peak of activity, a peak due to TL PST activity, has been found with platelet and

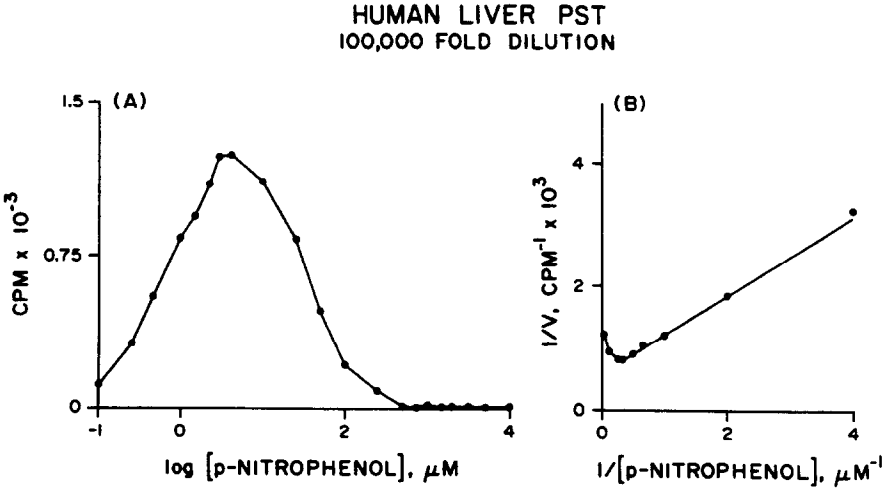

Fig. 1. Effect of *p*-nitrophenol concentration on human liver PST activity. (A) Relationship between *p*-nitrophenol concentration and PST activity. The data are plotted in a semilogarithmic fashion. (B) Double-reciprocal plot of the data in (A) for *p*-nitrophenol concentrations from 0.25 to 25 μM. Each point is the average of three determinations.

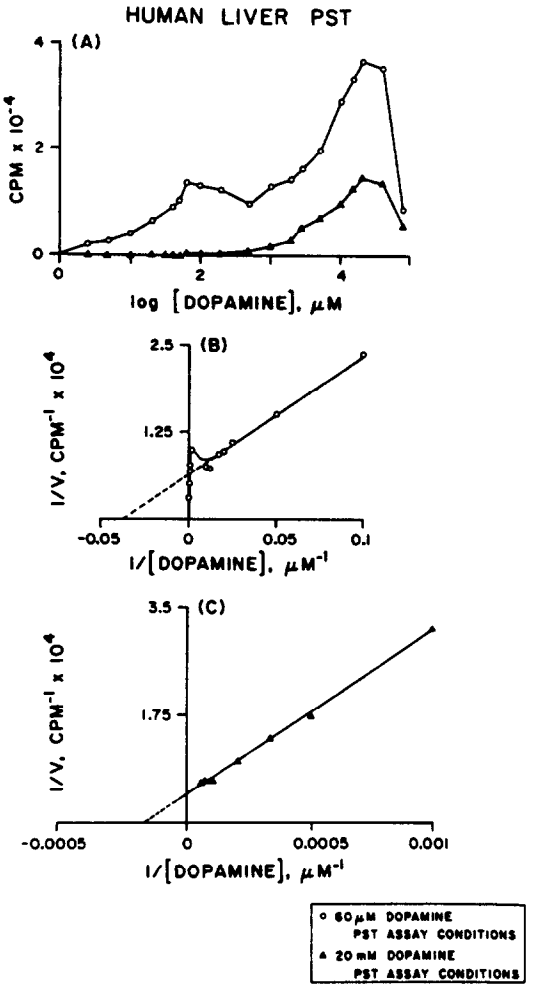

Fig. 2. Effect of dopamine concentration on human liver PST activity. (A) Relationships between dopamine concentration and PST activity under optimal assay conditions for 60 μM and 20 mM dopamine. The data are plotted in a semilogarithmic fashion. (B) Double-reciprocal plot of the data in (A) for dopamine concentrations from 10 to 3000 μM assayed under optimal conditions for 60 μM dopamine. (C) Double-reciprocal plot of the data in (A) for dopamine concentrations from 1 to 20 mM assayed under optimal conditions for 20 mM dopamine. Each point is the average of three determinations.

brain homogenates at *p*-nitrophenol concentrations of approximately 2–3 mM [6, 8]. Under the assay conditions used here, no second peak of *p*-nitrophenol sulfotransferase activity was found with liver homogenates.

The effect of dopamine concentration on PST activity was studied under optimal conditions of tissue dilution and buffer pH for both 60 μM and 20 mM dopamine (Fig. 2). When optimal assay conditions for 60 μM dopamine were used, there were two peaks of activity. One peak was present at a concentration of 60 μM. The apparent K_m for that activity was 27 μM. This value was calculated using six data points from 2.5 to 60 μM dopamine. The second peak was at a dopamine concentration of approximately 20 mM. The apparent K_m for the second peak was 8 mM, but that value was calculated with only four data points from 5 to 20 mM. Finally, under optimal conditions of tissue dilution and pH for the activity measured with 20 mM dopamine, there was a single peak of activity at a concentration of approximately 20 mM (Fig. 2A). The apparent K_m for that peak was 5 mM when calculated with seven data points from 1 to 20 mM (Fig. 2C).

Effect of PAPS concentration. The effect of PAPS concentration on PST activity was determined with 4 μM *p*-nitrophenol, 20 mM dopamine and 60 μM dopamine as substrates. Eight concentrations of PAPS from 0.05 to 4.0 μM were tested. Apparent K_m values for PAPS calculated from these data were 0.52, 0.56 and 0.29 μM with the three sulfate acceptor substrates respectively. A PAPS concentration of 0.4 μM was used in all routine assays, as it has been in other tissues [6–8, 14].

Effect of BSA and DTT. PST activity in these highly diluted liver homogenates increased in the presence of BSA with all three substrates tested. The increase was 100% for the 60 μM dopamine sulfotransferase activity, whereas activities measured with 4 μM *p*-nitrophenol and 20 mM dopamine could not be detected in the absence of BSA. A final BSA concentration of 1 mg/ml gave optimal activity for all three substrates. Concentrations of DTT from 0 to 30 mM had no effect on PST activity measured with 4 μM *p*-nitrophenol or 60 μM dopamine. However, activity measured with 20 mM dopamine was undetectable in the absence of DTT. This activity increased with increasing DTT concentrations up to 4 mM and then remained constant as the concentration was increased to 30 mM. A DTT concentration of 15 mM was used in the routine assays with all three substrates.

Identification of reaction products. The products of the PST reaction were identified by HPLC with 4 μM *p*-nitrophenol and with both 60 μM and 20 mM dopamine as substrates. HPLC was performed with a Waters C18 reversed phase column using the methods of Diamond and Quebbemann [21] and Sharpless *et al.* [22] as described in detail by Young *et al.* [8]. When 4 μM *p*-nitrophenol was used as a substrate, 93% of the radioactivity in the reaction product coeluted with *p*-nitrophenol sulfate. When 60 μM dopamine and 20 mM dopamine were used as substrates, 102 and 87%, respectively, of the radioactivity in the reaction products eluted with dopamine sulfate.

Thermal stability of liver homogenate PST. The thermal stability of PST activity was measured as described by Reiter *et al.* [6, 7] in 100,000 *g* supernatant fractions from human liver preparations with 4 μM *p*-nitrophenol and both 60 μM and 20 mM dopamine as substrates (Fig. 3A). The 50% inactivation temperatures after 15-min preincubations were 43 and 43.5° when assays were performed with 4 μM *p*-nitrophenol and 20 mM dopamine respectively. However, the activity measured with 60 μM dopamine was much more thermolabile, with a 50% inactivation temperature of approximately 36° (Fig. 3A).

Effect of DCNP. Human liver PST activity was measured in the presence of various concentrations of DCNP (Fig. 3B). The concentration of DCNP

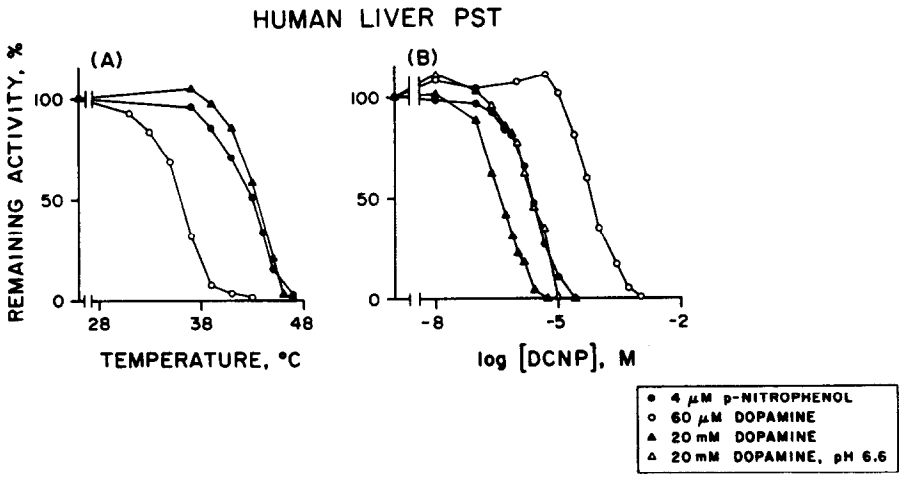

Fig. 3. Human liver PST thermal stability and inhibition by 2,6-dichloro-4-nitrophenol (DCNP). (A) PST activity was measured after preincubation for 15 min at the temperatures indicated. (B) PST activity was measured in the presence of the DCNP concentrations indicated. PST activity was measured with 20 mM dopamine as a substrate under optimal conditions for both 60 μM (pH 6.6) and 20 mM dopamine. Each point is the average of three determinations.

that inhibited by 50% (IC_{50}) was 2.3×10^{-6} M for the activity measured with 4 μM *p*-nitrophenol, while the IC_{50} value for inhibition of the activity measured with 60 μM dopamine was 6.6×10^{-5} M. The IC_{50} value was 4.4×10^{-6} M with 20 mM dopamine as a substrate under optimal assay conditions for that concentration of dopamine. However, when the buffer and reaction pH were changed so that they were identical to those used to measure 4 μM *p*-nitrophenol and 60 μM dopamine sulfotransferase activities, the IC_{50} for 20 mM dopamine was 2.3×10^{-6} M, the same as that found for 4 μM *p*-nitrophenol (Fig. 3B).

PST activity in individual human liver samples. PST activity was measured in samples of human hepatic tissue from thirteen male and seven female patients. Average activities were 35.8 ± 10.6, 23.3 ± 7.0, and 1.59 ± 0.87 units per mg protein (mean ± SEM) with 4 μM *p*-nitrophenol, 20 mM dopamine, and 60 μM dopamine as PST substrates respectively. PST activities in these individual samples measured with 4 μM *p*-nitrophenol and with 20 mM dopamine were significantly correlated ($r = 0.845$, $P < 0.01$). However, PST activities measured with 60 μM dopamine were not correlated significantly with activities measured with 20 mM dopamine ($r = 0.364$, $P > 0.05$) or with activities measured with 4 μM *p*-nitrophenol ($r = 0.491$, $P > 0.05$). Finally, there was not a significant correlation between enzyme activities and the ages of

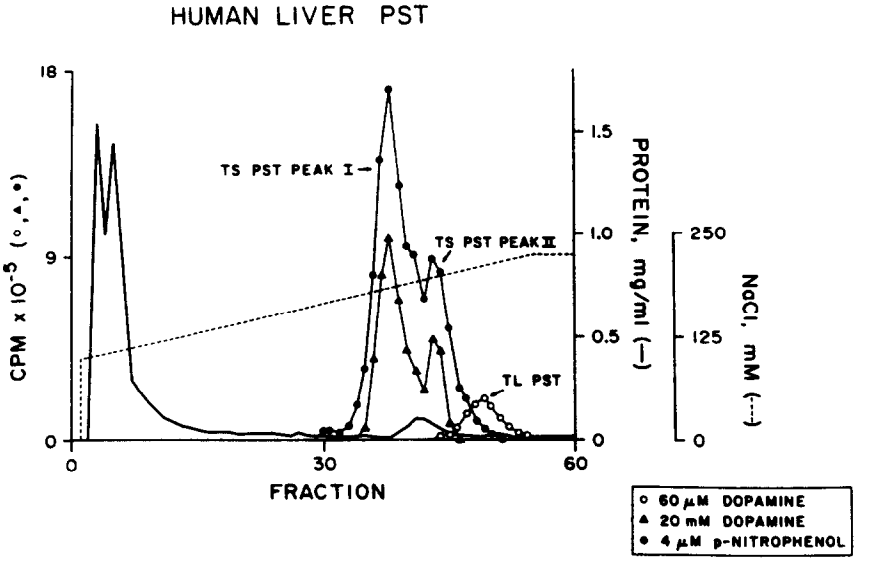

Fig. 4. Ion exchange chromatography of human liver PST performed with pooled 100,000 *g* supernatant fractions. PST activity was measured with 4 μM *p*-nitrophenol, 60 μM dopamine and 20 mM dopamine as substrates. See text for details.

the patients, nor were there significant differences between the sexes in hepatic PST activities.

Properties of purified human liver TS PST

Purification of human liver TS PST. Only the TS form of the enzymes was purified since its specific activity in liver was 20-fold greater than that of the TL form and because preliminary experiments had indicated that human small intestine would be a better source than liver from which to purify TL PST [18]. Ion exchange and gel filtration chromatography were used to partially purify TS PST from human liver. The use of additional purification steps was not possible because of instability of the enzyme activity. Two peaks of TS PST activity were always found during ion exchange chromatography of pooled liver preparations (Fig. 4). Both eluted before a late peak of TL PST activity (Fig. 4). In an attempt to determine whether each of the two TS PST peaks were present in samples from individual subjects, 100,000 *g* supernatant fractions from three individual liver samples were subjected to ion exchange chromatography (Fig. 5). One sample, "C", behaved like the pooled preparations and had two peaks of TS PST activity (Fig. 5B). Each of the other samples, "A" and "B", had only a single peak. However, the peaks in samples A and B eluted in different fractions (Fig. 5A). A mixture of supernatant fractions from livers A and B was also passed through the column. TS PST activity in the mixture eluted as two peaks with elution characteristics identical to those of peaks I and II in sample C (Fig. 5B).

The first peak of TS PST activity from a pooled preparation was then applied to a Sephadex G-100 gel filtration column. The fractions used were selected to avoid contamination by the second peak. The single peak that eluted from the gel filtration column had been purified at least 80-fold as compared with the 100,000 *g* supernatant fraction (Table 1). TS PST peak II was reapplied to the ion exchange column, and it eluted as a single peak. That material was also subjected to gel filtration chromatography with Sephadex G-100. The single peak resulting from gel filtration chromatography of peak II had been purified at least 36-fold (Table 1).

Purified human liver TS PST was very unstable during storage at 4°, the temperature at which the

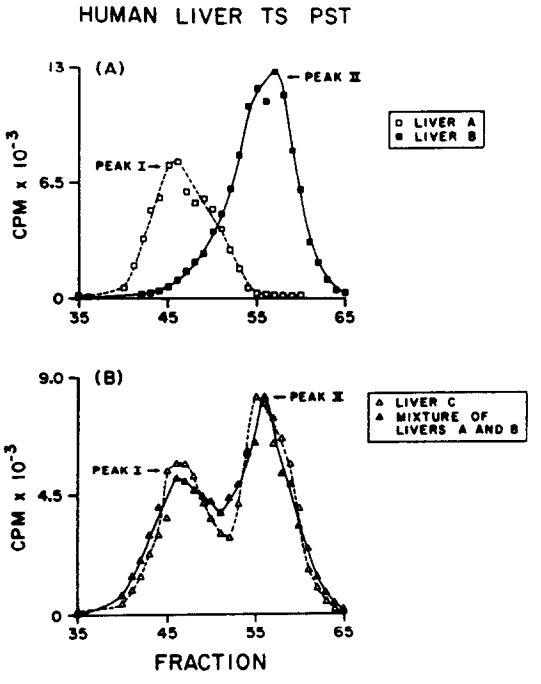

Fig. 5. Ion exchange chromatography of human liver PST performed with supernatant fractions from homogenates obtained from individual tissue samples. PST activity was measured with 4 μM *p*-nitrophenol as a substrate. See text for details.

Table 1. Purification of human liver TS PST

TS PST	Purification step	Specific activity (units/mg protein)	Recovery (%)	Purification (fold)
Peak I	100,000 *g* Supernatant	25.6	100	
	DEAE-Sepharose CL-6B	652	43.4	25.5
	Sephadex G-100 superfine	2050	18.9	80.1
Peak II	100,000 *g* Supernatant	22.4	100	
	DEAE-Sepharose CL-6B—Step I	307	11.3	13.7
	DEAE-Sepharose CL-6B—Step II	553	5.8	24.7
	Sephadex G-100 superfine	806	1.0	36.0

Peaks I and II refer to the two peaks of TS PST activity separated by ion exchange chromatography.

purification was conducted. After ion exchange chromatography, TS PST peak I lost 95% of its activity during storage at 4° for 48 hr. DTT, sucrose and glycerol at concentrations from 1 to 5.5 mM, 0.08 to 0.4 M, and 5 to 15%, respectively, reduced the instability to only a small extent. Ion exchange fractions did retain their activities during storage at −80° for at least 48 hr. After gel filtration chromatography, purified TS PST activity became even more unstable and lost approximately 24% of its activity daily, even when stored at −80°. Because of the enzyme's instability, additional purification beyond gel filtration chromatography was not attempted. However, addition of BSA at a final concentration of 0.75 mg/ml prevented instability of partially purified TS PST activity during storage at −80°. Therefore, the properties of TS PST peaks I and II were studied after purification by gel filtration chromatography using material that was stored at −80° with 0.75 mg/ml BSA.

Effect of pH on purified TS PST activity. The pH optimum for both peaks of partially purified TS PST activity assayed with 4 μM *p*-nitrophenol was approximately 6.6 in the presence of potassium phosphate buffer. When the assay was performed with 20 mM dopamine, the pH optimum was 7.15 in the presence of either Tris–HCl or potassium phosphate buffer.

Effect of substrate concentration on purified TS PST activity. The effects of *p*-nitrophenol, dopa-

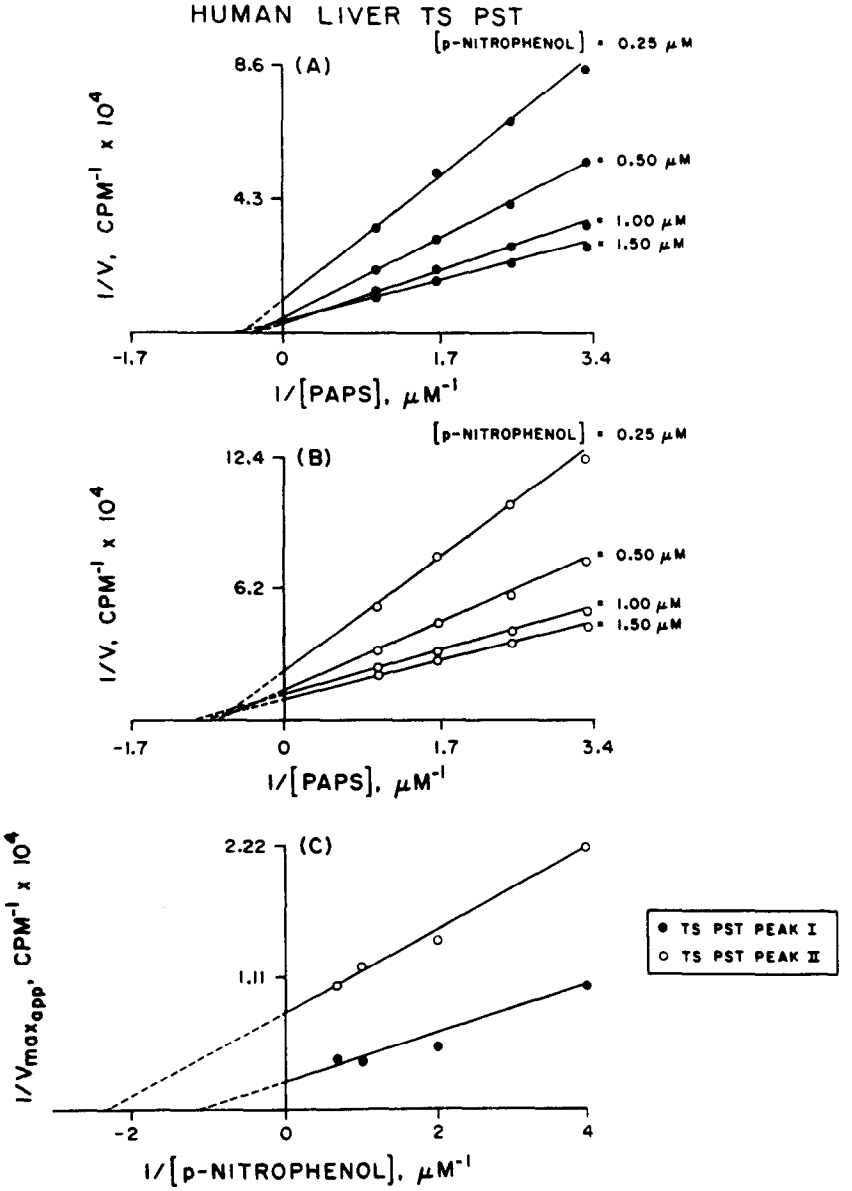

Fig. 6. Purified human liver TS PST substrate kinetics: Plots of 1/V vs 1/[PAPS] with various concentrations of *p*-nitrophenol for TS PST peak I (A) and peak II (B). Each point is the average of three determinations. (C) Plots of reciprocal apparent V_{max} values vs reciprocals of *p*-nitrophenol concentrations for TS PST peaks I and II.

Table 2. K_m Values for purified human liver TS PST

TS PST		K_m (μM)	
		Sulfate acceptor	PAPS
Peak I	*p*-Nitrophenol	0.91	0.86
	Dopamine	6,520	0.67
Peak II	*p*-Nitrophenol	0.43	0.64
	Dopamine	1,300	1.40

Peaks I and II refer to the two peaks of TS PST activity separated by ion exchange chromatography. Sulfate acceptor refers to either *p*-nitrophenol or dopamine. See text for details.

mine and PAPS concentrations on TS PST activities were determined for peaks I and II. These data were used to calculate "true" K_m values. Double-reciprocal plots of data obtained with different concentrations of *p*-nitrophenol in the presence of various concentrations of PAPS are shown in Fig. 6, panels A and B. Plots of reciprocals of *p*-nitrophenol concentrations versus reciprocals of apparent V_{max} values were also constructed for both peaks (Fig. 6C). K_m values were calculated from these plots and are listed in Table 2. Double-reciprocal plots of data obtained with different concentrations of dopamine in the presence of various concentrations of PAPS are shown in Fig. 7, panels A and B. Plots of recipro-

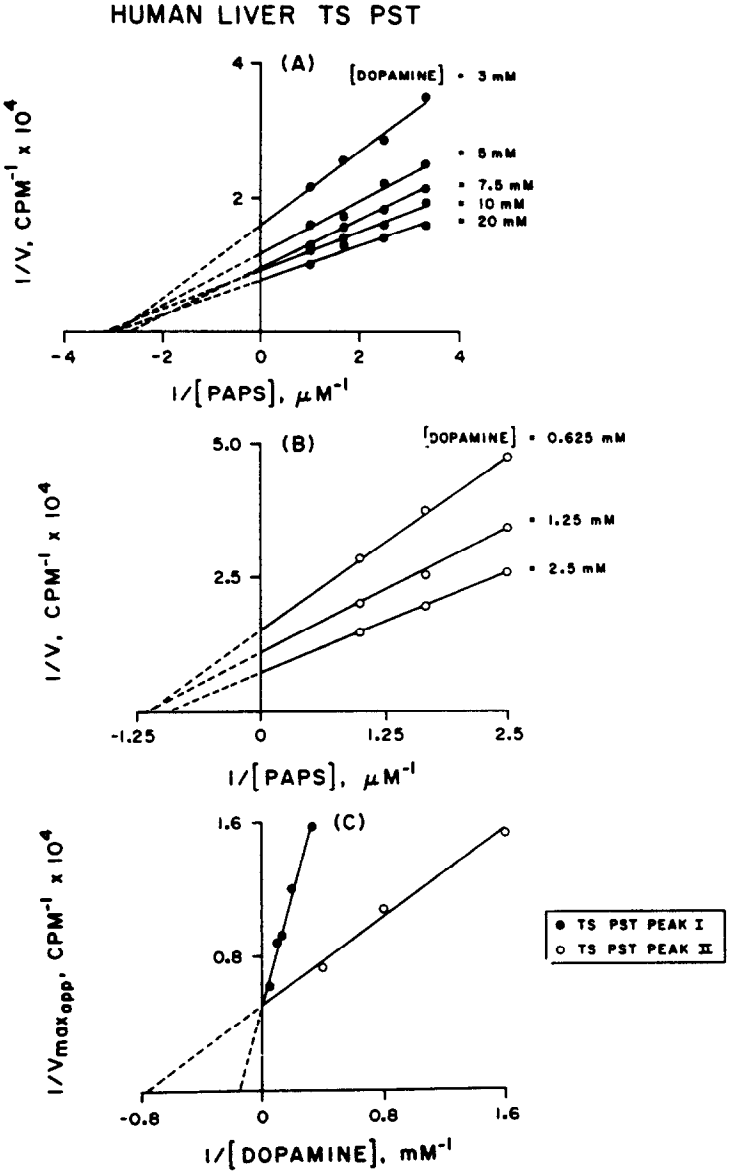

Fig. 7. Purified human liver TS PST substrate kinetics: Plots of 1/V vs 1/[PAPS] with various concentrations of dopamine for TS PST peak I (A) and peak II (B). Each point is the average of three determinations. (C) Plots of reciprocal apparent V_{max} values vs reciprocals of dopamine concentrations for TS PST peaks I and II.

cals of dopamine concentrations versus reciprocals of apparent V_{max} values were also constructed (Fig. 7C) and were used to calculate K_m values (Table 2). Finally, plots of the reciprocals of PAPS concentrations versus the reciprocals of apparent V_{max} values were constructed for data obtained with both *p*-nitrophenol and dopamine as substrates (plots not shown). Replots of these data were used to calculate K_m values for PAPS with both sulfate acceptor substrates (Table 2). When dopamine was used as a substrate, concentrations from 1 to 40,000 μM were tested with both peaks I and II. There was no dopamine sulfotransferase activity with either peak at 60 μM, the concentration of dopamine at which TL PST in liver homogenates showed maximal activity.

Effect of DCNP on purified TS PST activity. The effects of various concentrations of DCNP on TS PST activities in peaks I and II were determined in the presence of different concentrations of *p*-nitrophenol. Double-reciprocal plots of these data are shown in Fig. 8, panels A and B. DCNP inhibited PST activities in both peaks noncompetitively. Replots of DCNP concentrations versus the inverse of apparent V_{max} values were constructed for both peaks (Fig. 8C). These plots were used to calculate K_{ii} values of 2.5 and 1.5 μM for peaks I and II respectively. K_{is} values of 2.3 and 0.95 μM were also calculated for peaks I and II respectively (plots not shown).

Molecular weight of purified TS PST. The molecular weights of TS PST peaks I and II were esti-

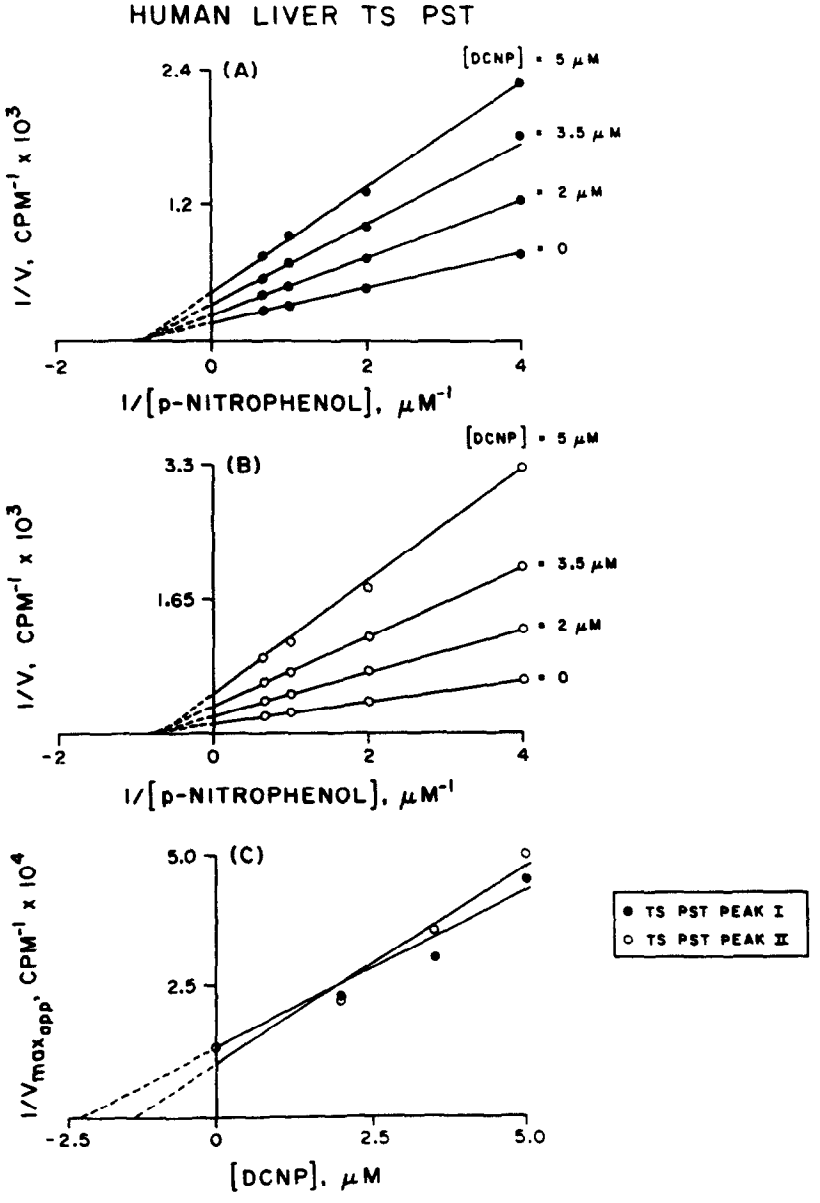

Fig. 8. Inhibition of purified human liver TS PST by DCNP. Plots of 1/V vs 1/[*p*-nitrophenol] with various concentrations of DCNP are shown for TS PST peaks I (A) and II (B). (C) Plots of reciprocal apparent V_{max} values vs concentrations of DCNP for TS PST peaks I and II.

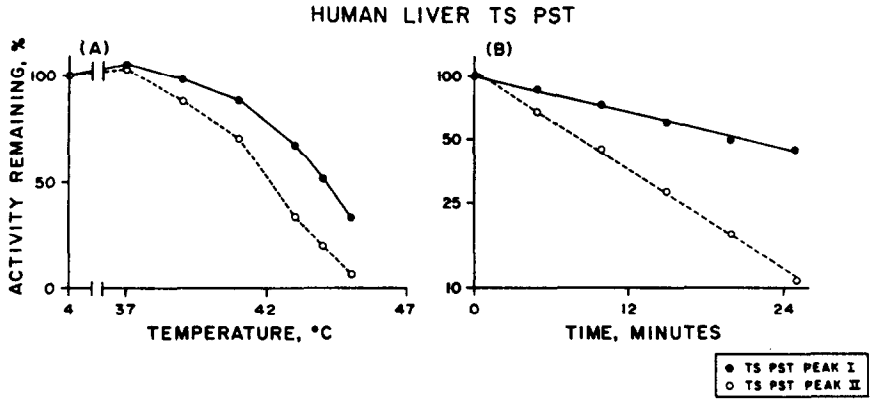

Fig. 9. Thermal inactivation of purified human TS PST. (A) Purified TS PST peaks I and II were preincubated for 15 min at various temperatures prior to assay. (B) Purified TS PST peaks I and II were preincubated at 43° for various times prior to assay. The data in (B) are plotted in a semilogarithmic fashion. PST activity was measured with 4 μM *p*-nitrophenol as a substrate.

mated by gel filtration chromatography using the method of Whitaker [23]. Molecular weight estimates were 65,000 and 60,000 daltons for peaks I and II respectively.

Thermal stability of purified TS PST. The thermal stabilities of TS PST in peaks I and II were determined with 4 μM *p*-nitrophenol as substrate (Fig. 9). The 50% inactivation temperatures after 15-min preincubations were 44° and 42° for peaks I and II respectively (Fig. 9A). Thermal stability was also determined after preincubation at 43° for various times (Fig. 9B). After 15 min at 43°, 66% of the peak I activity remained, whereas only 34% of the enzyme activity was retained in peak II (Fig. 9B). Slopes of semilogarithmic plots of thermal inactivation data for peaks I and II differed significantly ($P < 0.005$) and were -0.0145 ± 0.00057 and -0.0389 ± 0.00098 log % per min (mean ± SEM) respectively (Fig. 9B).

The thermal inactivation studies were extended to include supernatant fractions from individual liver samples that contained either peak I or II, i.e. samples A and B (Fig. 5). Supernatant fractions from livers A and B were heated at 43° for 15 min, and the proportion of enzyme activity remaining, a heated/control or "H/C" ratio [6, 7], was determined. The H/C ratio for liver sample A, the sample that contained only peak I, was 0.57, while that for sample B, the sample that contained only peak II, was 0.22. Finally, thermal inactivation studies were performed with peak I and II fractions isolated by ion exchange chromatography from liver samples A, B and C. The H/C ratio after preincubation at 43° for 15 min for peak I from sample A was 0.81, while that for peak II isolated from sample B was 0.36. The H/C ratio for peak I from sample C was 0.61, and the H/C ratio for peak II isolated from sample C was 0.41. These results supported the conclusion that TS PST peak II was more thermolabile than TS PST peak I.

Mixing experiments with purified TS PST. Differences in thermal stability may be properties of PST itself, or they may result from the presence of "labilizing" or "stabilizing" factors in the enzyme preparations. Mixing experiments were performed with partially purified PST in an attempt to choose between those two possibilities. Partially purified peak I and II preparations and mixtures of the two were either kept at 4° or were preincubated at 43° for 15 min prior to the measurement of TS PST activity with 4 μM *p*-nitrophenol as substrate. The results of mixing experiments performed with these partially purified peaks are shown in Table 3. H/C ratios in the mixtures were similar to the expected arithmetic means of H/C values in the two separate preparations. Therefore, differences in the thermal stabilities of peaks I and II were apparently properties of PST itself.

Table 3. Thermal inactivation mixing experiments performed with the two peaks of partially purified human liver TS PST

Peak I: Peak II ratio	Control activity (cpm)			Heated activity (cpm)			Heated/Control ratio		
	Expected	Observed	% of Expected	Expected	Observed	% of Expected	Expected	Observed	% of Expected
Peak I		5173			3515			0.679	
3:1	5276	5192	98.4	3149	2947	93.6	0.601	0.568	94.5
1:1	5378	5276	98.1	2783	2676	96.1	0.523	0.507	96.9
1:3	5481	5243	95.7	2417	2347	97.1	0.445	0.448	101
Peak II		5583			2051			0.367	

Control enzyme activities and heated/control ratios in mixtures of Peaks I and II are shown. Partially purified TS PST Peaks I and II were mixed in various proportions. The effects of preincubation at 43° for 15 min on activities in Peak I, Peak II, and mixtures of the two were determined. See text for details.

DISCUSSION

We have found that homogenates of human liver contain at least two forms of PST with properties similar to those of TS and TL PST in the platelet and brain [6–9]. Optimal conditions were determined for the measurement of TS and TL PST activities in human liver homogenates. It should now be possible to determine whether individual differences in levels of PST activity in an easily obtainable human tissue, the platelet, are correlated significantly with individual differences in PST activity in a less accessible tissue, the liver, as they are for TS PST activities in cerebral cortex [12]. That hypothesis is worthy of pursuit since it has already been shown that platelet PST activities are correlated significantly with the extent of sulfation of orally administered drugs such as acetaminophen and methyldopa [24, 25].

We also partially purified human liver TS PST by ion exchange and gel filtration chromatography. Three peaks of PST activity were separated by ion exchange chromatography of hepatic preparations. The first two peaks had properties similar to those of TS PST in supernatant fractions of liver homogenates. Both peaks of TS PST activity catalyzed the sulfate conjugation of millimolar concentrations of dopamine as well as that of micromolar concentrations of *p*-nitrophenol. When supernatant fractions from individual liver samples were subjected to ion exchange chromatography, they contained one, the other or both peaks of TS PST activity. The only characteristic in which human liver TS PST peaks I and II differed significantly, other than behavior during ion exchange chromatography, was thermal stability. The activity in peak II was more thermolabile than was that in peak I, although neither activity was as thermolabile as is TL PST. The fact that these two apparent "isoenzymes" of human liver TS PST differ in their thermal stabilities may be of importance. Familial variations in the thermal stability of TS PST are found in the human platelet [11], and these variations are correlated with individual differences in thermal stability of TS PST in cerebral cortical tissue [12]. Additional experiments will be required to study the relationship, if any, between the two peaks of TS PST activity in human liver and the familial trait of thermolabile TS PST in the platelet.

The presence of multiple forms of PST in human liver raises the question of their possible relationship to the multiple forms of PST that are present in rat liver. Based on their sequential elution from an ion exchange column, at least four "forms" of rat liver PST have been described [26–29]. Only three of these four forms have been well characterized. Forms "I" and "II" in rat liver have molecular weights of approximately 64,000 daltons and very similar substrate specificities [27, 29]. They catalyze the sulfate conjugation of many simple phenols, and, at higher pH values, of catechols. Rat liver PST form "IV" has a slightly lower apparent molecular weight than do forms I and II, and it may have a higher affinity for catechol substrates [27–29]. Rat liver PST form "III" is so rapidly inactivated after purification that it has not been well characterized [27]. It would be premature to speculate about the possible relationship between TS PST peaks I and II in human liver and forms I and II in rat liver. For example, K_m values of the two peaks of human liver TS PST for *p*-nitrophenol are approximately 3 orders of magnitude lower than are those of rat liver PST forms I and II [27, 29].

In summary, human liver homogenates, like other human tissues that have been studied carefully [4–8], contain at least two forms of PST. When human liver PST was partially purified, two peaks of TS PST activity could be separated by ion exchange chromatography. These two isozymes of TS PST also differed in their thermal stabilities. It should now be possible to test the hypothesis that individual variations in the properties and regulation of PST in an easily accessible human tissue, the platelet, may reflect individual differences in the properties and regulation of this important drug- and neurotransmitter-metabolizing enzyme in the human liver.

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REFERENCES

1. K. S. Dodgson, in *Drug Metabolism from Microbe to Man* (Eds. D. V. Parke and R. L. Smith), p. 91. Francis Ltd., London (1977).
2. A. B. Roy, in *Sulfation of Drugs and Related Compounds* (Ed. G. J. Mulder), p. 83. CRC Press, Boca Raton, FL (1981).
3. R. M. Weinshilboum, *Fedn Proc.* **45**, 2223 (1986).
4. G. Rein, V. Glover and M. Sandler, in *Phenolsulfotransferase in Mental Health Research* (Eds. M. Sandler and E. Usdin), p. 98. Macmillan, London (1981).
5. G. Rein, V. Glover and M. Sandler, *Biochem. Pharmac.* **31**, 1893 (1982).
6. C. Reiter, G. Mwaluko, J. Dunnette, J. Van Loon and R. Weinshilboum, *Naunyn-Schmiedeberg's Archs Pharmac.* **324**, 140 (1983).
7. C. Reiter and R. Weinshilboum, *J. Pharmac. exp. Ther.* **221**, 43 (1982).
8. W. F. Young Jr., H. Okazaki, E. R. Laws Jr. and R. Weinshilboum, *J. Neurochem.* **43**, 706 (1984).
9. G. Rein, V. Glover and M. Sandler, *J. Neurochem.* **42**, 80 (1984).
10. R. Sundaram and R. Weinshilboum, *Fedn Proc.* **44**, 1821 (1985).
11. J. Van Loon and R. M. Weinshilboum, *Biochem. Genet.* **22**, 997 (1984).
12. W. F. Young Jr., E. R. Laws, Jr., F. W. Sharbrough and R. M. Weinshilboum, *J. Neurochem.* **44**, 1131 (1985).
13. A. Foldes and J. L. Meek, *Biochim. biophys. Acta* **327**, 365 (1973).
14. R. J. Anderson and R. M. Weinshilboum, *Clinica chim. Acta* **103**, 79 (1980).
15. J. A. Fuentes and N. H. Neff. *Neuropharmacology* **14**, 819 (1975).
16. M. M. Bradford, *Analyt. Biochem.* **72**, 248 (1976).
17. G. N. Wilkinson, *Biochem. J.* **80**, 324 (1961).
18. W. W. Cleland, *Nature, Lond.* **198**, 463 (1963).
19. J. R. Florini and C. S. Vestling, *Biochim. biophys. Acta* **25**, 575 (1957).
20. M. Dixon, *Biochem. J.* **55**, 170 (1953).

21. G. Diamond and A. J. Quebbemann, *J. Chromat.* **177**, 368 (1979).
22. N. S. Sharpless, G. M. Tyce, L. J. Thal, J. M. Waltz, K. Tabaddor and L. I. Wolfson, *Brain Res.* **217**, 107 (1981).
23. J. R. Whitaker, *Analyt. Chem.* **35**, 1950 (1963).
24. C. Reiter and R. Weinshilboum, *Clin. Pharmac. Ther.* **32**, 612 (1982).
25. N. R. C. Campbell, R. S. Sundaram, P. J. Werness, J. Van Loon and R. M. Weinshilboum, *Clin. Pharmac. Ther.* **37**, 308 (1985).
26. R. D. Sekura and W. B. Jakoby, *J. biol. Chem.* **254**, 5658 (1979).
27. W. B. Jakoby, R. D. Sekura, E. S. Lyon, C. J. Marcus and J. L. Wang, in *Enzymatic Basis of Detoxication* (Ed. W. B. Jakoby), Vol. II, p. 199. Academic Press, New York (1980).
28. R. D. Sekura and W. B. Jakoby, *Archs Biochem. Biophys.* **211**, 352 (1981).
29. R. D. Sekura, M. W. Duffel and W. B. Jakoby, in *Methods in Enzymology* (Ed. W. B. Jakoby), Vol. 77, p. 197. Academic Press, New York (1981).