

Characterization of Human Iodothyronine Sulfotransferases*

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ABSTRACT

Sulfation is an important pathway of thyroid hormone metabolism that facilitates the degradation of the hormone by the type I iodothyronine deiodinase, but little is known about which human sulfotransferase isoenzymes are involved. We have investigated the sulfation of the prohormone T_4 , the active hormone T_3 , and the metabolites rT_3 and $3,3'$ -diiodothyronine ($3,3'$ - T_2) by human liver and kidney cytosol as well as by recombinant human SULT1A1 and SULT1A3, previously known as phenol-preferring and monoamine-preferring phenol sulfotransferase, respectively. In all cases, the substrate preference was $3,3'$ - $T_2 \gg rT_3 > T_3 > T_4$. The apparent K_m values of $3,3'$ - T_2 and T_3 [at 50 $\mu\text{mol/L}$ 3'-phosphoadenosine-5'-phosphosulfate (PAPS)] were 1.02 and 54.9 $\mu\text{mol/L}$ for liver cytosol, 0.64 and 27.8 $\mu\text{mol/L}$ for kidney cytosol, 0.14 and 29.1 $\mu\text{mol/L}$ for SULT1A1, and 33 and 112 $\mu\text{mol/L}$ for SULT1A3, respectively. The

apparent K_m of PAPS (at 0.1 $\mu\text{mol/L}$ $3,3'$ - T_2) was 6.0 $\mu\text{mol/L}$ for liver cytosol, 9.0 $\mu\text{mol/L}$ for kidney cytosol, 0.65 $\mu\text{mol/L}$ for SULT1A1, and 2.7 $\mu\text{mol/L}$ for SULT1A3. The sulfation of $3,3'$ - T_2 was inhibited by the other iodothyronines in a concentration-dependent manner. The inhibition profiles of the $3,3'$ - T_2 sulfotransferase activities of liver and kidney cytosol obtained by addition of 10 $\mu\text{mol/L}$ of the various analogs were better correlated with the inhibition profile of SULT1A1 than with that of SULT1A3. These results indicate similar substrate specificities for iodothyronine sulfation by native human liver and kidney sulfotransferases and recombinant SULT1A1 and SULT1A3. Of the latter, SULT1A1 clearly shows the highest affinity for both iodothyronines and PAPS, but it remains to be established whether it is the prominent isoenzyme for sulfation of thyroid hormone in human liver and kidney. (*J Clin Endocrinol Metab* 84: 1357–1364, 1999)

SULFATION is a detoxication reaction that increases the water solubility of a variety of endogenous and exogenous lipophilic compounds, thus facilitating their excretion in bile and/or urine (1–3). Sulfation is also an important pathway for the metabolism of thyroid hormone, increasing the hydrophilicity and the biliary excretion of the hormone. However, the major purpose of sulfation of thyroid hormone is to facilitate its degradation by the type I iodothyronine deiodinase (D1) (4, 5). This selenoenzyme catalyzes the outer ring deiodination (ORD) as well as the inner ring deiodination (IRD) of different iodothyronines, including the ORD of the prohormone T_4 to the active hormone T_3 and the IRD of T_4 and T_3 to the inactive metabolites rT_3 and $3,3'$ -diiodothyronine ($3,3'$ - T_2), respectively (6, 7). The preferred substrate for D1 is rT_3 , which is converted by ORD to $3,3'$ - T_2 (6, 7).

An intriguing characteristic of D1 is that its deiodination of a number of iodothyronines is accelerated by sulfation of their phenolic hydroxyl group (4, 5). Thus, IRD of both T_4

sulfate (T_4S) and T_3 sulfate (T_3S) by rat D1 is 40–200 times faster than deiodination of the nonsulfated substrates. In contrast, ORD of T_4 by rat D1 is completely blocked by sulfation (4, 5). This is not a general phenomenon, as ORD of rT_3 by rat D1 is not affected by sulfation, whereas ORD of $3,3'$ - T_2 by rat D1 is accelerated about 50-fold by sulfation of this compound (4, 5). Similar findings have been obtained with human and dog D1 (8, 9). The facilitated deiodination of sulfated iodothyronines is a unique property of D1. Neither the type II iodothyronine deiodinase (D2), which catalyzes only ORD, e.g. T_4 to T_3 and rT_3 to $3,3'$ - T_2 , nor the type III iodothyronine deiodinase (D3), which catalyzes only IRD, e.g. T_4 to rT_3 and T_3 to $3,3'$ - T_2 , is capable of catalyzing the deiodination of sulfated iodothyronines (10, 11).¹

Serum concentrations of T_4S , T_3S , rT_3S , and $3,3'$ - T_2S are low in normal human subjects, but they are high in fetal and cord blood, in patients with nonthyroidal illness, and in patients treated with propylthiouracil or iopanoic acid, inhibitors of D1 (12–19). The serum T_3S/T_3 ratio is also increased in hypothyroid patients (13). High serum T_4S , T_3S , rT_3S , and $3,3'$ - T_2S levels have also been detected in serum, bile, allantoic fluid, and amniotic fluid of fetal sheep (19–22). The high serum iodothyronine sulfate levels during nonthyroidal illness, hypothyroidism, and fetal development have

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¹ Visser, T. J., and E. Kaptein, unpublished work.

been ascribed to a low peripheral D1 activity in these conditions (4, 5, 11). These results are in accordance with experimental findings in rats showing marked increases in the serum concentration and biliary excretion of iodothyronine sulfates in animals with impaired hepatic and renal D1 activities due to administration of D1 inhibitors or selenium deficiency (23–27). These changes are not caused by an increased sulfation of iodothyronines, but, rather, by a decreased clearance of the sulfated iodothyronines by D1 (24, 28). Thus, sulfation is a primary step leading to the irreversible degradation of T_4 and T_3 by D1. However, if D1 activity is low, inactivation of thyroid hormone by sulfation is reversible due to expression of sulfatases in different tissues and by intestinal bacteria (11, 29–31). It has been speculated that especially in the fetus, T_3S has an important function as a reservoir from which active T_3 may be released in a tissue-specific and time-dependent manner (5, 11).

Sulfation of the hydroxyl group of a variety of substrates is catalyzed by a family of homologous sulfotransferases located in the cytoplasmic fraction of different tissues, such as liver, kidney, intestine, and brain (1–3). All of these isoenzymes use 3'-phosphoadenosine-5'-phosphosulfate (PAPS) as sulfate donor (1–3). On the basis of substrate specificity and amino acid sequence homology, two sulfotransferase families have been recognized in human tissues, *i.e.* phenol sulfotransferases (including estrogen sulfotransferases) and hydroxysteroid sulfotransferases (1–3). It is not known which sulfotransferases are involved in the sulfation of iodothyronines in human tissues. Previous studies have suggested a role for the enzymes termed phenol-preferring phenol sulfotransferase (P-PST) and monoamine-preferring phenol sulfotransferase (M-PST), in the sulfation of T_3 in human liver and intestine (32, 33). Recently, a large number of human and rat sulfotransferases have been cloned and characterized, including human SULT1A1 and SULT1A3, which represent P-PST and M-PST, respectively (34–36), under a new nomenclature system. Here we report the results of a comparison of the kinetic profiles of the sulfation of iodothyronines by human liver and kidney cytosol and by recombinant preparations of human SULT1A1 and SULT1A3.

Materials and Methods

Materials

$[3',5',^{125}I]T_4$ and $[3',^{125}I]T_3$ were obtained from Amersham (Aylesbury, UK); T_4 , T_3 , and PAPS from Sigma Chemical Co. (St. Louis, MO); rT_3 , 3,5- T_2 , 3,3'- T_2 , 3',5'- T_2 , 3- and 3'-iodothyronine (T_1), and thyronine (T_0) from Henning (Berlin, Germany); and Sephadex LH-20 from Pharmacia (Woerden, The Netherlands). $3, [3',^{125}I]T_2$ and $[3',5',^{125}I]rT_3$ were prepared by radioiodination of 3- T_1 and 3,3'- T_2 , respectively, as previously described (37).

Normal adult human liver and kidney tissues were obtained at surgery for liver and kidney tumors. Approval was obtained from the medical ethical committee of Erasmus University Medical School and Hospital. Tissue was homogenized in 0.25 mol/L sucrose, 10 mmol/L HEPES (pH 7.0), and 1 mmol/L dithiothreitol, and cytosol was prepared as previously described (8). SULT1A1 complementary DNA (cDNA) cloned by Wilborn *et al.* (34) and SULT1A3 cDNA cloned by Ganguly *et al.* (36) were provided by Dr. C. N. Falany (University of Alabama, Birmingham, AL) and expressed in *Salmonella typhimurium* as previously described (38). Human SULT1A3 cDNA was also cloned from human platelets and expressed in V79 cells (35). Bacterial and V79 cell cytosols

were prepared for characterization of recombinant sulfotransferase activities (35, 38). Protein was measured with the Bio-Rad protein assay (Bio-Rad, Veenendaal, The Netherlands), using BSA as the standard.

Sulfotransferase assay

Iodothyronine sulfotransferase activities were analyzed by incubation of 0.1 $\mu\text{mol/L}$ T_4 , T_3 , rT_3 , or 3,3'- T_2 and 100,000 cpm of the ^{125}I -labeled compound for 30 min at 37 C with the indicated amounts of liver or kidney cytosol or recombinant sulfotransferase preparation in the presence or absence (blank) of 50 $\mu\text{mol/L}$ PAPS in 0.2 mL 0.1 mol/L phosphate (pH 7.2) and 2 mmol/L ethylenediamine tetraacetate (39). Similar results were obtained in the absence of ethylenediamine tetraacetate. The reactions were started by the addition of enzyme diluted in ice-cold buffer and were stopped by the addition of 0.8 mL 0.1 mol/L HCl. The mixtures were analyzed for sulfoconjugate formation by chromatography on Sephadex LH-20 minicolumns as previously described (39). Sulfation in reaction mixtures with PAPS was corrected for background radioactivity detected in the corresponding Sephadex LH-20 fractions of the blanks. Incubations were carried out in triplicate, and the coefficient of variation was less than 10%.

Results

Figure 1 shows the sulfation of 0.1 $\mu\text{mol/L}$ T_4 , T_3 , rT_3 , and 3,3'- T_2 by human liver and kidney cytosol, SULT1A1, and SULT1A3 in the presence of 50 $\mu\text{mol/L}$ PAPS. All enzyme preparations display a strong substrate preference for 3,3'- T_2 , which is sulfated approximately 2 orders of magnitude more rapidly than T_3 and rT_3 , whereas T_4 is a poor substrate for these human sulfotransferases.

Figure 2 presents the sulfation of 3,3'- T_2 by human liver cytosol in the presence of PAPS as a function of incubation time and cytosolic protein concentration. Under the conditions used, 3,3'- T_2 sulfation was linear with incubation time up to 45 min, when about 50% of the substrate was converted (Fig. 2A). The subsequent decrease in sulfation rate was probably due to depletion of substrate rather than depletion of cofactor, because PAPS was added in large excess (50 $\mu\text{mol/L}$). Initially, 3,3'- T_2 sulfation showed a more than proportional increase with the cytosolic protein concentration (Fig. 2B). For instance, an increase in the cytosolic protein

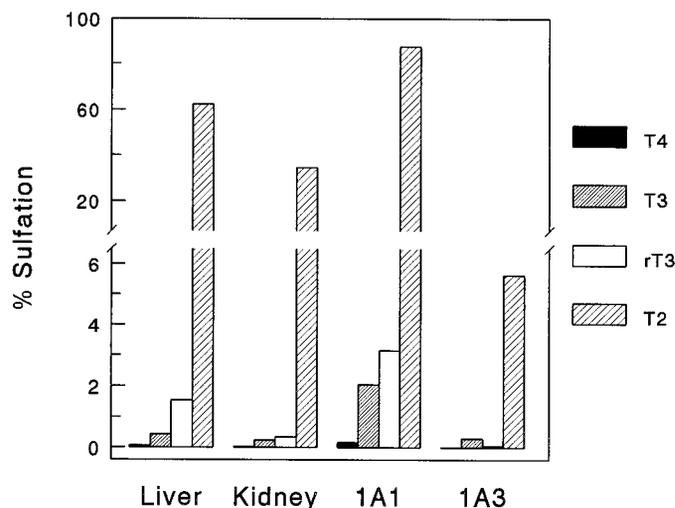
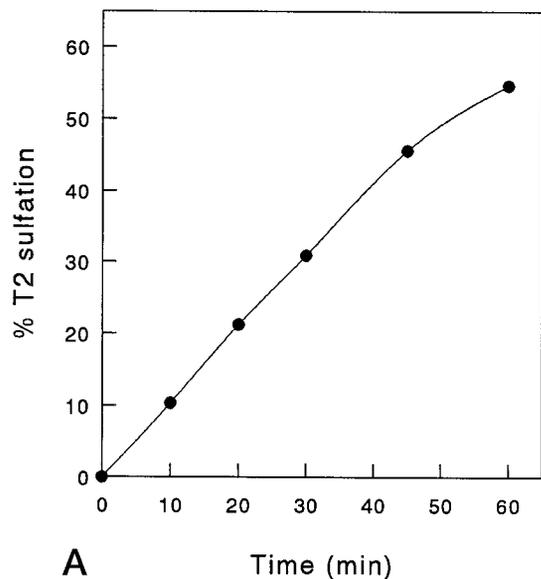
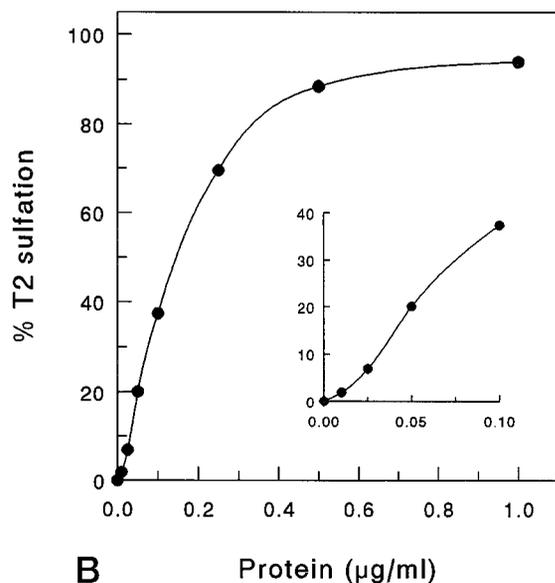


FIG. 1. Sulfation of iodothyronines by human liver and kidney cytosol, SULT1A1, and SULT1A3. Reaction conditions were 0.1 $\mu\text{mol/L}$ ^{125}I -labeled T_4 , T_3 , rT_3 , or 3,3'- T_2 ; 0.1 mg protein/mL; 50 $\mu\text{mol/L}$ PAPS; and 30-min incubation. Results are the means of triplicate determinations from a representative experiment.



A Time (min)

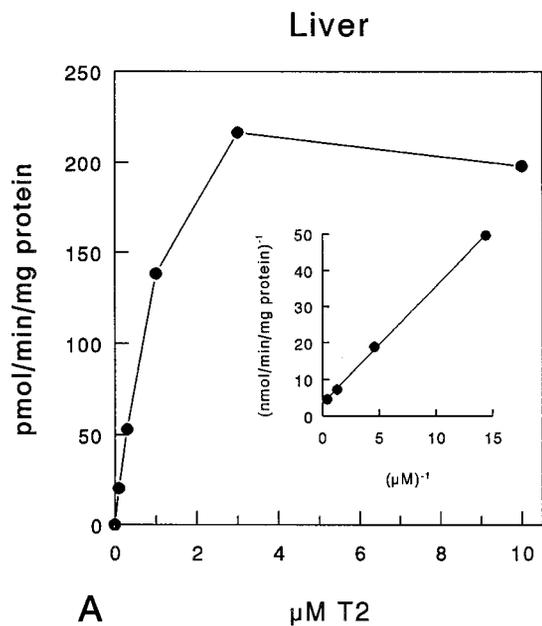


B Protein (µg/ml)

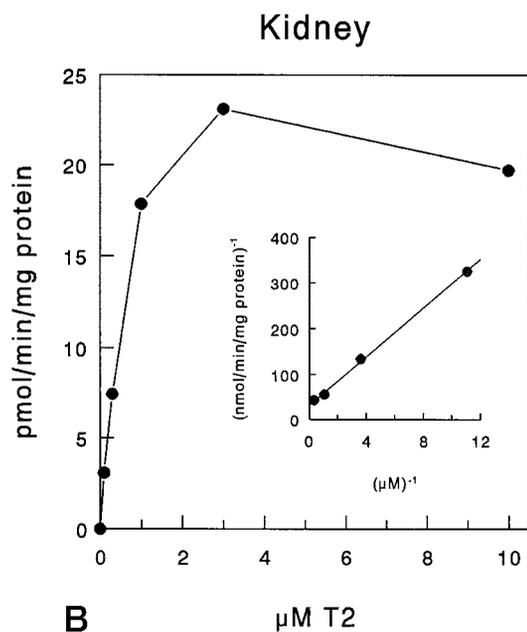
FIG. 2. Effects of incubation time (A) and protein concentration (B) on the sulfation of 3,3'-T₂ by human liver cytosol. Reaction conditions were 1 µmol/L 3,[3'-¹²⁵I]T₂, 50 µg protein/mL (A), 50 µmol/L PAPS, and 20-min incubation (B). Results are the means of triplicate determinations from a representative experiment.

concentration from 25 to 50 µg/mL resulted in a 3-fold increase in 3,3'-T₂S formation. Further increases in protein concentration resulted in roughly linear increases in 3,3'-T₂ sulfation until significant substrate depletion occurred. Similar results were obtained when 3,3'-T₂ sulfation by human kidney cytosol was analyzed as a function of the cytosolic protein concentration (data not shown).

Figure 3 shows the sulfation of 3,3'-T₂ by human liver (A) and kidney (B) cytosol as a function of the substrate concentration. In both tissues, saturation kinetics were observed



A µM T₂



B µM T₂

FIG. 3. Effects of substrate concentration on the sulfation of 3,3'-T₂ by human liver (A) or kidney cytosol (B). The insets show the double reciprocal plot. Reaction conditions were 0.1–3 µmol/L 3,[3'-¹²⁵I]T₂, 50 (A) or 100 (B) µg protein/mL, 50 µmol/L PAPS, and 60-min incubation.

in the range of the 3,3'-T₂ concentrations tested, with maximum sulfation rates obtained at 3 µmol/L 3,3'-T₂. At higher 3,3'-T₂ concentrations, a decrease in the sulfation rate was observed, suggesting substrate inhibition. The double reciprocal plots of sulfation rate vs. 3,3'-T₂ concentration were linear, allowing the calculation of apparent K_m values for 3,3'-T₂ and maximal velocity (V_{max}) values. Table 1 presents the kinetic parameters for 3,3'-T₂ sulfation by human liver and kidney cytosol at 50 µmol/L PAPS determined in dif-

TABLE 1. Kinetic parameters of human iodothyronine sulfotransferases

Enzyme source	K_m ($\mu\text{mol/L}$)	V_{max} (pmol/min · mg protein)
Variable substrate: 3,3'-T ₂ (50 $\mu\text{mol/L}$ PAPS)		
Liver cytosol	1.02 ± 0.11	337 ± 88
Kidney cytosol	0.64 ± 0.17	38.5 ± 18.1
SULT1A1 (<i>Salmonella</i>)	0.12 ± 0.05	465 ± 184
SULT1A3 (<i>Salmonella</i>)	31.2 ± 2.0	782 ± 239
SULT1A3 (V79 cells)	34.7 ± 6.1	2097 ± 474
Variable substrate: T ₃ (50 $\mu\text{mol/L}$ PAPS)		
Liver cytosol	54.9 ± 2.6	22.7 ± 8.6
Kidney cytosol	27.8 ± 2.6	2.7 ± 0.1
SULT1A1 (<i>Salmonella</i>)	29.1 ± 12.3	239 ± 82
SULT1A3 (<i>Salmonella</i>)	112 ± 23	158 ± 94
Variable substrate: PAPS (0.1 $\mu\text{mol/L}$ 3,3'-T ₂)		
Liver cytosol	6.00 ± 0.25	21.4 ± 3.4
Kidney cytosol	8.95 ± 0.39	5.8 ± 5.2
SULT1A1 (<i>Salmonella</i>)	0.65 ± 0.12	177 ± 34
SULT1A3 (V79 cells)	2.70 ± 0.19	4.9 ± 0.2

Data are presented as the mean ± SD of two to five experiments.

ferent experiments. The mean apparent K_m for 3,3'-T₂ was 1.02 $\mu\text{mol/L}$ in liver and 0.64 $\mu\text{mol/L}$ in kidney cytosol. The data presented were obtained using three different kidney samples and two different cytosol preparations from the same liver, whereas very similar results were obtained with 59 other liver samples.² Table 1 also gives the kinetic parameters for the sulfation of T₃ determined under similar conditions. Compared with 3,3'-T₂, apparent K_m values for T₃ were approximately 50-fold higher, *i.e.* 54.9 $\mu\text{mol/L}$ in liver and 27.8 $\mu\text{mol/L}$ kidney, whereas apparent V_{max} values were roughly 10-fold lower.

Figure 4 depicts the sulfation of 3,3'-T₂ by SULT1A1 (A) and SULT1A3 (B) as a function of the substrate concentration. Maximum sulfation rates were obtained at lower 3,3'-T₂ concentrations for SULT1A1 than for SULT1A3, with SULT1A1 showing clear substrate inhibition at 3,3'-T₂ levels above 1 $\mu\text{mol/L}$. From the linear double reciprocal plots, apparent K_m and V_{max} values were calculated. As the enzymes expressed in *Salmonella* and V79 cells were tested as crude cytosol, the maximum sulfation rates determined in these experiments are not representative of the differences in k_{cat} values between the different isoenzymes. The kinetic parameters determined in different experiments are summarized in Table 1, showing mean K_m values of 0.14 and 33 $\mu\text{mol/L}$ for 3,3'-T₂ sulfation by SULT1A1 and SULT1A3, respectively. Apparent K_m values were identical for SULT1A3 expressed in either *Salmonella* or V79 cells. Again, T₃ sulfation was characterized by much higher apparent K_m values, *i.e.* 29.1 $\mu\text{mol/L}$ for SULT1A1 and 112 $\mu\text{mol/L}$ for SULT1A3, and lower apparent V_{max} values compared with those for 3,3'-T₂ (Table 1).

Figure 5 shows the effects of increasing PAPS concentration on the sulfation of 0.1 $\mu\text{mol/L}$ 3,3'-T₂ by human liver (A) and kidney (B) cytosol. Sulfation approached maximum rates at PAPS concentrations of 30 $\mu\text{mol/L}$ or more. The double reciprocal plots of these data were linear, from which K_m and V_{max} values (at 0.1 $\mu\text{mol/L}$ 3,3'-T₂) were calculated. Table 1

² Gilissen, R. A. H. J., M. W. H. Coughtrie, E. Kaptein, and T. J. Visser, unpublished work.

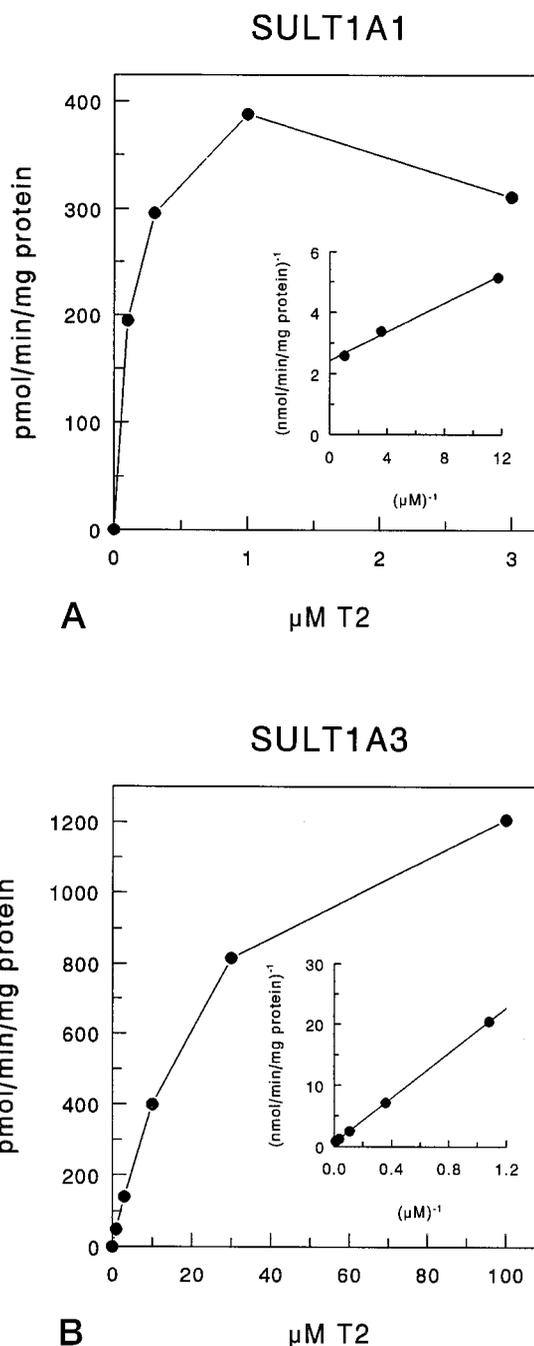


FIG. 4. Effects of substrate concentration on the sulfation of 3,3'-T₂ by SULT1A1 (A) and SULT1A3 (B). The insets show the double reciprocal plot. Reaction conditions were 0.1–100 $\mu\text{mol/L}$ [3,3'-¹²⁵I]T₂, 5 (A) or 100 (B) μg protein/mL, 50 $\mu\text{mol/L}$ PAPS, and 30-min incubation.

summarizes the results from different experiments. The mean apparent K_m value for PAPS was 6 $\mu\text{mol/L}$ in liver and 9 $\mu\text{mol/L}$ in kidney cytosol.

Figure 6 presents the sulfation of 0.1 $\mu\text{mol/L}$ 3,3'-T₂ by SULT1A1 (A) and SULT1A3 (B) as a function of the PAPS concentration. The PAPS concentration required for maximum sulfation rates was lower for SULT1A1 than for SULT1A3. The apparent K_m values for PAPS (at 0.1 $\mu\text{mol/L}$

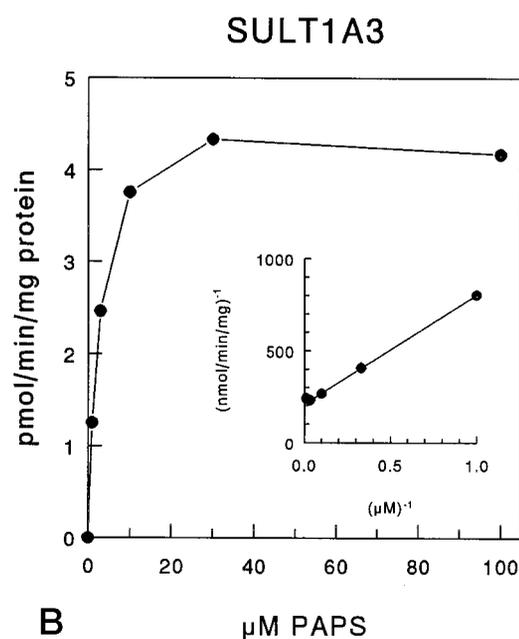
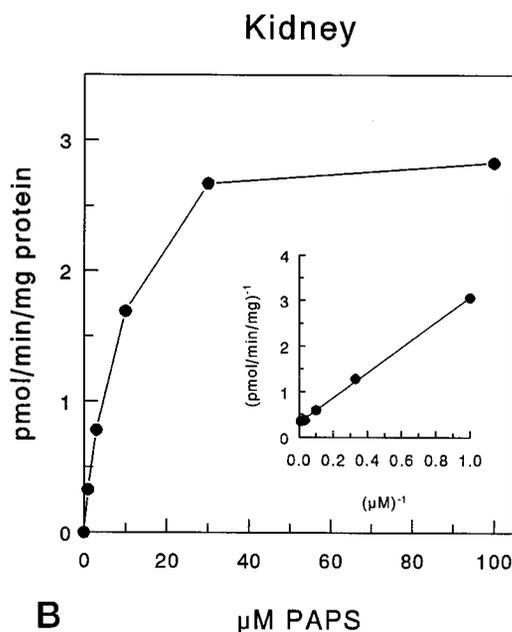
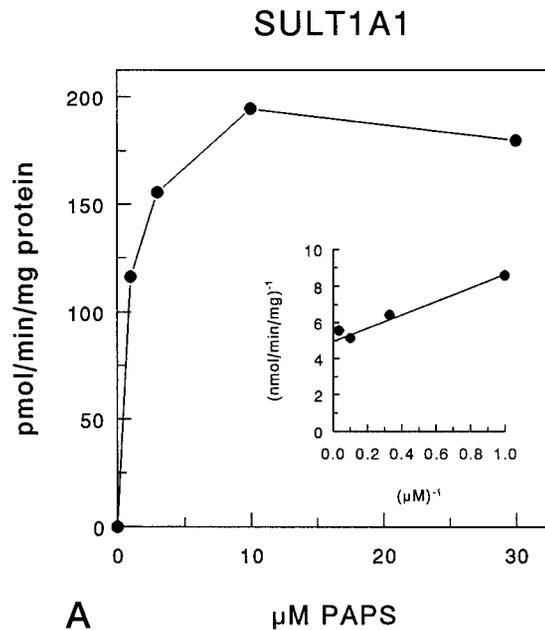
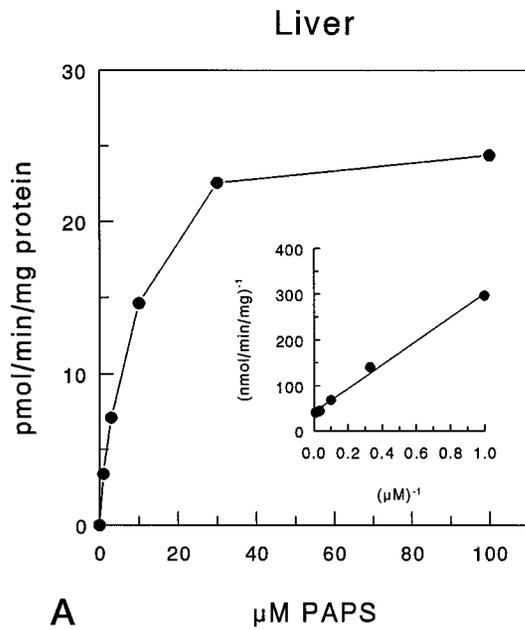


FIG. 5. Effects of cofactor concentration on the sulfation of 3,3'-T₂ by human liver (A) or kidney (B) cytosol. The insets show the double reciprocal plot. Reaction conditions were 0.1 μmol/L 3,[3'-¹²⁵I]T₂, 50 (A) or 100 (B) μg protein/mL, 1–100 μmol/L PAPS, and 30-min incubation.

FIG. 6. Effects of cofactor concentration on the sulfation of 3,3'-T₂ by SULT1A1 (A) and SULT1A3 (B). The insets show the double reciprocal plot. Reaction conditions were 0.1 μmol/L 3,[3'-¹²⁵I]T₂, 5 (A) or 100 (B) μg protein/mL, 1–100 μmol/L PAPS, and 30-min incubation.

3,3'-T₂) calculated from the linear Lineweaver-Burk plots were 0.65 and 2.7 μmol/L, respectively.

Figure 7 demonstrates the effects of increasing concentrations (1–100 μmol/L) of unlabeled iodothyronines on the sulfation of 3,[3'-¹²⁵I]T₂ (1 μmol/L) by human liver cytosol. T₀ had no effect, whereas 3-T₁ and 3,5-T₂ produced only 10–20% inhibition at the highest concentration tested. All other iodothyronines inhibited the sulfation of labeled 3,3'-T₂

dose dependently, with potencies decreasing in the order 3,3'-T₂ > rT₃ > 3',5'-T₂ > 3'-T₁ > T₃ ≈ T₄.

Table 2 compares the effects of unlabeled iodothyronines (10 μmol/L) on the sulfation of 3,[3'-¹²⁵I]T₂ (1 μmol/L) by human liver and kidney cytosol, SULT1A1, and SULT1A3. In general, the magnitude of inhibition of the sulfotransferase activities by the various iodothyronine analogs decreased in the order SULT1A1 > liver ≈ kidney > SULT1A3. The inhibition profiles of the tissue sulfotransferase activities were

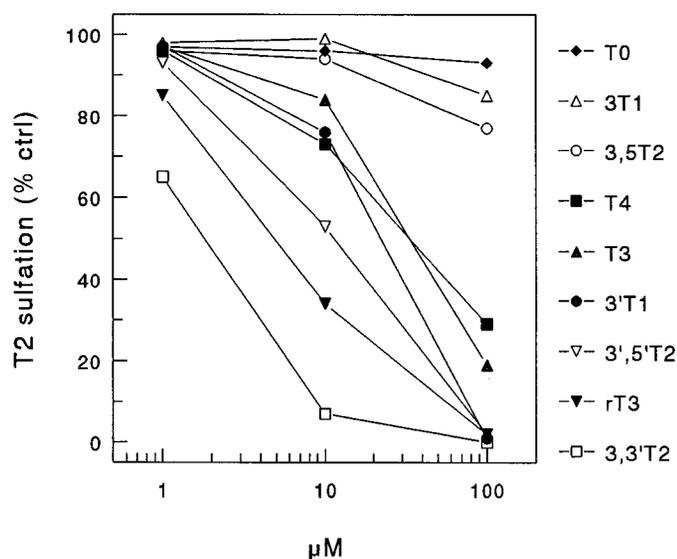


FIG. 7. Effects of 1–100 $\mu\text{mol/L}$ unlabeled iodothyronines on the sulfation of $3,3\text{'-}^{125}\text{I}]\text{T}_2$ by human liver cytosol. Reaction conditions were 1 $\mu\text{mol/L}$ $3,3\text{'-}^{125}\text{I}]\text{T}_2$, 50 μg protein/mL, 50 $\mu\text{mol/L}$ PAPS, and 30-min incubation. Results are the means of triplicate determinations from a representative experiment.

better correlated with the inhibition profile of SULT1A1 than with that of SULT1A3 (liver *vs.* SULT1A1: $r = 0.936$; $P < 0.01$; liver *vs.* SULT1A3: $r = 0.793$; $P < 0.01$; kidney *vs.* SULT1A1: $r = 0.920$; $P < 0.01$; kidney *vs.* SULT1A3: $r = 0.751$; $P < 0.01$).

Discussion

Several human phenol sulfotransferases have been cloned and characterized, including SULT1A1, SULT1A2, SULT1A3, SULT1B1, and SULT1C1 (1–3, 34–36, 40–50). SULT1A1 represents what has been known for some time as the P-PST, whereas SULT1A3 was previously known as the M-PST (1–3). The SULT1A isoenzymes are equally large proteins consisting of 295 amino acids. A significant degree of homology exists between the human SULT1A proteins, with 92% amino acid identity between SULT1A1 and SULT1A3 and an even greater degree of homology between SULT1A1 and SULT1A2, the genes of which are located close together on chromosome 16 (2, 34–36, 40–46). Specifically, SULT1A1 and SULT1A2 differ in 11–15 amino acid positions, SULT1A1 and SULT1A3 differ in 20–23 amino acid positions, and SULT1A2 and SULT1A3 differ in 30–31 amino acid positions. Different allelic variants have been identified for SULT1A1 and SULT1A2 but to date not for SULT1A3 (2, 34–36, 40–46).

Sulfation of T_3 by P-PST and M-PST purified from human liver and intestine has been reported previously (32, 33), but it remains to be determined which SULT1A isoenzyme is most important for thyroid hormone sulfation in human liver and other tissues. In addition to the members of the SULT1A family, two other human phenol sulfotransferases, SULT1B1 (49, 50) and SULT1C1 (47, 48), have been cloned recently. Whereas it is unknown whether isoenzymes homologous to SULT1A2 and SULT1A3 exist in rats, the rat homologs of human SULT1A1, SULT1B1, and SULT1C1 have been cloned and characterized regarding their activity toward iodothyronines (51–53). These studies have demonstrated that both

TABLE 2. Inhibition of the sulfation of $3,3\text{'-T}_2$ by iodothyronine analogs

Iodothyronine	% Inhibition			
	Liver	Kidney	SULT1A1	SULT1A3
T_4	17 ± 3	7 ± 5	32 ± 7	0 ± 1
T_3	4 ± 2	3 ± 4	24 ± 4	1 ± 2
$r\text{T}_3$	43 ± 4	42 ± 10	86 ± 1	1 ± 1
$3',5\text{'-T}_2$	47 ± 7	41 ± 14	86 ± 2	6 ± 1
$3,3\text{'-T}_2$	87 ± 2	88 ± 2	95 ± 1	19 ± 2
$3,5\text{-T}_2$	5 ± 7	-2 ± 2	5 ± 2	-1 ± 1
3'-T_1	71 ± 12	61 ± 18	99 ± 1	38 ± 2
3-T_1	5 ± 10	1 ± 1	3 ± 2	-1 ± 1
T_0	-1 ± 2	0 ± 1	-2 ± 6	-2 ± 1

$3,3\text{'-}^{125}\text{I}]\text{T}_2$ (1 $\mu\text{mol/L}$) was incubated for 30 min at 37 C with appropriate amounts of enzyme protein in the absence or presence of 10 $\mu\text{mol/L}$ unlabeled iodothyronine. The percent inhibition is calculated from the $3,3\text{'-}^{125}\text{I}]\text{T}_2$ sulfation rate in the presence *vs.* that in the absence of analog. Data are presented as the mean \pm SD of three to six experiments using three liver and three kidney samples.

rat SULT1B1 and SULT1C1 catalyze the sulfation of different iodothyronines, in particular $3,3\text{'-T}_2$, whereas rat SULT1A1 is completely inactive. Human SULT1B1 has recently also been shown to have sulfotransferase activity toward iodothyronines (50), but sulfation of iodothyronines by human SULT1C1 has not yet been reported.

We demonstrate that both human SULT1A1 and SULT1A3 are capable of catalyzing the sulfation of iodothyronines. This is not surprising, as the sulfation of T_3 by P-PST and M-PST purified from human liver and intestine has been reported previously (32, 33). We have also recently demonstrated effective sulfation of iodothyronines by human SULT1A2.³ It appears that small differences in amino acid sequence can effect large differences in sulfotransferase activity. The high activity of human SULT1A1 in contrast to the complete lack of iodothyronine sulfotransferase activity of rat SULT1A1 is remarkable, considering the high degree of amino acid sequence identity (80%) between these orthologous proteins (2, 51). Likewise, the smaller (8%) difference in amino acid sequence between human SULT1A1 and SULT1A3 (34–36, 46) is associated with a more than 200-fold difference in the K_m value for $3,3\text{'-T}_2$, a 4-fold difference in the K_m value for T_3 , and a 4-fold difference in the K_m value for PAPS. It should be noted that the apparent K_m value of $3,3\text{'-T}_2$ for SULT1A1 presented here is about 10-fold lower than that mentioned previously (53), which may be due to partial inactivation through oxidation (54) of the enzyme preparation used previously.

The main purpose for comparing the substrate specificities and kinetic parameters of native iodothyronine sulfotransferase activities in human liver and kidney with these properties of recombinant sulfotransferases is to try to identify the isoenzymes that contribute most to the sulfation of thyroid hormone in these tissues. The iodothyronine sulfotransferase activities of human liver and kidney cytosol are characterized by similar apparent K_m values for both $3,3\text{'-T}_2$ and PAPS as well as similar substrate specificities, suggesting the involvement of similar isoenzymes. The substrate specificities of the hepatic and renal sulfotransferase activities showed a

³ Kester, M. H. A., M. W. H. Coughtrie, H. Glatt, and T. J. Visser, unpublished work.

better correlation with SULT1A1 than with SULT1A3, suggesting that SULT1A1 is a prominent iodothyronine sulfotransferase in human liver and kidney. However, the different iodothyronines showed a lower apparent affinity for the native sulfotransferases than for recombinant SULT1A1, which may be due to the presence of iodothyronine-binding proteins in the tissues. Sulfation of thyroid hormone in human liver and kidney (and possibly other tissues) involves contributions of at least SULT1A1, SULT1A2, SULT1A3, and SULT1B1 and perhaps also SULT1C1. The complexity is further increased by the polymorphic variation in these isoenzymes (2, 55) and their tissue-specific expression (56). In addition, it has been demonstrated that functional rat phenol sulfotransferases may consist of either two identical (homodimer) or two different subunits (heterodimer) (57). Our findings of a more than linear increase in iodothyronine sulfotransferase activity with an increase in hepatic or renal cytosolic protein concentration may reflect this requirement for protein dimerization.

The native and recombinant sulfotransferases tested in this study show a marked preference for 3,3'-T₂ as the substrate. Both SULT1A1 and SULT1A3 are much less efficient in catalyzing the sulfation of T₃, which does not imply that these isoenzymes are not important for T₃ sulfation *in vivo*. This is supported by the significant sulfation of T₃ in both human liver and kidney cytosol. Sulfation of T₄ is almost undetectable, not only with recombinant SULT1A1 and SULT1A3 but also in human liver and kidney. Nevertheless, high serum T₄S levels have been detected in human newborns (14, 15), suggesting sulfation of T₄ by other isoenzymes.

In conclusion, we have identified SULT1A1 and SULT1A3 as low K_m and high K_m human iodothyronine sulfotransferases, respectively, and obtained evidence that the sulfation of iodothyronines in human liver and kidney is catalyzed by similar enzymes. Further investigations are required to determine the possible importance of other isoenzymes, such as SULT1A2, SULT1B1, and SULT1C1, and of polymorphic variations in the different sulfotransferases for the sulfation of thyroid hormone in human tissues. This information is essential for investigation of the regulation of this important pathway of thyroid hormone metabolism under (patho)physiological conditions, in particular during fetal development.

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