Molecular cloning and expression of a cDNA encoding an olfactory-specific mouse phenol sulphotransferase

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Previously we demonstrated the presence of phenol sulphotransferase (P-ST) in mouse nasal cytosols and identified its zonal location in mouse nasal cavity by staining with an antiserum raised against a rat liver P-ST isoenzyme, PSTG. In the present study a cDNA was isolated from a mouse olfactory cDNA library by immunological screening with the antiserum. The isolated cDNA consisted of 1347 bp with a 912 bp open reading frame encoding a 304-residue polypeptide. Both the nucleotide and deduced amino acid sequences of the cDNA were 94% identical with those of a rat liver P-ST isoenzyme, ST1C1. The expressed enzyme in *Escherichia coli* displayed high P-ST activity towards phenolic odorants such as eugenol and guaiacol, and it showed a high *N*-hydroxy-2-acetylaminofluorene sulphation activity in comparison with the rat ST1C1 enzyme. These results indicate that the olfactory P-ST encoded by the cDNA is a mouse orthologue of rat ST1C1; however, expression of the olfactory P-ST mRNA is specific for nasal tissues as revealed by reverse transcriptase-mediated PCR (RT–PCR).

INTRODUCTION

Sulphotransferases (STs) are phase II drug-metabolizing enzymes that catalyse the transfer of a sulphonate group from 3′-phosphoadenosine 5′-phosphosulphate (PAPS) to xenobiotics and endogenous compounds possessing phenols, enols, alcohols or amines [1–5]. STs are cytosolic enzymes found in several tissues, including liver, intestine, brain, adrenals and platelets [1,2]. In liver, sulphate conjugation confers greater polarity and water solubility on the parent molecules, thereby facilitating biliary or urinary excretion and detoxification. However, in some instances sulphate conjugation results in the generation of reactive intermediates that are capable of covalently binding DNA, RNA and proteins, causing a carcinogenic response [1–5]. The STs exist as a multi-gene family; a number of subfamilies have been identified on the basis of enzyme purification and molecular cloning studies [3–5].

Biotransformation enzymes present in the olfactory systems are thought to provide clearance and detoxification of odorants (olfactory perireceptor process). It is known that the nasal mucosa has nasal-specific phase I (e.g. cytochrome *P*-450) and phase II (e.g. UDP-glucuronosyltransferase, glutathione S-transferase) enzymes [6–9]. In our previous studies we have immunolocalized phenol ST (P-ST) polypeptide in mouse nasal sustentacular cells and demonstrated that mouse nasal cytosols showed comparatively high P-ST activities towards phenolic aromatic odorants similar to that shown by mouse liver cytosol [10,11]. Interestingly, the topographical immunopositive region was identical with that for odorant receptors, of which localization is symmetrical in a dorso-medial region in the nasal cavity [10,12]. To elucidate the molecular nature and the functional roles of olfactory ST, we isolated a cDNA from a λgt11 cDNA library of mouse nasal tissues by immunological screening and characterized it.

MATERIALS AND METHODS

Materials

[^{35}S]PAPS (82.78 Ci/mmol) and various aromatic odorants were purchased from NEN–Dupont and Aldrich respectively. The prokaryotic expression vector pTrc99A was purchased from Pharmacia Japan. Reagents for reverse transcriptase-mediated PCR (RT–PCR) were purchased from Stratagene (La Jolla, CA, U.S.A.). *Taq* polymerase was purchased from Perkin-Elmer, Japan. *N*-Hydroxy-2-acetylaminofluorene (*N*-OH-AAF) was kindly provided from Dr. M. Mochizuki (Kyoritsu College of Pharmacy, Tokyo, Japan) and Dr. Y. Yamazoe (Tohoku University, Sendai, Japan). 2-Acetylaminofluorene (AAF) was obtained from Sigma–Aldrich Japan K. K.

Immunological screening of λgt11 cDNA library and DNA sequence analysis

A λgt11 cDNA expression library was constructed from poly(A)$^+$ RNA of male ddY mouse nasal tissues. The library (3.6×10^5 plaque-forming units) was screened with a rabbit antiserum raised against PSTG, a P-ST isoenzyme purified from Wistar rat livers [13]. The cDNA insert was excised from the positive phage

Abbreviations used: AAF, 2-acetylaminofluorene; DCNP, 2,6-dichloro-4-nitrophenol; mOlfST, mouse olfactory ST; *N*-OH-AAF, *N*-hydroxy-2-acetylaminofluorene; P-ST, phenol ST; PAPS, 3′-phosphoadenosine 5′-phosphosulphate; RT–PCR, reverse transcriptase-mediated PCR; ST, sulphotransferase; TBA, tetra-n-butylammonium chloride; TEA, triethylamine.

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The nucleotide sequence data reported will appear in DDBJ, EMBL and GenBank Nucleotide Sequence Databases under the accession number O33653.

DNA by *Eco*RI, subcloned into pUC118 and sequenced by the dideoxy chain termination method [14]. DNA sequence analyses were performed by the GENETYX-Mac DNA analysis software (version 7.3) and GenBank Rel.97 database.

Expression of olfactory P-ST in *Escherichia coli*

The coding sequence was amplified by PCR with appropriate primers containing sequences to add restriction endonuclease sites for cloning at both ends of the sequence. The primers used were 5′-GGAATTCATGCCCTTGGAAAAATTG-3′ (forward) and 5′-GGATCCTCAGATCTCTGTGCGG-3′ (reverse) (underlining indicates the *Eco*RI and *Bam*HI sites for cloning respectively). The amplified fragment was digested with *Eco*RI and *Bam*HI and ligated into a prokaryotic expression vector, pTrc99A, and then used for transformation of *E. coli* JM109. The transformed cells were cultivated in Luria–Bertani broth containing 50 μg/ml ampicillin for 2 h at 37 °C and grown for a further 3 h after the addition of 1 mM isopropyl β-D-thiogalactopyranoside. The cells were harvested by centrifugation at 6500 ***g*** for 10 min, washed with 0.9 % NaCl and resuspended in buffer A [10 mM Tris/HCl (pH 7.4)/250 mM sucrose/5 μg/ml antipain/5 μg/ml pepstatin]. After sonication, unbroken cells were removed by centrifugation and the lysate was centrifuged at 105000 ***g*** for 60 min; the resulting supernatant was used for assays and other enzyme characterizations.

Assay of P-ST activity

The P-ST activity in the lysates was determined by using [^{35}S]PAPS as the sulphate donor and 2-naphthol or other odorants as the sulphate acceptor, in accordance with the procedure of Foldes and Meek [15] with a slight modification [10,11]. In brief, the reaction mixture (500 μl) consisted of 10 mM phosphate buffer, pH 6.4, 50 μM 2-naphthol or other phenolic odorants, 1.0 μM [^{35}S]PAPS (0.1 μCi) and lysates (5–20 μg of proteins). The mixture was incubated for 15 min at 37 °C and the reaction was stopped by the addition of 0.1 ml of cold 0.1 M barium acetate. The unconverted [^{35}S]PAPS was precipitated by the addition of 0.1 ml each of 0.1 M $Ba(OH)_2$ and 0.1 M $ZnSO_4$, and the precipitate was removed by centrifugation at 12000 ***g*** for 5 min. This precipitation procedure was repeated once. The supernatant (300 μl) after the second precipitation was transferred to 3 ml of liquid scintillator and counted for radioactivity. Blanks were monitored by omitting acceptor substrate. Under these incubation conditions, sulphate formation was linear with time and protein concentration. For assays at different pH values, phosphate buffer was replaced by 10 mM sodium acetate, pH 5.5, or 10 mM Tris/HCl, pH 7.4. In the assays for measurement of the K_m and V_{max} values for 2-naphthol and guaiacol, the concentration of PAPS was increased to 10 μM (0.1 μCi).

Assay of *N*-OH-AAF sulphation

Sulphation of *N*-OH-AAF was measured by the method of Yamazoe et al. [16] based on the reductive formation of AAF from the O-sulphonyl derivative of *N*-OH-AAF in the presence of dithiothreitol. The reaction mixture consisted of 50 mM bicine/KOH, pH 7.8, 250 μM PAPS, 1 mM dithiothreitol, *E. coli* lysate (40 μg of protein) and 20 μM *N*-OH-AFF, in a final volume of 100 μl. The reaction was terminated after incubation for 20 min at 37 °C by the addition of 200 μl of acetonitrile, after which the mixture was centrifuged at 180000 ***g***. The metabolites were separated by HPLC with a Nucleosil $_7C_{18}$ column (4×250 mm) and detected by the absorbance at 280 nm. A mixture of acetonitrile and 20 mM potassium dihydrogen phosphate (60:40) was used as the mobile phase. In the assays to determine the K_m and V_{max} values for *N*-OH-AAF, the concentration of PAPS was decreased to 25 μM.

RNA isolation and RT–PCR

Tissues were excised from male ddY mice (10 weeks old) and total RNA was isolated by the guanidinium thiocyanate phenol/chloroform extraction method [17]. RNA quality was evaluated by the integrity of ribosomal RNA bands of the denaturing agarose-gel electrophoresis of the total RNA. The first strand of cDNA was synthesized from 2.5 μg of total RNA by 1 unit of M-MLV reverse transcriptase with oligo(dT) primers in accordance with the manufacturer's protocol (Stratagene). PCR was performed with the single-stranded cDNA as a template for *Taq* DNA polymerase. Primers used for PCR for the olfactory P-ST were 5′-G^{271}GAACTTCCAAGCAAAGCCCG^{290}-3′ (forward) and 5′-T^{1067}CAGATCTCTGTGCGGAAGG^{1048}-3′ (reverse) (with nucleotide numbers shown in Figure 1). Primers for PCR of β-actin and *m*ST*p1* were designed from the published sequences [18,19]. Primers for *m*ST*p1* were 5′-T^{248}ATCAGGGTGGCAAGCTAG^{266}-3′ (forward) and 5′-A^{805}ACTTCAGTTGGGATGGTTG^{786}-3′ (reverse) (numbered from [19]). Cycling conditions (30–40 cycles) were programmed to 1 min at 94 °C, 2 min at 54 °C and 2 min at 72 °C for each cycle.

Other procedures

Protein concentrations were determined by the method of Bradford [20] with BSA as a standard. SDS/PAGE was performed by the method of Laemmli [21]. Immunoblot analysis was performed as described previously [10].

RESULTS

Cloning and sequence analysis of olfactory ST cDNA

The mouse olfactory λgt11 expression library (3.6×10^5 plaque-forming units) was screened with the anti-serum raised against the purified rat liver P-ST isoenzyme, PSTG [13]. An inserted cDNA of the positive phage clone was excised by *Eco*RI, then subcloned and sequenced. The cDNA (designated mOlfST cDNA) was 1347 bp in length and had a 912 bp open reading frame beginning at base position 153 and encoding a 304-residue polypeptide (Figure 1). Sequence similarities to other ST enzymes are listed in Table 1. Surprisingly, both the nucleotide and amino acid sequences are 94 % identical with those of a male-dominant rat liver P-ST isoenzyme, ST1C1 [22] (Figure 2). Rat liver ST1C1 is known to catalyse the sulphation of *N*-OH-AAF, by which the substrate is activated to a carcinogenic compound [22–26]. Figure 3 shows the molecular phylogenetic tree of related STs of mouse, rat, human and plant, calculated from the amino acid sequences of the STs.

Expression of mOlfST in *E. coli*

An expression vector for mOlfST cDNA was constructed by introducing the entire coding sequence into a prokaryotic expression vector, pTrc99A. As shown in Table 2, the lysate of *E. coli* expressing mOlfST cDNA displayed high P-ST activity towards 2-naphthol (0.64 nmol/min per mg) at pH 6.4, comparable with those of mouse liver (0.77 nmol/min per mg) and olfactory (0.91 nmol/min per mg) cytosols [10,11]. Immunoblot analysis probed with anti-PSTG antiserum identified a 34 kDa polypeptide in the mOlfST-expressing lysate, as in the mouse olfactory cytosol (Figure 4).

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CTCATGCATCCGGCAGAACTCAAAAGCTATCCTGGAAGATCCGTCTAAAGACAGTGAAGT   60
CATCAACGGTCAACAAACAGGGAACAGGGAACACAGGCCAAGGTAGAGATCGGTCCTCAC  120

CTGACATCCTAGCCTGTCAAGTCCCCATTCTAAGATGCCCTTGGAAAAATTGAAAGACCT  180
                                  M  P  L  E  K  L  K  D  L     9

CCACCTCGATGAACAGAACATGCAGCCAGAAACCAGGGAAGTGAATGGGATTCTAATGTC  240
  H  L  D  E  Q  N  M  Q  P  E  T  R  E  V  N  G  I  L  M  S    29

CAAGATGATGAGTGAAAACTGGGACAAAATTTGGAACTTCCAAGCAAAGCCCGATGATCT  300
  K  M  M  S  E  N  W  D  K  I  W  N  F  Q  A  K  P  D  D  L    49

CCTCATTGCAACCTATGCAAAGGCAGGTACCACCTGGACACAGGAAATTGTGGACATGAT  360
  L  I  A  T  Y  A  K  A  G  T  T  W  T  Q  E  I  V  D  M  I    69

CCAAAATGATGGGGATGTTCAAAAGTGCCAGCGGGCCAATACCTATGACCGACACCCTTT  420
  Q  N  D  G  D  V  Q  K  C  Q  R  A  N  T  Y  D  R  H  P  F    89

CATTGAGTGGACTTTGCCTCCACCCCTCAACTCAGGTCTGGATCTGGCTAACAAAATGCC  480
  I  E  W  T  L  P  P  P  L  N  S  G  L  D  L  A  N  K  M  P   109

TTCACCTAGAACTCTGAAGACTCATCTGCCTGTTCAAATGCTTCCACCTTCCTTTTGGAA  540
  S  P  R  T  L  K  T  H  L  P  V  Q  M  L  P  P  S  F  W  K   129

AGAAAACTCAAAGATTATCTATGTGGCCAGAAATGCCAAGGACTGCCTGGTATCTTACTA  600
  E  N  S  K  I  I  Y  V  A  R  N  A  K  D  C  L  V  S  Y  Y   149

CTATTTCTCAAGAATGAATAAAATGCTGCCTGACCCTGGTACCCTGGGAGAATACATTGA  660
  Y  F  S  R  M  N  K  M  L  P  D  P  G  T  L  G  E  Y  I  E   169

AACCTTCAAAGCTGGAAAAGTGCTGTGGGGCTCCTGGTATGACCATGTAAAGGGATGGTG  720
  T  F  K  A  G  K  V  L  W  G  S  W  Y  D  H  V  K  G  W  W   189

GGATGTGAAAGACAAACACCGTATTCTGTACCTCTTCTATGAAGACATGAAAGAGGACCC  780
  D  V  K  D  K  H  R  I  L  Y  L  F  Y  E  D  M  K  E  D  P   209

TAAAAGGGAAATTAAGAAGATAGTAAAATTCCTGGAAAAAGACATTTCAGAGGAAGTTCT  840
  K  R  E  I  K  K  I  V  K  F  L  E  K  D  I  S  E  E  V  L   229

AAATAAAATCATCCATCACACCTCCTTTGATGTAATGAAGCAGAACCCAATGGCCAACTA  900
  N  K  I  I  H  H  T  S  F  D  V  M  K  Q  N  P  M  A  N  Y   249

TACCACTCTACCCTCCAGTATCATGGACCACTCTATATCTCCTTTCATGAGAAAAGGGAT  960
  T  T  L  P  S  S  I  M  D  H  S  I  S  P  F  M  R  K  G  M   269

GCCTGGAGACTGGAAGAACTACTTTACCGTGGCACAAAGTGAGGATTTTGATGAAGACTA 1020
  P  G  D  W  K  N  Y  F  T  V  A  Q  S  E  D  F  D  E  D  Y   289

CCGGAAGAAGATGGCAGGGAGCACTATTACCTTCCGCACAGAGATCTGAGAGCAGTAGGG 1080
  R  K  K  M  A  G  S  T  I  T  F  R  T  E  I  *               304

TAGAGGGAATCCCTAGATGTCCTGACTGTATGCTTTAGCTATTTGAGCCTCCATTCCTAA 1140
GCGGTTGCACGGTTCTATGTGTCCTATGATTCTATTTCATCAAAAGGTAATCAGACCTTC 1200
TACAGAAATTTTTGGTTACACTATGTGAATATTATTGATACCTACCACACAATCATGTAC 1260
TTCACTACACTTGGGCAAAATAAACGGAATGCCTTCACTAGCCAAAAAAAAAAAAAAAAA 1320
AAAAAAAAAAAAAAAAAAAAAAAAAA                                    1347
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Figure 1 Nucleotide and deduced amino acid sequences of mouse olfactory P-ST cDNA

The stop codon is indicated by an asterisk. The consensus polyadenylation signal is underlined.

Table 1 Amino acid sequence similarities between mOlfST and other STs

All amino acid sequences except for olfactory ST were taken from the GenBank database [GenBank accession numbers O33653 (mOlfST), L02335 (mSTa1), L27121 (mSTa2), L02331 (mSTp1), M31363 (ST-20), M33329 (ST-40), X52883 (ST1A1), U38419 (DTST), L22339 (ST1C1), U08024 (hDHEAST), L19956 (hTLPST), L10819 (hTSPST), U66036 (hST1C1), M84135 (FST3)]. Abbreviations: P-ST, phenol ST; HS-ST, hydroxysteroid ST; F-ST, flavonol ST; DTST, dihydroxyphenylalanine/tyrosine ST; rEST, rat oestrogen ST; hDHEAST, human dehydroepiandrosterone ST; hTLPST, human thermolabile P-ST; hTSPST, human thermostable P-ST; hST1C1, human SULT1C1; FST3, flavonol ST3.

Species	STs[a]	St family	Similarity (%)
Mouse	mOlfST	P-ST	100
	mSTa1	HS-ST	40.8
	mSTa2	HS-ST	41.2
	mSTp1	P-ST	55.0
Rat	ST-20	HS-ST	39.4
	ST-40	HS-ST	39.6
	ST1A1	P-ST	53.1
	ST1B1/DTST	P-ST	52.7
	ST1C1	P-ST	94.4
	rEST	P-ST	48.4
Human	hDHEAST	HS-ST	39.0
	hTLPST	P-ST	51.0
	hTSPST	P-ST	50.7
	hST1C1	P-ST	61.6
Plant	FST3	F-ST	29.2

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mOlfST    1:MPLEKLKDLHLDEQNMQPETREVNGILMSKMMSENWDKIWNFQAKPDDLLIATYAKAGTT 60
rST1C1    1:MSLEKMKDLHLGEQDLQPETREVNGILMSKLMSDNWDKIWNFQAKPDDLLIATYAKAGTT 60
hST1C1    1:M------ALT-SDLGKQIKLKEVEGTLLQPATVDNWSQIQSFEAKPDDLLICTYPKAGTT 53
            * ... . *.. ..  *....**.*.*... .. **..*..*.********.**.*****

mOlfST   61:WTQEIVDMIQNDGDVQKCQRANTYDRHPFIEWTLPPPLNSGLDLANKMPSPRTLKTHLPV 120
rST1C1   61:WTQEIVDMIQNDGDVQKCQRANTYDRHPFIEWTLPSPLNSGLDLANKMPSPRTLKTHLPV 120
hST1C1   54:WIQEIVDMIEQNGDVEKCQRAIIQHRHPFIEWARP-PQPSGVEKAKAMPSPRILKTHLST 112
            *.*******...***.*****....********..* *..**...*..*****.*****..

mOlfST  121:QMLPPSFWKENSQIIYVARNAKDCLVSYYYFSRMNKMLPDPGTLGEYIETFKAGKVLWGS 180
rST1C1  121:HMLPPSFWKENSKIIYVARNAKDCLVSYYYFSRMNKMLPDPGTLGEYIEQFKAGKVLWGS 180
hST1C1  113:QLLPPSFWENNCKFLYVARNAKDCMVSYYHFQRMNHMLPDPGTWEEYFETFINGKVVWGS 172
             .******..*. ..*********.****.*.***.*******..**.* *..***.***

mOlfST  181:WYDHVKGWWDVKDKHRILYLFYEDMKEDPKREIKKIVKFLEKDISEEVLNKIIHHTSFDV 240
rST1C1  181:WYDHVKGWWDVKDQHRILYLFYEDMKEDPKREIKKIAKFLEKDISEEVLNKIIVHTSFDV 240
hST1C1  173:WFDHVKGWWEMKDRHQILFLFYEDIKRDPKHEIRKVMQFMGKKVDETVLDKIVQETSFEK 232
            *.*******..** *.**.*****.*.***.**.*. .*..*...*.**.**. .***..

mOlfST  241:MKQNPMANYTTLPSSIMDHSISPFMRKGMPGDWKNYFTVAQSEDFDEDYRKKMAGSTITF 300
rST1C1  241:MKENPMANYTTLPSSIMDHSISPFMRKGMPGDWKNYFTVAQSEDFDEDYRRKMAGSNITF 300
hST1C1  233:MKENPMTNRSTVSKSILDQSISSFMRKGTVGDWKNHFTVAQNERFDEIYRRKMEGTSINF 292
            ** ***.*..*...**.*.***.*****..*****.*****.*.***.** **.*. *.*

mOlfST  301:RTEI
rST1C1  301:RTEI
hST1C1  293:CMEL
            ..*.
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Figure 2 Alignment of amino acid sequences of mouse olfactory P-ST (mOlfST), rat ST1C1 (rST1C1) and human SULT1C1 (hST1C1)

Identical amino acids are donated by asterisks, and dots represent amino acids identical between mOlfST and rat ST1C1.

In previous reports we showed the presence of high ST activities towards phenolic odorants in the mouse olfactory tissues [10,11]. To discover whether the ST enzyme encoded by the mOlfST cDNA is active towards phenolic odorants, we measured the activity of the expressed enzyme towards several phenolic odorants. As shown in Table 3, the expressed enzyme displayed high activities towards phenolic odorants such as eugenol and guaiacol at pH 6.4, which were 6-fold that towards 2-naphthol. As reported previously, olfactory and liver cytosols showed very weak or no activities towards alcoholic aromatic odorants [10,11]. The expressed enzyme showed no activity towards a representative alcoholic aromatic odorant, 3-phenylpropan-1-ol (Table 3). We have reported a unique ST activity in the olfactory cytosols towards cinnamyl alcohol at neutral pH [11]. As shown in Table 3, the expressed enzyme also displayed significant activity towards cinnamyl alcohol at pH 6.4, as in nasal cytosols. No activity was detectable towards cinnamyl alcohol at pH 5.5, as observed in the olfactory cytosols [11].

We previously reported effects of inhibitors on the nasal ST activity towards phenolic odorants [11]. 2,6-Dichloro-4-nitrophenol (DCNP), a P-ST specific inhibitor, inhibited nasal ST activities completely at 10 μM; however, the hydroxysteroid ST-specific inhibitors triethylamine (TEA) and tetra-n-butylammonium chloride (TBA) did not inhibit these activities at all. We measured the effects of these inhibitors on the expressed mOlfST enzyme activity. More than 95% of the activity toward cinnamyl alcohol, guaiacol, 4-isopropyl phenol or 2-naphthol

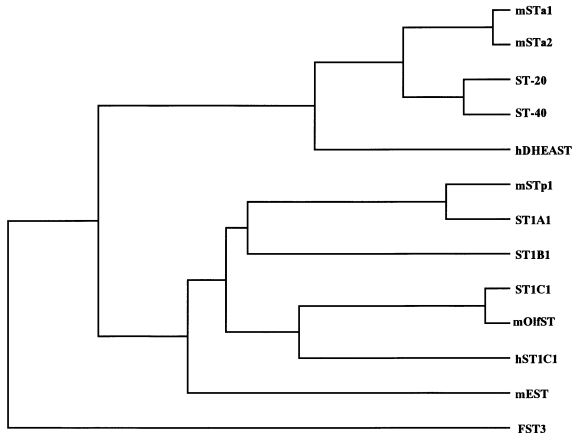

Figure 3 Molecular phylogenetic tree of mouse, rat, human and plant ST enzymes

Genetic distance was calculated by GENETYX-Mac DNA analysis software (Macintosh) with amino acid sequences of the ST enzymes. All amino acid sequences except for mOlfST were taken from the GenBank DNA database. Abbreviations and accession numbers are as shown in Table 1.

Table 2 P-ST activity in the lysate of *E. coli*-expressed mOlfST

The activity toward 2-naphthol was assayed at pH 6.4 by using lysates of 10 μg of protein. All values are the averages of duplicate assays. Deviations are within 5% of the values. Abbreviation: n.d., not detected.

Species	Lysate	P-ST activity (nmol/min per mg of protein)
E. coli	pTrc-*m*olfST	0.64
	pTrc99A	n.d.
Mouse	Olfactory	0.91
	Liver	0.77

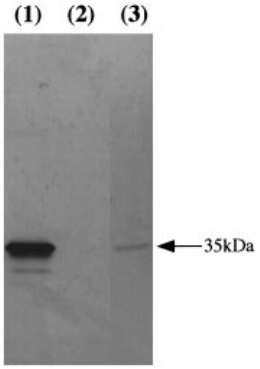

Figure 4 Western blot analysis of the *E. coli*-expressed olfactory P-ST proteins

Lysates were separated by SDS/PAGE and blotted on a nitrocellulose membrane. The membrane was immunostained with anti-P-ST_G antiserum (1:5000 dilution). The same amount of protein (10 μg) of each lysate was loaded. Lane 1, lysate of *E. coli* expressing mOlfST; lane 2, pTrc99A-harbouring *E. coli* lysates; lane 3, mouse nasal cytosols.

Table 3 ST activities of the *E. coli*-expressed mOlfST towards aromatic odorants and *N*-OH-AAF

ST activity was assayed as described in the Materials and methods section. All values are means of duplicated assays. Deviations are within 5% of the values. Abbreviation: n.d., not detected.

Substrate	ST activity (nmol/min per mg of protein)	
	mOlfST-*E. coli* lysate	Nasal cytosol
3-Phenylpropan-1-ol	n.d.	n.d.
Cinnamyl alcohol	0.65	0.10
Eugenol	2.66	1.26
Guaiacol	2.62	1.46
2-Naphthol	0.43	0.98
N-OH-AAF	1.38	0.25

was inhibited by 10 μM DCNP, whereas TEA and TBA showed no inhibitory effect even at 1 mM, except on cinnamyl alcohol. The sulphation of cinnamyl alcohol at pH 7.4 was moderately inhibited by TEA (30%) and TBA (18%), as in the nasal cytosols reported previously [11].

N-OH-AAF sulphation activity of the expressed mOlfST enzyme

Rat liver ST1C1 is thought to be responsible for *N*-OH-AAF sulphation in male rat liver [22]. On sulphation, the compound initiates carcinogenic events by binding DNA, RNA and proteins [22–26]. As the amino acid sequence of mOlfST is highly similar to that of ST1C1, we measured *N*-OH-AFF sulphation activity in the lysates of mOlfST-expressing *E. coli* by the method of Yamazoe et al. [16]. We detected high *N*-OH-AAF sulphation activity in the mOlfST-expressing lysates (1.38 nmol/min per mg of protein), comparable to that for odorant sulphation and 3-fold that for 2-naphthol (0.43 nmol/min per mg of protein) (Table 3). No activity was detectable in the control lysates. Nasal cytosol also diaplayed significant *N*-OH-AAF sulphation activity, but the activity (0.25 nmol/min per mg of protein) was one-quarter of that for 2-naphthol (0.98 nmol/min per mg of protein) (Table 3).

Determination of kinetic parameters of the expressed mOlfST enzyme

To characterize the expressed enzyme further, we determined the kinetic constants for three representative substrates. On the basis of the Lineweaver–Burk plots, the apparent K_m values of the three substrates were calculated to be almost the same (2.60–3.36 μM), altough the apparent V_{max} value for *N*-OH-AAF was small (0.709 nmol/min per mg of protein) compared with those for 2-naphthol and guaiacol (36.1 and 40.7 nmol/min per mg respectively) (Table 4). The lower apparent V_{max} for *N*-OH-AAF might be ascribable to the decreased PAPS concentration used in the kinetic study.

Tissue specificity of mOlfST expression

As a first step towards characterizing olfactory ST expression, we surveyed the expression of the isolated cDNA in other tissues by RT–PCR using primer sets specific for mOlfST cDNA. We designed primers to avoid cross-annealing to three other known mouse STs, *m*STp1, *m*STa1 and *m*STa2 [27,28]. As shown in

Table 4 Kinetic parameters of the expressed mOlfST enzyme

The ST activities for three representative substrates were measured as described in the Materials and methods section. The apparent K_m and V_{max} values were calculated on the basis of the Lineweaver–Burk plots. The ranges of substrate concentrations used for the assays were 0.1–2 μM (2-naphthol), 1–5 μM (guaiacol) and 1–10 μM (*N*-OH-AAF) respectively. All values are means ± S.D. ($n = 3$).

Substrate	K_m (μM)	V_{max} (nmol/min per mg)
2-Naphthol	3.36 ± 0.43	36.1 ± 5.5
Guaiacol	3.15 ± 0.37	40.7 ± 3.7
N-OH-AAF	2.60 ± 0.02	0.709 ± 0.02

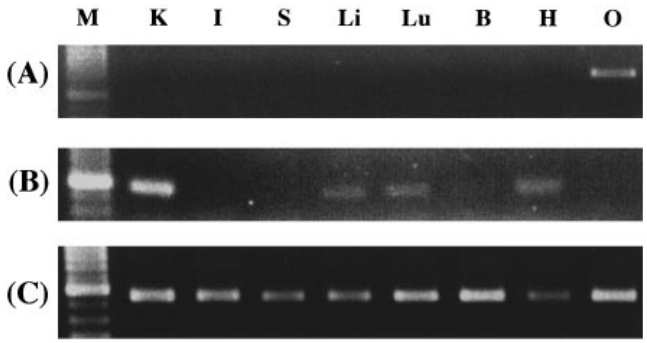

Figure 5 Tissue specificity of mOlfST mRNA expression

Total RNA species were isolated from tissues of 10-week-old ddY mice (male) and 2.5 μg of total RNA was subjected to each RT–PCR specific for (**A**) mOlfST, (**B**) *m*ST*p1* or (**C**) β-actin. Abbreviations: M, DNA size markers; K, kidney; I, intestine; S, spleen; Li, liver; Lu, lung; B, brain; H, heart; O, olfactory.

Figure 5(A), an 800 bp PCR product was detected only in the RNA isolated from olfactory tissues, indicating that the expression of mOlfST mRNA is specific for olfactory tissues out of eight mouse tissues examined (kidney, intestine, spleen, liver, lung, brain, heart and olfactory). Even increasing the cycle number of PCR (from 30 to 40 cycles) did not produce any corresponding bands in other tissue RNA species. As controls, cDNA species of β-actin (ubiquitously expressed) and *m*ST*p*1, a P-ST isoenzyme cloned from mouse liver [19], were amplified by RT–PCR with specific primer sets (Figures 5B and 5C). The levels of β-actin expression differed slightly between tissues; however, this did not account for the olfactory-specific expression of the isolated cDNA. Expression of *m*ST*p1* was detected in kidney, lung, brain and heart in addition to liver, but no expression was observed in olfactory tissues (Figure 5B).

DISCUSSION

Here we report the isolation and characterization of a P-ST cDNA (mOlfST cDNA) obtained from a mouse olfactory cDNA library. The nucleotide and amino acid sequences of the cDNA are highly similar to rat liver P-ST isoenzyme ST1C1 (Table 1). The high similarity (94 %) between the two STs suggests that ST encoded by the isolated cDNA is a mouse orthologue of rat ST1C1. However, the tissue specificity of expression of these two STs is quite different, because the expression of mOlfST is highly specific for nasal tissues (Figure 5), whereas that of ST1C1 is for livers. Recently Her et al. [29] have reported the cloning of a human orthologue of rat ST1C1 from a fetal liver–spleen cDNA library. Its expression is specific for human adult stomach, kidney and thyroid as well as fetal liver and kidney. Its amino acid sequence similarities to rat ST1C1 and mOlfST are 62 % and 61.6 % respectively. As shown in Figure 3, these three enzymes belong to the same subfamily.

We expressed the mOlfST cDNA in *E. coli*. The expressed enzyme catalysed the sulphation of phenolic odorants such as eugenol and guaiacol as efficiently as that of 2-naphthol, a typical substrate for P-ST (Table 3). Furthermore the expressed enzyme showed a significant activity towards cinnamyl alcohol, an alcoholic aromatic odorant, at neutral pH, as did the nasal cytosols reported previously [11]. The sulphation activity of the expressed enzyme was inhibited by a low concentration (10 μM) of DCNP, but not by TEA and TBA except for the sulphation of cinnamyl alcohol. All these properties of the expressed enzyme are similar to those of nasal cytosol P-ST activities, suggesting that mOlfST enzyme should be involved, at least in part, in the sulphation of aromatic odorants in the mouse olfactory tissues *in vivo*.

We detected significant *N*-OH-AAF sulphation activity by the expressed mOlfST, as by rat ST1C1 [22] (Table 3). The sulphation form of *N*-OH-AAF is known to degrade into a reactive electrophile capable of binding covalently to DNA, RNA and protein [16,22–26], and the importance of sulphation in arylamine hepatocarcinogenesis in experimental animals has been well documented [30,31]. At the moment it is unclear whether the sulphation activity of mOlfST for carcinogenic *N*-OH-AAF is correlated to the risk of nasal carcinoma or other mouse tumours. Further characterization of the mOlfST activity towards other odorants and carcinogenic *N*-OH-derivatives of arylamine and heterocyclic amine carcinogens must be performed to understand the physiological function of the enzyme in olfactory perception, detoxification and carcinogenesis.

We measured kinetic parameters for three representative substrates and found that the K_m values for these compounds are essentially the same (approx. 3 μM; Table 4). These values are comparable to those of other mammalian P-ST isoenzymes, although the results for ST1C1 are not available. These results indicate that the expressed mOlfST has a great affinity for phenolic compounds as well as for *N*-OH-AAF.

The expressed enzyme reacted with the anti-PSTG antiserum. However, the band intensities in the immunoblot analysis differed between *E. coli* lysates and nasal cytosols (Figure 4, lanes 1 and 3), although the P-ST activities in both extracts were compatible (Table 2). As the observed difference was reproducible, this suggests the presence of other unknown P-ST(s) that did not react with the antiserum. Our preliminary cloning experiments suggest the expression of several novel P-ST cDNA species in the olfactory tissues (results not shown). Characterization of these cDNA species would help our understanding of the molecular nature and function of olfactory ST enzymes.

We have previously reported a zonal distribution of immunopositive regions in the nasal cavity stained with antiserum raised against a rat P-ST isoenzyme, PSTG [10]. The zonal pattern resembled that of one of the olfactory receptors [12]. Zonal specification could involve transcription factors that are differentially expressed in different zones. In this report we have shown, by RT–PCR, nasal-specific expression of the isolated cDNA (Figure 5). These results suggest that olfactory P-ST expression might be controlled in a specific manner for nasal neuronal differentiation [10,12,32]. The isolation of genomic clones and identification of the regulatory elements will define the molecular mechanism of the regulation of nasal P-ST expression during nasal neuronal differentiation.

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