Mutational Analysis of the Substrate Binding/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) are greater than 93% identical in their primary sequences and yet display distinct substrate specificities and other enzymatic properties. Through the generation and characterization of a series of chimeric PSTs, we have previously demonstrated two highly variable regions within their sequences to be responsible for determining their substrate phenotypes (Sakakibara, Y., Takami, Y., Nakayama, T., Suiko, M., and Liu, M.-C. (1998) J. Biol. Chem. 273, 6242–6247). By employing the site-directed mutagenesis technique, the present study aims to identify and quantitatively evaluate the specific amino acid residues critical to the substrate binding and catalysis in these two enzymes. Twelve mutated M-PSTs and seven mutated P-PSTs were generated, expressed, and purified. Enzymatic characterization showed that, of the twelve mutated M-PSTs, mutations at residues Asp-86, Glu-89, and Glu-146 resulted in a dramatic decrease in $V_{\text{max}}/K_m$ with dopamine as substrate, being greater than 450 times for the D86A/E89I/E146A mutated M-PST. With p-nitrophenol as substrate, the $V_{\text{max}}/K_m$ determined for the D86A/E89I/E146A-mutated M-PST increased more than 25 times and approached that determined for the wild-type P-PST. These results indicated that the concerted action of the three mutated residues (D86A, E89I, and E146A) is sufficient for the conversion of the substrate phenotype of M-PST to that of P-PST. Among the mutated M-PSTs and P-PSTs, the I89E- and A146E-mutated P-PSTs demonstrated two highly variable regions within their sequences to be responsible for determining their substrate phenotypes (Sakakibara, Y., Takami, Y., Nakayama, T., Suiko, M., and Liu, M.-C. (1998) J. Biol. Chem. 273, 6242–6247). By employing the site-directed mutagenesis technique, the present study aims to identify and quantitatively evaluate the specific amino acid residues critical to the substrate binding and catalysis in these two enzymes. Twelve mutated M-PSTs and seven mutated P-PSTs were generated, expressed, and purified. Enzymatic characterization showed that, of the twelve mutated M-PSTs, mutations at residues Asp-86, Glu-89, and Glu-146 resulted in a dramatic decrease in $V_{\text{max}}/K_m$ with dopamine as substrate, being greater than 450 times for the D86A/E89I/E146A mutated M-PST. With p-nitrophenol as substrate, the $V_{\text{max}}/K_m$ determined for the D86A/E89I/E146A-mutated M-PST increased more than 25 times and approached that determined for the wild-type P-PST. These results indicated that the concerted action of the three mutated residues (D86A, E89I, and E146A) is sufficient for the conversion of the substrate phenotype of M-PST to that of P-PST. Among the mutated P-PSTs, the I89E- and A146E-mutated P-PSTs displayed considerable deviations in $V_{\text{max}}/K_m$ with dopamine or p-nitrophenol as substrate. No corresponding changes, however, were detected with the opposite compound as substrate. These results indicated that, in contrast to M-PST, mutations at Ala-86, Ile-89, and Ala-146 to the corresponding residues in M-PST are not sufficient for rendering the change of P-PST substrate phenotype to that of M-PST. For both M-PSTs and P-PSTs, mutations at Lys-48 or His-108 led to the loss of sulfotransferase activities, indicating their importance in the catalytic mechanism.

Sulfation, or more precisely sulfonation, represents 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). The responsible enzymes, collectively called the cytosolic sulfotransferases, in general catalyze the transfer of a sulfonate group from the active sulfate, 3′-phosphoadenosine 5′-phosphosulfate (PAPS),1 to a substrate compound containing a hydroxyl or an amino group (4). Based on their amino acid sequences, the cytosolic sulfotransferases found in vertebrates have been classified into two gene families: the phenol sulfotransferase (PST) family (designated SULT1) and the hydroxyxysteroid sulfotransferase family (designated SULT2) (5, 6). The PST family presently consists of four subfamilies, PSTs (SULT1A), Dopa/tyrosine (or thyroid hormone) sulfotransferases (SULT1B), hydroxarylamine (or acetylaminofluorene) sulfotransferases (SULT1C), and estrogen sulfotransferases (SULT1E) (5, 6). With the recent discovery of two new human hydroxyxysteroid sulfotransferases (7), the hydroxyxysteroid sulfotransferase family now comprises two subfamilies designated, respectively, SULT2A and SULT2B.

Although the various cytosolic sulfotransferases mentioned above utilize preferentially different compounds as substrates, they share the common requirement for PAPS being the sulfate donor (4). These enzymes, therefore, must in their structures contain binding sites for PAPS and specific substrate compounds. The structural determinants for the PAPS binding activity of the cytosolic sulfotransferases have been rather well characterized. Based on the analysis of aligned sulfotransferase sequences, two highly conserved regions (YPKSGTXX close to the N terminus and RKGXXGDWKNXT near the C terminus) have been identified (6). Of these two regions, the latter resembles the so-called “P-loop” motif found in the sequences of many ATP- and GTP-binding proteins (8). Considering that PAPS is a co-substrate for the sulfation reactions catalyzed by all cytosolic sulfotransferases, it has been proposed that these highly conserved regions may be involved in PAPS binding (5, 6, 8). Some supporting evidence came from an affinity labeling study using a rat aryl sulfotransferase (9), which showed the labeling of two amino acid residues (Lys-65 and Cys-66) proximal to the conserved region near 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 a dramatic decrease in sulfotransferase activity and an inability to photoaffinity label with [32P]PAPS (8). 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). More recently, studies employing affinity chromatography in conjunction with 31P NMR further demon-

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1 The abbreviations used are: PAPS, 3′-phosphoadenosine 5′-phosphosulfate; PST, phenol sulfotransferase; M, monoamine; P, phenol; pNP, p-nitrophenol; Taps, 3-[N-tris-(hydroxymethyl)methylamino]-propanesulfonic acid.
**Mutational Analysis of Human Phenol Sulfotransferases**

**Table I**

Mutagenic oligonucleotide primers used for the generation of mutated human M-PSTs and P-PSTs

| For mutated M-PSTs | Mutated nucleotide residues are bold and underlined. Only the sequence of the sense primer of each mutagenic primer set is shown. |
|--------------------|----------------------------------------------------------------------------------------------------------------------------------|
| N85K               | 5'-CCCTCCTGAGGAATGTTG           |
| D86A               | 5'-CCCTGAGGAATGTTG |
| E89I               | 5'-CCCTGAGGAATGTTG |
| N85K/D86A          | 5'-CCCTGAGGAATGTTG |
| D86A/E89I          | 5'-CCCTGAGGAATGTTG |
| N85K/E89I          | 5'-CCCTGAGGAATGTTG |
| E146A              | 5'-CCCTGAGGAATGTTG |
| D86A/E89I/E146A    | 5'-CCCTGAGGAATGTTG |
| H140Y              | 5'-CCCTGAGGAATGTTG |
| K48M               | 5'-CCCTGAGGAATGTTG |
| H108A              | 5'-CCCTGAGGAATGTTG |
| K48M/H108A         | 5'-CCCTGAGGAATGTTG |

**For mutated P-PSTs**

| I89E               | 5'-GATGTTCAGCCGGGCAAAGGGGAGGAC-3' |
| A146E              | 5'-GATGTTCAGCCGGGAGGAC-3' |
| K48M               | 5'-GATGTTCAGCCGGGAGGAC-3' |
| H108A              | 5'-GATGTTCAGCCGGGAGGAC-3' |
| A86D/I89E          | 5'-GATGTTCAGCCGGGAGGAC-3' |
| I89E/A146E         | 5'-GATGTTCAGCCGGGAGGAC-3' |
| A86D/I89E/A146E    | 5'-GATGTTCAGCCGGGAGGAC-3' |

A. Generated by using D86A/E89I- or K48M-mutated M-PST cDNA packaged in pGEX-2TK as template in conjunction with E146A or H108A mutagenic primers.

B. Generated by using I89E- or A86D/I89E-mutated P-PST cDNA packaged in pGEX-2TK as template in conjunction with A146E mutagenic primers.

strated the involvement of Lys-59, Arg-141, and Arg-277 of flavonol 3-sulfotransferase in the orientation/binding of PAPS (11).

Compared with the PAPS binding site, less information is available regarding the substrate binding domains of sulfotransferase enzymes. Studies using plant flavonol 3-sulfotransferase and flavonol 4-sulfotransferase, which display distinct specificity (12). Another study using rat liver hydroxysteroid sulfotransferase as being responsible for its substrate and position specificity (13). Precise structural determinants for the substrate specificity and/or catalytic activity for both these two sulfotransferase models, however, remain largely unknown. Through the generation and characterization of a series of point-mutated M-PSTs and P-PSTs, we have recently demonstrated two highly variable regions (Fig. 1, designated I and II) present within the sequence spanning amino acid residues 84–148 to be critical for their distinct substrate phenotypes (14).

By employing the site-directed mutagenesis technique, we have embarked on the analysis of amino acid residues that are important for the substrate binding/catalysis of M-PST and P-PST. We report in this communication the results on the generation and characterization of a series of point-mutated M-PSTs and P-PSTs.

**EXPERIMENTAL PROCEDURES**

**Materials**—Dopamine, p-nitrophenol (pNP), ampicillin, aprotinin, thiomalin, adenosine 5'-triphosphate (ATP), sodium dodecyl sulfate (SDS), Taps, Trizma base, and isopropyl-1-thio-β-D-galactopyranoside were products of Sigma. The QuikChange site-directed mutagenesis kit from Stratagene was used for the generation of point-mutated PSTs. Briefly, wild-type M-PST or P-PST cDNA packaged in pGEX-2TK prokaryotic expression vector was used as the template in conjunction with specific mutagenic primers (see Table I for the mutagenic primers used). The amplification conditions were 12 cycles of 30 s at 95 °C, 1 min at 55 °C, and 1.5 min at 68 °C. The mutated M-PST and P-PST sequences were verified by nucleotide sequencing (16). pGEX-2TK vector harboring individual mutated PST sequence was transformed into competent XL1-Blue E. coli cells. The transformed cells, grown to an _A_ 

**Enzymatic Assay**—Sulfotransferase activities of purified point-mutated M-PSTs and P-PSTs were assayed using [[35S]]PAPS as the sulfate donor. The standard assay mixture, with a final volume of 30 μl, contained 50 mM potassium phosphate buffer, pH 7.0 (or 50 mM Tris-Cl, pH 8.0, or 100 mM NaAc, 1 mM EDTA) using an Aminco French press. The crude enzyme preparation, allowed to proceed for 10 min at 37 °C, and terminated by heating at 100 °C for 2 min. The precipitates formed were cleared by centrifugation. The clear supernatant was subjected to the analysis of [[35S]]-labeled pNP or dopamine based on the thin-layer chromatography procedure previously established (17).

**Miscellaneous Methods**—[[35S]]PAPS (15 Ci/mmol) and 10 μM pNP (10 μM dopamine). For kinetic studies, varying concentrations of pNP or dopamine were used. The reaction was started by the addition of the enzyme preparation, allowed to proceed for 10 min at 37 °C, and terminated by heating at 100 °C for 2 min. The precipitates formed were cleared by centrifugation. The clear supernatant was subjected to the analysis of [[35S]]-labeled pNP or dopamine based on the thin-layer chromatography procedure previously established (17). Sulfotransferase activities of purified point-mutated M-PSTs and P-PSTs were assayed using [[35S]]PAPS as the sulfate donor. The standard assay mixture, with a final volume of 30 μl, contained 50 mM potassium phosphate buffer, pH 7.0 (or 50 mM Tris-Cl, pH 8.0, or 100 mM NaAc, 1 mM EDTA) using an Aminco French press. The crude enzyme preparation, allowed to proceed for 10 min at 37 °C, and terminated by heating at 100 °C for 2 min. The precipitates formed were cleared by centrifugation. The clear supernatant was subjected to the analysis of [[35S]]-labeled pNP or dopamine based on the thin-layer chromatography procedure previously established (17).
RESULTS AND DISCUSSION

Cytosolic sulfotransferase enzymes in general catalyze the transfer of a sulfonate group from PAPS, the sulfate donor, to the hydroxyl groups or amino groups of substrate compounds. 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). Whereas the structural determinants for the former, designated the “signature sequences” (TYPKSGTFV and RKGXXGDWXXPT) (6), have been rather well documented, much information is currently available concerning the latter. In view of the fact that the human M-PST and P-PST are >93% identical in their primary sequences (20, 21) and yet display distinct substrate specificity, manganese dependence, stereoselectivity, inhibitor sensitivity, and thermostability (2, 3, 22, 23), these two enzymes serve as an excellent model for investigating the structural basis for their functional differences. The underlying rationale for the present study is that the distinct amino acid residues may be responsible for their differential substrate specificity and other properties. Whereas amino acid residues that are conserved not only between these two enzymes but also among the sulfotransferases from other vertebrates may be critical to the general catalytic process. Examining the aligned amino acid sequences of M-PST and P-PST (20, 21), two variable regions, designated I and II, were noted (Fig. 1). We have therefore hypothesized that these two regions may contain the structural determinants for the substrate specificity and other distinct properties of these two homologous enzymes. Through the generation and characterization of a series of chimeric PSTs, in comparison with wild-type M-PST and P-PST, we have demonstrated unequivocally the substrate phenotypes of the two PSTs to be indeed determined by the two variable regions (14). A distinct feature of the two variable regions, upon closer examination of the aligned M-PST and P-PST sequences (cf. Fig. 1), is the presence of a good number of charged amino acid residues, e.g. Asp-86, Glu-89, His-143, Arg-144, Glu-146, and Lys-147 of M-PST, and His-144, and Lys-147 of P-PST. In view of the fact that the M-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 α-amino group and a negatively charged α-carboxyl group) and that the P-PST utilizes preferentially uncharged phenolic compounds as substrates, it is possible that these charged residues may in fact account for their differential enzymatic properties. By employing the site-directed mutagenesis technique, we attempted in the present study to verify the roles of these charged amino acid residues in determining the distinctive substrate phenotypes of M-PST and P-PST, as well as those that are important for the general catalytic process of the sulfation reaction.

Preparation of Point-mutated M-PSTs and P-PSTs—Based on the procedure described under “Experimental Procedures,” wild-type M-PST and P-PST cDNAs and the point-mutated PST cDNAs were individually transformed into E. coli host cells for expressing the recombinant enzymes. As shown in Fig. 2, the recombinant wild-type and point-mutated PSTs fractionated from the homogenates of transformed E. coli cells using glutathione-Sepharose and cleaved off the bound fusion proteins by thrombin digestion appeared to be highly homogeneous upon SDS-polyacylamide gel electrophoresis. It was noted that, despite their similar predicted molecular masses, there were considerable differences in electrophoretic mobility among wild-type M-PST and P-PST and the various point-mutated PSTs (cf. Fig. 1). It is at present unknown whether these differential electrophoretic mobilities are because of the charge differences between wild-type M-PST and P-PST, as well as the various mutated PSTs with charged amino acid residues being replaced by uncharged residues (or vice versa), resulting in the gain or loss of positive or negative charges. Another possibility is that, despite the SDS-denaturation in the presence of 2-mercaptoethanol, the various wild-type and mutated M-PSTs and P-PSTs may still possess conformational differences that in turn contribute to their distinct electrophoretic mobilities. Although the reasons are unclear, this aberrant phenomenon has been well documented for wild-type, chimeric, and mutated human PSTs (14, 24–27). It will be interesting to examine whether these differences in electrophoretic mobility correlate with the distinct thermostability and/or other enzymatic properties of the mutated M-PSTs and P-PSTs.

Sulfotransferase Activities of Point-mutated M-PSTs and P-PSTs—To characterize the point-mutated PSTs prepared, we first assayed the sulfotransferase activities of the mutated PSTs using dopamine (a preferred substrate for M-PST) and n-p-NP (a preferred substrate for P-PST). Of the nine mutated M-PSTs containing mutations within the two variable regions, three (D86A/E89I, E146A, and D86A/E89I/E146A) displayed dramatically lower activities toward dopamine. Interestingly, whereas the activity of D86A/E89I toward p-NP remained comparable to that of wild-type M-PST, E146A and D86A/E89I/E146A became nearly an order of magnitude more active. These results suggested that Glu-146 may play a critical role in determining the substrate phenotype of M-PST. The three mu-
tated M-PSTs with mutations at Lys-48 and/or His-108 exhibited little or virtually no sulfotransferase activities toward either dopamine or pNP, indicating the possible involvement of these two residues in the catalytic mechanism of the sulfation reaction. It is to be pointed out that analysis of the crystal structure of mouse estrogen sulfotransferase has revealed that His-108 may act as the proton acceptor in the reaction mechanism of the sulfation of 17β-estradiol (28). Site-directed mutagenesis of Lys-48 (Lys-48 in both M-PST and P-PST) of plant flavonol 3-sulfotransferase suggested its involvement in the proper orientation of the phosphosulfate group of the co-substrate, PAPS, for catalysis (11). Of the seven mutated P-PSTs, three (A146E, I89E/A146E, and A86D/I89E/A146E) displayed dramatically lower activities toward pNP. In contrast to the mutated M-PSTs containing the E146A mutation, the mutated P-PSTs with the A146E mutation exhibited no increase in their activities toward dopamine. These results indicated that, unlike in the case of M-PST, mutation at Ala-146 is not sufficient to render any significant change in substrate phenotype of P-PST to that of M-PST. Similar to M-PST, however, the two mutated P-PSTs with mutations at Lys-48 or His-108 also resulted in the complete loss of the activities toward either dopamine or pNP.

To examine more closely the mechanistic roles of the specific amino acid residues in these mutated PSTs, their kinetic constants were determined. As shown in Table II, wild-type M-PST and P-PST, as anticipated, displayed distinct substrate specificities, with the former having higher $V_{max}$ and lower $K_m$ values toward dopamine and the latter having higher $V_{max}/K_m$ for p-nitrophenol. Of the twelve mutated M-PSTs, the nine mutated at residues present in the two variable regions exhibited different $V_{max}/K_m$ values (with either dopamine or pNP as substrate) because of variation in $K_m$ and $V_{max}$ values in comparison with those of wild-type M-PST. Mutations at residues Asp-86, Glu-89, and Glu-146 resulted in an increase in $K_m$, while with varying effects on $V_{max}$, with dopamine as substrate. It is particularly notable that the mutation of Glu-146 to Ala resulted in a greater than 54 times increase in $K_m$ and two times decrease in $V_{max}$. With combined mutations at Asp-86, Glu-89, and Glu-146 to the corresponding residues found in wild-type P-PST, the increase in $K_m$ and decrease in $V_{max}$ were even more dramatic, and the values determined with dopamine as substrate were very close to those determined for wild-type P-PST (cf. Table II). With pNP as substrate, the $K_m$ value decreased more than 22 times for both E146A- and D86A/E89I/E146A-mutated M-PSTs. For the D86A/E89I/E146A-mutated M-PST, the catalytic efficiency, as reflected by $V_{max}/K_m$, with pNP as substrate increased by 25 times and approached that determined for wild-type P-PST. These results combined indicated that residue Glu-146, similar to that reported in two other studies (25, 27), plays an important role in determining the substrate phenotype of M-PST. The concerted action of three mutated residues (D86A, E89I, and E146A), however, is required for the complete change in substrate phenotype of M-PST to that of P-PST. Of the two residues hypothesized to be involved in catalysis, mutation of Lys-48 to Met in M-PST resulted in a complete loss of sulfotransferase activity toward either dopamine or pNP. Mutation of His-108 to Ala, although exerting only a small increase in $K_m$, resulted in an 18 times decrease in $V_{max}$ with dopamine as substrate, supporting its involvement in the catalytic process. Similar to the K48M and H108A mutated M-PSTs, mutations at Lys-48 and His-108 in P-PST also resulted in a complete loss of sulfotransferase activity toward either pNP or dopamine. Of the other five mutated P-PSTs, mutation of Ile-89 to Glu, whereas exerting little effects on the activity toward pNP, resulted in a greater than two times increase in $V_{max}$ with dopamine as substrate. Mutation of Ala-146 to Glu resulted in a greater than five times decrease in $V_{max}/K_m$ with pNP as substrate. Interestingly, combined mutations at Ile-89 and Ala-146 resulted in a tremendous decrease of sulfotransferase activity toward pNP, causing difficulty in accurately determining $V_{max}$ and $K_m$ with pNP as substrate. With an additional mutation at Ala-86, the A86D/I89E/A146E-mutated P-PST no longer displayed high enough sulfotransferase activities for reliable kinetic constant determination with either pNP or dopamine as substrate. These results combined indicated that, in contrast to M-PST, mutations at Ala-86, Ile-89, and Ala-146 to the corresponding residues found in M-PST are not sufficient for rendering the change in substrate phenotype of P-PST to that of M-PST.

### Table II

| Enzyme          | *V*<sub>max</sub> (dopamine) | *V*<sub>max</sub>/*K*<sub>m</sub> (dopamine) | *K*<sub>m</sub> (dopamine) | *K*<sub>m</sub> (pNP) | *V*<sub>max</sub> (pNP) | *V*<sub>max</sub>/*K*<sub>m</sub> (pNP) |
|-----------------|-------------------------------|------------------------------------------|---------------------------|----------------------|------------------------|--------------------------------------|
| Wild-type M-PST | 0.44                          | 12.1                                     | 27.4                      | 28.2                 | 16.6                   | 0.37                                 |
| D86A            | 1.33                          | 5.00                                     | 3.76                      | 7.40                 | 8.20                   | 0.29                                 |
| E89I            | 1.11                          | 12.5                                     | 11.3                      | 20.6                 | 6.06                   | 0.29                                 |
| N85K/D86A       | 3.64                          | 7.69                                     | 2.11                      | 21.5                 | 8.02                   | 0.37                                 |
| D86A/E89I       | 1.55                          | 22.2                                     | 1.55                      | 24.1                 | 4.44                   | 0.18                                 |
| N85K/E89I       | 0.54                          | 10.20                                    | 18.9                      | 34.5                 | 6.89                   | 0.20                                 |
| E146A           | 23.8                          | 5.88                                     | 0.25                      | 1.27                 | 4.12                   | 3.24                                 |
| D86A/E89I/E146A | 44.9                          | 2.74                                     | 0.06                      | 1.21                 | 11.4                   | 9.39                                 |
| H143Y           | 1.18                          | 13.7                                     | 11.60                     | 31.3                 | 10.9                   | 0.35                                 |
| K48M            | 0.55                          | 0.67                                     | 1.22                      | —                    | —                      | —                                    |
| H108A           | 0.58                          | 0.67                                     | 1.22                      | —                    | —                      | —                                    |
| N85K/D86A       | 3.64                          | 7.69                                     | 2.11                      | 21.5                 | 8.02                   | 0.37                                 |
| D86A/E89I       | 1.55                          | 22.2                                     | 1.55                      | 24.1                 | 4.44                   | 0.18                                 |
| N85K/E89I       | 0.54                          | 10.20                                    | 18.9                      | 34.5                 | 6.89                   | 0.20                                 |
| E146A           | 23.8                          | 5.88                                     | 0.25                      | 1.27                 | 4.12                   | 3.24                                 |
| D86A/E89I/E146A | 44.9                          | 2.74                                     | 0.06                      | 1.21                 | 11.4                   | 9.39                                 |
| H143Y           | 1.18                          | 13.7                                     | 11.60                     | 31.3                 | 10.9                   | 0.35                                 |
| K48M/H108A      | 0.58                          | 0.67                                     | 1.22                      | —                    | —                      | —                                    |
| A86D/I89E       | 59.9                          | 3.87                                     | 0.06                      | 0.49                 | 5.50                   | 11.2                                 |
| I89E/A146E      | 61.7                          | 4.29                                     | 0.07                      | —                    | —                      | —                                    |
| A86D/I89E/A146E | —                             | —                                        | —                         | —                    | —                      | —                                    |

*Not determined due to undetectable or low level of activity.*
In conclusion, the results derived from the present study, in support of our previous investigation using chimeric PSTs (14), have confirmed the importance of charged amino acid residues present in the two variable regions (cf. Fig. 1) in determining the substrate phenotypes of M-PST and P-PST. Previous studies by us and others have demonstrated that M-PST and P-PST differ not only in substrate phenotype but also in thermostability, inhibitor sensitivity, manganese dependence, and stereoselectivity (2, 3, 22, 23). It will be interesting to investigate whether (some of) the amino acid residues examined in the present study are also critical in determining these latter distinct properties between the two enzymes.

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