A novel sulfotransferase was purified from the rat liver cytosol to electrophoretic homogeneity via five column chromatography steps (hydroxylapatite I, DEAE Bio-Gel, ATP-agarose I, hydroxylapatite II, and ATP-agarose II). The minimum molecular weight of the purified enzyme was determined by sodium dodecyl sulfatel polyacrylamide gel electrophoresis to be ~33,000. Gel filtration chromatography revealed a native molecular weight of ~34,000, indicating the enzyme being present in the monomeric form. The purified sulfotransferase displayed enzymatic activities, with a pH optimum of 9.25, toward various tyrosine and 3,4-dihydroxyphenylalanine (Dopa) isomers, except D-ortho-tyrosine. Thyroid hormones, as well as dopamine and p-nitrophenol, could also be used as substrates. The apparent $K_m$ value of the enzyme (designated the Dopa/tyrosine sulfotransferase) for L-Dopa, determined at a constant 14 μM of 3'-phosphoadenosine 5'-phosphosulfate, was 0.76 mM. The intact enzyme was found to be N-blocked when subjected to N-terminal sequencing. Three internal partial amino acid sequences, obtained by analyzing its proteolytic fragments, were found to be distinct from the homologous sequences of other known rat liver sulfotransferases. The deduced amino acid sequence of a full-length cDNA isolated from a rat liver cDNA library confirmed the identity of the Dopa/tyrosine sulfotransferase as a new type of aryl sulfotransferase. Upon transfection of COS-7 cells with an expression vector (pMSG-CMV) harboring the full-length cDNA, a 33-kDa protein displaying enzymatic and immunological properties similar to those of the purified Dopa/tyrosine sulfotransferase was expressed.

Sulfation represents an important mechanism in vivo for the biotransformation and excretion of a variety of compounds (1–3). Upon sulfation, some peptides or proteins undergo changes in their biological activities to fulfill particular biochemical/physiological needs (3). For low molecular weight xenobiotics or endogenous compounds such as steroid hormones, catecholamines, and bile acids, sulfation may increase the water solubility and facilitate their excretion from the body by endowing them with (additional) charged properties (1, 2). In relation to this latter aspect, the biochemistry and functional relevance of the excretion of free tyrosine-O-sulfate (TyrS) in mammalian urine have remained intriguing questions for the past 40 years.

Free TyrS was first reported to be present in human urine by Tallan et al. (4). Similar findings were made subsequently for other mammalian species including rat, rabbit, and mouse (5, 6). Because none of the mammalian arylsulfatas could effectively catalyze its desulfation (7, 8), the free TyrS produced by mammalian cells has been generally considered a modified amino acid destined to be excreted. Concerning the biochemical origin of the free TyrS excreted, two distinct mechanisms for its generation have been suggested: i) the enzymatic sulfation of L-tyrosine forming free TyrS and ii) the turnover (degradation) of tyrosine sulfated proteins, thereby releasing free TyrS. Aiming at demonstrating the first of these two mechanisms, a great number of studies using various cell homogenates or purified aryl sulfotransferases have, however, persistently failed to reveal the enzymatic activity that catalyzes the sulfation of free L-tyrosine (1, 9–12). Among the different mammalian aryl sulfotransferases characterized, only the rat liver type IV aryl sulfotransferase, also named the “tyrosine-ester sulfotransferase,” can use tyrosine esters or N-terminally located tyrosine residues as substrates. This enzyme, however, cannot catalyze the sulfation of unmodified L-tyrosine (13). In view of the widespread occurrence of the post-translational tyrosine sulfation among proteins of multicellular eukaryotic organisms (14), it has become increasingly accepted that the free TyrS excreted in mammalian urine is probably derived exclusively from the degradation of tyrosine sulfated proteins (1, 6, 15–17). In support of this hypothesis, free TyrS$^{35}$S was shown to be generated when tyrosine $^{35}$S-sulfated peptides or proteins were either injected into rabbits (15) or added to the medium of cultured cells (18). However, to quantitatively account for the excreted TyrS reported to be approximately 28 mg/day in normal adult human (4), the turnover of a large amount of tyrosine sulfated proteins would be needed. This has therefore continued to raise the question whether the turnover of tyrosine sulfated proteins
is truly the sole source of the free TyrS produced and released by mammalian animals. By employing radioactive 3'-phosphoadenosine 5'-phospho-[35S]sulfate (PAP[35S]) as the sulfate donor, we have recently obtained evidence showing the enzymatic sulfation of l-tyrosine in several mammalian cell lines (19, 20). We have further demonstrated that 3,4-dihydroxyphenylalanine (Dopa) and L-tyrosine can be sulfated more efficiently by the sulfotransferase(s) present in the cytosol of HepG2 human hepatoma cells. An important question therefore is whether, in mammalian cells, there is a single enzyme catalyzing the sulfation of all Dopa and tyrosine isomers or if there are distinct sulfotransferases responsible for the sulfation of different Dopa and tyrosine isomers. Furthermore, is (are) the enzyme(s) different from the previously reported sulfotransferase(s) (21–24)?

In this paper, we report the purification, characterization, and molecular cloning of a single species of the enzyme, designated the “Dopa/tyrosine sulfotransferase,” from the rat liver. Comparison of the nucleotide sequence and the deduced amino acid sequence of the cloned cDNA with sequences of known aryl (phenol) sulfotransferases, as well as the data from the biochemical characterization, demonstrated the Dopa/tyrosine sulfotransferase to be a novel enzyme.

**EXPERIMENTAL PROCEDURES**

**Materials**—l-Dopa, d-Dopa, l-D-p-tyrosine, d-L-tyrosine, DL-tyrosine, o-nitrophenol, acrylamidophenylalanine, N-prenylated benzamidine, soybean trypsin inhibitor, phenylmethylsulfonyl fluoride, 2,6-dichloro-4-nitrophenol (DCNP), ATP, adipic acid dihydrazide-agarose, 5′-AMP, Hepes, Tris, 3-[dimethyl(hydroxymethyl)methylamino]-2-hydroxypropanesulfonic acid (Ampso), and Caps were products of Sigma. 4-(2-Hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), Ches, Taps, 3-[dimethyl(hydroxymethyl)methylamino]-2-hydroxypropanesulfonic acid (Ampso), and Caps were products of Sigma. PAPS was a generous gift from Unitika, Ltd. (I. Japan). l-Tyrosine (disodium salt) was purchased from Research Organics, Inc. Bio-Gel HTP hydroxyapatite and DEAE Bio-Gel A were from Bio-Rad Laboratories. ATP-agarose was prepared by coupling sodium periodate-oxidized ATP to adipic acid dihydrazide-agarose using the procedure of Lamed et al. (25). TyrS, dopamine-O-sulfate, and a mixture of l-Dopa 3-sulfate and l-Dopa 4-O-sulfate (collectively referred to as DopaS) were synthesized according to the procedure developed by Jevons (26). Rat liver Lambda ZAP II cDNA library and XL-blue MRF2 Escherichia coli host strain were purchased from Stratagene. SuperScript Preamplification System and LipofectAMINE were from Life Technologies, Inc. Taq polymerase was purchased from Perkin-Elmer. Cycle sequencing kits were products of Applied Biosystems, Inc. The mammalian expression vector, pMSG-CMV, was kindly provided by Dr. Nakayama at Miyazaki Medical College. BacBEST labeling kit, Exoneuclease III, mung bean nuclease, and DNA ligation kit were products of Takara Shuzo. All restriction endonucleases were from New England Biolabs. Carrier-free sodium [35S]sulfate and [α-32P]dCTP (3,000 Ci/mmol) were from ICN Biomedicals. Chromatogram cellulose TLC plates were from Eastman Kodak Company. COS-7 SV40 transformed African green monkey kidney cells (ATCC CRL 1651) were obtained from the American Type Culture Collection. Rabbit antiserum against the rat liver Dopa/tyrosine sulfotransferase was prepared according to the procedure previously described (27). All other chemicals were of the highest grades commercially available.

**Preparation of the Rat Liver Cytosol**—Rat liver (190 g) rinsed thoroughly with ice-cold phosphate-buffered saline was ground through a 40-mesh U.S. standard testing sieve (35 mesh) and made into a 1.2 (w/v) suspension in 0.5 ml of 0.1 M Tris-HCl buffer containing 50 mM NaCl, 140 mM potassium chloride, 14 mM sodium dihydrogen phosphate, and 1 mM magnesium chloride. The homogenate was centrifuged at 15,000 × g for 20 min at 4°C, and the supernatant collected was further subjected to ultracentrifugation at 140,000 × g for 2 h at 4°C. The supernatant containing cytosolic proteins was used for the purification as described below.

**Purification of the Rat Liver Dopa/Tyrosine Sulfotransferase**—Unless otherwise indicated, all buffer solutions used in the purification were at pH 7.4 and supplemented with 1 mM dithiothreitol. All operations described below were carried out at 4°C.

**First Bio-Gel HTP Hydroxyapatite Column Chromatography**—The rat liver cytosol (400 ml) was applied onto a Bio-Gel HTP column (4.5 × 25 cm) pre-equilibrated with 10 mM Tris-HCl. After loading, 200 ml of 10 mM Tris-HCl was passed through the column to remove unbound proteins. The proteins bound on the column were eluted using a linear potassium phosphate buffer gradient composed of 100 and 400 mM potassium phosphate buffer. The eluted fractions (spanning from 150 to 300 mM) containing the Dopa/tyrosine sulfotransferase activity were combined and dialyzed overnight against 10 mM Tris-HCl.

**DEAE Bio-Gel A Anion Exchange Chromatography**—The dialyzed fraction was applied onto a DEAE Bio-Gel A column (4.5 × 25 cm) pre-equilibrated with 10 mM Tris-HCl. The bound proteins were eluted with an NaCl gradient composed of 350 ml each of 0 and 350 mM NaCl solution containing 10 mM Tris-HCl. The eluted fractions (spanning from 130 to 250 mM) containing the Dopa/tyrosine sulfotransferase activity were combined and dialyzed overnight against 10 mM Tris-HCl.

**First ATP-agarose Column Chromatography**—The dialyzed fraction was loaded onto an ATP-agarose column (2.5 × 4 cm). After loading, the bound proteins were eluted with an NaCl gradient composed of 320 ml each of 0 and 350 mM NaCl solution containing 10 mM Tris-HCl. The eluted fractions (spanning from 125 to 200 mM) containing the Dopa/tyrosine sulfotransferase activity were pooled.

**Second Bio-Gel HTP Column Chromatography**—The Dopa/tyrosine sulfotransferase eluate from the first ATP agarose column was directly applied on a Bio-Gel HTP hydroxyapatite column (1.5 × 5 cm) pre-equilibrated with 10 mM Tris-HCl. The bound proteins were eluted with a linear gradient composed of 100 ml each of 0 and 75 mM PAPS in 10 mM Tris-HCl. The Dopa/tyrosine sulfotransferase eluted throughout the entire PAPS concentration range was finally purified by electrophoretically homogenous solution (0.5%).

**Enzymatic Assay**—The activities of the Dopa/tyrosine sulfotransferase were assayed using PAP[35S] as the sulfate donor. The standard assay mixture, with a final volume of 50 μl, contained 50 mM Ampso, NaOH (pH 9.25), 250 mM sucrose, 25 mM NaF, 1 mM 5′-AMP, protease inhibitors (30 μg/ml aprotinin, 30 μg/ml antipain, 300 μg/ml benzamidine, and 30 μg/ml soybean trypsin inhibitor), 14 μg PAPI[35S] (4.4 Ci/mmol), and 1 mM substrate (Dopa, tyrosine, etc.). The reaction was started by the addition of the enzyme preparation, allowed to proceed for 60 min at 37°C, and terminated by heating at 100°C for 3 min. The precipitates were formed by centrifugation. The clear supernatant was subjected to the analysis of 35S-sulfated product as described below. For the Km determination, the enzymatic assay was performed at different concentrations of PAP[35S] with varying concentrations of Dopa/tyrosine. The sulfated product was collected and used as a substrate for the next stage of the assay. To examine the pH dependence of the Dopa/tyrosine sulfotransferase activity, different buffers (50 mM Hepes at pH 7.0, 7.5, or 8.0; Taps at pH 8.0, 8.25, 8.5, 8.75, or 9.0; Amino at pH 8.5, 8.75, 9.0, 9.25, 9.5, 9.75, or 10.0; or Caps at pH 10, 10.25, 10.5, or 11) instead of 50 mM Ampso, NaOH (pH 9.25) were used in the reaction mixtures.

**Analysis of 35S-Sulfated Compound**—For the analysis of Tyr[35S], Dopa[35S], dopamine[35S], or other 35S-sulfated products, 5 μl of the clear reaction mixture were mixed with 10 μg of synthetic standard (TyrS, DopaS, or dopamine-O-sulfate), spotted onto a 20 × 20-cm cellulose TLC plate, and analyzed according to a two-dimensional thin-layer chromatography procedure previously developed in this laboratory. The plate was first subjected to high voltage electrophoresis (1,000 V for 70 min) in 7.8% (v/v) acetic acid/2.5% (v/v) 88% formic acid (pH 1.9). After electrophoresis, the plate was air-dried and subjected in the second dimension to ascending chromatography in n-butanol/acetic acid/88% formic acid (3:1:1, v/v/v). Upon completion of the chromatography, the ninhydrin plate was sprayed with 1% ninhydrin in acetone. The ninhydrin-stained spot of the sulfated product was scraped off, suspended in 0.5-ml aliquots of H2O, and mixed with 4 ml of scintillation mixture (Eclumix, ICN Radiochemicals). The radioactivity associated with Tyr[35S], Dopa[35S], dopamine[35S], or other 35S-sulfated product was counted.

N-terminal and Internal Partial Amino Acid Sequence Analysis—N-terminal amino acid sequence determination was performed according to the method of Matsudaira (29). Briefly, the purified Dopa/tyrosine sulfotransferase was subjected to SDS-PAGE (30) and electrophoresis and transfer to nitrocellulose. Rat Liver Dopa/Tyrosine Sulfotransferase

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2 M. Suiko, Y. Sakakibara, Y. Takami, C. Zwieb, T. Nakayama, H. Nakajima, and M.-C. Liu, unpublished data.
ferred onto a Millipore Immobilon-P membrane. The blotting was performed at a constant 200 mA for 6 h in 10 mM Caps-NaOH (pH 11). The blotted membrane was briefly stained with 0.1% Ponceau S in 5% acetic acid to reveal the protein band. After extensive washing with water, the membrane piece containing the bound Dopa/tyrosine sulfotransferase was used for the analysis of the N-terminal sequence. To determine the internal amino acid sequences, the Dopa/tyrosine sulfotransferase bound on the Immobilon-P membrane was digested with endoproteinase Lys-C, and the peptide fragments were purified using high performance liquid chromatography (HPLC). Purified peptide fragments were subjected to the N-terminal sequence determination according to the manufacturer's instructions. The transfected cells were incubated at 37 °C in Dulbecco's modified Eagle's medium containing 10% fetal bovine serum. At the end of a 48-h incubation, the cells were washed twice with phosphate-buffered saline and homogenized in buffer A containing 10 mM Tris-HCl (pH 7.4), 250 mM sucrose, 10 μg/ml aprotinin, and 1 mM phenylmethylsulfonfyl fluoride. Aliquots of the homogenates prepared were assayed for the Dopa/tyrosine sulfotransferase activities or subjected to the Western blot analysis for the presence of the immunoreactive enzyme using rabbit anti-rat liver Dopa/tyrosine sulfotransferase antisemur. The conditions for the Western blot analysis were as described previously (33).

### RESULTS

Purification of the Rat Liver Dopa/Tyrosine Sulfotransferase—Preliminary experiments showed that similar to several mammalian cell lines previously studied (19, 20), the Dopa/tyrosine sulfotransferase was present predominantly in the cytosolic fraction of the rat liver. The specific activity of the enzyme purified from the rat liver cytosol, with L-Dopa as the substrate, was determined to be 2,153 pmol/min/mg protein, indicating a 760-fold purification over its specific activity in the rat liver cytosol (Table I). It was noted that, during the elution from the DEAE Bio-Gel A anion exchange chromatography step, the Dopa/tyrosine sulfotransferase activity was well separated from the major phenol sulfotransferase activity (data not shown). As shown in Fig. 1, the purified Dopa/tyrosine sulfotransferase migrated as a single protein band upon SDS-PAGE under reducing conditions. A key step during the purification of the Dopa/tyrosine sulfotransferase was the first ATP-agarose affinity chromatography, which furnished a specific activity of 4.3. ATP-agarose was used for the analysis of the N-terminal sequence of the purified Dopa/tyrosine sulfotransferase by removing denatured enzymes and, at the same time, improve the specific activity of the enzyme. SDS-PAGE was performed in a 5% polyacrylamide gel using the method of Laemmli (30). The native molecular weight (Mn) of the purified Dopa/tyrosine sulfotransferase was determined by gel filtration chromatography using a Sephacryl S-200 column (2.6 × 90 cm). Molecular weight standards including bovine serum albumin (Mn 67,000), ovalbumin (Mn 43,000), carbonic anhydrase (Mn 29,000), chymotrypsinogen A (Mn 25,800), and cytochrome c (Mn 12,400) were used for calibration. Protein determination was based on the method of Bradford (35) with bovine serum albumin as the standard.

### Purification of the Rat Liver Dopa/tyrosine Sulfotransferase

| Purification step | Protein | Total activity | Yield | Specific activity* | Purification |
|-------------------|---------|----------------|-------|-------------------|-------------|
| Cytosol           | 13,436  | 37,890         | 100   | 2.8 ± 0.3         | 1           |
| First Bio-Gel HTP | 2,813   | 33,990         | 89.7  | 32.6 ± 1.3        | 11.5        |
| DEAE Bio-Gel A    | 710.4   | 23,140         | 61.1  | 241.8 ± 6.1       | 85.8        |
| First ATP-agarose | 37.3    | 9,020          | 23.8  | 436.7 ± 7.4       | 154.9       |
| Second Bio-Gel HTP| 16.9    | 7,380          | 19.5  | 2,153.4 ± 47.1    | 763.6       |
| Second ATP-agarose| 1.3     | 2,781          | 7.2   |                   |             |

*The results are expressed as the means ± S.D. derived from three experiments.
fied Dopa/tyrosine sulfotransferase to lose its enzymatic activity.

Characterization of the Purified Rat Liver Dopa/Tyrosine Sulfotransferase—The purified rat liver Dopa/tyrosine sulfotransferase was subjected to characterization with respect to its physicochemical and enzymatic properties as described below.

Molecular Weight—Based on its electrophoretic mobility relative to the molecular weight standards co-electrophoresed during the SDS-PAGE under reducing conditions (Fig. 1), the minimum molecular weight of the purified Dopa/tyrosine sulfotransferase was determined to be approximately 33,000. Gel filtration chromatography revealed a molecular weight of approximately 34,000 for the native Dopa/tyrosine sulfotransferase (figure not shown). These results combined indicate that the purified Dopa/tyrosine sulfotransferase was present in monomeric form.

Partial Amino Acid Sequences—Repeated attempts to determine the N-terminal amino acid sequence of the purified Dopa/tyrosine sulfotransferase were unsuccessful, indicating that the enzyme, similar to some other cytosolic sulfotransferases (36, 37), is N-blocked. Upon digestion with endoproteinase Lys-C, three proteolytic fragments were purified by HPLC and sequenced. Fig. 2 shows the alignment of the three partial amino acid sequences with the homologous sequences of other sulfotransferases reported previously. Although the Dopa/tyrosine sulfotransferase displayed higher degrees of sequence homology to phenol (aryl) sulfotransferases from mammalian animals, same degrees of homology to the sequences of even the other cytosolic sulfotransferases. Boxed residues indicate the amino acid residues identical to those of the rat liver Dopa/tyrosine sulfotransferase. The enzymes listed include the rat liver phenol sulfotransferase (rPST), mouse liver phenol sulfotransferase (mPST) (56), human liver thermostable phenol sulfotransferase (hPST) (57), rat liver ST1B1 gene product (rST(TL)) (58), rat liver ST1B1 gene product (rPST) (59), rat liver hydroxyarylamine sulfotransferase (rHAST) (59), bovine placental estrogen sulfotransferase (bEST) (60), guinea pig adrenal corticosteroid sulfotransferase (gpEST) (61), rat liver estrogen sulfotransferase (rEST) (62), human liver estrogen sulfotransferase (hEST) (63), human liver hydroxysteroid sulfotransferase (hHSST) (64), mouse liver hydroxysteroid sulfotransferase (mHSST) (65), guinea pig adrenal hydroxysteroid sulfotransferase (gHAST) (66), rat liver senescence marker protein (rSM2) (67), rat liver hydroxysteroid sulfotransferase I (rHST(I)) (68), rat liver hydroxysteroid sulfotransferase II (rHST(II)) (69), Flaveria chloroelcia flavonol 3-sulfotransferase (fCFST3), and flavonol 4'-sulfotransferase (fCFST4) (70).

Table II. Of the Dopa and tyrosine isomer tested, both L-Dopa and D-tyrosine as the substrate was 2 orders of magnitude higher than those observed with L-tyrosine or D-tyrosine as the substrate. Both p-nitrophenol, a substrate for the P form phenol sulfotransferase, and dopamine, a substrate for the M form phenol sulfotransferase, yielded specific activities of the same order of magnitude.
and dopamine, calculated from the Lineweaver-Burk plots, that although the apparent values of the Dopa/tyrosine sulfotransferase for substrates is almost the same. With triiodothyronine as the substrate, increasing concentrations of NaCl or KCl resulted in the inhibition of the Dopa/tyrosine sulfotransferase activity. A 50% decrease in the enzymatic activity was found when the concentration of NaCl or KCl in the reaction mixture was increased to 200 mM.

Effect of DCNP on the Enzymatic Activity—DCNP, a commonly used inhibitor for aryl (phenol) sulfotransferases (1, 2, 21), was tested for its inhibitory effect on the Dopa/tyrosine sulfotransferase activity. It was found that the purified Dopa/tyrosine sulfotransferase was inhibited by submillimolar levels of DCNP, with an \( IC_{50} \) of approximately \( 7 \times 10^{-5} \) M. A similar \( IC_{50} \) value for DCNP has previously been determined for the thermolabile M form but not the thermostable P form of human phenol sulfotransferase (1, 22).

Molecular Cloning of the Rat Liver Dopa/Tyrosine Sulfotransferase—To determine unequivocally the identity of the rat liver Dopa/tyrosine sulfotransferase as a novel enzyme, we have cloned and sequenced its cDNA. Repeated screening of the rat liver Lambda ZAP II cDNA library yielded 14 positive cDNA clones. The three largest cDNA inserts, ranging from 1,500 to 2,400 base pairs, were subjected to preliminary nucleotide sequencing. The analysis revealed that one of them, designated clone D/TST-11, contained an initiation codon and a 3' region encoding the poly(A) tail and thus appeared to contain the full-length sequence. The nucleotide and deduced amino acid sequences are presented in Fig. 4. Because the purified Dopa/tyrosine sulfotransferase was found to be N-blocked, the ATG codon encoding the N-terminal methionine residue was assigned based on i) the predicted molecular weight that matches the data from SDS-PAGE and gel filtration chromatography and ii) the sequence alignment in comparison with known aryl sulfotransferases (see below). The open reading frame, beginning at base residue 91, encompasses 897 nucleotides and encodes a 299-amino acid polypeptide. The predicted molecular weight, 34,762, is in agreement with the results (33,000 and 34,000, respectively) obtained through SDS-PAGE and gel filtration chromatography using the purified Dopa/tyrosine sulfotransferase. The termination codon, located at nucleotide residues 988-990, was followed by a 292-nucleotide 3'-untranslated sequence that includes a poly(A) tract. Two polyadenylation signals (ATTAAA) (39) located 180 and 19 nucleotides, respectively, upstream from the poly(A) tract were found. The authenticity of the cDNA was indicated by the inclusion of the three partial amino acid sequences obtained through direct amino acid sequencing of the purified Dopa/tyrosine sulfotransferase, and by the expression of the functionally active recombinant enzyme that cross-reacted with the substrate, a strong substrate inhibition effect was observed when the concentration of triiodothyronine was greater than 0.5 mM.

Effects of Cationic Salts on the Enzymatic Activity—The effects of cationic salts on the Dopa/tyrosine sulfotransferase activity were measured. The addition of different divalent cationic salts, such as MgCl\(_2\), MnCl\(_2\), and CoCl\(_2\), exerted virtually no effects on the activities of the purified enzyme. With l-Dopa as the substrate, increasing concentrations of NaCl or KCl resulted in the inhibition of the Dopa/tyrosine sulfotransferase activity. A 50% decrease in the enzymatic activity was found when the concentration of NaCl or KCl in the reaction mixture was increased to 200 mM.

## Table III

| Substrate       | \( K_m \) (mM) | \( V_{max} \) (pmol/min/mg) |
|-----------------|----------------|----------------------------|
| L-Dopa          | 0.76           | 3,521                      |
| D-Dopa          | 3.44           | 13,699                     |
| p-Nitrophenol   | 30.9           | 125,000                    |
| Dopamine        | 0.24           | 5,435                      |
| Triiodothyronin (inhibited at 250 \( \mu M \)) | 21,156 (at 250 \( \mu M \)) |
antiserum against the purified Dopa/tyrosine sulfotransferase (see below). As shown in Fig. 5, the deduced amino acid sequence of the rat liver Dopa/tyrosine sulfotransferase (rD/TST) cDNA displays 72.2/52.6, 72.6/52.4, 72.5/51.9, and 71.9/53.6% similarity/identity to the amino acid sequences of rat liver Dopa/tyrosine sulfotransferase, human thermolabile phenol sulfotransferase, human thermostable phenol sulfotransferase, and mouse phenol sulfotransferase, human thermolabile phenol sulfotransferase, and human thermostable phenol sulfotransferase. As shown in Fig. 6, a 33-kDa protein cross-reactive toward the antiserum against the purified Dopa/tyrosine sulfotransferase was expressed specifically when the COS-7 cells were transfected with an expression vector (pSMG-CMV) that contained the full-length cDNA encoding the Dopa/tyrosine sulfotransferase. When the cell homogenates were assayed for the Dopa/tyrosine sulfotransferase activity, it was found that the sample prepared from the cells transfected with the expression vector inserted with the full-length cDNA indeed exhibited a highly elevated Dopa/tyrosine sulfotransferase activity (Table IV).

**DISCUSSION**

Since the discovery of the excretion of free TyrS in human urine (4), the questions concerning the functional relevance and the formation of TyrS by the enzymatic sulfation of tyrosine have remained unresolved for nearly forty years. A consensus formed (following Huttner’s discovery of the widespread occurrence of the post-translational tyrosine sulfation of eukaryotic proteins (14)) is that free TyrS is generated primarily through the turnover of tyrosine sulfated proteins in vivo. We have indeed demonstrated earlier (16) that exogenous tyrosine 35S-sulfated proteins added to the medium could be endocytosed by cultured cells and degraded intracellularly to generate free Tyr(35S). A metabolic labeling experiment using the same cells, however, showed a considerable discrepancy between the amount of the free Tyr(35S) generated and the amount of tyrosine 35S-sulfated proteins turned over during a 48-h time course monitored. This finding had prompted our interest in investigating further the possibility of the sulfation of free tyrosine.

In our recent studies (19, 20), we have obtained conclusive evidence that sulfation of L-p-tyrosine does occur in several mammalian cell lines. It is, however, unclear why mammalian cells should carry out the sulfation of an amino acid needed for protein synthesis. To convert L-p-tyrosine to L-p-TyrS, a compound destined for excretion (4), would seem to be counterproductive in terms of cellular economy. The question that should be raised then is whether L-p-tyrosine truly represents the physiological substrate of the enzyme, initially designated the “tyrosine sulfotransferase” (19). Using HepG2 human hepatoma cells as a model, we have demonstrated in a more recent study (20) that other tyrosine derivatives, e.g., Dopa and m-tyrosine isomers, are in fact better substrates for sulfation than is L-p-tyrosine. These results are summarized in the schematic diagram shown in Fig. 7. In view of the large number of aryl sulfotransferases that have been identified, it is tempting to ask whether, in mammalian cells, there is one single enzyme catalyzing the sulfation of all Dopa and tyrosine isomers or instead that there are multiple sulfotransferases responsible for the sulfation of individual Dopa and tyrosine isomers. To find an answer to this question, we have decided to isolate the enzyme(s) from rat liver for further characterization.

The rat liver has been more exhaustively studied with regard to aryl sulfotransferases (1, 2) than other mammalian tissues. At least six different types of aryl sulfotransferases have been identified and characterized (13, 23, 24). The rat liver is therefore the best model for investigating whether the Dopa/tyrosine sulfotransferase activities are associated with a new enzyme(s) or instead a known aryl sulfotransferase(s). In the present study, a single Dopa/tyrosine sulfotransferase was purified from the rat liver. It was noted that for all five chromatography steps during the purification, single symmetric peaks of elution of the Dopa/tyrosine sulfotransferase, as monitored by standard assays using either L-Dopa or DL-m-tyrosine as substrate, were observed. The existence of multiple enzymes catalyzing the sulfation of individual Dopa and tyrosine isomers, therefore, seemed unlikely. SDS-PAGE and gel filtration chromatography revealed the enzyme to be present in the monomeric form. In contrast to these data obtained with the purified
Most, if not all, of the known aryl sulfotransferases were shown to be present in the dimeric form (21, 24). The purified rat liver Dopa/tyrosine sulfotransferase was found to be capable of catalyzing the sulfation of all Dopa and tyrosine isomers, except DL-Dopa. The specific activity of the purified enzyme with L-Dopa as the substrate was 2,153.4 pmol/min/mg protein. This value is considerably lower than those previously reported for phenol sulfotransferases from rat liver with either simple phenols or monoamines as substrates (1, 2, 21, 22). However, some sulfotransferases that utilize endogenous compounds as substrates, e.g., the tyrosylprotein sulfotransferase (5,700 pmol/min/mg) (40) and the dopamine-sulfating sulfotransferase (7,735 pmol/min/mg) (41), also displayed specific activities in the same order of magnitude as that determined for the Dopa/tyrosine sulfotransferase. Furthermore, because tyrosine and Dopa are important precursors for the synthesis of proteins and/or catecholamines, there may be regulatory mechanisms in vivo for the Dopa/tyrosine sulfotransferase activity. Thyroid hormones...
triodothyronine and thyroxine, as well as dopamine and p-nitrophenol, could also be used as substrates by the purified Dopa/tyrosine sulfotransferase. Although the broad substrate specificity seems to be a rule rather than exception for aryl sulfotransferases that have been studied, it should be pointed out that these latter compounds (thyroid hormones, dopamine, and p-nitrophenol) are more effectively used by other aryl (phenol) sulfotransferases (42, 43) with K_m values 2–3 orders of magnitude lower than those determined for the purified rat liver Dopa/tyrosine sulfotransferase. Furthermore, the Dopa/tyrosine sulfotransferase represents the only known enzyme that is capable of catalyzing the sulfation of Dopa and tyrosine isomers. In contrast to the HepG2 Dopa/tyrosine sulfotransferase, which showed higher activities toward D-form Dopa and tyrosine isomers and a remarkable divalent cation dependence, the rat liver enzyme displayed higher activities toward the L-form substrates and showed no significant changes in activity in the presence of a variety of divalent cations.

Initial attempts to determine the N-terminal amino acid sequence of the purified Dopa/tyrosine sulfotransferase showed it to be N-blocked. Three internal partial amino acid sequences were obtained by sequencing the HPLC-purified fragments derived from the digestion of the purified enzyme with endoproteinase Lys-C. The alignment of the partial amino acid sequences of the rat liver Dopa/tyrosine sulfotransferase with the homologous sequences from other sulfotransferases provided the first clue that the Dopa/tyrosine sulfotransferase is a novel enzyme. The three partial amino acid sequences, however, completely matched those found in the deduced amino acid sequence of an unidentified sulfotransferase cDNA clone (ST1B1) reported by Yamazoe et al. (38). Because the ST1B1 cDNA clone encodes the only rat liver sulfotransferase that remains unidentified to date, we decided to clone and express it in COS-7 cells for functional characterization with respect to its identity as the Dopa/tyrosine sulfotransferase. Two regions, WDNKCKM and WKNYFTM, of the deduced amino acid sequence of the ST1B1 cDNA clone were chosen for designing degenerate oligonucleotide primers for reverse transcriptase-PCR. Using the 452-nucleotide PCR product as the probe for screening, a full-length cDNA clone was isolated and sequenced. The deduced amino acid sequence of the isolated cDNA contained the three partial amino acid sequences derived from the protein sequencing of the purified Dopa/tyrosine sulfotransferase and was found to be identical to that of clone ST1B1 except for a glycine residue instead of a glutamic acid residue at position 68. Whether this difference reflects the presence of isoforms in the rat liver remains to be clarified. The identity of the cDNA isolated was further verified by the expression in transfected COS-7 cells of a functional 33-kDa Dopa/tyrosine sulfotransferase that displayed immunologic cross-reactivity toward the antiserum against the purified rat liver Dopa/tyrosine sulfotransferase. These results have thus unequivocally confirmed the identity of the rat liver Dopa/tyrosine sulfotransferase as a novel enzyme, being distinct from all known sulfotransferases previously characterized.

The important question remains whether the sulfation of Dopa and tyrosine isomers is functionally relevant. Although the precise physiological involvement of the Dopa/tyrosine sulfotransferase still awaits further clarification, some possibilities could be put forth by taking into account the metabolic roles of its substrates, in particular L-Dopa and L-m-tyrosine. L-Dopa is generally known as the biosynthetic precursor of catecholamines including dopamine, norepinephrine, and epinephrine (44). L-meta-Tyrosine has been shown to be present in vivo (45, 46) and is capable of crossing the blood-brain barrier (47). Quantitative analysis showed that although L-p-tyrosine represents the predominant species, L-m-tyrosine constitutes a significant amount (2.8%) of the total tyrosine circulating in blood (46). Using bovine adrenal medulla extract or rat brain homogenate, it has been demonstrated that L-m-tyrosine was produced through the meta-hydroxylation of L-phenylalanine (48, 49). Furthermore, in vivo studies have shown that L-m-tyrosine could be converted to L-Dopa (50–52) or m-tyramine (47, 53), a decarboxylated product of L-m-tyrosine with neurotransmitter activity. Considering that L-Dopa and L-m-tyrosine are both involved in the biosynthesis of neurotransmitters, it would be important to regulate the concentrations of these compounds in vivo. A hypothetical role for the sulfation of L-Dopa and L-m-tyrosine therefore is that under normal circumstances, sulfation may be employed as a safeguard against the overproduction of L-Dopa and L-meta-tyrosine that if not prevented, might lead to the overproduction of catecholamines and consequently some neurological problems. When L-Dopa or L-m-tyrosine exceeds the normal concentration range, sulfation reaction may provide a mechanism by which they can be readily excreted. In this regard, the Dopa/tyrosine sulfotransferase may occupy a unique position related to the neurotransmitter metabolism. For other Dopa and tyrosine isomers, as well as thyroid hormones (triiodothyronine and thyroxine), a similar role for sulfation in facilitating their excretion can also be proposed. It is to be pointed out that the sulfation of dopamine and other catecholamines has been reported (2, 54). The enzyme responsible for the sulfation of catecholamines, the M form phenol sulfotransferase, has been shown to be predominantly present in neuronal cells (41). Whereas the M form phenol sulfotransferase serves to catalyze the sulfation of catecholamines that may have already exerted their neurotransmitter function, the Dopa/tyrosine sulfotransferase discovered in our studies functions to catalyze the sulfation of their biosynthetic precursors (L-Dopa, L-p-tyrosine, and L-m-tyrosine), thereby preventing the overproduction of catecholamines.

Finally, it is to be noted that the co-elution with synthetic L-p-TyrS standard upon ion-exchange column chromatography was the major, if not exclusive, criteria used for the identification of free TyrS excreted in mammalian urine (4–6). This procedure, however, is unlikely to provide enough resolution needed to distinguish between different sulfated tyrosine and/or Dopa isomers. It will be important to investigate, using more precise methods, the true identity (or identities) of the TyrS excreted in mammalian urine. Such information will be valuable in delineating the real substrate(s) for the Dopa/tyrosine sulfotransferase in vivo.

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