Purification and Kinetic Characterization of a Dopamine-Sulfating Form of Phenol Sulfotransferase from Human Brain†

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ABSTRACT: The kinetic and biochemical properties of a purified, monoamine-sulfating form of phenol sulfotransferase (M-PST) from human brain are described. M-PST activity was separated and purified from phenol-sulfating activity by anion-exchange chromatography on DEAE-cellulose and subsequently purified on AffiGel Blue and Sephacryl S-200, routinely giving a final purification of over 20 000-fold, with approximately a 3% yield. The molecular weight of the active species, as estimated by gel filtration chromatography, was 250 000. The purified enzyme was inhibited by NaCl (50% at 325 mM) and showed an optimum for dopamine sulfation at pH 7.0. Of the monoamine substrates examined, 4-methoxytyramine was the most extensively sulfated at 20 μM, while at higher substrate concentrations (200 μM), tyramine was the apparent preferred substrate. Kinetic analysis demonstrated that sulfation by M-PST proceeds via an ordered, bisubstrate reaction mechanism, where 3′-phosphoadenosine 5′-phosphosulfate (PAPS) is the leading substrate. True K_m values for dopamine and PAPS were 2.9 and 0.35 μM, respectively. The product inhibitor 3′-phosphoadenosine 5′-phosphate possessed a K_i of 0.07 μM, while the dead-end inhibitor ATP exhibited a K_i of 170 μM.

The presence of a metabolic pathway that catalyzes the sulfate conjugation of catecholamine neurotransmitters and their metabolites in humans has been known for over 40 years (Richter, 1940; Richter & MacIntosh, 1941). More recent studies have demonstrated that the process of sulfoconjugation may play an important role in biogenic amine metabolism in the central nervous system of several species (Dencker et al., 1967; Schanberg et al., 1968; Chase et al., 1973; Karoum et al., 1977; Tyce et al., 1980; Elchisak et al., 1982), including man, where it has been suggested sulfoconjugation may contribute to as much as 15% of total dopamine metabolism in brain (Rivett et al., 1982; Roth & Rivett, 1982; Rivett et al., 1984).

The enzyme responsible for the formation of sulfated catecholamines, phenol sulfotransferase (PST)[1] (EC 2.8.2.1), has been localized in the soluble fraction of brain tissue from rats (Meek & Neff, 1973) as well as human platelets (Hart et al., 1979) and brain (Foldes & Meek, 1974; Wong, 1976; Renskers & Roth, 1979). Multiple forms of PST have been demonstrated in the liver of guinea pig (Banerjee & Roy, 1966), rats (Sekura & Jakoby, 1979), dogs (Romain et al., 1982), human brain (Rein et al., 1981c; Whittemore et al., 1983), and platelets (Rein et al., 1981a). The two forms of the enzyme in human tissue have been designated M- and P-PST based on their preferential sulfation of monoamines (e.g., dopamine) and phenol, respectively (Rein et al., 1981a).

Substantial differences in the biochemical properties of PST from many species have been documented. Changes in substrate specificity and pH optima have been reported for rat brain (Foldes & Meek, 1973; Meek & Neff, 1973) and liver PST (Sekura & Jakoby, 1979) when compared with those of human platelets (Hart et al., 1979; Rein et al., 1981b,c) and brain (Renskers et al., 1980; Rivett et al., 1982). Similarly, the K_m value of dopamine (DA) varies over 100-fold between human PST and several animal species (Roth et al., 1982). These variations in K_m values may also point to more fundamental species differences in the kinetic mechanisms for PST, as a random bireactant mechanism has been proposed for rat liver aryl sulfotransferase IV (Sekura & Jakoby, 1979), and on the basis of preliminary studies in our laboratory, an ordered mechanism is thought to describe sulfation by human PST (Roth et al., 1982). Since the properties of human brain M-PST may be different from those described for other species, it was of interest to further characterize the biochemical and kinetic properties of a purified preparation of this sulfotransferase from human brain.

Experimental Procedures

Tissue Preparation. Sections of frontal cortex from human brain were obtained at autopsy within 12 h of death. Fresh, unfrozen tissue was used whenever possible but was occasionally stored at −80 °C until use. Postmortem changes in activity were expected to be minimal, as it has been previously demonstrated that no correlation exists between enzyme activity and the length of storage time (Foldes & Meek, 1974). Tissue was disrupted in 5 mL of homogenization buffer [10 mM triethanolamine (TEA), pH 7.4, 0.25 M sucrose, and 5 mM 2-mercaptoethanol] per gram of tissue, using a motorized, glass–Teflon homogenizer. The crude homogenate was subsequently centrifuged at 12000*g* for 30 min, and the resulting supernatant solution was recentrifuged at 100000*g* for 60 min to obtain the soluble enzyme fraction. All tissue isolation procedures and subsequent purification steps were carried out at 4 °C.

DEAE-cellulose Chromatography. The soluble enzyme fraction (800–900 mL) was applied to a 2.5 × 13 cm column of DEAE-cellulose (DE-52, Whatman; equilibrated in homogenization buffer) at a flow rate of 42 mL/h. The column

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[1] Abbreviations: PST, phenol sulfotransferase; DA, dopamine; DA-SO_4, dopamine sulfate; PAPS, 3′-phosphoadenosine 5′-phosphosulfate; PAP, 3′-phosphoadenosine 5′-phosphate; TEA, triethanolamine.

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Table I: Purification of Human Brain M-PST

step	total volume (mL)	protein (mg/mL)	sp act. [pmol (30 min)$^{-1}$ mg^{-1}]	yield (%)	purification (x-fold)
crude homogenate	960	14.08	9.15	100	
12000g supernatant	700	2.92	43.35	72	4.7
10000g	650	2.13	63.30	71	6.9
DEAE-cellulose	294	0.25	755.0	45	82.5
AffiGel Blue	297	0.017	10590	9	1157.4
Sephacryl S-200	55	3.1×10^{-4}	232050	3	25360

was then washed in sequence with 500 mL of buffer followed by a buffered, linear NaCl gradient (0–250 mM, 1600 mL). Fractions of 10 mL were collected and examined for M-PST (dopamine sulfation) and P-PST (phenol sulfation) activity. The fractions containing dopamine-sulfating activity were pooled for subsequent purification on AffiGel Blue.

AffiGel Blue Chromatography. The pooled fractions of M-PST activity obtained by anion-exchange chromatography were applied to a 1.5 × 16 cm column of AffiGel Blue (preequilibrated in homogenization buffer) at a flow rate of 30 mL/h. The column was eluted in sequence with 300 mL of buffer and 300 mL of 350 mM NaCl in buffer. Following these washes, M-PST activity was eluted with a linear gradient consisting of 400 mL each of 350 mM NaCl and 750 mM NaCl in buffer. Fractions of 10 mL were collected, and those containing PST activity were pooled for positive pressure concentration and subsequent gel filtration.

Gel Filtration Chromatography. In preparation for gel filtration chromatography, pooled AffiGel Blue fractions were concentrated under nitrogen by positive pressure ultrafiltration (60 psi, Amicon PM-10 membrane). Samples were concentrated 10–12-fold over a period of 3–5 h, with approximately a 5% loss of activity. Concentrated enzyme from the AffiGel purification step was applied to a 2.5 × 157 cm column of Sephacryl S-200 which had been equilibrated in 100 mM TEA buffer (pH 7.4) containing the same concentrations of mercaptoethanol and sucrose described previously. The pressure-concentrated enzyme solution was applied to the Sephacryl S-200 at a flow rate of 12 mL/h and was eluted from the column at the same flow rate with the 100 mM TEA buffer described above.

Assays for PST Activity. Incubation mixtures routinely consisted of 10 μM dopamine (DA) to measure M-PST activity, or 10 μM phenol to measure P-PST, 1 μM 3′-phosphoadenosine 5′-phospho[^{35}S]sulfate ([^{35}S]PAPS), 1 mM pargyline (a monoamine oxidase inhibitor), and 250 μL of enzyme in a final volume of 500 μL. The barium precipitation method of Foldes & Meek (1973) was used in the assay of all purification steps and the salt inhibition experiments. In this method, incubations were terminated by the addition of 100 μL of 0.1 M barium acetate followed immediately by the addition of 100 μL of $Ba(OH)_2$. Samples were vortexed, and 100 μL of $ZnSO_4$ was added. Following vortexing, the precipitate formed was removed by centrifugation at 1500g for 5 min and the entire procedure repeated following transfer of the supernatant solution to fresh culture tubes. The resultant ^{35}S-labeled product was retained for liquid scintillation counting. All other studies were performed by using a modification of the ion-exchange chromatography procedure of Borchardt et al. (1983) (Whittemore & Roth, 1985). Ecteola–cellulose was prepared by using a modification of the method of Balasubramanian et al. (1967), with conversion of the cellulose to the formate counterion form and subsequent equilibration with 5 mM NH_4HCO_3. When the ecteola–cellulose assay was employed, incubations were terminated by placing samples in boiling water for 1 min. After these samples were placed on ice, aliquots of the reaction mixtures were applied to 0.5 × 2.0 cm ecteola–cellulose columns prepared in $5^3/_4$-in. Pasteur pipets. ^{35}S-Labeled dopamine sulfate ($DASO_4$) was eluted from the column into scintillation vials with a 4-mL water wash. Radioactivity was monitored by using a Beckman LS-6800 liquid scintillation spectrophotometer.

Pargyline hydrochloride, dopamine hydrochloride, triethanolamine hydrochloride, 3′-phosphoadenosine 5′-phosphosulfate, 3′-phosphoadenosine 5′-phosphate, 2-mercaptoethanol, dithiothreitol, norepinephrine hydrochloride, normetanephrine hydrochloride, epinephrine hydrochloride, tyramine hydrochloride, 3,4-dihydroxybenzylamine hydrobromide, octopamine hydrochloride, 6-hydroxydopamine hydrochloride, 5-hydroxytryptamine (creatinine sulfate salt), and all molecular weight calibration proteins were obtained from Sigma Chemical Co., St. Louis, MO. 3- and 4-methoxytyramines were purchased from Aldrich Chemical Co., Milwaukee, WI. AffiGel Blue (100–200 mesh) was purchased from Bio-Rad Laboratories, Rockville Center, NY, while phenol and TEA free base were obtained from J. T. Baker Chemical Co., Phillipsburg, NJ. Sucrose, citric acid, and Na_2CO_3 were obtained from Fisher Chemicals, Fairlawn NJ, and Formula 963 liquid scintillation cocktail and [^{35}S]PAPS (1.0–5.0 Ci/mmol) were obtained through New England Nuclear, Boston, MA. DEAE-cellulose (DE-52) and Sephacryl S-200 were purchased through Whatman Chemical Separations, Ltd, Clifton, NJ, and Pharmacia Fine Chemicals, Piscataway, NJ, respectively.

Results

Figure 1 depicts the elution profiles of M- and P-PST activities from a DEAE-cellulose column following application of a linear NaCl gradient to the column (see Experimental Procedures). Two P-PST activities (as measured by the sulfation of 10 μM phenol) eluted from the column, one (P_I) at approximately 100 mM and the other (P_{II}) at 150 mM NaCl. In contrast, M-PST activity (determined by the conjugation of 10 μM dopamine) began to appear in those fractions corresponding to a salt concentration of about 120 mM, with maximal activity occurring at around 160 mM NaCl. Fractions that contained M- and P_{II}-PST activity were pooled for subsequent purification with AffiGel Blue. As indicated in Table I, the M-PST as measured by dopamine sulfation was purified by this procedure approximately 12-fold over the high-speed supernatant solution.

During purification on AffiGel Blue, M-PST activity did not elute from the column during washes with homogenization buffer or buffer containing 0.35 M NaCl. M-PST activity was eluted from the AffiGel column using a linear 0.35–0.75 M NaCl gradient and was localized in a broad peak of activity between 0.4 and 0.55 M NaCl. The enzyme was purified 14-fold with respect to the previous step (Table I). Pooled samples were concentrated by positive pressure ultrafiltration to a volume suitable for gel filtration chromatography. The elution profile of M-PST from the Sephacryl S-200 column

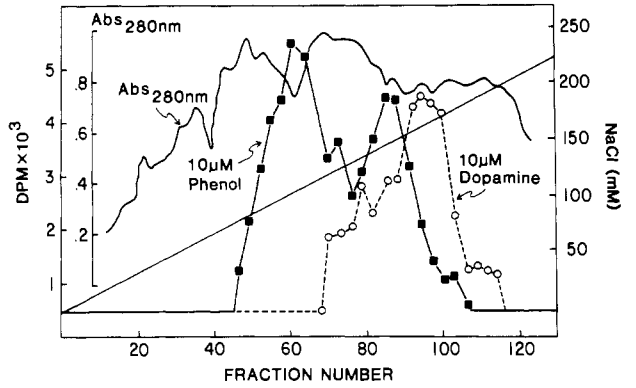


FIGURE 1: Elution of PST activities from DEAE-cellulose. Assays were carried out by using the barium precipitation assay method, as described in the text. (—) Absorbance at 280 nm; (○) activity in the presence of 10 μM DA; (■) activity in the presence of 10 μM phenol.

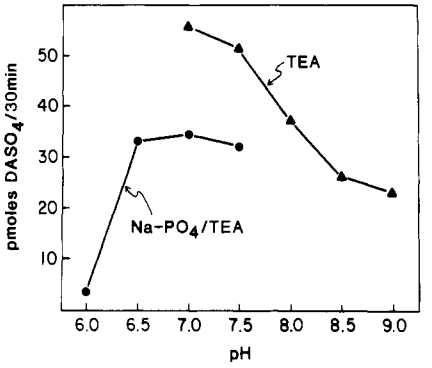


FIGURE 2: Effect of varied pH on purified M-PST activity. Enzyme was incubated with 10 μM DA in the presence of 50 mM TEA or 50 mM sodium phosphate–TEA buffer. Other details were as described in the text.

reveals that this form of PST has a molecular weight of approximately 250 000. As noted in Table I, the apparent increase in the specific activity of this form of PST was by a factor of approximately 20-fold and yielded an overall purification greater than 25 000-fold.

The relationship between pH and enzyme activity in TEA and TEA–sodium phosphate buffers is illustrated in Figure 2. It was not possible to examine the activity of purified M-PST in sodium phosphate buffer alone, as the purified enzyme exhibited poor stability during dialysis, when desalted on Sephadex G-25, and during positive pressure ultrafiltration. Examination of M-PST activity in these buffer systems showed that the enzyme had an optimum for dopamine sulfation at approximately pH 7.0, with higher absolute activity present when the enzyme was assayed in TEA.

Previous studies of rat liver aryl sulfotransferases have revealed a differential sensitivity of the multiple forms of this enzyme to NaCl (Sekura & Jakoby, 1979, 1981). Accordingly, the effects of NaCl on M-PST and the P_I form isolated from human brain were examined. As shown in Figure 3, inhibition of DA sulfation was observed over a range of 0.2–0.5 M NaCl, with 50% inhibition occurring at 325 mM NaCl. In contrast to the M form of the enzyme, P_I-PST exhibited a significantly greater sensitivity to NaCl, 50% inhibition occurring at 100 mM NaCl, while 325 mM NaCl inhibited this form of the enzyme greater than 90%.

The ability of M-PST to conjugate a variety of biogenic amines is outlined in Table II. At a concentration of 20 μM, 4-methoxytyramine was found to be the best substrate, with norepinephrine, dopamine, and normetanephrine being sulfated to a comparable extent. However, when substrate concentrations were increased to 200 μM, tyramine and 3,4-dihydroxybenzylamine were the preferred substrates. With the exception of 3,4-dihydroxybenzylamine, sulfation of the catecholamines and their O-methylated metabolites declined substantially at the higher substrate concentration. The indoleamine 5-hydroxytryptamine was the poorest substrate at both concentrations examined.

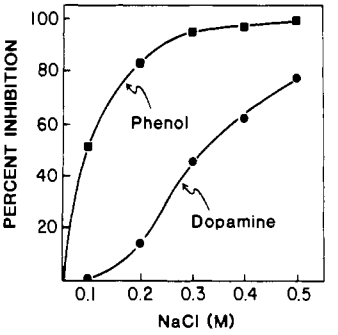


FIGURE 3: Effect of NaCl on M- and P-PST activities. Enzymes were incubated with 10 μM DA or phenol by using the barium precipitation assay procedure (see Experimental Procedures). Other details were as described in the text.

Table II: Sulfation of Various Amines by M-PST[a]

substrate	activity (pmol/30 min) 20 μM	200 μM
4-methoxytyramine	63.3	38.1
norepinephrine	57.6	22.7
dopamine	43.3	17.0
normetanephrine	41.1	24.5
3-methoxytyramine	26.7	6.5
epinephrine	24.8	8.1
tyramine	20.5	47.5
3,4-dihydroxybenzylamine	16.5	41.9
octopamine	10.3	31.5
6-hydroxydopamine	3.4	15.3
5-hydroxytryptamine	0.5	7.3

[a] All incubations were carried out in the presence of 1 μM PAPS, at 37 °C for 30 min. The ecteola–cellulose assay was employed for all substrates, as described in the text. The values presented are the average from two experiments each performed in duplicate.

Previous kinetic studies have suggested that rat PST proceeds by either an ordered (Pennings et al., 1978) or a random reaction mechanism (Duffel & Jakoby, 1981), while preliminary studies from this laboratory on human brain PST are consistent with an ordered mechanism (Roth et al., 1982). For these reasons, a more complete kinetic study of purified human brain M-PST was undertaken in order to establish the mechanism by which this form of PST conjugates dopamine. Double-reciprocal plots obtained by varying dopamine and PAPS demonstrate a pattern of converging lines that intersect at a common point below the x axis. Replots of these data revealed that the true K_m values for DA and PAPS were 2.9 and 0.35 μM, respectively, while the K_{ia} for PAPS was determined to be 0.12 μM. Double-reciprocal plots obtained for product inhibition of purified M-PST by PAP with dopamine as the varied substrate (Figure 4) indicate a mixed pattern of inhibition, with a K_{iq} value for PAP of 0.07 μM. When PAP was used as a product inhibitor with respect to PAPS, a competitive pattern of inhibition was observed (data not shown).

While the above data are consistent with an ordered reaction mechanism and rule out a simple random mechanism for M-PST, they do not eliminate the random mechanism as proposed by Duffel & Jakoby (1981), as this mechanism is characterized by enzyme–DA–PAP and enzyme–PAPS–

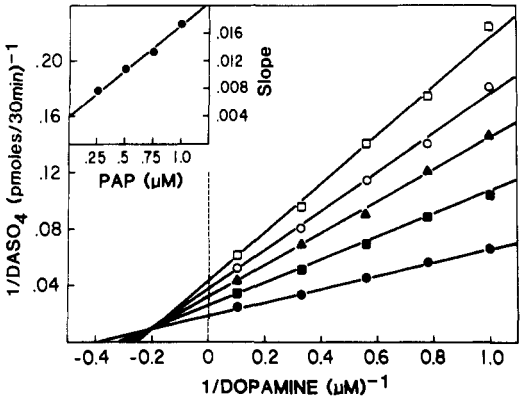


FIGURE 4: Inhibition of purified M-PST by PAP: dopamine as varied substrate. Assays were performed in the presence of 0.5 μM PAPS, and the PAP concentrations were 0 (●), 0.25 (■), 0.50 (▲), 0.75 (○), and 1.0 μM (□). Other details were as described in the text.

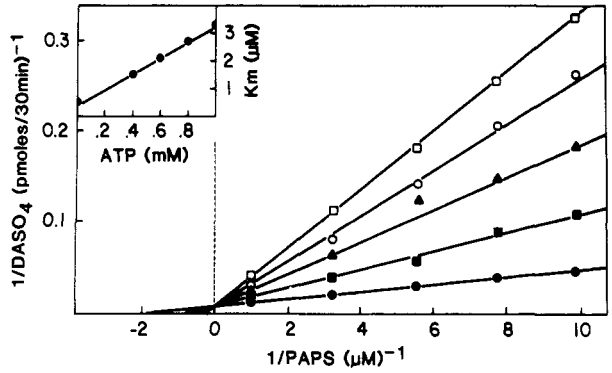


FIGURE 5: Inhibition of purified M-PST by ATP: PAPS as varied substrate. Assays were performed in the presence of 10 μM DA, and ATP concentrations were 0 (●), 0.4 (■), 0.6 (▲), 0.8 (○), and 1.0 mM (□). Other details were as described in the text.

$DASO_4$ dead-end complexes. To rule out this random mechanism, ATP was used as a dead-end inhibitor with respect to PAPS. The results of these experiments, illustrated in Figure 5, reveal a competitive pattern of inhibition and are consistent with M-PST proceeding via an ordered mechanism (see Discussion). Replots of the primary kinetic data indicate that the K_i for ATP was 170 μM.

Discussion

The results presented in this report demonstrate the existence of at least two and possibly three forms of PST in human brain, corresponding to the so-called M and P forms of PST described in human platelets (Rein et al., 1981a). The apparent molecular weight of the M form of the sulfotransferase, as determined by gel filtration chromatography, was approximately 250 000. This molecular weight is substantially different from those reported for PST from a variety of other species and tissues. Sekura & Jakoby (1981) have reported an apparent molecular weight of 61 000 for aryl sulfotransferase IV (which conjugates dopamine at alkaline pH) from rat liver, while Romain et al. (1982) and Borchardt & Schasteen (1982) have reported molecular weights for dopamine-conjugating sulfotransferases from dog and rat liver of 60 000 and 69 000, respectively. More recently, Butler et al. (1983) have demonstrated a molecular weight of 65 000 for partially purified PST from human platelets. Because phenol sulfotransferases seem to share a common molecular weight (irrespective of species) in the range of 60 000–70 000, this would suggest that the active species eluted from Sephacryl S-200 in this report represents an oligomer of smaller molecular weight species, possibly a tetrameric aggregate. Formation of the putative aggregate could be the result of pressure concentration of the enzyme, or it may represent a spontaneous process, as the rat liver enzyme has been found to form aggregates of M_r 300 000 upon storage at 0 °C (Carroll & McEvoy, 1970).

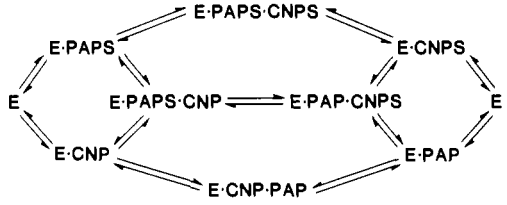
Further differences between purified human brain M-PST and PST from other species are apparent when pH optima for DA sulfation are compared. The optimal pH for DA sulfation by human brain M-PST is approximately 7.0. In contrast, Foldes & Meek (1973) report an optimum for DA sulfation of 9.0 in rat brain, and Sekura & Jakoby (1981) report an identical optimum for purified aryl sulfotransferase IV from rat liver. Romain et al. (1982) have also reported a similar optimum (pH 9.2) for DA sulfation by PST from dog liver. The results reported in this study are in close agreement with data previously published in other studies using crude or partially purified human PST. Butler et al. (1983) describe an optimum for DA conjugation of 6.8 in human platelets, while Rein et al. (1984) have also reported an optimum of 7.0 for partially purified P-PST from human brain.

Examination of the effects of NaCl on M- and P_1-PST (Figure 4) suggests that salt inhibition may be a useful tool in differentiating the two forms of PST in human brain, as the NaCl concentration which inhibits the M form 50% produces essentially complete inhibition of phenol sulfation. Considerable heterogeneity exists in the salt sensitivity of multiple forms of PST from other species as well. Sekura & Jakoby (1979) have demonstrated that aryl sulfotransferases I and II from rat liver exhibit significant activation over a broad range of NaCl concentrations, with inhibition only becoming apparent above 0.8 M NaCl. In contrast, aryl sulfotransferase IV from rat liver is activated at 100 mM NaCl, yet the IC_{50} for this form of the enzyme is only 200 mM (Sekura & Jakoby, 1981).

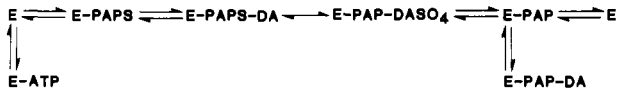
When the sulfation of various amines is examined at a substrate concentration of 20 μM, a number of relationships become apparent. Both shortening of the alkyl side chain (3,4-dihydroxybenzylamine vs. DA) and N-methylation (epinephrine vs. norepinephrine) appear to decrease the ability of M-PST to sulfate biogenic amines, while β-hydroxylation seems to increase the capacity of the enzyme to conjugate a given molecule (norepinephrine vs. DA and normetanephrine vs. 3-methoxytyramine). However, this relationship appears only to hold for catecholamine-like molecules: when octopamine and tyramine are compared, β-hydroxylation actually decreases the sulfation of octopamine at 20 μM. Similar trends have also been noted for the M form of PST in human platelets (Rein et al., 1981c). In addition, sulfation of the 3-OH moiety occurs preferentially over the 4-OH derivative (4-methoxytyramine vs. 3-methoxytyramine and DA). This finding is consistent with the observation that the formation of dopamine 3-*O*-sulfate is favored over that of 4-*O*-sulfate by a factor of 4 in high-speed supernatant fractions from human brain (Renskers et al., 1980). Similar high ratios of 3-*O*- to 4-*O*-sulfate have also been reported in human urine (Bronaugh et al., 1975). When substrate concentrations are increased to 200 μM, apparent substrate inhibition is observed for all of the catecholamines and their O-methylated metabolites, with the exception of 3,4-dihydroxybenzylamine. These results are in agreement with previously published reports demonstrating substrate inhibition for those compounds with K_m values below 20 μM (Rein et al., 1981c; Roth et al., 1980).

The kinetic studies presented in this report are consistent with the M form of human brain PST proceeding by an ordered reaction mechanism, where PAPS is the leading sub-

Scheme I



Scheme II



strate. Data obtained by varying concentrations of DA and PAPS with purified human brain M-PST are consistent with either a rapid-equilibrium, random-order mechanism or a steady-state, compulsory-order ternary complex mechanism. Product inhibition studies with PAP exclude the simple, random mechanism and are also inconsistent with a Theorell–Chance mechanism. The competitive pattern of inhibition observed when PAP is used as an inhibitor with respect to PAPS suggests that PAPS is the leading substrate in the proposed ordered mechanism. Further evidence in support of an ordered mechanism for human brain M-PST is indicated by the uncompetitive pattern of inhibition by the substrate DA with respect to PAPS (Roth et al., 1982). Although compulsory-ordered mechanisms where PAPS is the leading substrate have been proposed in the past for rat (Pennings et al., 1978; Pennings & van Kempen, 1982) and human brain PST (Roth et al., 1982), none of these studies have ruled out the mechanism for PST described for the substrate 2-chloro-4-nitrophenol (CNPS) by Duffel & Jakoby (1981) (Scheme I). While product inhibition studies with $DASO_4$ would, in theory, differentiate an ordered mechanism from that shown in Scheme I, unpublished studies in our laboratory have shown that $DASO_4$ is not an inhibitor of M-PST, even in millimolar concentrations, implying that the formation of the E–PAP–$DASO_4$ ternary complex may possibly be an irreversible step. In contrast, Duffel & Jakoby (1981) have reported that rat liver aryl sulfotransferase IV readily catalyzes the reverse reaction with *p*-nitrocatechol sulfate and PAP as substrates. These authors also have reported that ATP is an effective inhibitor of PST, presumably acting as a dead-end inhibitor by competing with PAPS for the nucleotide-binding site of the enzyme. In an ordered-reaction mechanism where PAPS is the leading substrate, ATP can only bind to free enzyme. In contrast, for the random mechanism shown above, ATP can form three dead-end complexes (with free enzyme, E–DA, and E–$DASO_4$ species). Thus, a mixed pattern of inhibition with respect to PAPS would be predicted with ATP inhibition, assuming the mechanism proposed by Duffel & Jakoby (1981), while a simple, competitive inhibition pattern would be obtained if human brain M-PST proceeded via an ordered mechanism. Therefore, the competitive pattern of inhibition observed in Figure 5 is only consistent with an ordered, bireactant mechanism, as shown in Scheme II.

In summary, the purified dopamine-sulfating form of PST isolated from human brain possesses a pH optimum, salt sensitivity, and kinetic mechanism that differ markedly from sulfotransferases isolated from guinea pig (Banerjee & Roy, 1966), rat (Sekura & Jakoby, 1979, 1981; Duffel & Jakoby, 1981; Borchardt & Schasteen, 1982), and dog liver (Romain et al., 1982), as well as rat brain (Foldes & Meek, 1973; Meek & Neff, 1973; Pennings et al., 1978). Both the biochemical characteristics and the kinetic mechanism strongly suggest that human brain M-PST is unique among the sulfotransferases that have been characterized to date.

Registry No. PST, 9026-09-9; DA, 51-61-6; PAPS, 482-67-7; PAP, 1053-73-2; ATP, 56-65-5; 4-methoxytyramine, 3213-30-7; norepinephrine, 51-41-2; normetanephrine, 97-31-4; 3-methoxytyramine, 554-52-9; epinephrine, 51-43-4; tyramine, 51-67-2; 3,4-dihydroxybenzylamine, 37491-68-2; octopamine, 104-14-3; 6-hydroxydopamine, 1199-18-4; 5-hydroxytryptamine, 50-67-9.

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Characterization of an Inorganic Phosphate Binding Site on the Isolated, Reconstitutively Active β Subunit of $F_0 \cdot F_1$ ATP Synthase†

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ABSTRACT: One binding site for phosphate (P_i) has been demonstrated on the reconstitutively active β subunit that has been removed from the *Rhodospirillum rubrum* membrane-bound ATP synthase ($RrF_0 \cdot F_1$). Under optimal conditions 1 mol of P_i is bound per mole of β subunit with a K_d of 270 ± 30 μM and a half-time of 15 min. P_i binding to β is absolutely dependent on $MgCl_2$, and for its stable binding, $MgCl_2$ must be present not only during the binding step but also during the elution–centrifugation step used to separate the bound and free [^{32}P]P_i. The binding of P_i is inhibited by the presence of ATP or ADP. When present at low concentrations (5–50 μM) both nucleotides inhibit P_i binding to β in a noncompetitive manner with a K_i of 10 μM. At higher concentrations (0.1–10 mM) the inhibition becomes competitive with ATP being a much more effective inhibitor (K_i = 350 μM) than ADP (K_i = 10 mM). These results indicate that P_i binds to the $MgCl_2$-dependent low-affinity nucleotide binding site that has been demonstrated on the isolated *R. rubrum* β subunit [Gromet-Elhanan, Z., & Khananshvili, D. (1984) *Biochemistry 23*, 1022–1028] probably at the site occupied by the γ-phosphoryl group of ATP. The observation that estimated K_d values for binding of P_i, ADP, and ATP to this $MgCl_2$-dependent low-affinity binding site on β are very similar to the reported K_m for ATP hydrolysis and for P_i and ADP during photophosphorylation indicates that this site might be the catalytic site of the $RrF_0 \cdot F_1$ ATP synthase.

The molecular mechanism of ATP synthesis and hydrolysis by the proton-translocating reversible $F_0 \cdot F_1$ ATP synthase is still unknown, although a number of mechanisms have been proposed (Boyer et al., 1977; Cross, 1981). One possible approach to the elucidation of this problem is to identify and characterize the substrate binding sites on this enzyme complex. Previous studies have demonstrated the presence of several nucleotide binding sites that reside in the two larger subunits of the $F_0 \cdot F_1$ ATP synthase, α and β (Harris, 1978; Shavit, 1980; Cross, 1981; Senior & Wise, 1983). They seem to include both catalytic and regulatory sites, but the exact subunit location of each category is not clear. A number of F_1-ATPases have also been reported to bind P_i[1] (Penefsky, 1977; Kasahara & Penefsky, 1978) or its analogue 4-azido-2-nitrophenyl phosphate (Lauquin et al., 1980; Pougeois et al., 1983a,b). One (Penefsky, 1977) or possibly two (Kasahara & Penefsky, 1978) P_i binding sites have been found on MF_1. The binding site of the phosphate analogue has been located on the β subunit (Pougeois et al., 1983a,b).

A detailed characterization of individual substrate binding sites on the $F_0 \cdot F_1$ enzyme complex is, however, very difficult because (a) it contains two or three copies of $\alpha\beta$ pairs that could be in different conformational states in the catalytically active complex (Grubmeyer et al., 1982; O'Neal & Boyer, 1984) and (b) its catalytic activity leads to interconversion of the substrates. These problems could be circumvented by direct examination of substrate binding sites on isolated, purified, reconstitutively active α and β subunits. Such subunits have been obtained up to now only from three different bacterial sources, i.e., from a thermophilic bacterium (Yoshida et al., 1977), from *Escherichia coli* (Futai, 1977), and from *Rhodospirillum rubrum* (Philosoph et al., 1977; Khananshvili & Gromet-Elhanan, 1982a). The isolated subunits provide

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[1] Abbreviations: $RrF_0 \cdot F_1$, proton-translocating ATP synthase–ATPase complex of *R. rubrum*; RrF_1, soluble *R. rubrum* ATPase; CF_1, soluble chloroplast ATPase; MF_1, soluble mitochondrial ATPase; P_i, inorganic phosphate; Tricine, *N*-[tris(hydroxymethyl)methyl]glycine; Mes, 2-(*N*-morpholino)ethanesulfonic acid; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; EDTA, ethylenediaminetetraacetic acid; SDS, sodium dodecyl sulfate.

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