Functional characterization of two human sulphotransferase cDNAs that encode monoamine- and phenol-sulphating forms of phenol sulphotransferase: substrate kinetics, thermal-stability and inhibitor-sensitivity studies

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The present paper describes the functional characterization of two human aryl sulphotransferase (HAST) cDNAs, HAST1 and HAST3, previously isolated by us from liver and brain, respectively [Zhu, Veronese, Sansom, and McManus (1993) Biochem. Biophys. Res. Commun. **192**, 671–676; Zhu, Veronese, Bernard, Sansom and McManus (1993) Biochem. Biophys. Res. Commun. **195**, 120–127]. These appear to encode the two major forms of phenol sulphotransferase (PST) characterized in a number of human tissue cytosols, these being the phenol-sulphating (P-PST) and monoamine-sulphating (M-PST) forms of phenol sulphotransferase. HAST1 and HAST3 cDNAs were functionally expressed in COS-7 cells and kinetically characterized using the model substrates for P-PST and M-PST, *p*-nitrophenol and dopamine (3,4-dihydroxyphenethylamine) respectively. COS-expressed HAST1 was shown to be enzymically active in sulphating *p*-nitrophenol with high affinity (K_m 0.6 μM), whereas dopamine was the preferred substrate for HAST3 (K_m 9.7 μM). HAST1 could also sulphate dopamine, as could HAST3 sulphate *p*-nitrophenol, but the K_m for these reactions were at least two orders of magnitude greater than for the preferred substrates. COS-expressed HAST1 and HAST3 displayed inhibition profiles with the ST inhibitor 2,6-dichloro-4-nitrophenol (DCNP), identical with human liver cytosolic P-PST and M-PST activities respectively. Thermal-stability studies with the expressed enzymes showed that HAST1 was considerably more thermostable (TS) than HAST3, which is consistent with P-PST being termed the TS PST and M-PST being termed the thermolabile (TL) PST. Western immunoblot analyses of the expressed PST proteins using an antibody generated to a bacterially expressed rat liver aryl/phenol ST showed that HAST1 and HAST3 migrated as single proteins with different electrophoretic mobilities (32 versus 34 kDa). This is consistent with the differences in electrophoretic mobilities observed for P-PST and M-PST in a variety of tissues reported by other workers. This report on the functional characterization of P-PST and M-PST cDNAs provides important information on the structural as well as functional relationships of human PSTs, which sulphate a vast array of exogenous and endogenous compounds.

INTRODUCTION

Sulphotransferases (STs) exist as a family of functionally related forms which are involved in the sulphation of a diverse range of compounds including biogenic amines, steroids, hormones, bile acids and a variety of xenobiotics including drugs and carcinogens (Mulder and Jakoby, 1990). A common feature of all reactions catalysed by the STs is the utilization of 3′-phosphoadenosine 5′-phosphosulphate (PAPS) as the sulphate donor. Although generally thought of as a detoxification pathway for many compounds, sulphation can also play a major role in the bioactivation of certain drugs to their therapeutically active form, such as minoxidil, and some *N*-hydroxy aromatic amines to their ultimate carcinogenic species (Thorgeirsson et al., 1983; Mulder and Jakoby, 1990; Falany, 1991). Because of the potential importance of these sulphation reactions there has been an increasing effort to characterize the individual members of the ST enzyme system over the past decade.

At least three major forms have been characterized in human tissues from purified preparations; a steroid/bile acid ST, also known as dehydroepiandrosterone ST (DHEA ST), and two forms of PST (Falany, 1991). The two forms of PST have been referred to on the basis of their substrate specificity, i.e. M-PST ('M' designating monoamine-metabolizing) preferentially catalyses the sulphation of monoamines such as dopamine (3,4-dihydroxyphenethylamine), whereas P-PST ('P' designating phenol-metabolizing), has a preference for sulphating simple phenols such as *p*-nitrophenol. These two forms of PST have also been differentiated according to their thermal stability. The thermolabile (TL) and thermostable (TS) forms correspond to M-PST and P-PST forms respectively (Reiter et al., 1983; Campbell et al., 1987).

Both forms of PST have been characterized in a number of human tissues, including liver (Campbell et al., 1987; Falany et al., 1990), brain (Whittmore et al., 1985, 1986), blood platelets (Reiter et al., 1983) and intestine (Sundaram et al., 1989). The functional characteristics of the two PSTs appear quite similar between each of these tissues. However, both forms appear independently regulated and large variations in the tissue expression of PSTs have been shown to be inherited (Weinshilboum,

Abbreviations used: ST, sulphotransferase; HAST, human aryl sulphotransferase; PST, phenol sulphotransferase; P-PST, phenol-sulphating form of phenol sulphotransferase; M-PST, monoamine-sulphating form of phenol sulphotransferase; PAPS, 3′-phosphoadenosine 5′-phosphosulphate; rPST, rat aryl/phenol sulphotransferase; DHEA, dehydroepiandrosterone; DCNP, 2,6-dichloro-4-nitrophenol; TS, thermostable; TL, thermolabile.

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1990). In order to elucidate the relationship between these enzymes as well as their structure–function relationships and regulation we have recently taken the molecular-cloning approach of isolating cDNAs encoding individual human PSTs. To date we have isolated three distinct human ST cDNAs, one from liver termed HAST1 and two new forms from brain (HAST2 and HAST3) (Zhu et al., 1993a,b). HAST3 was also identified in human liver (Zhu et al., 1993b). The HAST1 and 2 cDNAs, which differ in only their 5′ non-coding sequence, encode identical proteins which differ from the unique protein encoded by HAST3 by only 21 amino acids.

A human liver cDNA very similar to HAST1 (three amino acid differences) has been recently expressed in mammalian cell culture and was shown to have activity towards *p*-nitrophenol, but not dopamine (Wilborn et al., 1993). This evidence suggests that their encoded cDNA may represent P-PST; however, this expressed protein has not been completely characterized with respect to substrate kinetics, thermal stability and inhibitor-sensitivities. No other cDNAs encoding human PSTs have been reported to date, but cDNAs encoding another human ST, DHEA ST, have been reported by other workers (Otterness et al., 1992; Comer et al., 1993).

The present paper describes the first extensive functional characterisation of two human PST cDNAs (human liver HAST1 and human brain HAST3 cDNAs) by expression in the mammalian COS-7 expression system. Kinetic, inhibitor and thermal-inactivation experiments performed with the individual expressed PST enzymes demonstrated that HAST1 and HAST3 encode PST enzymes corresponding to P-PST and M-PST respectively. These data represent a major advance toward understanding the biochemical and molecular relationships between human PSTs.

MATERIALS AND METHODS

Materials

[^{35}S]PAPS was purchased from du Pont (Wilmington, DE, U.S.A.). PAPS, dopamine, *p*-nitrophenol and pargyline were obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.) and 2,6-dichloro-4-nitrophenol (DCNP) from ICN Biochemicals (Sydney, NSW, Australia). Restriction enzymes and other reagents used in molecular-biological techniques were obtained from New England Biolabs (Genesearch Pty. Ltd., Arundel, Qld., Australia) or Pharmacia LKB Biotechnology (North Ryde, NSW, Australia). Newborn-calf serum was obtained from GIBCO-BRL (Glen Waverly, Vic., Australia). The COS cell expression vector pCMV5 was kindly provided by Dr. Mark Stinsky, Department of Microbiology, School of Medicine, University of Iowa, Ames, IA, U.S.A.). All other reagents were of molecular-biology or analytical-reagent grade.

Preparation of antibody against bacterially expressed rat aryl ST

A polyclonal antibody was produced in rabbits to recombinant rat aryl/phenol ST using the previously isolated cDNA (Cruickshank et al., 1993). The rat cDNA was excised from the pBluescript construct (Cruickshank et al., 1993) using *Bam*HI and *Hin*dIII and subcloned into the similarly digested bacterial expression vector pQE11 (Qiagen Inc. Chatsworth, CA, U.S.A.) and transformed into the *Escherichia coli* strain SG13009 containing the *lac* repressor-producing plasmid pREP4 (Qiagen). This vector incorporates a $(His)_6$ tag sequence upstream of the inserted cDNA such that ST protein is synthesized with a $(His)_6$ tail at the N-terminal end. This allows purification from *E. coli* proteins by selective binding of the histidine-tagged protein to a Nickel-NTA resin (Qiagen). The protocol to obtain purified protein is essentially as described in the manufacturer's protocol (Qiagen). Briefly, an overnight culture of SG13009[pREP4] *E.coli* cells transformed with the rat aryl/phenol ST cDNA in the pQE11 expression vector was used to innoculate 250 ml of medium, which was grown to mid-exponential phase ($A_{600} \approx 0.7$–0.9) with shaking at 37 °C before inducing expression of ST protein by the addition of isopropyl β-D-thio-galactopyranoside to a final concentration of 2 mM and allowing the cells to grow for a further 3 h at 37 °C. The cells were then pelleted, solubilized in a 6 M guanidine/HCl buffer, pH 8.0, containing 0.1 M NaH_2PO_4 and 0.01M Tris/HCl, and the cleared lysate loaded on to a column containing 2 ml of Nickel-NTA resin. After *E. coli* proteins were washed away using the above buffer followed by an 8 M urea buffer, pH 6.3, also containing the salts as the previous buffer, the $(His)_6$-tagged rat ST protein was eluted with this latter buffer, which also contained 250 mM imidazole (pH 6.3). The eluted protein was essentially pure, migrating as a single band on SDS/polyacrylamide gels (≈41 kDa). This purified protein was then used as antigen to produce anti-(recombinant rat aryl/phenol ST) antisera in a rabbit as described previously (McManus *et al.*, 1987).

Expression and characterization in COS-7 cells

The human liver ST cDNA, HAST1 (Zhu et al., 1993a), was excised from a pBluescript construct using *Xba*I and *Cla*I and subcloned into the pCMV5 COS expression vector digested with the same enzymes. The human brain ST cDNA, HAST3 (Zhu et al., 1993b), was excised from a pBluescript construct using *Eco*RI and *Spe*I and subcloned into the COS vector digested with *Eco*RI and *Xba*I. Expression constructs were transformed into competent *E. coli* HB101 cells, and DNA was prepared by the alkaline-lysis method followed by two CsCl-gradient-density centrifugations. Transfections in COS-7 cells were carried out as previously described (Cruickshank et al., 1993); however, newborn-calf serum was used in place of the Nuserum. Mock-transfected cells were prepared by transfecting COS-7 cells with the COS vector that did not contain any ST cDNA insert. Cells were generally harvested by scraping approx. 50–70 h post-transfection, washed twice with phosphate-buffered saline, pH 7.4, and stored in 50 mM potassium phosphate buffer, pH 7.0, containing 10% glycerol at −70 °C until required for assay. Cells required for the heat-inactivation experiments were stored in 5 mM potassium phosphate buffer, pH 7.0, without glycerol. Preliminary experiments indicated that glycerol protected STs from heat inactivation (results not shown). Western immunoblots were also performed on these cell lysates as previously described (McManus et al., 1987), but with some minor modifications. First, the electrophoresis and protein-blotting system used was a Mini-PROTEAN II cell and a Mini Trans-Blot cell, respectively (Bio-Rad, North Ryde, NSW, Australia). Secondly, the colour developing system used was 3,3′,5,5′-tetramethylbenzidene-stabilized substrate (Promega, Madison, WI, U.S.A.) instead of diaminobenzidine. Antisera to recombinant rat aryl ST, produced as described above, was used to probe these immunoblots at a dilution of 1:200.

PST activity assays

PST activities were assayed by the radiochemical method of Foldes and Meek (1973), as previously described but with some minor modifications (Cruickshank et al., 1993). Briefly, the incubations (500 μl) contained 10 mM potassium phosphate buffer, pH 7.0, either dopamine or *p*-nitrophenol as substrate, 5–100 μg of COS-cell protein and 8 μM PAPS, of which 0.25 μM

was [^{35}S]PAPS. Reactions were initiated by the addition of substrate, and the mixtures were incubated for 10–30 min at 37 °C. Assays with dopamine as substrate also included the monoamine oxidase inhibitor pargyline (1 mM). Blanks were identical, except they contained no substrate. For both substrates incubation conditions were chosen that were optimal with respect to protein concentration and incubation time. All reactions were performed in duplicate, and the results presented are means.

Analysis of results

Initial estimates of the apparent K_m and $V_{max.}$ values for dopamine and *p*-nitrophenol sulphation were determined by linear regression analysis of the Lineweaver–Burk plots. These values were then used as initial estimates for Multifit 2.01, a Macintosh-based curve-fitting program (Cruickshank et al., 1993), using a single-enzyme Michaelis–Menten system. The standard errors for the computer-derived kinetic parameters were generally less than 10%.

RESULTS

In order to establish that HAST1 and HAST3 cDNAs encoded functional PST enzymes, we expressed them individually in COS-7 cells and determined their activity towards the model substrates, dopamine and *p*-nitrophenol, as used to characterize the PST forms in a variety of human tissues (Reiter et al., 1983; Young et al., 1984; Campbell et al., 1987; Sundaram et al., 1989). Both COS-expressed HAST1 and HAST3 catalysed the sulphation of *p*-nitrophenol and dopamine (Figure 1), but there were marked differences in both apparent K_m and $V_{max.}$ for each form towards both compounds (Table 1). Over the substrate range studied, single-enzyme Michaelis–Menten kinetics best described the data obtained for both expressed HAST proteins. The substrate, *p*-nitrophenol, was the preferred substrate for HAST1 as reflected in the 575-fold lower K_m and 50-fold higher $V_{max.}$ than for dopamine. These apparent K_m values are quite similar to the values obtained for P-PST characterized in a number of tissues (Reiter et al., 1983; Young et al., 1984; Campbell et al., 1987). In comparison, dopamine was the preferred substrate for HAST3, the K_m being 230-fold lower than for *p*-nitrophenol. However, for this expressed PST the $V_{max.}$ for dopamine sulphation was only marginally higher than for *p*-nitrophenol. The apparent K_m values obtained for these substrates for HAST3 were quite similar to those obtained for M-PST characterized in a number of human tissues, including brain (Reiter et al., 1983; Young et al., 1984; Whittmore et al., 1985; Sundaram et al., 1989).

Control transfected COS cells (i.e. transfected with pCMV5 vector without HAST cDNA insert) gave negligible activity with both phenol and dopamine. Interestingly, COS-expressed HAST1, but not HAST3, in the absence of either substrate did give some activity which was protein- and time-dependent and most likely represents the sulphation of a compound endogenous to the COS cells which can be sulphated by HAST1 but not HAST3. This endogenous COS-expressed HAST1 activity was generally less than 3% of the activity obtained for *p*-nitrophenol at $V_{max.}$ and in all cases was subtracted from the substrate activities obtained.

PST activity was also measured with various concentrations of the sulphate donor, PAPS, in the presence of the preferred substrate for both expressed HAST1 and HAST3, i.e. 2 μM *p*-nitrophenol and 60 μM dopamine respectively (Figure 2). The apparent K_m values for PAPS derived from these data were 0.98 μM for *p*-nitrophenol by HAST1 and 1.1 μM for dopamine by HAST3. These data indicate that, at least for the preferred substates, the PST reactions performed in the present study (which contained 8 μM PAPS) were at saturating PAPS concentrations.

The expressed enzymes were also characterized for their sensitivity to the specific inhibitor DCNP. The profile of in-

Table 1 Computer-derived Michaelis–Menten parameters for the sulphation of *p*-nitrophenol and dopamine by COS-expressed HAST1 and HAST3

All data shown are from COS cells obtained in one transfection experiment with both cDNAs so as to avoid batch-to-batch variability and were assayed in duplicate.

	p-Nitrophenol		Dopamine	
Enzyme	K_m (μM)	$V_{max.}$ (nmol/min per mg)	K_m (μM)	$V_{max.}$ (nmol/min per mg)
HAST1	0.60	7.5	345	0.16
HAST3	2200	2.6	9.7	2.8

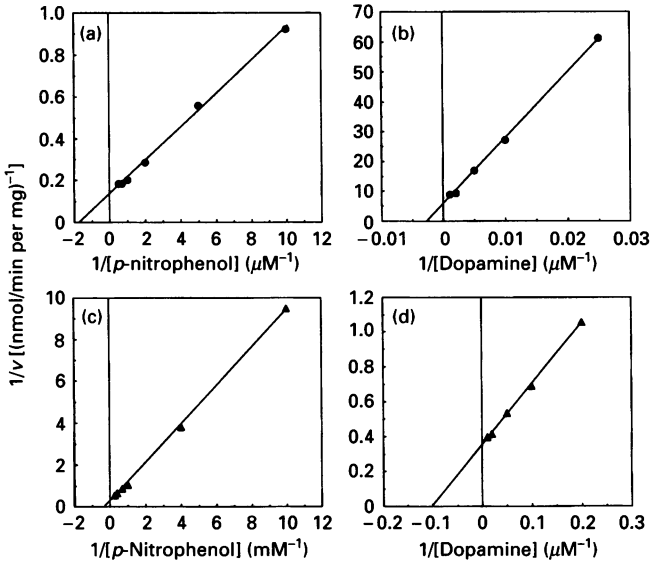

Figure 1 Lineweaver–Burk plots of the substrate-dependent sulphation of *p*-nitrophenol and dopamine by COS-expressed HAST1 (a and b respectively) and by COS-expressed HAST3 (c and d respectively)

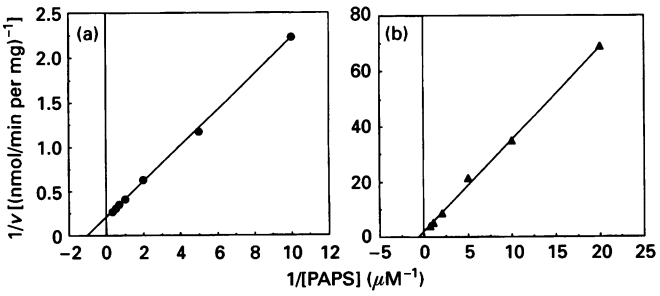

Figure 2 Lineweaver–Burk plots of the PAPS-dependent sulphation of *p*-nitrophenol by COS-expressed HAST1 (a) and dopamine by COS-expressed HAST3 (b)

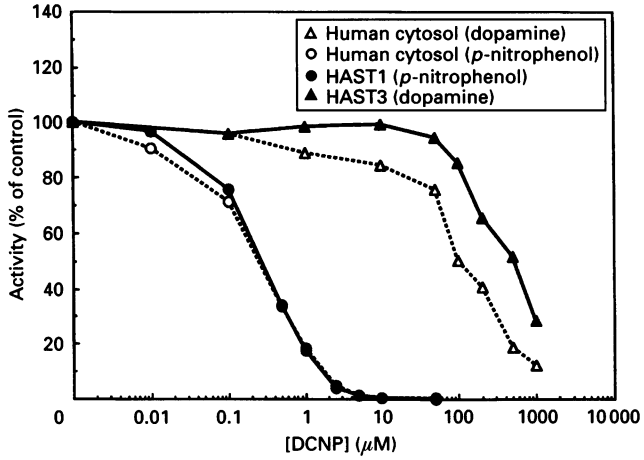

Figure 3 DCNP inhibition of *p*-nitrophenol sulphation by COS-expressed HAST1 and human liver cytosol and dopamine sulphation by COS-expressed HAST3 and human liver cytosol

The *p*-nitrophenol and dopamine concentrations were 2 μM and 60 μM respectively. The human liver cytosol data are the average of those determined in two different livers.

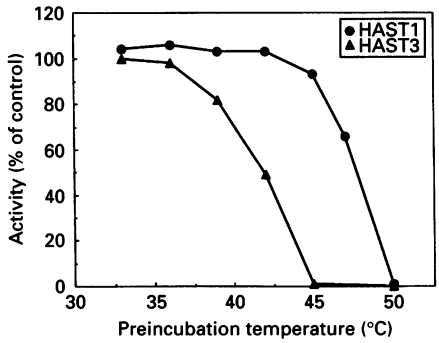

Figure 4 Thermal stability of COS-expressed HAST1 and HAST3

PST activity for HAST1 and HAST3 was determined using 2 μM *p*-nitrophenol and 60 μM dopamine respectively. PST activity was measured after preincubation for 15 min at the temperature shown.

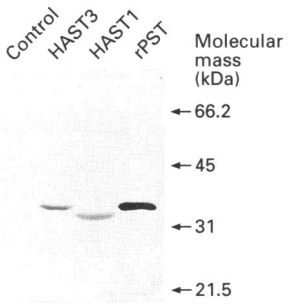

Figure 5 Western immunoblot of COS-expressed HAST1, HAST3 and rat liver aryl/phenol ST (rPST)

COS-7 cell homogenates transfected with HAST1, HAST3 and rPST (Cruickshank et al., 1993) cDNAs were subjected to electrophoresis on SDS/PAGE (10% acrylamide) and Western immunoblotting with anti-rPST antisera. The control consisted of COS cells transfected with the COS vector without any ST cDNA insert. Samples (50 μg) of COS cell homogenates expressing HAST1, HAST3 and control, and 2 μg of rPST were loaded. The migration of Bio-Rad molecular-mass markers are also indicated by arrows.

hibition of *p*-nitrophenol sulphation by HAST1 was identical to the P-PST activity determined in human liver cytosol using 2 μM *p*-nitrophenol (Figure 3). In comparison, high-affinity dopamine sulphation by HAST3 showed inhibition characteristic of M-PST activity in human liver cytosol as determined by using 60 μM dopamine (Figure 3).

Thermostability has also been used to differentiate the two major PST forms in a variety of human tissues by other workers (Campbell et al., 1987; Sundaram et al., 1989). They have consistently shown that M-PST is relatively more TL than P-PST. For these reasons M-PST has been termed the TL PST and P-PST the TS PST. In agreement with these workers we found that COS-expressed HAST1 (the putative P-PST form), was relatively more TL than COS-expressed HAST3 (the putative M-PST form) (Figure 4).

Successful expression of HAST1 and HAST3 in COS-7 cells was also confirmed by the presence of immunoreactive protein in these transfected cells that were subjected to SDS/PAGE and Western immunoblotting (Figure 5). No proteins of equivalent electrophoretic mobilities could be detected in control transfected COS-7 cells. Expressed HAST1 and HAST3 ran at estimated molecular masses of approx. 32 and 34 kDa respectiviely. However, these differences in electrophoretic mobilities would not be expected from the similarities in predicted molecular masses for these proteins based on their amino acid sequences [i.e. HAST1, 34178 Da; HAST3, 34196 Da (Zhu et al., 1993a,b). Unfortunately Western immunoblots of human liver cytosol preparations revealed multiple immunoreactive bands that could not be singled out as being equivalent to the COS-expressed HAST proteins (results not shown). This is probably a result of the antibody being prepared to a rat aryl/phenol ST protein which shares 79 and 76% amino acid similarity to the human HAST1 and HAST3 proteins respectively. This is highlighted by the fact that the antibody recognizes the expressed rat ST to a far greater degree than the expressed human proteins (Figure 5). This may also possibly reflect the low level of expression and the stability of these STs in subcellular fractions of human tissues. However, in Northern analysis we have shown that HAST-related transcripts could be detected in human liver and lung (Zhu et al., 1993a). Also, using a specific cDNA probe, we could detect HAST3 in the mRNA of human liver, colon, kidney and lung (Zhu et al., 1993b). These data indicate that these STs (or other highly related STs) are present in a number of human tissues.

DISCUSSION

The results presented here demonstrate for the first time the functional characteristics of two human sulphotransferase cDNAs, corresponding to the M and P forms of PST described in a number of human tissues. The HAST1 cDNA previously isolated in this laboratory from a human liver cDNA library (Zhu et al., 1993a) when expressed in the mammalian COS cell expression system was functionally similar to partially purified P-PST reported by other workers (Campbell et al., 1987). This is based on the ability of the COS-expressed HAST1 enzyme to preferentially metabolize the model phenolic substrate, *p*-nitrophenol, with high affinity, as well as being relatively sensitive to inhibition by DCNP. Furthermore, results of the thermal-stability experiments performed with COS-expressed HAST1 was consistent with this form being the TL form as has been suggested for purified P-PST (Campbell et al., 1987). These data are also consistent with recently published data on another very similar liver PST cDNA, P-PST-1 (Wilborn et al., 1993). Their clone encodes a protein which differs from our HAST1 protein

by three amino acids, these differences being His-213 → Arg, Glu-282 → Lys and Ser-290 → Thr. The P-PST-1 cDNA was expressed in COS cells and it also showed activity towards *p*-nitrophenol (using a concentration of 4 μM), but did not show activity towards dopamine using a concentration of 10 μM (Wilborn et al., 1993). The lack of activity towards dopamine at this low substrate concentration is not surprising, considering we observed the K_m towards dopamine for expressed HAST1 to be approx. 345 μM. Although these workers concluded that their cDNA encoded P-PST on the basis of a single observation of activity towards *p*-nitrophenol, we have definitively shown using a combination of substrate-kinetics, inhibitor-sensitivity and thermal-stability studies that COS-expressed HAST1 is functionally similar to human cytosolic P-PST.

At least two forms of P-PST have been reported in liver (Campbell et al., 1987), platelets (Reiter et al., 1983; Whittmore et al., 1983) and brain (Whittmore et al., 1986) on the basis of differences in thermal stabilities and their behaviour during anion-exchange chromatography. These differences in P-PST activity have been correlated with different allelic forms of the enzyme in human liver (Weinshilboum, 1990). However, it has been suggested that the thermostability differences of the two forms may be an artifact of the method of cytosol preparation (Falany et al., 1990). Whether the amino acid differences between the P-PST proteins encoded by our cDNA, HAST1 (Zhu et al., 1993a) and that of Falany's group, P-PST-1 (Wilbourn et al., 1993) represent the different allelic forms described from the purified tissue proteins and whether they are functionally dissimilar requires further functional characterization of the expressed PST proteins, in particular the P-PST-1 variant.

In contrast, the unique HAST3 cDNA previously isolated by us from human brain (Zhu et al., 1993b) when expressed in COS cells was shown to preferentially metabolize the model monoamine substrate dopamine with high affinity, as well as being relatively resistant to inhibition by DCNP. This form could also metabolize *p*-nitrophenol, but with an affinity approximately two orders of magnitude lower. Thermal-stability studies performed with expressed HAST3 confirmed that this form is relatively TL, consistent with M-PST being referred to as the TL form of PST. These data are consistent with COS-expressed HAST3 being functionally equivalent to M-PST characterized in a number of human tissues (Reiter et al., 1983; Campbell et al., 1987; Sundaram et al., 1989), including brain (Whittmore et al., 1985).

Results of this study are also consistent with the known immunological similarity between M- and P-PST. Falany et al. (1990) has shown that antibodies raised to M-PST cross-react strongly with purified P-PST, suggesting that the two PSTs are structurally closely related. Our Western blot of COS-expressed HAST1 and HAST3 probed with an antibody produced to rat aryl ST recognized equally both expressed human proteins, consistent with the findings of these workers. This immunological similarity should not be surprising, considering that the proteins encoded by these two cDNAs are only 21 amino acids different out of a total length of 295 amino acids. Although both cDNAs encode proteins of similar molecular mass (HAST1, 34178 Da; HAST3, 34196 Da), both proteins could be separated by SDS/PAGE with COS-expressed HAST3 running at a slightly higher electrophoretic mobility when compared with HAST1. This is consistent with Western immunoblots performed with purified PSTs, which show that M-PST has a slightly higher electrophoretic mobility than P-PST (Falany et al., 1990; Heroux et al., 1989). It should be noted that P- and M-PST have been shown to have molecular masses of approx. 64 and 69 kDa respectively, as determined by gel filtration (size-exclusion) chromatography, and suggests that these proteins are composed of two identical subunits (Falany et al., 1990; Sundaram et al., 1989). As the individual COS-expressed proteins have functional characteristics similar to the purified forms, it seems likely, as has been suggested by Wilborn et al. (1993) for P-PST, that the PST enzymes exist as homodimers. However, the possibility exists that heterodimers may also exist, as occurs for the cytosolic glutathione transferases family of xenobiotic-metabolizing enzymes, although there is no direct evidence to date to suggest of this occurrence.

It has been suggested that human liver P-PST is the homologue of the rat liver aryl ST, rat minoxidil-ST (Wilborn et al., 1993). This is based on the encoded cDNA to rat minoxidil-ST (Hirshey et al., 1992) showing 80% identity at the amino acid level to the human P-PST-1 encoded protein (Wilbourn et al., 1993). In addition, both expressed rat and human proteins are able to sulphate minoxidil and *p*-nitrophenol and show immunological cross-reactivity (Wilbourn et al., 1993). We have recently isolated and functionally characterized an identical rat liver aryl ST cDNA (Cruickshank et al., 1993) and shown that its product can metabolize dopamine, as can that of the expressed P-PST clone (HAST1) described here. However, the dopamine K_m obtained for the expressed rat enzyme (44 μM) was an order of magnitude lower than for P-PST (HAST1) described here. We have also determined that the human P-PST (HAST1) enzyme has a K_m towards phenol of 3.4 μM (M. E. Veronese, X. Zhu and M. E. McManus, unpublished work), this also being markedly higher than that obtained for the rat enzyme (K_m 0.87 μM; Cruickshank et al., 1993). Even though there appear to be functional similarities between the expressed rat aryl ST and human P-PST, subtle differences are apparent, and hence caution should be exercised in extrapolating results obtained with ST proteins from one species to another.

In summary, this paper has described the functional characteristics of two human ST cDNAs, HAST1 and HAST3, which appear to encode the major PST forms found in a number of human tissues, these being P-PST and M-PST respectively. The PST cDNAs were functionally expressed in COS cells and substrate kinetics and inhibition studies used to differentiate these forms. P-PST (HAST1) was shown to preferentially sulphate *p*-nitrophenol as well as being sensitive to DCNP inhibition. In contrast, M-PST (HAST3) was shown to preferentially sulphate dopamine as well as being quite resistant to DCNP inhibition. Thermal-stability studies were also consistent in recognizing P-PST as the TS form and M-PST as the TL form of PST. This study represents a major step towards understanding the functional and structural heterogeneity of human PSTs that catalyse the sulphation of an enormous array of exogenous and endogenous compounds.

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