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Human Platelet Phenol Sulfotransferase: Familial Variation in Thermal Stability of the TS Form

Jon Van Loon[1] and Richard M. Weinshilboum[1]

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Phenol sulfotransferase (PST) catalyzes the sulfate conjugation of catechol and phenolic drugs and xenobiotic compounds. Platelets and other tissues contain at least two forms of PST, forms that have been designated the "TL" and the "TS" forms. We measured the thermal stability of platelet TS PST in blood samples from 218 randomly selected unrelated subjects by heating platelet homogenates at 44° C for 15 min. Thermal stability was expressed as the ratio of the enzyme activity remaining after preincubation to that in an unheated sample, a heated/control (H/C) ratio. The frequency distribution of H/C ratios for this population sample was bimodal, with a nadir at an H/C ratio of 0.33. Of the 218 subjects studied, 29 (13.3%) had thermolabile TS PST (H/C < 0.33). Platelet samples were then obtained from subjects with thermolabile and thermostable TS platelet PST. PST activity in these platelet samples had similar apparent Km *constants for substrates.* IC_{50} *values for inhibition of TS PST by 2,6-dichloro-4-nitrophenol in these samples were also nearly identical. The results of experiments in which platelet homogenates from subjects with thermolabile and thermostable TS PST were mixed and the results of experiments in which platelet homogenates were subjected to gel filtration chromatography were compatible with the conclusion that individual differences in TS PST thermal stability were properties of PST itself. Finally, there was a significant familial aggregation of the trait of thermolabile TS PST when H/C ratios were measured in platelet homogenates from 231 members of 49 randomly selected families.*

KEY WORDS: phenol sulfotransferase; platelet enzymes; enzyme thermal stability.

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[1] Clinical Pharmacology Unit, Department of Pharmacology, Mayo Clinic/Mayo Foundation, Rochester, Minnesota 55905.

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INTRODUCTION

Phenol sulfotransferase (EC 2.8.2.1; PST) catalyzes the sulfate conjugation of catecholamines, catechol drugs, and phenolic drugs (Roy, 1981). PST is present in many human tissues including the blood platelet (Hart *et al.*, 1979; Anderson and Weinshilboum, 1980; Anderson *et al.*, 1981). Human platelets contain at least two independently regulated forms of PST, forms that differ in their physical properties, in their substrate specificities, and in their sensitivity to inhibitors (Rein *et al.*, 1981, 1982; Bonham-Carter *et al.*, 1981; Reiter and Weinshilboum, 1982a; Reiter *et al.*, 1983). The two forms of platelet PST have been separated by ion-exchange chromatography (Reiter *et al.*, 1983). One form is relatively thermolabile and catalyzes the sulfate conjugation of dopamine and of other monoamines. This form has been referred to as the "TL" (thermolabile) or "M" (monoamine metabolizing) form of PST (Rein *et al.*, 1981; Reiter and Weinshilboum, 1982a). The other form of platelet PST is relatively thermostable and catalyzes the sulfate conjugation of micromolar concentrations of phenol and *p*-nitrophenol. It has been referred to as either the "TS" (thermostable) or the "P" (phenol metabolizing) form (Rein *et al.*, 1981; Reiter and Weinshilboum, 1982a; Reiter *et al.*, 1983). At millimolar concentrations, phenol and *p*-nitrophenol are also substrates for the TL form of the enzyme (Reiter and Weinshilboum, 1982a; Reiter *et al.*, 1983; Bonham-Carter *et al.*, 1983).

PST activity in humans has been investigated most extensively in the platelet. Platelet PST has been studied largely because of the possibility that its biochemical properties and regulation might reflect those of the enzyme in other tissues more directly involved in drug and neurotransmitter metabolism (Anderson *et al.*, 1981). Some data have appeared in support of that hypothesis. For example, the relative level of platelet TS PST activity correlates significantly with the relative level of TS PST activity in human cerebral cortex obtained at the time of clinically indicated neurosurgery (Young *et al.*, 1984). In addition, variations in platelet PST activity are correlated significantly with individual differences in the sulfate conjugation of orally administered drugs such as acetaminophen—an observation which suggests that regulation of the platelet activity parallels regulation of PST activity at sites of drug metabolism (Reiter and Weinshilboum, 1982b).

The experiments described subsequently were performed to study the biological basis for individual variations in the activity of the TS form of PST in humans and as a first step toward biochemical genetic studies of PST. Thermal stability is a sensitive indicator of differences in protein structure (Langridge, 1968; Weinshilboum, 1981). Thermal stability experiments are widely used in biochemical genetic studies and have proven useful in studying the biochemical basis for variations in the regulation of other drug metabolizing enzymes in humans (Weinshilboum, 1981). Our experiments were

designed to determine whether there were differences among individuals in the thermal stability of the TS form of human platelet PST and, if so, to determine whether this trait showed familial aggregation. Wide individual variations in the thermal stability of TS PST were found, variations that appeared to be a property of the enzyme itself. These variations showed a strong familial aggregation.

MATERIALS AND METHODS

Subjects. Subjects for the study of platelet PST activity in a large population sample were 218 randomly selected unrelated adult blood donors at the Mayo Clinic in Rochester, Minnesota. The average age of these 119 male and 99 female subjects was 35.8 ± 0.7 years (mean ± SE). Blood samples for more extensive biochemical characterization of the thermal stability of platelet TS PST were obtained from three male volunteers. Family studies were performed with blood samples from 231 members of 49 randomly selected families. The manner in which the families were recruited has been described elsewhere (Keith *et al.*, 1983). Blood was obtained from only one parent in two of the families. The average age of the 96 parents studied was 40.8 ± 0.4 years, and the average age of the 135 offspring was 14.9 ± 0.5 years. The number of children per family varied from 1 to 7, with an average of 2.8 ± 0.1. All subjects were white and none was either acutely or chronically ill. These experiments were performed under guidelines approved by the Mayo Clinic Human Studies Committee.

Platelet Isolation. Blood samples were obtained in 7-ml Vacutainer tubes that contained 10.5 mg disodium EDTA. Platelets were isolated within 2 hr of the time that the blood samples were obtained. The blood was kept at room temperature prior to processing and was centrifuged at 200*g* for 10 min at room temperature to obtain platelet-rich plasma. Platelet number in the platelet-rich plasma was measured with a Coulter model ZBI particle counter. One milliliter of the platelet-rich plasma was then centrifuged at 16,000*g* for 10 min at 4°C. The supernatant plasma was aspirated, and 2 ml of 5 mM potassium phosphate buffer, *p*H 7.5, that contained 2.5 mM EDTA, 10 mM dithiothreitol (DTT), and 0.5 mg/ml bovine serum albumin (BSA) was added to the pellet. The platelets were homogenized with a Polytron tissue homogenizer, and homogenates were stored at −20°C. Freezing of the homogenates had no effect on the thermal stability characteristics of platelet PST.

Thermal Stability Experiments. PST thermal stability was measured as described by Reiter and Weinshilboum (1982a). Specifically, frozen platelet homogenates were thawed and were diluted 1:1 (v/v) with 5 mM potassium phosphate buffer, *p*H 7.5, that contained 10 mM DTT and 0.0625 mg/ml

BSA. Separate aliquots of the diluted homogenate were preincubated for 15 min in a shaker water bath at several different temperatures. In some studies the samples were preincubated for varying periods of time at 44°C. Aliquots were always kept at 4°C as "controls." All samples were placed on ice immediately after the preincubation step and prior to the measurement of PST activity.

PST Assay. TS PST activity was measured by the method of Foldes and Meek (1973) as modified by Anderson and Weinshilboum (1980) and by Reiter *et al.* (1983). Frozen platelet homogenates were thawed and were diluted by adding 1 vol of homogenate to 160 vol of 5 mM potassium phosphate buffer, *p*H 7.5, that contained 10 mM DTT and 0.0625 mg/ml BSA. After the preincubation step for the thermal stability experiments (see Thermal Stability Experiments), the already twofold diluted homogenate was diluted further by adding 1 vol to 80 vol of buffer. One hundred microliters of the final 160-fold diluted homogenate was incubated for 30 min at 37°C with 0.4 μM [^{35}S]3′-phosphoadenosine-5′-phosphosulfate ([^{35}S]PAPS) and with 4 μM *p*-nitrophenol. *p*-Nitrophenol is the preferred substrate for the measurement of TS PST activity in tissue homogenates (Reiter *et al.*, 1983). The enzyme reaction was terminated by the precipitation of PAPS and protein with barium acetate, barium hydroxide, and zinc sulfate as described in detail elsewhere (Anderson and Weinshilboum, 1980; Reiter and Weinshilboum, 1982a; Reiter *et al.*, 1983). After centrifugation of the samples, the supernatant containing the radioactive reaction product was aspirated, and its radioactivity was measured in a liquid scintillation counter. One unit of PST activity represented the formation of 1 nmol of product per hr of incubation. The results were expressed per 10^8 platelets since that method has been shown to result in less variation than expression of the activity per milligram platelet protein (Anderson *et al.*, 1981).

Gel Filtration Chromatography. Gel filtration chromatography of platelet homogenates was performed at 4°C with a 0.9 × 30.0-cm column of Sephadex G-100. The eluting buffer was 50 mM Tris–HCl, *p*H 7.5, that contained 10 mM DTT, 2.5 mM EDTA, and 0.5 mg/ml BSA. The flow rate was approximately 3 ml/hr. For these experiments, a platelet pellet was obtained from 8 ml of platelet-rich plasma, and the pellet was homogenized in 2 ml of buffer. After centrifugation at 100,000*g* for 1 hr at 4°C, the supernatant was passed through a 5-μm SMWP Millipore filter. One milliliter of the filtered supernatant was applied to the column, and 1.0-ml fractions were collected for measurement of PST activity. Protein concentrations in the fractions were estimated by measurement of absorbance at 280 nm.

Analysis of Data. Differences between group means were tested for statistical significance by Student's *t* test. Significant deviations of group

frequencies from expected values were tested by chi-square analysis with the Yates correction for continuity (Sokal and Rohlf, 1981).

Data from the population study were analyzed by performing a probit analysis. Probits were calculated by the use of the following equation (Finney, 1971):

$$y = 5 + \frac{(x - \mu)}{\sigma}.$$

In this equation, μ is the mean, σ is the standard deviation, and x is the value of each data point.

Apparent Michaelis–Menten (K_m) constants were calculated by the method of Wilkinson (1961) using a computer program written by Cleland (1963). A Hewlett–Packard 9845B computer was used to perform all calculations.

Materials. [^{35}S]PAPS (2.0–4.5 Ci/mmol) was purchased from New England Nuclear Corporation, Boston, Mass. Dithiothreitol (Cleland's reagent), *p*-nitrophenol, and BSA were obtained from Sigma Chemical Company, St. Louis, Mo. EDTA was purchased from Fisher Chemical Company, Fairlawn, N.J. 2,6-Dichloro-4-nitrophenol (DCNP) was obtained from ICN Pharmaceuticals, Inc., Plainview, N.Y. Sephadex G-100 was purchased from Pharmacia Fine Chemicals, Piscataway, N.J.

RESULTS

Preliminary Study of PST Thermal Stability. A preliminary experiment was performed to determine whether there might be large individual variations in the thermal stability of the TS form of human platelet PST. Samples from six randomly selected subjects were preincubated for 15 min at a variety of temperatures prior to measurement of TS PST activity. A wide range of thermal stabilities was found. Data from two samples with very different thermal inactivation curves are shown in Fig. 1. There was an approximately 2°C difference in the temperatures at which PST activity in these samples was reduced by 50%. However, PST activity in both of these samples was much more thermostable than is the TL form of PST, the form that catalyzes the sulfate conjugation of dopamine at micromolar concentrations. The TL form of platelet PST is inactivated 50% after 15 min of preincubation at 39.5°C (Reiter and Weinshilboum, 1982a).

These preliminary results indicated that there might be large individual variations in platelet TS PST thermal stability. Although complete thermal inactivation curves such as those shown in Fig. 1 are very informative, full thermal inactivation curves are impractical for use in studies of large

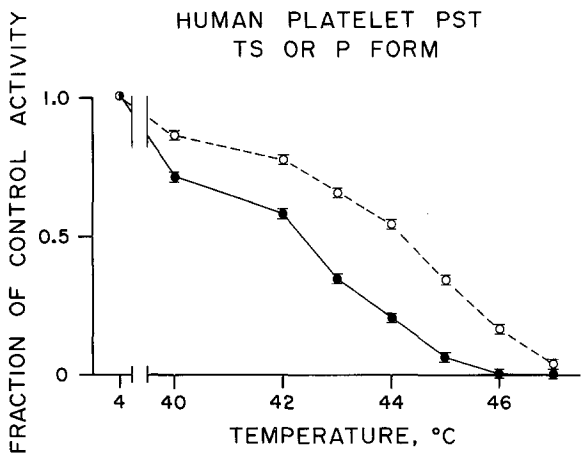

Fig. 1. Thermal inactivation of the TS form of PST activity in platelet samples from two individual subjects. Each value is the mean ± SE of three determinations.

population samples. One approach that has proven useful for the study of enzyme thermal stability in large samples is the measurement of the proportion of enzyme activity remaining after preincubation at a single temperature and time, a so-called heated/control, or H/C, ratio (Weinshilboum, 1981). Information obtained by measurement of H/C ratios may be confirmed by more extensive experiments performed with a few samples chosen for study on the basis of the results of a population experiment. As the next step in the study of platelet TS PST thermal stability, H/C ratios were measured in a large number of samples after preincubation of platelet homogenates for 15 min at 44°C, a temperature chosen on the basis of the data shown in Fig. 1.

TS PST Thermal Stability in a Large Population Sample. Platelet TS PST H/C ratios were measured in blood samples from 218 randomly selected unrelated blood donors. The relationship between basal levels of enzyme activity and H/C ratios in these samples is shown graphically in Fig. 2. Subjects with lower H/C ratios were found entirely among those individuals with lower levels of basal enzyme activity, but many subjects with equally low levels of enzyme activity had relatively high H/C ratios (Fig. 2). Basal enzyme activity levels and H/C ratios were very similar in male and female subjects. Average levels of TS PST activity were 0.58 ± 0.03 and 0.57 ± 0.03 $U/10^8$ platelets (mean ± SE) for male and female subjects, respectively. The lack of a sex difference in basal levels of enzyme activity confirms the results of earlier reports (Reiter and Weinshilboum, 1982a, b; Anderson and Jackson, 1984), reports which also demonstrated that TS PST activity remains constant over time in any given subject (Anderson and Jackson, 1984).

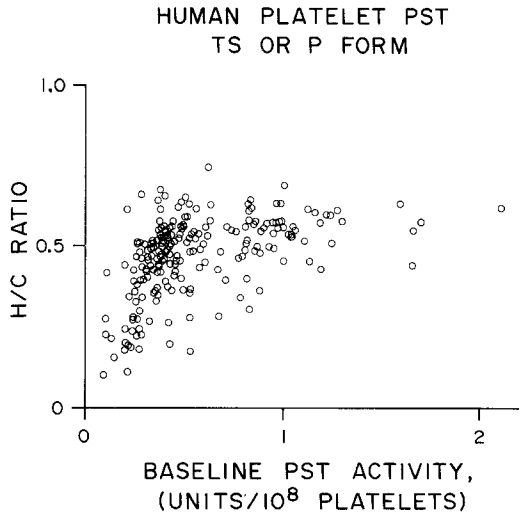

Fig. 2. Relationship between baseline TS PST activity and H/C ratios in platelet samples from 218 randomly selected unrelated subjects. Each value is the mean of three determinations.

Average H/C ratios for the two sexes in the randomly selected population sample were 0.46 ± 0.01 and 0.49 ± 0.01, respectively.

The frequency distribution histogram of H/C ratios in these samples was nongaussian and included an apparent subgroup of subjects with low H/C ratios (Fig. 3A). This conclusion was supported by the results of a probit analysis (Fig. 3B). The probit analysis was compatible with the presence of at least two subgroups in the population. Separate probit curves for male and female subjects were virtually identical. On the basis of the probit plot, an H/C ratio of 0.33 was chosen as the antimode of the distribution. This value served as a useful "cutoff" to separate subjects with "thermolabile" from those with "thermostable" TS PST (see arrows in Fig. 3). Classification was performed with the full knowledge that, if the population did include two subgroups, some individuals would be misclassified in both directions by the use of such a cutoff value. Of the 218 subjects studied, 13.3% (29/218) had thermolabile PST, i.e., they had TS PST H/C ratios of less than 0.33. The average basal enzyme activity in the platelets of individuals with thermolabile enzyme was 0.43 ± 0.05. This value was significantly lower than the average basal activity in the platelets of individuals with thermostable TS PST, 0.61 ± 0.02 ($P < 0.005$).

Properties of Thermolabile and Thermostable TS PST. It was important to study the properties of PST in platelets from subjects with both

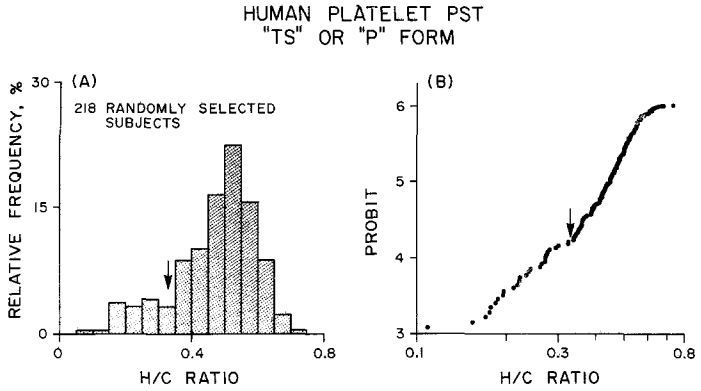

Fig. 3. (A) Frequency distribution of H/C ratios for TS PST in platelet samples from 218 randomly selected unrelated subjects. Each value is the mean of three determinations. (B) Probits of data shown in A are plotted against H/C ratios on a log scale. Arrows indicate the inflection point in the probit plot and the antimode of the frequency distribution histogram determined from the probit plot.

thermostable and thermolabile enzyme. Therefore, three subjects whose platelets had different combinations of PST thermal stabilities and basal activities were chosen for more detailed studies. These included the following: subject 1, thermostable TS PST with high basal activity (H/C = 0.75, 1.67 $U/10^8$ platelets); subject 2, thermostable TS PST with low basal activity (HC = 0.60, 0.21 $U/10^8$ platelets); and subject 3, thermolabile TS PST with low basal activity (H/C = 0.05, 0.19 $U/10^8$ platelets). These samples were chosen not only to represent extreme values of TS PST thermal stability, but also to include, as an additional control, a sample with low basal activity but with thermostable enzyme.

Thermal stability was the first property studied in these three samples. Differences in H/C ratios among the samples were confirmed in two separate experiments. In the first experiment aliquots of each sample were preincubated for 15 min at various temperatures. There was a greater than 2°C difference in the temperature that resulted in inactivation of 50% of the enzyme activity in the samples with thermolabile and thermostable enzyme (Fig. 4A). When aliquots of the same three platelet homogenates were incubated at 44°C for varying times, PST activity in the homogenate from subject 3 was inactivated 50% after only 8 min, while activity in homogenates from the other two subjects was inactivated 50% after 25 min (Fig. 4B). Semilogarithmic plots of the latter data showed no evidence of biphasic inactivation. Both of these findings confirmed the results obtained by measurements of H/C ratios.

Samples from the same three subjects were studied to determine whether

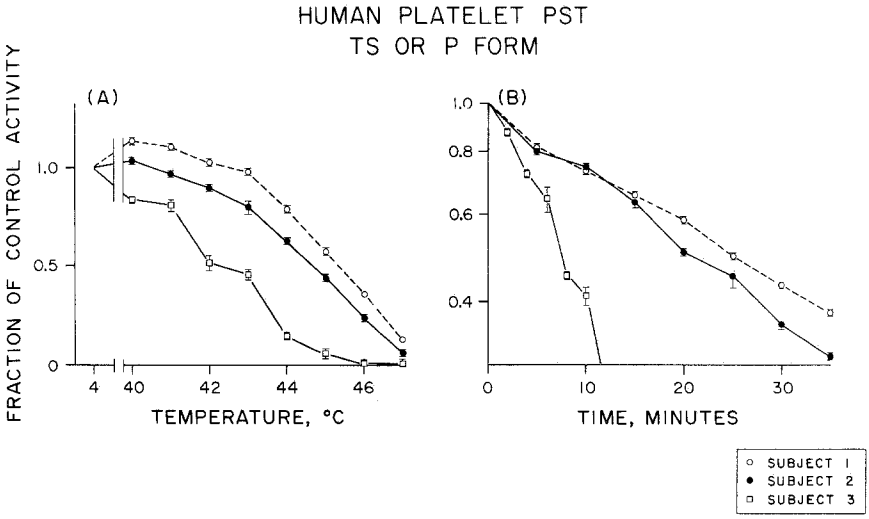

Fig. 4. Thermal stability of platelet TS PST from three subjects with different H/C ratios and different basal levels of activity. Levels of activity and H/C ratios for each subject are listed in the text. (A) Platelet homogenates were preincubated for 15 min at various temperatures. Each value is the mean ± SE of three determinations. (B) Platelet homogenates were preincubated at 44°C for increasing times. Each value is the mean ± SE of three determinations.

they might differ in properties other than thermal stability. Apparent K_m constants were determined for *p*-nitrophenol and PAPS, the two cosubstrates for the reaction. TS PST activity was measured in the presence of varying concentrations of either *p*-nitrophenol or PAPS and double inverse plots of the data were constructed (Fig. 5). These data were used to calculate apparent K_m constants. In the case of *p*-nitrophenol, substrate concentrations of greater than 0.4 μM were not used in the calculations because of substrate inhibition. Apparent K_m constants were very similar for both substrates in all three samples (Table I).

TS PST is inhibited by 2,6-dichloro-4-nitrophenol (DCNP) (Rein *et al.*, 1982; Reiter *et al.*, 1983). Therefore, IC_{50} values for inhibition of TS PST by DCNP were determined in these three platelet samples by measuring enzyme activity in the presence of various concentrations of DCNP (Fig. 6). IC_{50} values for DCNP inhibition in all of the samples were approximately 0.4 μM—a value identical to that found in previous experiments performed with pooled platelet homogenates (Reiter *et al.*, 1983).

Gel Filtration Chromatography. Individual differences in thermal stability might result either from variations in the properties of PST itself or from variations in the properties of other constituents of the platelet homogenates. In an attempt to choose between these two possibilities, homogenates from the

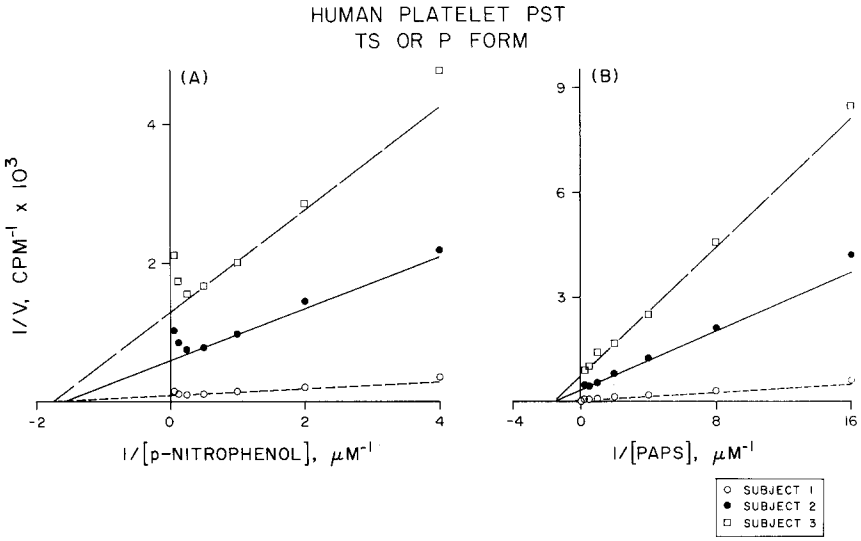

Fig. 5. Double inverse plots of the effects of various concentrations of *p*-nitrophenol (A) and PAPS (B) on TS PST activity in platelet homogenates from three subjects with different H/C ratios and different basal levels of activity. Each value is the mean of three determinations.

same three subjects were partially purified by gel filtration chromatography performed with Sephadex G-100. The purpose of this experiment was to remove homogenate constituents with molecular weights greater than and less than that of PST itself. However, the first time that platelet homogenates were applied to the gel filtration column, no detectable PST activity was recovered in any fraction. In an attempt to prevent loss of activity during chromatography, 0.5 mg/ml BSA was added to the elution buffer. In the presence of 0.5 mg/ml BSA, 42–57% of the enzyme activity applied to the column could be recovered. Elution patterns for PST activity and for non-BSA protein were similar for all samples. The elution pattern for the homogenate

Table I. Apparent K_m Constants for Platelet Homogenates from Three Subjects with Different TS PST Thermal Stabilities and Different Basal Levels of Enzyme Activity[a]

Subject	Apparent K_m (μM)	
	p-Nitrophenol	PAPS
1	0.60 ± 0.06	0.77 ± 0.02
2	0.62 ± 0.08	0.64 ± 0.04
3	0.57 ± 0.06	0.64 ± 0.09

[a]All values are means ± SE. See text for details.

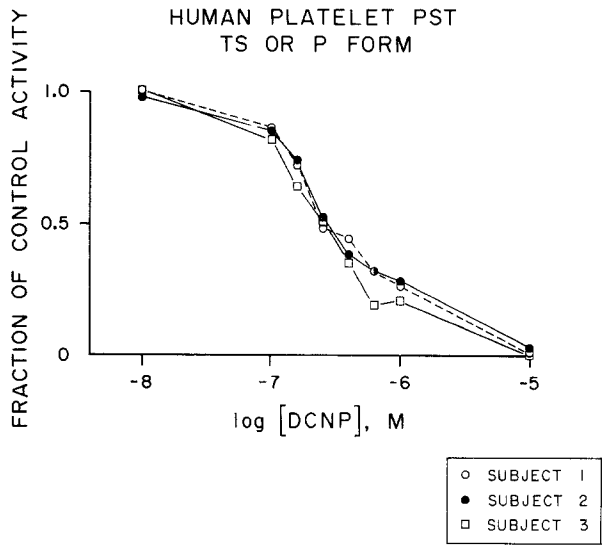

Fig. 6. Effect of various concentrations of 2,6-dichloro-4-nitrophenol (DCNP) on TS PST activities in platelet homogenates from three subjects with different H/C ratios and different basal levels of activity. Each value is the mean of three determinations.

from subject 1 is shown in Fig 7. Fractions 9, 10, and 11 were pooled and used in subsequent thermal stability experiments. It should be emphasized that these experiments were not designed to yield purified PST but only to remove higher and lower molecular weight homogenate constituents. When platelet PST preparations that had been subjected to gel filtration chromatography were studied by thermal inactivation, the rank order of thermal stabilities for the three samples was unchanged (Fig. 8). The sample from subject 1 was still the most thermostable, with 58% of basal activity remaining after 15 min of preincubation at 44°C—treatment that resulted in retention of less than 1% of the activity in the sample from subject 3 (Fig. 8). It is of interest that after gel filtration chromatography, the thermal inactivation curve for subject 2 was clearly intermediate to that for subjects 1 and 3. These results were consistent with the conclusion that individual differences in the thermal stability of human platelet TS PST were properties of the enzyme itself.

Mixing Experiments. Another way in which to determine whether variations in TS PST thermal stability represented a property of the enzyme itself was to measure thermal stability in mixtures of homogenates from subjects with thermostable and thermolabile enzyme. Such experiments might provide evidence for the existence of endogenous PST "stabilizing" or "labilizing" factors. Therefore, samples of platelet homogenates from three

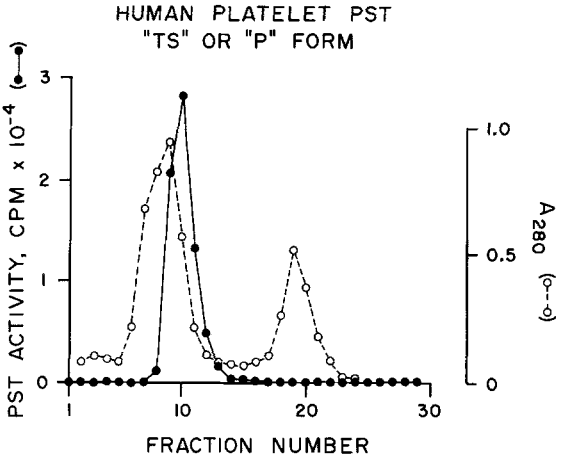

Fig. 7. Gel filtration chromatography of a platelet homogenate from subject 1. TS PST activity and absorbance at 280 nm are shown for each fraction.

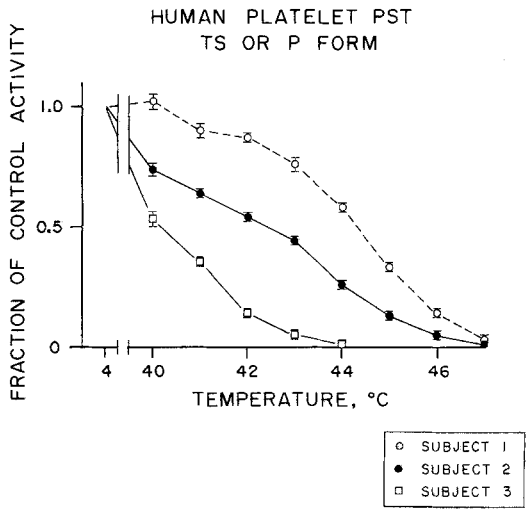

Fig. 8. Thermal inactivation of TS PST after partial purification by gel filtration chromatography. Platelet homogenates from three subjects with different H/C ratios and different basal levels of activity were heated at various temperatures for 15 min. Each value is the mean ± SE of three determinations.

Table II. Basal PST Activities, Activities After Heating, and H/C Ratios for Platelet Samples That Were Used to Perform "Mixing" Experiments[a]

Sample	TS PST activity			
	Basal ($U/10^8$ platelets)	Basal (cpm)	Heated (cpm)	H/C ratio
S1	0.34	1048	487	0.46
S2	0.30	1085	667	0.61
S3	0.27	896	426	0.48
L1	0.26	650	136	0.21
L2	0.35	727	206	0.28
L3	0.26	633	183	0.29

[a] Activities are expressed both as units per 10^8 platelets and as counts per minute (cpm). These samples were chosen from aliquots stored during the survey of H/C ratios in platelet homogenates from 218 randomly selected unrelated subjects (see Figs. 2 and 3). Care was taken to choose samples with similar levels of basal activity. Neither basal activities nor H/C ratios were changed significantly after storage of the samples at −20°C for 45 days. Each value is the mean of three determinations.

subjects with thermostable TS PST, H/C ratios > 0.45, and from three subjects with thermolabile TS PST, H/C < 0.30, were studied. The samples were obtained during the survey of H/C ratios in platelets obtained from 218 blood donors (Figs. 2 and 3). Basal PST activities and H/C ratios for platelet homogenates from these six subjects are listed in Table II. One to one (v:v) mixtures were made of samples from each subject with thermolabile and each subject with thermostable enzyme. Mixing was performed without preincubation and both before and after preincubation of the homogenates at 44°C for 15 min. When TS PST activity was measured in aliquots of each mixture, all final activities were very similar to the anticipated arithmetic mean values (Table III). On average, H/C ratios of the mixtures were slightly greater if mixing was performed after rather than before thermal pretreatment. Although this effect was statistically significant, it was quantitatively small and could account for only a minor part of the differences in H/C ratios between thermolabile and thermostable samples. These results also failed to indicate the existence of PST labilizing or stabilizing factors and supported the conclusion that individual variations in platelet TS PST thermal stability were properties of the enzyme itself.

Family Studies. Thermal stability of TS PST was measured in platelet samples from 231 first-degree relatives in 49 randomly selected families to determine whether thermolabile TS PST might be a familial trait. There were no significant differences between parents and children either in average basal

Table III. Mixing Experiments[a]

Mixture	Unheated activity			Thermal pretreatment				
				Mixed after pretreatment			Mixed before pretreatment	
	Expected	Observed	% of expected	Expected	Observed	% of expected	Observed	% of expected
S1 + L1	849	832	98	311	326	105	306	98
S1 + L2	887	927	105	346	338	98	308	89
S1 + L3	840	826	98	334	334	100	304	91
S2 + L1	867	858	99	401	360	90	376	94
S2 + L2	905	846	93	436	448	103	410	94
S2 + L3	858	849	99	424	447	105	406	96
S3 + L1	773	734	95	281	321	114	297	106
S3 + L2	811	757	93	316	310	98	284	90
S3 + L3	764	723	95	304	319	105	299	98

[a]Each mixture consisted of equal volumes of a platelet homogenate with thermolabile PST and a homogenate with thermostable PST. Characteristics of the individual homogenates are listed in Table II. Mixing was performed without heating and before and after heating at 44°C for 15 min. All values other than percentages are expressed as cpm and are means of three determinations.

Table IV. Family Studies of Platelet TS PST Thermal Stability[a]

		Number of subjects with thermolabile TS PST	
	N	Expected	Observed
All family members	231	31	32
All parents	96	13	15
All children	135	18	17
Children of parents with thermolabile PST	35	5	11*
Siblings of children with thermolabile PST	21	3	7**

[a] Data are shown for 231 first-degree relatives in 49 randomly selected families. The asterisk indicates that the observed frequency is significantly different from the expected frequency calculated on the basis of data from a randomly selected sample of unrelated adult subjects.
*$\chi^2_{[1]} = 7.06$, $P < 0.01$.
**$\chi^2_{[1]} = 4.76$, $P < 0.05$.

levels of enzyme activity or in average H/C ratios. Average basal TS PST activities were 0.58 ± 0.04 and 0.55 ± 0.03 U/10^8 platelets, and average H/C ratios were 0.46 ± 0.01 and 0.47 ± 0.01 for the 96 parents and the 135 offspring, respectively. Of the 231 family members studied, 32 (13.8%) had thermolabile TS PST (H/C < 0.33). This value was almost identical to the 13.3% found in the study of randomly selected unrelated subjects. Of the 96 parents studied, 15 (15.6%) had thermolabile platelet TS PST. In two families, a sample was available from only one parent. In no family did both parents share the trait of thermolabile enzyme. There were 35 offspring in the 15 families with parents who had thermolabile enzyme. Eleven (31.4%) of these children had thermolabile TS PST, a value significantly greater than the 13.3% anticipated among these 35 children ($\chi^2_{[1]} = 7.06$, $P < 0.01$). There were nine families in which at least one child had thermolabile TS PST. Seven of the 21 siblings (33%) of the nine "index" children also had thermolabile enzyme. This value also differed significantly from the 13.3% expected ($\chi^2_{[1]} = 4.76$, $P < 0.05$). Data from these 49 families are summarized in Table IV. All of these results were compatible with the conclusion that there was a high degree of familial aggregation of the trait of thermolabile platelet TS PST.

DISCUSSION

Sulfate conjugation catalyzed by PST plays an important role in the metabolism of many phenolic drugs and xenobiotic compounds (Roy, 1981). The platelet is an easily accessible human tissue that contains PST (Hart *et al.*,

1979; Anderson and Weinshilboum, 1980; Anderson *et al.*, 1981). The level of platelet TS PST activity is significantly correlated with the level of TS PST activity in the human cerebral cortex (Young *et al.*, 1984) and apparently reflects the level of enzyme activity in tissues involved in the metabolism of orally administered drugs such as acetaminophen (Reiter *et al.*, 1982b). Therefore, study of platelet PST activity may help us to understand the biological basis for individual variation in the regulation of this important drug metabolizing enzyme activity in humans.

Our experiments were performed to determine whether there might be large individual differences in one physical property of platelet TS PST, thermal stability. Thermal stability can be a sensitive indicator of structural differences among proteins (Langridge, 1968; Weinshilboum, 1981), and studies of thermal stability have enhanced our understanding of the biochemical basis for individual variations in the regulation of many enzymes.

There were large individual variations in the thermal stability of the TS or P form of PST in the human platelet. The results of mixing experiments and experiments in which the enzyme was partially purified by gel filtration chromatography indicated that these variations were properties of PST itself and were not due to differences in the properties of other constituents of the platelet homogenates. Definite proof of this hypothesis must await comparison of the properties of highly purified thermolabile and thermostable TS PST. Thermolabile and thermostable TS PST did not differ in their apparent K_m constants for the two cosubstrates for the enzyme reaction or in their sensitivity to inhibition by DCNP. Only subjects with lower basal levels of platelet PST had thermolabile enzyme. However, the majority of subjects with low basal levels of enzyme activity had thermostable TS PST. Finally, family studies demonstrated a significant familial aggregation of the trait of thermolabile platelet TS PST. Whether this familial aggregation resulted from the effects of inheritance or of shared environment must await the results of additional family studies. The family data presently available are insufficient for the performance of definitive segregation analysis. The experiments described here obviously represent only a first step in the study of possible genetic regulation of PST in humans.

The discovery of thermolabile and thermostable "subtypes" of a form of PST that has itself been named the TS (thermostable) form emphasizes the inadequacy of the existing nomenclature for PST in humans. The alternative system of nomenclature in which the two forms are named P, or phenol metabolizing, and M, or monoamine metabolizing, types is also inadequate. All simple phenols that have been studied carefully are substrates for both forms of platelet PST (Reiter and Weinshilboum, 1982a; Reiter *et al.*, 1983). In the case of compounds such as acetaminophen, K_m constants of the two forms for the compound are approximately equal (Reiter and Weinshilboum,

1982a; Reiter *et al.,* 1983), while K_m constants of the two forms for *p*-nitrophenol differ by three orders of magnitude (Reiter *et al.,* 1983). Conversely, it has recently been reported that dopamine, a monoamine, is also able to serve as a substrate for both forms of PST in humans (Campbell and Weinshilboum, 1984)—although K_m constants of the two forms for dopamine differ by three orders of magnitude. Obviously, neither the M and P nor the TS and TL nomenclature is optimal.

Finally, the results of recent studies show not only that basal levels of TS PST activity in the platelet correlate significantly with levels of the enzyme activity in human cerebral cortex, but also that TS PST thermal stability is correlated in these two tissues (Young *et al.,* 1984). Our observation of significant familial variations in the thermal stability of platelet TS PST represents a step toward understanding biochemical mechanisms responsible for regulating individual variations in the activity of this important drug metabolizing activity in humans.

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