Localization and Functional Analysis of the Substrate Specificity/Catalytic Domains of Human M-form and P-form Phenol Sulfotransferases*

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Human monoamine (M)-form and simple phenol (P)-form phenol sulfotransferases (PSTs), which are greater than 95% identical in their primary sequences, were used as models for investigating the structural determinants responsible for their distinct substrate specificity and other enzymatic properties. A series of chimeric PSTs were constructed by reciprocal exchanges of DNA segments between cDNAs encoding M-form and P-form PSTs. Functional characterization of the recombinant wild-type M-form, P-form, and chimeric PSTs expressed in Escherichia coli and purified to homogeneity revealed that internal domain-spanning amino acid residues 84–148 contain the structural determinants for the substrate specificity of either M-form or P-form PST. Data on the kinetic constants (Km, Vmax, and Vmax/Km) further showed the differential roles of the two highly variable regions (Region I spanning amino acid residues 84–89 and Region II spanning amino acid residues 143–148) in substrate binding, catalysis, and sensitivity to the inhibition by 2,6-dichloro-4-nitrophenol. In contrast to the differential sulfotransferase activities of M-form and P-form PSTs toward dopamine and n-nitrophenol, the Dopa/tyrosine sulfotransferase activities were found to be restricted to M-form, but not P-form PST. Furthermore, the variable Region II of M-form PST appeared to play a predominant role in determining the Dopa/tyrosine sulfotransferase activities of chimeric PSTs. Kinetic studies indicated the role of manganese ions in dramatically enhancing the binding of n-p-tyrosine to wild-type M-form PST. Taken together, these results pinpoint unequivocally the sequence encompassing amino acid residues 84–148 to be the substrate specificity/catalytic domain of both M-form and P-form PSTs and indicate the importance of the variable Regions I and II in determining their distinct enzymatic properties.

Sulfation is a major pathway for the biotransformation/excretion of drugs and xenobiotics as well as endogenous compounds such as catecholamines, cholesterol, steroid and thyroid hormones, and bile acids (1–3). In mammalian cells, the cytosolic sulfotransferases constitute a group of enzymes that catalyze the transfer of a sulfonate group from the active sulfate, PAPS,1 to a substrate compound containing either a hydroxyl or an amino group (4). Two essential components of their catalytic actions therefore are the PAPS binding activity (which is common among various sulfotransferases) and the substrate binding activity (which is unique for individual sulfotransferases). Through sequence comparison, two highly conserved regions (YPKSGTXW close to the N terminus and RKGXXGD-WKXNFT near the C terminus) among different cytosolic sulfotransferases had been identified (3). Of these two regions, the latter was shown to be similar to a motif, designated the P-loop, found in the sequences of many ATP- and GTP-binding proteins (5). It was further pointed out (6) that sequences homologous to the C-terminal conserved region are present in PAPS-synthesizing enzymes from Rhizobium meliloti (7) and Escherichia coli (8). Since PAPS is a co-substrate for the sulfation reactions catalyzed by all cytosolic sulfotransferases, it has been speculated that these highly conserved regions may be involved in PAPS binding (3, 5, 6). Some supporting evidence came from an affinity labeling study (9), which showed the labeling of two amino acid residues (lysine 65 and cysteine 66) that are proximal to the conserved region close to the N terminus as mentioned above. With regard to the conserved region near the C terminus, point mutations with alanines substituting for the critical glycine and lysine residues of the P-loop-related sequence in guinea pig estrogen sulfotransferase were shown to result in dramatic decrease in sulfotransferase activity and inability to photoaffinity label with [35S]PAPS (5). Another site-directed mutagenesis study using plant flavonol 3-sulfotransferase also revealed the importance of the invariant arginine residue of the P-loop-related sequence in PAPS binding (10).

Compared with the PAPS binding site, much less information is available regarding the structural determinants for the substrate binding sites of sulfotransferase enzymes. Studies using plant flavonol 3-sulfotransferase and flavonol 4-sulfotransferase, which display distinct substrate and position specificity, have revealed an internal region spanning amino acid residues 92–194 of flavonol 3-sulfotransferase also revealed the importance of the invariant arginine residue of the P-loop-related sequence in PAPS binding (10).

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1 The abbreviations used are: PAPS, adenosine 3′-phosphate 5′-phosphosulfate; PST, phenol sulfotransferase; Dopa, 3,4-dihydroxyphenylalanine; DCNP, 2,6-dichloro-4-nitrophenol; Taps, 3-[(4-hydroxymethyl)methylamino]propanesulfonic acid; PCR, polymerase chain reaction; APS, adenosine 5′-phosphosulfate.
f erases as model enzymes showed that the central region spanning amino acid residues 102–164 is essential for the hydroxysteroid sulfotransferase activity (12). Precise structural determinants for the substrate specificity and/or catalytic activity for both these two sulfotransferase models, however, remain largely unknown, except for a recent mutational analysis study suggesting that His-118 of flavonol 3-sulfotransferase might be involved in catalysis (13). More work and better sulfotransferase models are needed to pin down unequivocally the particular regions and specific amino acid residues therein that are responsible for the substrate binding and/or catalytic activities.

In searching for sulfotransferase models for investigating their substrate binding/catalytic sites, our attention has been focusing on human phenol sulfotransferases. Three distinct phenol sulfotransferases have been identified in human tissues (14). One of them is the so-called M-form PST that preferentially catalyzes the sulfation of monoamines such as dopamine (15). The other two phenol sulfotransferases, designated the P-form phenol sulfotransferases, preferentially catalyze the sulfation of simple phenolic compounds such as p-nitrophenol (16–18). In addition to their distinct substrate specificity, earlier studies have demonstrated M-form PST to be markedly more thermolabile and 3 orders of magnitude less sensitive to inhibition by 2,6-dichloro-4-nitrophenol (DCNP), a sulfation inhibitor (19). Our recent study also showed that, in contrast to P-form PSTs, M-form PST displayed stereoselective and manganese-dependent Dopa/tyrosine sulfotransferase activities (20). Since M-form and P-form PSTs are greater than 95% identical in their primary sequences (cf. Fig. 1A) and yet display distinct substrate specificity and other properties, these two enzymes serve as an excellent model for investigating the structural determinants for their substrate binding and catalytic activities.

In the present work, a series of chimeric PSTs were constructed by reciprocal exchanges of DNA fragments between wild-type M-form, or chimeric PSTs. To prepare the first set of chimeric PSTs, wild-type M-form and P-form PST cDNAs were used. Two chimeric PST cDNA clones, designated MPPM and PMMP, were generated by BanI restriction digestion of wild-type M-form and P-form PST sequences, followed by ligation of the middle 281-base pair fragment with the two flanking fragments derived from the opposite wild-type PST sequence. The chimeric PST sequences thus generated, as well as wild-type sequences were cloned into the pGEX-2TK prokaryotic expression vector. Construction of the four additional chimeric PST clones (MPPP, MPMMP, PMPM, and PPMM) were carried out similarly by reciprocal exchange between the two wild-type (M-PST and P-PST) and two chimeric PSTs (MPPP and PMPM) packaged in pGEX-2TK following Bou61I digestion. pGEX-2TK plasmids containing, respectively, the two wild-type and six chimeric PST constructs were transformed into E. coli BL21 host cells for the expression of the recombinant wild-type M-form, P-form, and chimeric PST enzymes.

**Bacterial Expression and Purification of the Wild-type and Chimeric PSTs—Competent E. coli BL21 cells transformed with pGEX-2TK harboring the cDNA encoding either wild-type or chimeric PST were grown to A600 free = 0.1 in 750 ml of LB medium supplemented with 100 μg/ml ampicillin. Upon induction with 0.1 mM isopropyl β-D-thiogalactopyranoside at room temperature, the cells were centrifuged and homogenized in 20 ml of an ice-cold lysis buffer (containing 10 mM Tris-HCl, pH 8.0, 150 mM NaCl, 1 mM EDTA) using an Amino French press. The crude homogenate thus prepared was subjected to centrifugation at 10,000 × g for 30 min at 4 °C. The supernatant collected was subjected to the analysis of 35S-sulfated product by either an isoform of P-form PST (23), were synthesized with the procedure previously developed (24).**

**EXPERIMENTAL PROCEDURES**

**Materials—**L-Dopa, D-Dopa, L-p-tyrosine, D-p-tyrosine, L-m-tyrosine, dopamine, p-nitrophenol, aprotinin, thrombin, ATP, AMP, SDS, Hepes, Taps, Trizma base (Tris base), dithiothreitol, DCNP, and isopropyl β-D-thiogalactopyranoside were products of Sigma. L-p-tyrosine-O-sulfate, D-p-tyrosine-O-sulfate, L-m-tyrosine-O-sulfate, dopamine-O-sulfate, and a mixture of L-Dopa-3-O-sulfate and L-Dopa-4-O-sulfate were synthesized according to the procedure of Jevons (21). pGEX-2TK gene fusion vector, E. coli BL21 host cells, and glutathione-Sepharose were products of Pharmacia Biotech Inc. pBluescript II SK (+) and XL1-Blue MRF’ E. coli host strain were purchased from Stratagene. An L.M.A.G.E. cDNA clone 229827 (GenBank™ Accession number H67938) encoding an isoform of P-form PST was from Genome Systems, Inc. AmpliTaq DNA polymerase was from Perkin-Elmer. Long and accurate polymerase chain reaction (PCR) kit version 2 was from Takara (Shiga, Japan). Taq dye primer cycle sequencing kits were products of Applied Biosystems, Inc. T4 DNA polymerase, T4 DNA ligase, and all restriction endonucleases used were from New England Biolabs. Sulfate-activating enzymes (ATP sulfurylase and APS kinase) from Bacillus stearothermophilus and all restriction endonucleases used were kindly provided by Dr. Hiroshi Nakajima at Unitika (Uji, Japan). Oligonucleotide primers were from Operon Technologies, Inc. Carrier-free sodium [35S]sulfate was from ICN Biomedicals. All other chemicals were of the highest grades commercially available.

**PCR Cloning of Human Liver Phenol Sulfotransferases—**A set of sense (5'-GGGAGATTCTCGCAAGCTCAAGGACACC-3') and antisense (5'-GGGCGACTTCTACAGCTCAGGGAACGT-3') oligonucleotide primers, based on 5'- and 3'- homologous regions of the reported nucleotide sequences encoding human liver M-form PST (22) and an isoform of P-form PST (23), were synthesized with BanI and EcoRI restriction sites incorporated at the ends. With these two oligonucleotides as primers, PCR in 100 μl reaction mixtures were carried out under the reaction conditions described using the human liver M-form PST or P-form PST cDNA as the template. Amplification conditions were 30 cycles of 1 min at 94 °C, 1 min at 55 °C, and 3 min at 72 °C. The final reaction mixtures were applied onto a 0.7% agarose gel, separated by electrophoresis, and visualized by ethidium bromide staining. The discrete 906-base pair PCR products detected were excised from the gel, and the DNA fragments therein were isolated by phenol precipitation. After BanI and EcoRI digestion, the PCR products were individually subcloned into the BanI/HindIII site of pScript II SK (+) and transformed into E. coli XL1-Blue MRF’. To verify their authenticity, the inserts were completely sequenced in both directions using automated cycle sequencing method.

**Preparation of Chimeric Constructs—**Plasmids containing chimeric constructs were prepared as reciprocal exchange of internal DNA fragments. Two wild type PST fragments, between the two wild-type (M-PST and P-PST) and two chimeric PSTs (MPPP and PMPM) packaged in pGEX-2TK following Bou61I digestion. pGEX-2TK plasmids containing, respectively, the two wild-type and six chimeric PST constructs were transformed into E. coli BL21 host cells for the expression of the recombinant wild-type M-form, P-form, and chimeric PST enzymes.

**Enzymatic Assay—**The sulfotransferase assays were performed using PAP[35S] as the sulfate donor. The standard assay mixture, with a final volume of 30 μl, contained 50 mM Hepes-NaOH, pH 7.0, 250 mM sucrose, 1 mM dithiothreitol, 14 μM PAP[35S] (15 Ci/mmol), and dopamine (25 μM) or p-nitrophenol (5 μM) as substrate. For assays examining the inhibitory effect of DCNP on the sulfation of dopamine or p-nitrophenol, the assay mixtures were supplemented with different concentrations of DCNP. For the assays using tyrosine or Dopa as substrates, the standard assay mixture, with a final volume of 30 μl, contained 50 mM Tris-HCl, pH 8.0, 150 mM NaCl, and 2.5 mM CaCl2. After a 1-h incubation at room temperature, 5 μg of aprotinin were added to inactivate thrombin, and the preparation was subjected to centrifugation. The chimeric enzyme present in the supernatant collected was used for the enzymatic characterization.

**Miscellaneous Methods—**PAP[35S] (15 Ci/mmol) was synthesized from ATP and [35S]sulfate using ATP sulfurylase and APS kinase from B. stearothermophilus as described previously (25). SDS-polyacrylamide gel electrophoresis was performed on 12% polyacrylamide gels using the method of Laemmli (26). Protein determination was based on the method of Lowry et al. (27). Protein determination was based on the meth
results

Since the human M-form and P-form PSTs are >93% identical in their primary sequences and yet display distinct substrate specificity and other properties, these two enzymes serve as an excellent model for investigating the structural determinants for the substrate binding specificity as well as the catalytic activity. Examining the aligned amino acid sequences of these two PSTs (22,23), two highly variable regions, designated Regions I and II, were noted (Fig. 1A). It was therefore hypothesized that these two regions may contain the structural determinants for the substrate specificity and/or catalytic activity of these two homologous enzymes. Taking advantage of two BanI restriction sites that are conveniently located close to the 5' and 3' side of the nucleotide sequences encoding Regions I and II (Fig. 1B), we decided to first prepare two chimeric cDNAs, designated MPPM and PMMP (Fig. 1B). To further narrow down the differential importance of Regions I and II, four more chimeric PST clones were prepared by making use of the Bsu36I restriction site located between Regions I and II (Fig. 1B). The recombinant chimeric PSTs as well as wild-type M-form and P-form PSTs were expressed for functional characterization.

Construction and Expression of Chimeric Phenol Sulfotransferases—Based on the procedure described under “Experimental Procedures,” wild-type M-form and P-form PST cDNAs and chimeric PST cDNAs constructed were individually subcloned into the pGEX-2TK prokaryotic expression vector and transformed into E. coli host cells for expressing the recombinant enzymes. As shown in Fig. 2, the recombinant wild-type and chimeric PSTs fractionated from the homogenates of transformed E. coli host cells using glutathione-Sepharose and cleaved off the fusion proteins by thrombin appeared to be highly homogeneous upon SDS-polyacrylamide gel electrophoresis. It was noted that there were some differences in electrophoretic mobility among M-form and P-form PSTs as well as chimeric PSTs despite the similar predicted molecular masses of these enzymes (cf. Fig. 1A). While the reasons are not clear, this aberrant phenomenon has previously been documented (17). It is to be pointed out that human M-form and P-form PSTs have also been expressed previously in either E. coli or COS cells (17, 22, 29–31).

Sulfotransferase Activities of Recombinant Wild-type M-form, P-form, and Chimeric PSTs toward Dopamine and p-Nitrophenol—To examine whether the sequence defined by the variable Regions I and II is indeed responsible for the substrate specificity, the recombinant wild-type and chimeric PSTs were subjected to sulfotransferase assays using dopamine (a typical substrate for M-form PST activities) and p-nitrophenol (a typical substrate for P-form PST activities). As shown in Table I, wild-type M-form and P-form PSTs, as expected, displayed distinct substrate specificities, with the former having higher \( \frac{V_{\text{max}}}{K_m} \) for dopamine and the latter having higher \( \frac{V_{\text{max}}}{K_m} \) for p-nitrophenol. Interestingly, when the regions coded by the 281-base pair BanI fragments (cf. Fig. 1) were reciprocally exchanged, the substrate phenotypes of the resulting chimeric PSTs (MPPM and PMMP) switched to the ones originally displayed by the opposite PSTs. The \( \frac{V_{\text{max}}}{K_m} \) values of PMMP for dopamine or p-nitrophenol were surprisingly close to those of the wild-type M-form PST. The \( \frac{V_{\text{max}}}{K_m} \) values of

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**Fig. 1. A.** Amino acid sequence comparison of the human M-form and P-form phenol sulfotransferases. Identical amino acid residues are boxed. **B.** Schematic representation of the molecular architecture of wild-type M-form, P-form, and chimeric PSTs.

**Fig. 2.** SDS gel electrophoretic patterns of purified wild-type M-form, P-form, and chimeric PSTs. Purified PST samples subjected to SDS-polyacrylamide gel electrophoresis followed by Coomassie Blue staining. Lane 1, wild-type M-form PST; lane 2, wild-type P-form PST; lane 3, MPPM; lane 4, PMMP; lane 5, MPMM; lane 6, MMPM; lane 7, PMPP; lane 8, PPMP.
MPPM for dopamine or p-nitrophenol, although showing some variations, were also close to those of the wild-type P-form PST. Based on these results, it can be concluded that the internal variations, were also close to those of the wild-type P-form PST. For all eight wild-type M-form, P-form, and chimeric PSTs, dopamine was used as the substrate. Furthermore, a dramatic decrease in \( K_m \) with p-nitrophenol as substrate was observed. These results may suggest that the incorporation of Region I or II sequence derived from M-form PST, which are intrinsically more efficient than those present in the Region I sequence of P-form PST. Taken collectively, the kinetic constant data compiled in Table I provide ample evidence indicating that the sequence coded by the 281-base pair BamI fragment indeed contains the structural determinants for the substrate specificity and, to a large extent, the catalytic activity of either M-form or P-form PST. This is also supported by the data on the Dopa/tyrosine sulfotransferase activities (cf. Fig. 1) of MPMM, MMPM, PMPP, and PPMP that have been subjected to reciprocal exchanges of shorter sequences encompassing either Region I or Region II, the results were also informative, although somewhat more complicated. Their kinetic constants compiled in Table I indicate the importance of intrinsically more efficient than those present in the Region I sequence of P-form PST. Taken collectively, the kinetic constant data compiled in Table I provide ample evidence indicating that the sequence coded by the 281-base pair BamI fragment contains the structural determinants for both the substrate specificity and the catalytic activity of either M-form or P-form PST.

### Table I

| Enzyme | \( K_m \) (\( \mu \text{M} \)) | \( V_{\text{max}} \) (nmol/min/mg) | \( V_{\text{max}}/K_m \) | \( K_m \) (\( \mu \text{M} \)) | \( V_{\text{max}} \) (nmol/min/mg) | \( V_{\text{max}}/K_m \) |
|--------|-----------------|-------------------------------|-----------------|-----------------|-------------------------------|-----------------|
| M-PST  | 1.36 ± 0.05     | 17.3 ± 0.8                    | 12.7            | 166.5 ± 15.8    | 12.5 ± 0.9                    | 0.08 |
| P-PST  | 87.5 ± 3.9      | 4.04 ± 0.14                   | 0.05            | 0.36 ± 0.03     | 9.95 ± 0.25                   | 25.6 |
| MPPM   | 91.4 ± 7.9      | 2.47 ± 0.84                   | 0.03            | 0.21 ± 0.01     | 3.05 ± 0.01                   | 14.5 |
| MMPM   | 1.48 ± 0.16     | 17.9 ± 1.5                    | 12.1            | 127.3 ± 19.8    | 12.6 ± 1.4                    | 10.0 |
| MMPPM  | 611.3 ± 15.7    | 3.72 ± 0.09                   | 0.01            | 102.6 ± 6.5     | 2.06 ± 0.06                   | 0.02 |
| MPPM   | 34.8 ± 1.0      | 8.28 ± 0.22                   | 0.24            | 19.4 ± 1.0      | 7.96 ± 0.34                   | 0.41 |
| PMPPM  | 98.4 ± 25.4     | 7.58 ± 1.59                   | 0.08            | 39.5 ± 2.8      | 10.5 ± 0.4                    | 0.27 |
| PPMPM  | 59.0 ± 6.7      | 6.01 ± 0.49                   | 0.10            | 25.6 ± 2.9      | 2.49 ± 0.11                   | 0.10 |

Specific activity refers to activity/mg protein of purified wild-type M-form, P-form, or chimeric PST. Data shown represent means ± S.D. derived from five determinations.

### Table II

| Enzyme | L-Dopa | D-Dopa | DL-\( \text{-m-Tyrosine} \) | L-\( \text{-p-Tyrosine} \) | D-\( \text{-p-Tyrosine} \) |
|--------|--------|--------|-----------------|-----------------|-----------------|
| M-PST  | 70.3 ± 1.9 | 91.3 ± 2.6 | 34.6 ± 1.6 | 0.82 ± 0.25 | 8.70 ± 0.81 |
| P-PST  | ND     | ND     | ND              | ND              | ND              |
| MPPM   | ND     | ND     | ND              | ND              | ND              |
| MMPM   | 57.1 ± 1.7 | 88.3 ± 2.6 | 27.1 ± 1.4 | 1.00 ± 0.27 | 6.87 ± 0.73 |
| MMPPM  | 32.5 ± 1.7 | 61.6 ± 2.5 | 10.7 ± 0.9 | 6.06 ± 1.2 | 6.76 ± 0.96 |
| MPPM   | ND     | ND     | ND              | ND              | ND              |
| PMPP   | ND     | ND     | ND              | ND              | ND              |
| PPMP   | 27.4 ± 1.2 | 67.6 ± 2.3 | 11.1 ± 0.9 | ND              | ND              |

Specific activity refers to activity/mg protein of purified wild-type M-form, P-form, or chimeric PST. Data shown represent means ± S.D. derived from five determinations.

**Fig. 3.** Effect of increasing concentrations of DCNP on the activities of wild-type M-form, P-form, and chimeric PSTs. For wild-type M-form and chimeric PMPP PSTs, dopamine was used as the substrate. For wild-type P-form and other chimeric PSTs, p-nitrophenol was used as the substrate. ○, M-PST; □, P-PST; ×, MPPM; ■, MMPM; ▲, MMPPM; ▼, PMPP; □, PPMP.
Regions I and II may in fact account for the distinct substrate binding and catalytic activities between M-form and P-form PSTs. It is interesting to note that analysis of aligned amino acid sequences has revealed a low degree of homology in the central region among cytosolic sulfotransferases (3, 32). It is possible, therefore, that the central region may also constitute the substrate specificity domain for other sulfotransferase enzymes. The results presented above indeed provide support for this hypothesis.

**DCNP Inhibition of Wild-type M-form, P-form, and Chimeric PSTs**—Sensitivity to the inhibition by DCNP, a sulfotransferase inhibitor (19), has been widely used to distinguish M-form and P-form PSTs present in a variety of human tissues (33, 34). We were interested in investigating the structural basis for the differential sensitivity of these two highly homologous sulfotransferases to DCNP. As shown in Fig. 3, similar to the results previously reported (33, 34), recombinant P-form PST was approximately 3 orders of magnitude more sensitive to the inhibition by DCNP than wild-type M-form PST. For the two chimeric PSTs (MPPM and PMPP) with the regions coded by the 281-base pair PST. For the two chimeric PSTs (MMPM and PMPP) with the regions coded by the 281-base pair PST. Reciprocally exchanged, the DCNP inhibition phenotype changed to that of the opposite PST. For chimeric PSTs (MPPM, MMPM, PMPP, and PMPP) that have been subjected to reciprocal exchange of a shorter sequence encompassing either Region I or Region II, the sensitivities lie in between those of the M-form and P-form PSTs. Their IC_{50} values, however, were closer to those of the parental sources of the newly incorporated sequences that contained Region II. These results collectively indicate that for both M-form and P-form PSTs, the sequence defined by the variable Regions I and II (cf. Fig. 1) also contains the structural determinants for the sensitivity to the inhibition by DCNP. Moreover, of the two variable regions, Region II appeared to play a more important role in determining this property.

**Dopa and Tyrosine Sulfation**—In a recent study (20), we have demonstrated that M-form PST exhibited unique Dopa/tyrosine sulfotransferase activities. This finding led us to hypothesize that M-form PST, among other physiological involvement, may also contribute to the homeostatic regulation of Dopa and tyrosine by converting the excess of these compounds to the more water-soluble sulfated forms, thereby facilitating their removal from the body. Since Dopa and tyrosine serve as precursors for the biosynthesis of catecholamines (including dopamine, norepinephrine, and epinephrine), the M-form PST may be important in vivo for the normal functioning of the nervous system and/or the endocrine system. To investigate the structural determinants for the Dopa/tyrosine sulfotransferase activities of M-form PST, we decided to characterize the enzymatic activities of the wild-type M-form, P-form, and chimeric PSTs. As shown in Table II, in contrast to the differential activities of M-form and P-form PSTs toward dopamine or p-nitrophenol, only M-form, but not P-form, PST could catalyze the sulfation of Dopa and tyrosine isomers. When the regions coded by the 281-base pair PST. Reciprocally exchanged, the chimeric PSTM, but not MMPM, displayed the similar Dopa/tyrosine sulfotransferase activities. These results provided the most clear-cut evidence indicating that the sequence defined by the variable Regions I and II in M-form PST contains the structural determinants for the substrate specificity for Dopa and tyrosine. Of the other four chimeric PSTs, MPMM and PPMP, but not MMPM and PMPP, displayed lower yet comparable, Dopa/tyrosine sulfotransferase activities for L-Dopa, d-Dopa, and D,L-L-tyrosine. No sulfotransferase activities were detected for MPMM and PPMP with D-p-tyrosine or L-p-tyrosine as substrate. Although it is possible that their activities might have been below the detection limit of the analytical method used, the results may indicate that the presence of the M-form PST Region II sequence alone may allow only the sulfation of m-hydroxyl group, but not p-hydroxyl group. A combination of the M-form PST Regions I and II may be required for the sulfation of p-hydroxyl group. In another study, we have demonstrated unequivocally the sulfation of Dopa, as catalyzed by M-form PST, being exclusively on the m-hydroxyl group.2 Taken together, the results compiled in Table II indicate that variable Region II of M-form PST plays a predominant role in the sulfotransferase activities for Dopa and tyrosine, although variable Region I may be needed for the sulfotransferase activity for L- or D-p-tyrosine.

**Effects of Manganese Ions on the Kinetic Constants of Wild-type M-form PST with D-p-tyrosine as substrate**

| Assay Condition | K_m (mM) | V_{max} (nmol/min/mg) | V_{max}/K_m |
|-----------------|----------|-----------------------|-------------|
| Without Mn^{2+} | 28.67 ± 9.17 | 2.52 ± 0.78 | 0.09 |
| With 10 mM Mn^{2+} | 0.36 ± 0.06 | 8.44 ± 1.10 | 23.44 |

2 M. Suiko, Y. Sakakibara, H. Sakaida, H. Yoshikawa, and M.-C. Liu, unpublished results.
and l-m-tyrosine, which serve directly or indirectly as biosynthetic precursors for catecholamines (37), may become sulfated and excreted. The lowered levels of these Dopa and Tyrrosine isomers in turn may result in decreased production of dopamine and other catecholamines. Indeed, individuals afflicted with manganese poisoning have been reported to display symptoms resembling those of Parkinson’s disease (38), and reduction or even elimination of some of these symptoms has been observed in those receiving treatment with L-Dopa (28, 38). In a recent study (20), we have demonstrated unequivocally that the manganese-dependent Dopa/tirosine sulfotransferase activities found in HepG2 cells in fact are associated with M-form PST. The stimulatory effect of manganese ions on M-form PST was found to be the strongest with d-p-tyrosine as substrate. To investigate the mechanistic basis of the manganese stimulation of the Dopa/tirosine sulfotransferase activities of M-form PST, we therefore decided to examine the kinetics of the sulfation reaction with d-p-tyrosine as substrate. As shown in Fig. 4, the Lineweaver-Burk double reciprocal plot derived from the data obtained indicated that, although the $V_{\text{max}}$ of the sulfation of d-p-tyrosine in the presence or absence of manganese ions remained in the same order of magnitude, the $K_m$ values appear to be dramatically different. The calculated $V_{\text{max}}$, $K_m$, and $V_{\text{max}}/K_m$ values under these two assay conditions are compiled in Table III. Based on these results, it can be concluded that the presence of manganese ions greatly enhanced the binding of d-p-tyrosine and the catalytic efficiency (by more than 260-fold as reflected by $V_{\text{max}}/K_m$) of M-form PST. Exactly how this is achieved at the molecular level, however, remains to be elucidated.

The results presented in this paper indicate that, for both M-form and P-form PSTs, the sequence spanning amino acid residues 84–148 as defined by the variable Regions I and II (cf. Fig. 1A) is indeed responsible for the substrate specificity phenotype of these two enzymes. This central domain, to a large extent, is also critical for the catalytic activities of these two model enzymes. To elucidate precisely the key structural elements responsible for its functional activities, it will be necessary to identify specific amino acid residues in this domain that are involved in the substrate binding and/or catalytic activities. Examining the aligned amino acid sequences of M-form and P-form PSTs (Fig. 1A), a distinct feature noted is that the two enzymes contain a good number of charged amino acid residues, e.g. Asp86, Glu89, His143, Arg144, Glu146, and Lys147 of M-form PST and Lys85, His143, and Lys147 of P-form PST, within the variable Regions I and II. Since the M-form PST is more capable of catalyzing the sulfation of charged compounds such as dopamine (which contains a positively charged amino group) and Dopa (which contains both a positively charged $\alpha$-amino group and a negatively charged $\alpha$-carboxyl group) and since the P-form PST utilizes preferentially uncharged phenolic compounds as substrates, it is possible that these charged residues may in fact account for their differential enzymatic properties. Site-directed mutagenesis studies are currently under way to change those charged amino acid residues to uncharged or oppositely charged residues for evaluating their possible involvement in substrate binding and/or catalytic activities.

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