Biochemical and Biophysical Research Communications **254,** 65–69 (1999)
Article ID bbrc.1998.9872, available online at http://www.idealibrary.com on IDEAL®

Bacterial Expression, Purification, and Characterization of a Novel Mouse Sulfotransferase That Catalyzes the Sulfation of Eicosanoids

Ming-Cheh Liu,*[,1] Yoichi Sakakibara,* and Chau-Ching Liu†
**Department of Biochemistry, University of Texas Health Center, Tyler, Texas 75708; and †Department of Medicine, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania 15213*

Received November 17, 1998

Analysis of the nucleotide sequence of a recently cloned mouse sulfotransferase cDNA (clone 679153) revealed the presence in its 3′-untranslated sequence of an AT-rich region which contains four ATTTA motifs and an TTATTTAT-like sequence, commonly found among those encoding inflammation-related proteins. The recombinant enzyme expressed in *Escherichia coli* and purified to near electrophoretic homogeneity displayed strong sulfotransferase activities toward various prostaglandins, thromboxane B_2, and leukotriene E_4. These results mark the first discovery of the sulfation of eicosanoids catalyzed by a distinct sulfotransferase.

In mammals, sulfation 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, the so-called "cytosolic sulfotransferases," constitute a superfamily of enzymes that catalyze the transfer of a sulfonate group from the active sulfate, 3′-phosphoadenosine 5′-phosphosulfate (PAPS), to a substrate compound containing a hydroxyl or an amino group [1]. Based on the amino acid sequences of known mammalian cytosolic sulfotransferases, two gene families, the phenol sulfotransferase (PST) family (designated SULT1) and hydroxysteroid sulfotransferase (HSST) family (designated SULT2), have been categorized [4,5]. The PST family presently consists of four sub-families, PSTs (SULT1A), Dopa/tyrosine (or thyroid hormone) sulfotransferases (SULT1B), hydroxyarylamine (or acetylaminofluorene) sulfotransferases (SULT1C), and estrogen sulfotransferases (SULT1E) [5]. Taking into account the two recently discovered human hydroxysteroid sulfotransferases [6,7], the HSST family now comprises two subfamilies, SULT2A and SULT2B. In a recent study, we have discovered a novel mouse sulfotransferase (clone 679153) that belongs to the PST family [8]. Because of its low % identity to other members of the family, however, this new sulfotransferase has been placed independently from the four known sub-families of the phenol sulfotransferase family mentioned above.

We report in this communication the sequence analysis and identification of an AT-rich region and its constituent sequence motifs present in the 3′-untranslated sequence of the cDNA encoding this novel mouse sulfotransferase, as well as the bacterial expression, purification, and characterization of the recombinant enzyme.

EXPERIMENTAL PROCEDURES

Materials. Serotonin (5-hydroxytryptamine), p-nitrophenol, dopamine, histamine, β-naphthol, β-naphthylamine, aprotinin, thrombin, adenosine 5′-triphosphate (ATP), sodium dodecyl sulfate (SDS), *N*-2-hydroxylpiperazine-*N*′-2-ethanesulfonic acid (Hepes), 3-[*N*-tris-(hydroxymethyl)methylamino]-propanesulfonic acid (Taps), Trizma base, dithiothreitol (DTT), and isopropyl β-D-thiogalactopyranoside (IPTG) were products of Sigma Chemical Co. Prostaglandins (A_1, B_2, D_2, E_1, E_2, $F_{1\alpha}$, and $F_{2\alpha}$), thromboxane B_2, and leukotriene E_4 were from Cayman Chemical Company. Serotonin-*O*-sulfate was synthesized according to the procedure of Jevons [9]. *Pfu* DNA polymerase, pBluescript II SK(+), and XL1-Blue MRF′ *E. coli* host strain were from Stratagene. Ampli-*Taq* DNA polymerase was product of Perkin–Elmer. T4 DNA polymerase, T4 DNA ligase, and all restriction endonucleases were from New England Biolabs. Oligonucleotide primers were synthesized by Operon Technologies, Inc. pGEX-2TK glutathione *S*-transferase (GST) gene fusion vector, *E. coli* BL21, and glutathione Sepharose 4B were products of Pharmacia Biotech. A pcDNA3 mammalian cell expression vector harboring the full-length sequence of mouse sulfotransferase clone 679153 (pcDNA3 · mST 679153) was prepared as previously described [8]. All restriction endonucleases were from New England Biolabs. Sulfate-activating enzymes, ATP sulfurylase and adenosine 5′-phos-

[1] To whom correspondence should be addressed at Department of Biochemistry, University of Texas Health Center, 11937 U.S. Highway 271, Tyler, TX 75708-3154. Fax: (903) 877-2863. E-mail: liu@uthct.edu.

Abbreviations used: PST, phenol sulfotransferase; PAPS, 3′-phosphoadenosine 5′-phosphosulfate; SDS-PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis.

0006-291X/99 $30.00

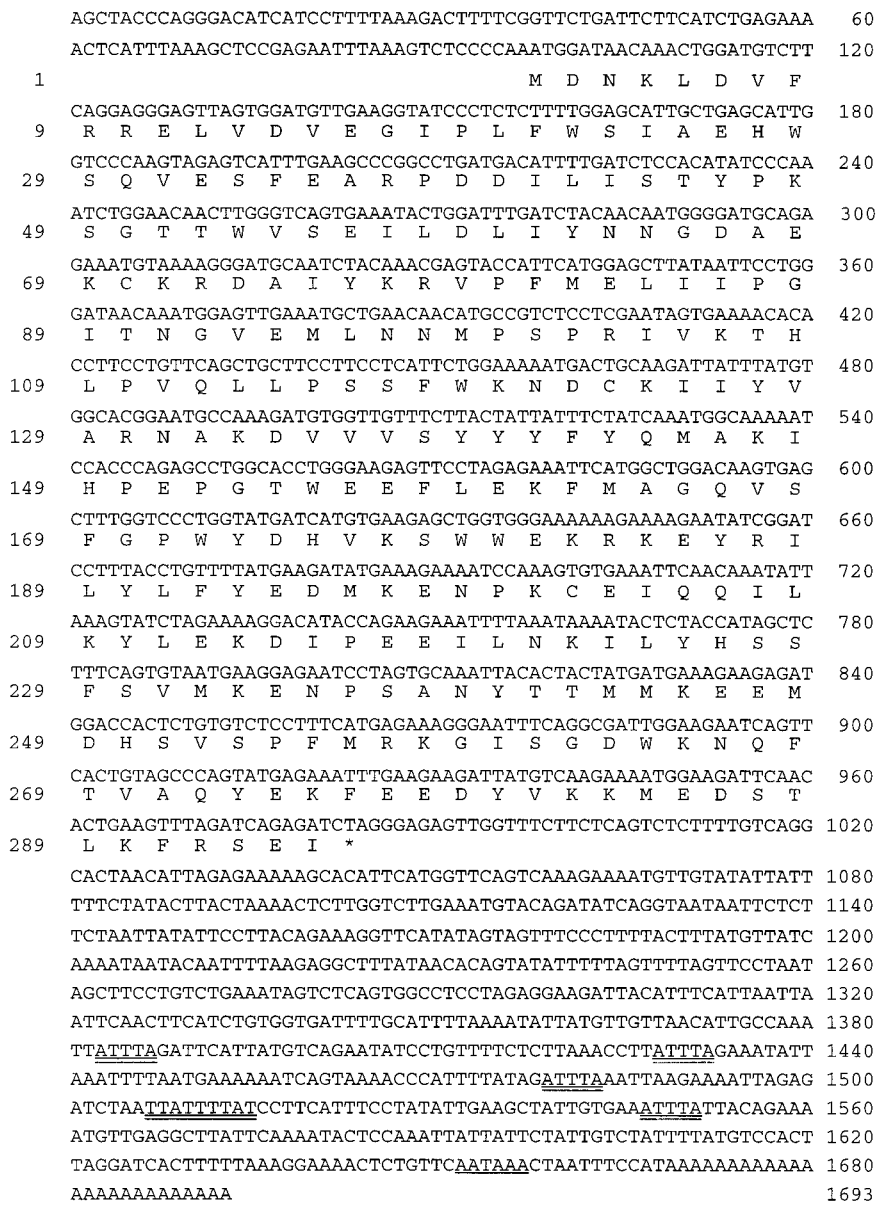

FIG. 1. Nucleotide and deduced amino acid sequences of the mouse sulfotransferase clone 679153 cDNA. Nucleotides are numbered in the 5′ to 3′ direction with the adenosine of the translation initiation codon designated as +1. The translation stop codon is indicated by an asterisk. The polyadenylation signal is singly underlined, and the ATTTA and TTATTTAT-like motifs are doubly underlined.

phosulfate kinase, from *Bacillus stearothermophilus* were kindly provided by Dr. Hiroshi Nakajima at Unitika, Ltd. (Uji, Japan). Chromatogram cellulose thin-layer chromatography (TLC) plates were products of Eastman Kodak Company. Carrier-free sodium [^{35}S]sulfate was from ICN Biomedicals. All other reagents were of the highest grades commercially available.

Bacterial expression and purification of recombinant mouse sulfotransferase clone 679153. To amplify the mouse sulfotransferase clone 679153 sequence for subcloning into a prokaryotic expression vector pGEX-2TK, a set of sense and antisense oligonucleotide primers (5′-CGCGGATCCATGGATAACAAACTGGATGTC-3′ and 5′-GGCGAATTCCTAGATCTCTGATCTAAACTT-3′), based on 5′- and 3′-regions of the nucleotide sequence encoding the mouse sulfotransferase clone 679153, were synthesized with *Bam*HI and *Eco*RI restriction sites incorporated at the ends. With these two oligonucleotides as primers, a PCR in a 100-μl reaction mixture was carried out under the action of *Pfu* DNA polymerase using pcDNA3 · mST 679153 as the template. Amplification conditions were 27 cycles of 1 min at 94°C, 1 min at 56°C, and 2 minutes at 72°C. The final reaction mixture was applied onto a 1.2% agarose gel and separated by electrophoresis. The discrete PCR product band, visualized upon ethidium bromide staining, was excised from the gel and the DNA fragment therein was isolated by spin filtration. After *Bam*HI/*Eco*RI digestion, the PCR product was subcloned into the *Bam*HI/*Eco*RI site of pGEX-2TK and transformed into *E. coli* BL21. To verify its authenticity, the cDNA insert was subjected to nucleotide sequencing.

Competent *E. coli* BL21 cells were transformed with pGEX-2TK harboring the full-length cDNA encoding the mouse sulfotransferase clone 679153. The transformed cells, grown to $OD_{600\,nm} = \sim 0.5$ in 1 liter LB medium supplemented with 100 μg/ml ampicillin and induced with

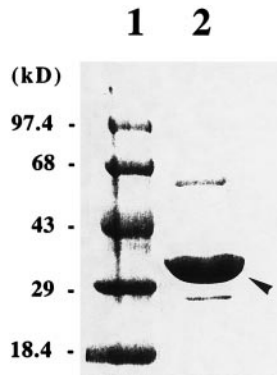

FIG. 2. SDS gel electrophoretic pattern of purified recombinant mouse sulfotransferase clone 679153. Purified recombinant mouse sulfotransferase sample (lane 2) was subjected to SDS–PAGE on a 12% gel, followed by Coomassie blue staining. Protein molecular weight markers coelectrophoresed on lane 1 are β-lactoglobulin (M_r = 18,400), carbonic anhydrase (M_r = 29,000), ovalbumin (M_r = 43,000), bovine serum albumin (M_r = 68,000), phosphorylase B (M_r = 97,400).

0.1 mM IPTG overnight at room temperature, were collected by centrifugation and homogenized in 20 ml ice-cold STE (10 mM Tris–HCl, pH 8.0, 150 mM NaCl, 1 mM EDTA) using an Aminco 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 fractionated using 0.5 ml of glutathione Sepharose, and the bound GST fusion protein was treated with 2 ml of a thrombin digestion buffer (50 mM Tris–HCl, pH 8.0, 150 mM NaCl, and 2.5 mM $CaCl_2$) containing 5 unit/ml bovine thrombin. Following a 30-min incubation at room temperature with constant agitation, 5 μg of aprotinin were added to inactivate thrombin and the preparation was subjected to centrifugation. The recombinant enzyme present in the supernatant collected was analyzed by SDS–gel electrophoresis for purity and examined with respect to its enzymatic properties.

Enzymatic assay. The sulfotransferase activities were assayed using 3′-phosphoadenosine 5′-phospho[^{35}S]sulfate (PAP[^{35}S]) as the sulfate donor. The standard assay mixture, with a final volume of 30 μl, contained 50 mM Taps (pH 8.25), 14 μM PAP[^{35}S] (15 Ci/mmol), and specified concentration of substrate. 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, and the supernatant was subjected to the analysis of [^{35}S]sulfated product based on the thin-layer chromatography separation procedure using n-butanol/isopropanol/88% formic acid/water (3:1:1:1; by volume) as the solvent system [10].

Miscellaneous methods. PAP[^{35}S] (15 Ci/mmol) was synthesized from ATP and [^{35}S]sulfate using the sulfate-activating enzymes, ATP sulfurylase and adenosine 5′-phosphosulfate kinase, from *Bacillus stearothermophilus* as described previously [11]. Protein determination was based on the method of Bradford [12] with bovine serum albumin as the standard.

RESULTS AND DISCUSSION

Figure 1 shows the nucleotide and deduced amino acid sequences of the cDNA encoding mouse sulfotransferase clone 679153 (GenBank Accession No. AF026073). The open reading frame, beginning at base residue 98, encompasses 885 nucleotides and encodes a 295-amino acid polypeptide. The predicted molecular weight, 35,099, falls within the molecular weight range (33,000–36,000) generally found for cytosolic sulfotransferase enzymes [1–3]. The termination codon, located at nucleotide residues 983–985, is followed by a 708-nucleotide 3′-untranslated sequence which includes a poly(A) tract. Examining the 3′-untranslated region, it was interesting to note the presence of four ATTTA sequence motifs (with their first A residues being located at, respectively, positions 1383, 1427, 1479, and 1547; as underlined). The region between the first and the fourth motifs is highly AT-rich, being composed of 79.9% A/T. Previous studies have shown that AU-rich elements are found in the 3′-untranslated region of mRNAs encoding, in particular, inflammatory mediators, transcription factors, or proto-oncogenes [13]. The constituent sequence motifs, AUUUA, therein are believed to act as destabilizing sequences, conferring short half-lives upon those mRNAs [14]. In addition to the four ATTTA motifs, there is also a TTATTTTAT sequence observed at nucleotide residues 1507–1515 in the 3′-untranslated region of the sulfotransferase Clone 679153 cDNA (as underlined in Fig. 1). This sequence is similar to the previously identified sequence (TTATTTAT) present near the 3′ end of the cDNAs coding for inflammatory mediators [15]. That both the ATTTA motif and the TTATTTAT-like motif are present in the 3′-untranslated region raises the issue whether the sulfotransferase encoded by the clone 679153 cDNA is functionally related to the inflammation process *in vivo*. Such a possibility prompted our interest in preparing recombinant enzyme coded by

TABLE 1

Substrate Specificity of the Recombinant Mouse Sulfotransferase Clone 679153

Substrate[a]	Specific activity (nmol/min/mg protein)[b]
p-Nitrophenol	1.92 ± 0.04
Dopamine	2.40 ± 0.04
β-Naphthol	2.53 ± 0.03
β-Naphthylamine	0.20 ± 0.01
Serotonin	1.65 ± 0.08
Histamine	N.D.[c]
Prostaglandin A_1	0.91 ± 0.06
Prostaglandin B_2	0.86 ± 0.01
Prostaglandin D_2	0.92 ± 0.02
Prostaglandin E_1	0.81 ± 0.08
Prostaglandin E_2	0.94 ± 0.03
Prostaglandin $F_{1\alpha}$	0.87 ± 0.03
Prostaglandin $F_{2\alpha}$	0.89 ± 0.02
Thromboxane B_2	0.91 ± 0.02
Leukotriene E_4	0.71 ± 0.03

[a] The concentrations of substrates were 10 μM for *p*-nitrophenol and β-naphthol; 50 μM for dopamine and β-naphthylamine; 200 μM for prostaglandins, thromboxane B_2, and leukotriene E_4; and 1 mM for serotonin and histamine.

[b] Data shown represent means ± SD derived from three experiments.

[c] Activity not detected.

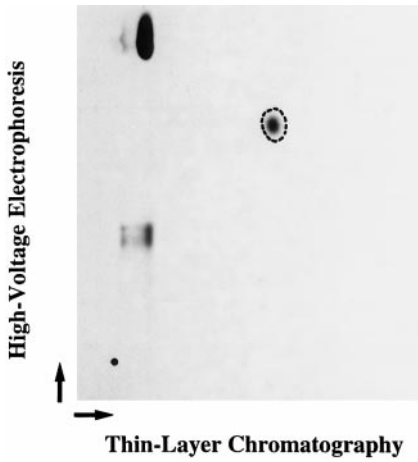

FIG. 3. Enzymatic sulfation of prostaglandin E_2 using PAP[^{35}S] as the sulfate donor. The figure shows the autoradiograph taken from the TLC plate used for the two-dimensional thin-layer analysis of the reaction mixture. The dashed-line circle indicates the position of [^{35}S]sulfated prostaglandin E_2. The strong radioactive spot located close to the upper left corner corresponds to the unused PAP[^{35}S] present in the reaction mixture.

this cDNA for investigating its potential activity toward eicosanoid compounds of which some have been shown to be involved in inflammatory response [16].

The full-length cDNA encoding the mouse sulfotransferase clone 679153 was subcloned into pGEX-2TK, a prokaryotic expression vector, for the expression of the recombinant enzyme in *E. coli*. As shown in Fig. 2, recombinant mouse sulfotransferase clone 679153, cleaved from the GST fusion protein fractionated from the *E. coli* homogenate using glutathione Sepharose, appeared to be highly purified and migrated at approximately 35 kDa position upon SDS–PAGE. The recombinant enzyme was subjected to functional characterization with respect to its sulfotransferase activities. Since the mouse sulfotransferase clone 679153 is categorized into the phenol sulfotransferase gene family based on sequence comparison with other mammalian cytosolic sulfotransferases [8], we decided to first test its activities toward a number of compounds previously shown to be substrates for phenol sulfotransferases. In view of the inflammation-related (ATTTA and TTATTTAT-like) motifs present in the 3′-untranslated region of the mouse sulfotransferase clone 679153 cDNA, a variety of eicosanoid compounds, as well as serotonin, were also tested as substrates. Activity data compiled in Table 1 revealed that the recombinant mouse sulfotransferase clone 679153 indeed was able to catalyze the sulfation of a number of known substrates, including serotonin, p-nitrophenol, dopamine, histamine, β-nitrophenol, and β-naphthylamine, for enzymes of the PST family. More importantly, the enzyme displayed strong activities catalyzing the sulfation of various prostaglandins (cf. Fig. 3), thromboxane B_2, and leukotriene E_4. These results mark the first discovery of sulfotransferase activities toward eicosanoids and their association with a distinct mammalian sulfotransferase.

It is interesting to point out that our earlier study had demonstrated that the mouse sulfotransferase clone 679153 is expressed predominantly in mouse kidney and at a lower level in uterus [8]. Uterus has previously been shown to be an organ heavily influenced by the endocrinologic effects of eicosanoids, especially during parturition [17]. Furthermore, prostaglandins have been used as therapeutic agents in some clinical aspects [17]. For kidney, the roles of eicosanoids in its pathophysiology has also been well documented [17]. The tissue-specific expression of the mouse sulfotransferase clone 679153 in these two mouse organs therefore appears to provide additional support for the functional involvement of this novel enzyme in the eicosanoid metabolism.

Considering that eicosanoids (prostaglandins, thromboxanes, hydroxyeicosatetranoic acids, and leukotrienes) have been implicated in various physiological processes such as inflammation, pain and fever production, blood pressure regulation, platelet aggregation, induction of labor, and regulation of sleep/wake cycle [18], the sulfation of eicosanoids, possibly catalyzed by other types of sulfotransferases as well, may have broad functional implications *in vivo*. Some possibilities of the functional relevance of the sulfation of eicosanoids can be put forth by drawing analogy to other systems. First, sulfation may be employed for the inactivation and/or excretion of eicosanoids. Second, sulfation of eicosanoids may generate products that are biologically active in ways different from their parental compounds. Third, sulfation of eicosanoids may serve for their transport inside the body. Fourth, sulfated eicosanoids may represent a reserve *in vivo* which can be called upon when needed. Finally, sulfation may be used as a means for the reversible modulation of the activities of eicosanoids. One requirement for the latter two possibilities, however, is the existence of a corresponding sulfatase that can hydrolyze the sulfated eicosanoids. Further studies are needed in order to clarify these various possibilities.

ACKNOWLEDGMENTS

This work was supported in part by a Grant-in-Aid (95G-564) from the American Heart Association, Texas Affiliate (M.C.L.); a JSPS Postdoctoral Fellowship for Research Abroad from the Japan Society for the Promotion of Sciences (Y.S.); and an Established Investigatorship from the American Heart Association, National Center (C.C.L.).

REFERENCES

1. Mulder, G. J., and Jakoby, W. B. (1990) *in* Conjugation Reactions in Drug Metabolism (Mulder, G. J., Ed.), pp. 107–161, Taylor and Francis, London.
2. Falany, C., and Roth, J. A. (1993) *in* Human Drug Metabolism: From Molecular Biology to Man (Jeffery, E. H., Ed.), pp. 101–115, CRC Press, Boca Raton.

3. Weinshilboum, R., and Otterness, D. (1994) *in* Conjugation–Deconjugation Reactions in Drug Metabolism and Toxicity (Kaufmann, F. C., Ed.), pp. 45–78, Springer-Verlag, Berlin.
4. Yamazoe, Y., Nagata, K., Ozawa, S., and Kato, R. (1994) *Chem.-Biol. Interact.* **92,** 107–117.
5. Weinshilboum, R. M., Otterness, D. M., Aksoy, I. A., Wood, T. C., Her, C., and Raftogianis, R. B. (1997) *FASEB J.* **11,** 3–14.
6. Her, C., Wood, T. C., Eichler, E., Mohrenweiser, H. W., Siciliano, M. J., Ramagli, L. S., and Weinshilboum, R. M. (1997) GenBank Accession No. U92314.
7. Her, C., Wood, T. C., Eichler, E., Mohrenweiser, H. W., Siciliano, M. J., Ramagli, L. S., and Weinshilboum, R. M. (1997) GenBank Accession No. U92315.
8. Sakakibara, Y., Yanagisawa, K., Takami, Y., Nakayama, T., Suiko, M., and Liu, M.-C. (1998) *Biochem. Biophys. Res. Commun.* **247,** 681–686.
9. Jevons, F. R. (1964) *Biochem. J.* **89,** 621–624.
10. Liu, M.-C., and Lipmann, F. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 3695–3698.
11. Fernando, P. H. P., Karakawa, A., Sakakibara, Y., Ibuki, H., Nakajima, H., Liu, M.-C., and Suiko, M. (1993) *Biosci. Biotech. Biochem.* **5,** 1974–1975.
12. Bradford, M. M. (1976) *Anal. Biochem.* **72,** 248–254.
13. Chen, C.-Y. A., and Shyu, A.-B. (1995) *Trends Biochem. Sci.* **20,** 465–470.
14. Shaw, G., and Kamen, R. (1986) *Cell* **46,** 659–667.
15. Caput, D., Beutler, B., Hartog, K., Thayer, R., Brown-Shimer, S., and Cerami, A. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 1670–1674.
16. Samuelsson, B. (1983) *Science* **220,** 568–575.
17. Wilson, J. D., Foster, D. W., Kronenberg, H. M., and Larsen, P. D. (1998) Williams Textbook of Endocrinology, 9th ed., Saunders, Philadelphia.
18. Goetzl, E. J., An, S., and Smith, W. L. (1995) *FASEB J.* **9,** 1051–1058.