0026-895X/88/020194-06$02.00/0

MOLECULAR PHARMACOLOGY, 34:194-199

Physical Characterization of a Monoamine-Sulfating Form of Phenol Sulfotransferase from Human Platelets

JEFFREY A. HEROUX and JEROME A. ROTH

Department of Pharmacology and Therapeutics, State University of New York at Buffalo, School of Medicine and Biomedical Sciences, Buffalo, New York 14214

Received April 15, 1988; Accepted May 15, 1988

SUMMARY

The purification to homogeneity and physical characterization of a monoamine-sulfating form of phenol sulfotransferase (PST) from human platelets is described. DEAE-cellulose chromatography of a 100,000 × *g* supernatant solution of homogenized human platelets revealed the presence of two peaks of both dopamine- and phenol-sulfating activity, termed M- and P-PST, respectively. The latter dopamine-sulfating form eluting from the ion exchange column, M_{II}-PST, was purified approximately 10,000-fold to electrophoretic homogeneity by Sephacryl S-200 HR and 3′-phosphoadenosine-5′-phosphate-agarose chromatography. The final specific activity of the enzyme was 930 nmol/min/mg of protein. As determined by the hydrodynamic properties of M_{II}-PST, the native M_r was approximately 69,000. The frictional ratio (f/f_o) was estimated to be 1.28, indicating that the enzyme possesses a relatively low degree of asymmetry. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis analysis of the affinity-purified enzyme revealed the presence of single M_r species of approximately 34,000, suggesting that M_{II}-PST exists as a homodimer *in vivo*. Isoelectric focusing of purified M_{II}-PST yielded a single protein species with a pI of 4.7. The sulfhydryl-modifying reagent *N*-ethylmaleimide (50 μM) was found to inactivate M_{II}-PST in a time-dependent manner. This inactivation was totally prevented by saturating concentrations of 3′-phosphoadenosine-5′-phosphosulfate, whereas dopamine bestowed only partial protection to the enzyme. These results suggest that at least one sulfhydryl moiety is present at the active site of M_{II}-PST.

Sulfation is a reaction whereby biologically active catechol and phenolic compounds of endogenous and exogenous origin are inactivated *in vivo*. (for review, see Ref. 1). PST (EC 2.8.2.1), the enzyme responsible for this process, catalyzes the transfer of a sulfonate moiety from PAPS to a wide variety of acceptor molecules including the catecholamine neurotransmitters. PST has been widely investigated in many animal species including rats (2–4) guinea pigs (5), dogs (6), and humans (7–13). Studies with rat liver PST (2–4) have demonstrated the presence of four separate isozymes of which three of the forms have been purified to homogeneity. PST has been partially purified from a variety of human tissues including brain (10–12), liver (14), and platelets (7, 15). Human PST exists in at least two functionally distinct forms (7), termed M- and P-PST, which are distinguishable by their selective activities towards monoamines (e.g., dopamine) and phenol, respectively. These forms can also be characterized by their differential sensitivities to thermal inactivation (16) and to inhibition by DCNP (17) with the M form being both more thermally labile and DCNP resistant.

Recent studies of the enzymatic properties of PST have suggested that the enzymes from human sources are fundamentally different from those isolated from a number of animal species. For example, human M-PST has a 100-fold lower K_m for dopamine and a markedly different pH profile than the equivalent rat sulfotransferase (18, 19). In addition, there appear to be multiple forms of both M- and P-PST in human brain (11, 12) and liver (14) and it is unclear what the relationship is between these forms and those isolated from animal tissues. Given these preliminary data, it was of interest to further purify and characterize the various isozymes of PST from human tissues. In this report, we describe the purification to homogeneity and physical characterization of a monoamine-sulfating form of PST from human platelets and present evidence of a dimeric subunit composition for this enzyme.

Experimental Procedures

Materials

PAPS, PAP, 3′-AMP, NEM, cytochrome *c* (horse heart, type II), peroxidase (horseradish, type II), alcohol dehydrogenase (horse liver),

This work was supported by Grants GM 7145 and NS20530 from the National Institutes of Health.

ABBREVIATIONS: PST, phenol sulfotransferase; NEM, *N*-ethylmaleimide; PAPS, 3′-phosphoadenosine-5′-phosphosulfate; PAP, 3′-phosphoadenosine-5′-phosphate; DCNP, 2,6-dichloro-p-nitrophenol; BSA, bovine serum albumin; TEA, triethanolamine; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; IEF, isoelectric focusing.

BSA, ovalbumin, catalase, and horse γ-globulin were purchased from Sigma Chemical Company, St. Louis, MO. Adenosine 3′,5′-diphosphate-hexane-agarose and Sephacryl S-200 HR were obtained from Pharmacia, Piscataway, NJ, whereas Whatman DE52 anion exchanger was acquired from Krackler Scientific, Inc., Albany, NY. [^{35}S]PAPS (specific activity 1.1 Ci/mmol) was purchased from New England Nuclear, Boston, MA, and ultrapure sucrose was obtained from Schwarz/Mann Biotech, Cleveland, OH.

PST Assay

Reaction mixtures routinely consisted of various preparations of enzyme, either 10 μM dopamine or phenol, and 1 μM PAPS (approximately 0.2 μCi/nmol, final specific activity) in a final volume of 0.5 ml of 50 mM TEA buffer, pH 7.4. The reaction was performed at 37° and terminated by placing the tubes in a boiling water bath for 2 min and then immediately onto ice. Radiolabeled sulfated products were separated from reactants using Ecteola cellulose as described by Whittemore and Roth (20).

Purification of PST

Outdated human platelets were obtained from the American Red Cross, Rochester, NY, stored at 4°, and used within 1 month after the expiration date. All purification procedures were carried out at 4°.

Step 1. Homogenization. A total of 200 units of outdated human platelets were pelleted at 8,000 × *g* and washed with 2 liters of phosphate-buffered saline, pH 7.4. The resulting pellet was resuspended in 450 ml of 10 mM TEA buffer, pH 7.4, containing 0.25 M sucrose and 5 mM 2-mercaptoethanol. The platelet suspension was homogenized in 35-ml aliquots with a Brinkman Polytron (setting 6) for 15 sec and centrifuged for 60 min at 100,000 × *g* in a Beckman L5-50B ultracentrifuge.

Step 2. Anion exchange chromatography. The 100,000 × *g* supernatant solution was loaded at 80 ml/hr onto a DEAE cellulose column (2.5 cm × 18 cm) and the column was washed with 2 liters of homogenization buffer. PST activities were eluted with a 2-liter linear NaCl gradient (0–250 mM) in homogenization buffer at a flow rate of 45 ml/hr. Fractions of 11 ml were collected and assayed for both dopamine- and phenol-sulfating activity as described above. The appropriate fractions of dopamine-sulfating activity as indicated in Results were pooled and concentrated to approximately 12 ml using an ultrafiltration-stirred cell with a PM30 membrane (Amicon, Danvers, MA).

Step 3. Gel filtration chromatography. The DEAE cellulose concentrate was loaded onto a Sephacryl S-200 HR column (2.5 cm × 106 cm) preequilibrated with 200 mM TEA buffer (pH 7.4) and eluted at a flow rate of 10 ml/hr with the same buffer. Fractions of 4 ml were collected and assayed for dopamine-sulfating activity. The appropriate fractions were pooled and concentrated to a final volume of approximately 1.2 ml using a Centriprep-30 concentrator (Amicon). The column was calibrated for molecular weight determination of PST using γ-globulin, BSA, cytochrome *c*, peroxidase, and ovalbumin as markers. Stokes radii for these standards were calculated using the expression $A = kT/6\pi\eta D_{20,w}$ where A = Stokes radius, k = Boltzman constant, T = absolute temperature, η = viscosity of the medium, and $D_{20,w}$ = diffusion coefficient. Values for $D_{20,w}$ and molecular weights were obtained from published sources (21).

Step 4. Affinity chromatography. The Sephacryl S-200 HR concentrate was loaded (4 ml/hg) onto a PAP-agarose column (1.5 cm × 2.5 cm) equilibrated with 100 mM TEA buffer, pH 7.4. The column was washed sequentially (30 ml/hr) with 20 ml of TEA buffer, 20 ml of this buffer containing 50 mM NaCl, and 20 ml of buffer containing 50 mM NaCl and 500 μM 3′-AMP. Enzyme activity was eluted with a 20-ml linear PAP (0–1000 μM) gradient in buffer containing 50 mM NaCl at a flow rate of 20 ml/hr. Because PAP is a potent inhibitor of PST activity (K_i = 0.1 μM), the following dilution protocol became necessary for the measurement of enzyme activity in the affinity-eluted fractions. Each fraction (2 ml) was initially concentrated to a volume of approximately 60 μl using a Amicon PM30 microconcentrator. A 10-μl aliquot was then removed and diluted to a final volume of 1 ml with 10 mM TEA buffer, pH 7.4, and 10 μl of this solution was used to assay each fraction. The overall PAP concentration in each fraction was diluted 5000-fold by this procedure.

Sucrose Density Gradient Ultracentrifugation

Sedimentation coefficients were determined by the method of Martin and Ames (22). A 300-μl aliquot of partially purified M_{II}-PST was gently layered over a 12-ml linear sucrose gradient (5–20%) containing 10 mM TEA buffer, pH 7.4. Centrifugation was performed at 4° for 24 hr at 40,000 rpm using a Beckman SW 41 rotor. The bottom of each centrifuge tube was punctured via a gradient fractionator (Hoefer Scientific Instruments, San Francisco, CA) and 35 to 40 fractions of five drops each were collected and assayed for M-PST activity. Cytochrome *c*, peroxidase, alcohol dehydrogenase, and catalase were used as external standards. Cytochrome *c* was detected by absorbance at 550 nm whereas the other markers were measured enzymatically by previously published methods (23–25). Catalase was included as an internal standard when PST was sedimented. Values for sedimentation coefficients of standard proteins were obtained from published sources (21).

Gel Electrophoresis and IEF

For SDS-PAGE, samples obtained from PAP affinity chromatography were boiled for 5 min in an equal volume of buffer containing 0.125 M Tris·HCl (pH 6.8), 4% SDS, 20% glycerol, and 10% 2-mercaptoethanol. SDS-PAGE was performed by the method of Laemmli (26) using a 10% acrylamide resolving gel. The gel was calibrated by running proteins of known M_r as indicated. IEF was performed for 45 min at 10 W constant power using Isogel Agarose pH 3–7 IEF plates (FMC Bioproducts, Rockland, ME). IEF plates were calibrated for pH with pI 2.5–6.5 markers (Pharmacia). Proteins for both SDS-PAGE and IEF were detected by Coomassie blue staining.

NEM and Phenylglyoxal Inactivation

Sephacryl S-200-purified M_{II}-PST was preincubated at 24° in the presence and absence of 50 μM NEM or 5 mM phenylglyoxal in a total volume of 50 mM TEA buffer, pH 7.4. At various times, 25-μl aliquots were removed and mixed with an equal volume of cold 1 mM 2-mercaptoethanol to stop the NEM reaction. For substrate protection experiments, either 4μM PAPS or 10 μM dopamine was included in the preincubation mixture. Appropriate controls were performed in each case.

Other Methods

Protein concentrations were determined as described by Bradford (27) using BSA as standard. For determination of the protein concentration of affinity-purified PST, individual SDS-PAGE lanes were analyzed for Coomassie blue-stained protein using an LKB Ultroscan XL scanning densitometer. Varying amounts (0.1 to 1.0 μg) of BSA were similarly electrophoresed and scanned to generate a standard curve.

Results

The elution profile of PST activity from a DEAE cellulose column is illustrated in Fig. 1. For both M- and P-PST, two peaks of activity were observed. The fractions containing dopamine-sulfating activity eluting at a NaCl concentration greater than 130 mM had essentially no P-PST activity and were selectively pooled in order to obtain an enzyme preparation devoid of this form of PST. The enzyme form present in this pooled fraction will be referred to as M_{II}-PST, corresponding to the order of elution of the two M activities from the DEAE column. The apparently low yield of only 31% for M_{II}-PST activity obtained by this purification step was largely due

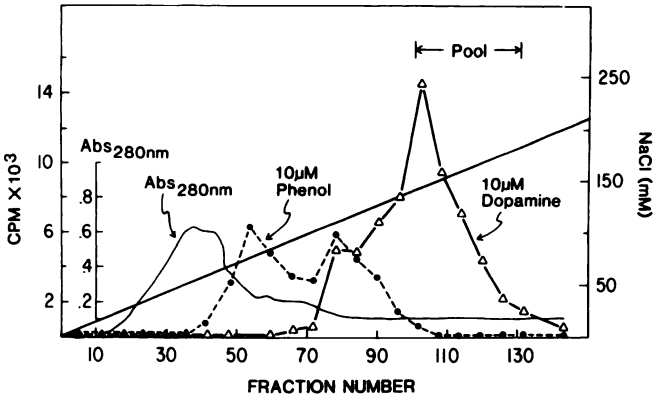

Fig. 1. DEAE cellulose chromatography of 100,000 × *g* platelet supernatant solution. PST activities were eluted with a 2-liter linear 0 to 250 mM NaCl gradient in 10 mM TEA buffer, pH 7.4, containing 0.25 M sucrose and 5 mM 2-mercaptoethanol. Fractions of dopamine-sulfating activity were pooled as indicated.

to the highly selective pooling of the fractions containing only a single species of M-PST, rather than actual loss of activity during the chromatographic procedure. However, the specific activity of the enzyme was increased 23-fold as a result of this purification step (Table 1).

The M_{II}-PST activity eluted as a single symmetrical peak upon Sephacryl S-200 HR chromatography with an apparent M_r of about 72,000 and Stokes radius of 38 Å (Table 2 and Fig. 2). A 5-fold purification with a 68% yield with respect to the pooled DEAE fractions was observed using this procedure.

As indicated in Table 1, affinity chromatography using PAP-agarose resulted in a 138-fold purification over the previous step with an overall purification of approximately 10,000-fold. Data illustrated in Fig. 3 demonstrate that M_{II}-PST eluted as a single peak of activity at a PAP concentration of approximately 500 μM. Although not indicated, more than 50% of the PST activity originally applied to the PAP-agarose column was eluted during the loading and washing stages, resulting in a yield of only 8% for this step. SDS-PAGE analysis of the affinity-eluted fractions showed a single protein band of M_r 34,400, which coincided with the elution of M_{II}-PST activity from the column (Fig. 3). As illustrated in Fig. 4, IEF of affinity-purified M_{II}-PST produced a single protein band with a pI of 4.7.

The results of sucrose gradient analysis, illustrated in Fig. 5, yielded a $S_{20,w}$ value of 4.4 for M_{II}-PST. The M_r as calculated using the sedimentation coefficient and Stokes radius is approximately 69,000. The hydrodynamic properties of M_{II}-PST are summarized in Table 2.

The effect of NEM on Sephacryl-purified M_{II}-PST is shown in Fig. 6. Preincubation of the enzyme with this sulfhydryl-modifying reagent at a concentration of 50 μM for 5 min decreased activity approximately 60%. This inactivation was time-dependent with greater than 95% of the enzyme activity being lost after a 15-min preincubation (data not shown). Inactivation followed apparent pseudo-first order kinetics with a k_{obs} of −0.09. Inclusion of a near-saturating concentration of dopamine in the preincubation mixture only partially protected PST from NEM inactivation whereas addition of PAPS afforded complete protection to the enzyme at any preincubation time. In contrast, preincubation with the arginine-modifying reagent phenylglyoxal (5 mM) for 15 min had no effect on enzyme activity (data not shown).

Discussion

We have previously reported a 5000-fold purification of human brain M-PST (10); however, this enzyme was shown to be impure upon gel electrophoresis. In the present report, M_{II}-PST was purified from human platelets by a simple three-step procedure using anion exchange, gel filtration, and affinity chromatography, producing an overall purification of approximately 10,000-fold and resulting in a homogeneous preparation as evaluated by both SDS-PAGE and IEF. As illustrated in Fig. 1, DEAE chromatography revealed the presence of two peaks for both dopamine- and phenol-sulfating activity. This chromatographic profile is similar to that obtained with human brain homogenate (10), suggesting that four distinct forms of PST may be present in both human brain and platelets. Gel filtration of M_{II}-PST yielded an apparent M_r of about 72,000, similar to previous estimates of 62,000 and 68,000 for human brain (12) and platelet (28) PST, respectively. However, because molecular weights based on gel filtration are influenced by the degree of molecular asymmetry, we analyzed the hydrodynamic properties of the enzyme as described by Siegel and Monty (29). The M_r as calculated from the Stokes radius and sedimentation coefficient of the enzyme is approximately 68,000, corroborating the estimate obtained from Sephacryl chromatography. In addition, a calculated f/f_o of 1.28 reveals M_{II}-PST to possess a relatively low degree of asymmetry.

As indicated by Fig. 3, SDS-PAGE analysis revealed a single protein species with a M_r of approximately 34,000. These data from hydrodynamic studies and gel electrophoresis together strongly suggest that the native enzyme exists as a homodimer *in vivo*. Furthermore, no change in subunit M_r was observed when mercaptoethanol was omitted from the SDS-PAGE analysis, indicating that the subunits are not held together by disulfide bridges (data not shown). Yu *et al.* (12) have previously reported a monoamine-sulfating sulfotransferase from human brain with M_r estimates of 64,000 and 62,000 by gel filtration chromatography and SDS-PAGE, respectively, suggesting that the brain enzyme is not composed of subunits. The reason for the discrepancy when compared with our data is unclear because both brain and platelet PST appear to be similar enzymes based on kinetic parameters and chromatographic profiles (10,

TABLE 1
Purification of human platelet M_{II}-PST

Step	Protein	Total activity	Specific activity	% Yield	Purification
	mg	*pmol/min*	*pmol/min/mg of protein*		
100,000 × *g* supernatant	2728	254,144	93.2	100	
DEAE-Cellulose	36.8	77,668	2110	30.6	22.6
Sephacryl S-200 HR	5.06	52,808	1.0×10^4	20.8	107.3
PAP-Agarose	4.3×10^{-3}	3,999	9.3×10^5	1.6	9978

TABLE 2
Physical properties of M_{II}-PST

Parameter	Value
Stokes radius (Å)	38
Sedimentation coefficient ($S_{20,w}$)	4.4
Experimentally determined M_r[a]	71,928
Calculated M_r[b]	68,763
Frictional ratio (f/f_0)[c]	1.28
Subunit M_r[d]	34,359

[a] Determined by Sephacryl S-200 HR chromatography.
[b] M_r was calculated from the relationship

$$M_r = \frac{6\pi\eta NAS_{20,w}}{(1 - \bar{\upsilon}\rho)}$$

where η = solvent viscosity, ρ = solvent density, and υ = partial specific volume of the enzyme. with an assumed value of 0.725.
[c] The fractional ratio was calculated from the relationship

$$\frac{f}{f_0} = A\left[\frac{4\pi N}{3M_r(\bar{\upsilon} + \delta/\rho)}\right]^{1/3}$$

where δ = solvation factor, with an assumed value of 0.2.
[d] Determined by SDS-PAGE as described in Experimental Procedures.

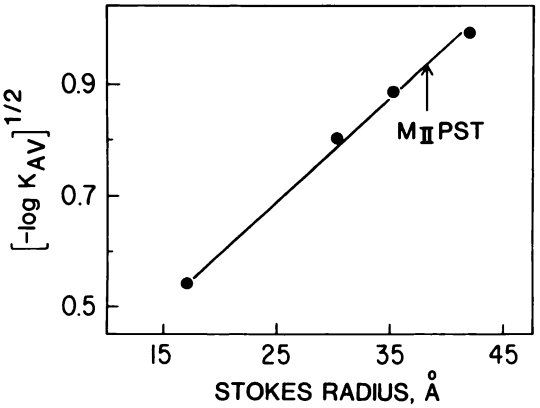

Fig. 2. Determination of the Stokes radius for M_{II}-PST. PST activity was eluted from a Sephacryl S-200 HR column at a flow rate of 10 ml/hr in 200 mM TEA buffer, pH 7.4. The column was calibrated with standard proteins as indicated in the text.

30). The purified brain enzyme was reported to have a specific activity of only 0.375 nmol/min/mg of protein, whereas the purified platelet M_{II}-PST reported here had a specific activity approximately 2,480 times greater (930 nmol/min/mg of protein). This wide disparity in specific activities suggests that the 62,000 M_r protein species in brain identified by Yu and coworkers (12) by SDS-PAGE analysis may likely have been a contaminating protein that is often observed in cruder enzyme preparations from either brain or platelets. We have often observed contaminating proteins of M_r 60,000 to 66,000 that copurify with PST during the first two purification steps and that are subsequently lost during PAP affinity chromatography. However, we cannot rule out the possibility that the human brain enzyme has a different molecular composition than that of the platelet enzyme.

In another study (13), a 38,000 M_r sulfotransferase from human lung was purified to homogeneity and shown to be monomeric by SDS-PAGE analysis. It is uncertain what type of sulfotransferase was isolated from lung tissue because the native M_r and substrate specificity of the purified enzyme are markedly different from what has been observed in a number of laboratories (10–12, 14, 28) for PSTs from other human tissues.

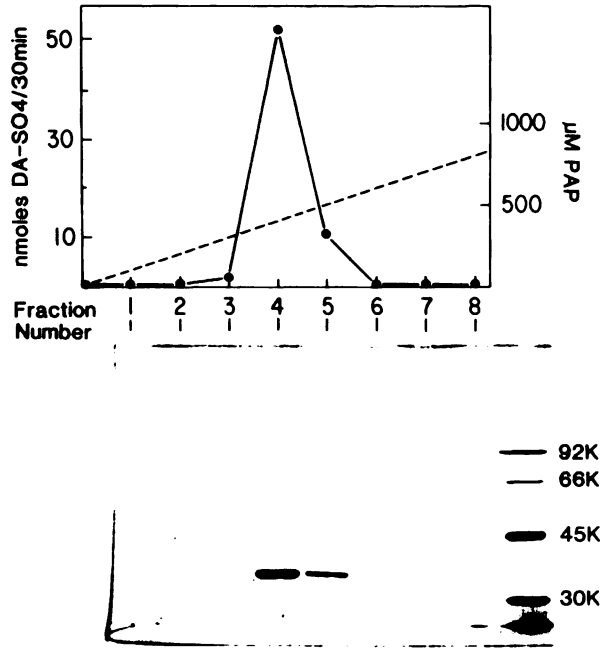

Fig. 3. PAP-agarose chromatography of M_{II}-PST activity. M_{II}-PST was processed through affinity chromatography as described in the text. The *lower panel* shows the corresponding affinity-eluted M_{II}-PST fractions analysed by SDS-PAGE.

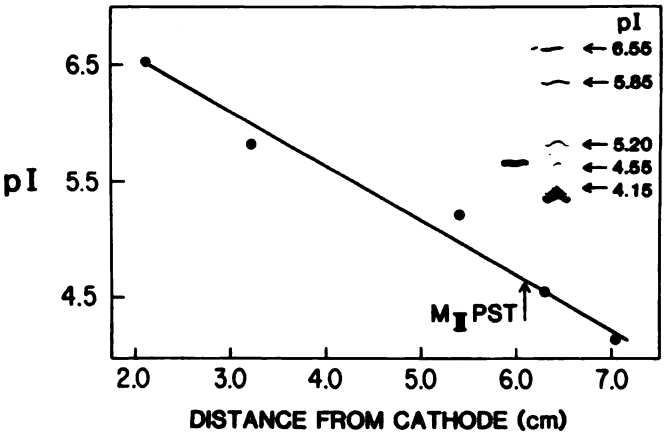

Fig. 4. IEF of M_{II}-PST affinity purified was electrophoresed on a pH 3 to 7 agarose gel as described in the text. The *inset* shows the position of M_{II}-PST relative to various pI standard proteins (*right*).

Results from IEF (Fig. 4) show the affinity-purified M_{II}-PST to focus as a slightly broadened protein band with a pI of approximately 4.7. One possible reason that M_{II}-PST did not focus sharply during the IEF run is that multiple allelic forms of the enzyme are present in our purified enzyme preparation, because platelet-rich plasma from 200 individuals are routinely used during each purification procedure. Other investigators have reported pI values of 5.8 and 4.7 for monoamine-sulfating sulfotransferases in rats (2) and dogs (6), respectively. It is interesting to note that the sulfotransferase responsible for sulfation of catecholamines in rats is very similar to M_{II}-PST in both native and subunit M_r with values of 60,000 and 33,500, respectively (2). However, a marked difference is observed in pI, suggesting variations in amino acid sequences between the human and rat enzymes. In another study (31), a *N*-hydroxy-2-acetylaminofluorene sulfotransferase was isolated from rat liver with a native and subunit M_r of 68,000 and 38,250,

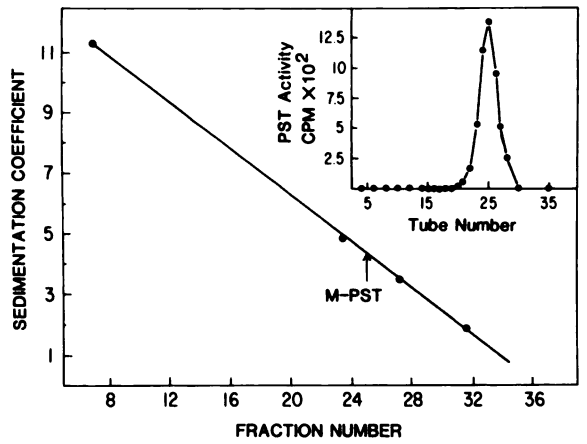

Fig. 5. Sucrose gradient analysis of M_r-PST. A total of 300 μl of concentrated Sephacryl-purified M_r-PST was layered over a 12-ml linear (5–20%) sucrose gradient in 10 mM TEA buffer, pH 7.4. Centrifugation was performed for 24 hr at 40,000 rpm in a Beckman SW 41 rotor. The tubes were then punctured at the bottom and 35 to 40 fractions of five drops each were collected and assayed for M-PST activity. $S_{20,w}$ standard proteins were detected as described in the text.

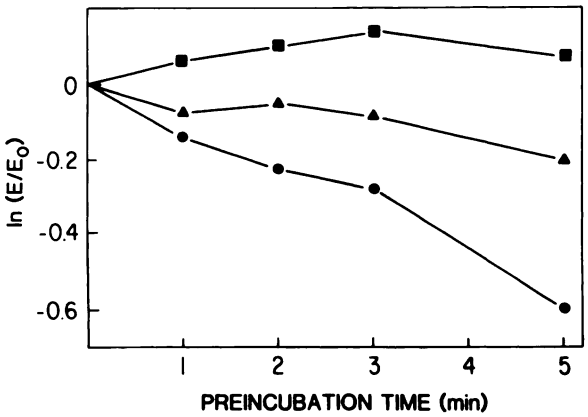

Fig. 6. Inactivation and substrate protection of M_r-PST. Sephacryl-purified M_r-PST (100 μg) was preincubated for various times at 25° as indicated in the presence of 50 μM NEM (●), 50 μM NEM and 4 μM PAPS (■), or 50 μM NEM and 10 μM dopamine (▲). The reaction was terminated by the addition of mercaptoethanol. PST activity was determined as described in Experimental Procedures.

respectively. Again, although similarities appear to exist in regard to native and subunit M_r when compared with human M_{II}-PST, a significant difference is noted in pI with a value of approximately 5.7 being obtained for the rat enzyme.

Although the kinetics of the human M-PST reaction have been well characterized (10), little information is available concerning the amino acid sequence of the active site of this sulfotransferase. Experiments described in this report suggest that at least one sulfhydryl moiety may be present in the active site of human M_{II}-PST inasmuch as PAPS was capable of completely protecting the enzyme from inactivation by the sulfhydryl-modifying reagent NEM. Similarly, a rat liver sulfotransferase, capable of sulfating tyrosine methyl ester, was found to be partially protected from sulfhydryl group inactivation by iodoacetate when the cosubstrate PAPS was present during the experiment (32). In addition, other studies (33) have shown that rat liver PST is totally protected from sulfhydryl group inactivation by the binding of the cosubstrates *p*-nitrophenol and PAPS, also suggesting that sulfhydryl moieties are present in the active site of rat liver sulfotransferase. Although similarities appear to exist between rat and human PST with respect to enzyme inactivation caused by sulfhydryl group modification, fundamental differences have been previously observed in salt sensitivity, kinetic reaction mechanism, and substrate specificity (1, 19). In addition, human M-PST has a 100-fold lower K_m for dopamine than does the equivalent rat enzyme. This disparity suggests differences in substrate recognition sites due to variations in the amino acid sequence of the active sites. In support of this are the results from the present study, which demonstrate that phenylglyoxal had no effect on the catalytic activity of human M_{II}-PST whereas this arginine-modifying reagent was previously reported to produce both a time- and concentration-dependent inactivation of rat liver sulfotransferase (32). These observations serve to highlight fundamental differences in amino acid sequence at the active site that may exist between the rat and human enzymes.

References

1. Roth, J. A., and A. J. Rivett. Does sulfate conjugation contribute to the metabolic inactivation of catecholamines in humans? *Biochem. Pharmacol.* **31**:3017–3021 (1982).
2. Sekura, R. D., and W. B. Jacoby. Phenol sulfotransferases. *J. Biol. Chem.* **254**:5658–5663 (1979).
3. Sekura, R. D., and W. B. Jacoby. Aryl sulfotransferase IV from rat liver. *Arch. Biochem. Biophys.* **211**:352–359 (1981).
4. Duffel, M. W., and W. B. Jacoby. On the mechanism of aryl sulfotransferase. *J. Biol. Chem.* **256**:11123–11127 (1981).
5. Banerjee, R. K., and A. B. Roy. The sulfotransferases of guinea pig liver. *Mol. Pharmacol.* **2**:56–66 (1966).
6. Romain, Y., S. Demassieux, and S. Carriere. Partial purification and characterization of two isozymes involved in the sulfurylation of catecholamines. *Biochem. Biophys. Res. Commun.* **106**: 999–1005 (1982).
7. Rein, G., V. Glover, and M. Sandler. Evidence for multiple forms of human phenolsulfotransferase. *Psychopharmacol. Bull.* **17**:43–45 (1981).
8. Rein, G., V. Glover, and M. Sandler. Sulphate conjugation of biologically active monoamines and their metabolites by human platelet phenolsulphotransferase. *Clin. Chim. Acta* **111**:247–256 (1981).
9. Renskers, K. J., K. D. Feor, and J. A. Roth. Sulfation of dopamine and other biogenic amines by human brain phenol sulfotransferase. *J. Neurochem.* **34**:1362–1368 (1980).
10. Whittemore, R. M., L. B. Pearce, and J. A. Roth. Purification and kinetic characterization of a dopamine-sulfating form of phenol sulfotransferase from human brain. *Biochemistry* **24**:2477–2482 (1985).
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. Yu, P. H., B. Rozdilsky, and A. A. Boulton. The sulfate conjugation of monoamines in human brain: purification and some properties of an arylamine sulfotransferase from cerebral cortex. *J. Neurochem.* **45**:836–843 (1985).
13. Baranczyk-Kuzma, A. Phenol sulfotrnasferase in human lung. *Biochem. Med. Metab. Biol.* **35**:18–30 (1986).
14. 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).
15. Hart, R. F., K. J. Renskers, E. B. Nelson, and J. A. Roth. Localization and characterization of phenol sulfotransferase in human platelets. *Life Sci.* **24**:125–130 (1979).
16. Reiter, C., G. Mwaluko, J. Dunnette, J. Vanloon, and R. Weinshilboum. Thermolabile and thermostable human platelet phenol sulfotransferase: substrate specificity and physical separation. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **324**:140–147 (1983).
17. Rein, G., V. Glover, and M. Sandler. Multiple forms of phenolsulphotransferase in human tissues: selective inhibition by dichloronitrophenol. *Biochem. Pharmacol.* **31**:1893–1897 (1982).
18. Roth, J. A. Phenol sulfotransferase, in *Neuromethods, Neurotransmitter Enzymes* (A. A. Boulton, G. B. Baker, and P. H. Yu, eds.), Vol. 5. Humana Press Inc., Clifton, New Jersey, 575–604 (1986).
19. Roth, J. A. Sulfoconjugation: role in neurotransmitter and secretory protein activity. *Trends Pharmacol. Sci.* **7**:404–407 (1986).
20. Whittemore, R. M., and J. A. Roth. A modified ecteola cellulose assay for M and P phenol sulfotransferase. *Biochem. Pharmacol.* **43**:1647–1652 (1985).
21. Smith, M. H. in *Handbook of Biochemistry*, (H. A. Sober, ed.). The Chemical Rubber Co., Cleveland, Ohio, C10–C25 (1970).
22. Martin, R. G., and B. N. Ames. A method for determining the sedimentation behavior of enzymes: application to protein mixtures. *J. Biol. Chem.* **236**:1372–1379 (1961).
23. Beers, R. F., and I. W. Sizer. A spectrophotometric method for measuring

the breakdown of hydrogen peroxide by catalase. *J. Biol. Chem.* **195**:133–140 (1952).
24. Maehly, A. C., and B. Chance. The assay of catalases and peroxidases. *Methods Biochem. Anal.* **1**:357–424 (1954).
25. Valee, B. L., and F. L. Hoch. Zinc, a component of yeast alcohol dehydrogenase. *Proc. Nat. Acad. Sci. USA* **41**:327–338 (1955).
26. Laemmli, U. K. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* (*Lond.*) **227**:680–685 (1970).
27. Bradford, 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).
28. Butler, P. R., R. J. Anderson, and D. L. Venton. Human platelet phenol sulfotransferse: partial purification and detection of two forms of the enzyme. *J. Neurochem.* **41**:630–639 (1983).
29. Siegel, L. M., and K. J. Monty. Determination of molecular weights and frictional ratios of proteins in impure systems by use of gel filtration and density gradient centrifugation: application to crude preparations of sulfate and hydroxylamine reductases. *Biochim. Biophys. Acta* **112**:346–362 (1966).
30. Picotti, G. B., A. M. Cesura, M. D. Galva, P. Mantegazza, R. Kettler, and M. Da Prada. Monoamine sulphoconjugation by human platelets, in *Phenolsulfotransferase in Mental Health Research* (M. Sandler and E. Usdin, eds.). MacMillian, London 44–54 (1981).
31. Wu, S. G., and K. D. Straub. Purification and characterization of *N*-hydroxy-2-acetylaminofluorene sulfotransferase from rat liver. *J. Biol. Chem.* **251**:6529–6539 (1976).
32. Mattock, P., D. J. Barford, J. M. Basford, and J. G. Jones. Partial purification and properties of an enzyme from rat liver that catalyzes the sulphation of L-tyrosyl derivatives. *J. Biochem.* **116**:805–810 (1970).
33. Borchardt, R. T., C. S. Schasteen, and S. Wu. Phenol sulfotransferase. II. Inactivation by phenylglyoxal, *N*-ethylmaleimide, and 2,3-dialdehydes. *Biochem. Biophys. Acta* **708**:280–293 (1982).

Send reprint requests to: Dr. Jerome A. Roth, Department of Pharmacology and Therapeutics, 127 Farber Hall, SUNY at Buffalo, Buffalo, NY 14214.