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Characterization of Human Iodothyronine Sulfotransferases*

MONIQUE H. A. KESTER, ELLEN KAPTEIN, THIRZA J. ROEST, CAREN H. VAN DIJK, DICK TIBBOEL, WALTER MEINL, HANSRUEDI GLATT, MICHAEL W. H. COUGHTRIE, AND THEO J. VISSER

Department of Internal Medicine III, Erasmus University Medical School (M.H.A.K., E.K., T.J.R., C.H.v.D., T.J.V.), and the Department of Pediatric Surgery, Erasmus University Medical School and Sophia Children Hospital (M.H.A.K., D.T.), 3000 DR Rotterdam, The Netherlands; the Department of Toxicology, German Institute of Human Nutrition (W.M., H.G.) D-14558, Potsdam-Rehbrucke, Germany; and the Department of Molecular and Cellular Pathology, University of Dundee (M.W.H.C.), Dundee DD1 9S4, Scotland

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 μmol/L 3′-phosphoadenosine-5′-phosphosulfate (PAPS)] were 1.02 and 54.9 μmol/L for liver cytosol, 0.64 and 27.8 μmol/L for kidney cytosol, 0.14 and 29.1 μmol/L for SULT1A1, and 33 and 112 μmol/L for SULT1A3, respectively. The apparent K_m of PAPS (at 0.1 μmol/L 3,3′-T_2) was 6.0 μmol/L for liver cytosol, 9.0 μmol/L for kidney cytosol, 0.65 μmol/L for SULT1A1, and 2.7 μmol/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 μmol/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 iodothyonine 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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Address all correspondence and requests for reprints to: Dr. Theo J. Visser, Department of Internal Medicine III, Erasmus University Medical School, Room Bd 234, P.O. Box 1738, 3000 DR Rotterdam, The Netherlands. E-mail: visser@inw3.azr.nl.

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[1] Visser, T. J., and E. Kaptein, unpublished work.

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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 μmol/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 μmol/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 μmol/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 μmol/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 μmol/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

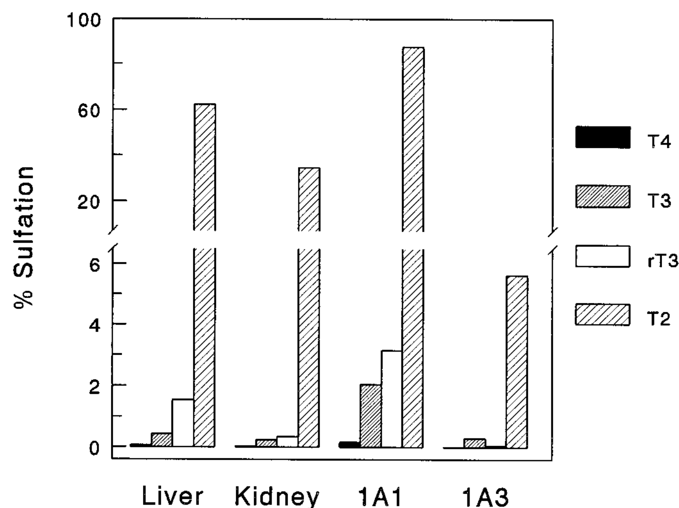

FIG. 1. Sulfation of iodothyronines by human liver and kidney cytosol, SULT1A1, and SULT1A3. Reaction conditions were 0.1 μmol/L ^{125}I-labeled T_4, T_3, rT_3, or 3,3′-T_2; 0.1 mg protein/mL; 50 μmol/L PAPS; and 30-min incubation. Results are the means of triplicate determinations from a representative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

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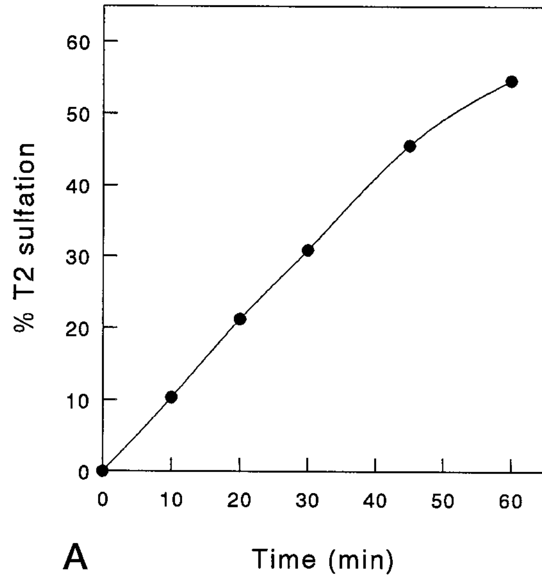


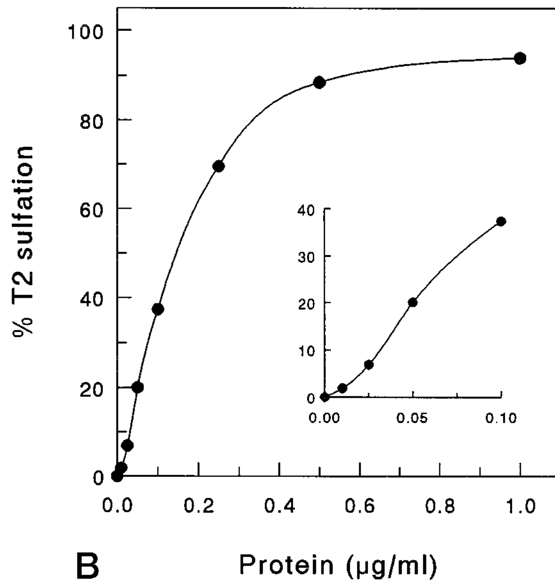
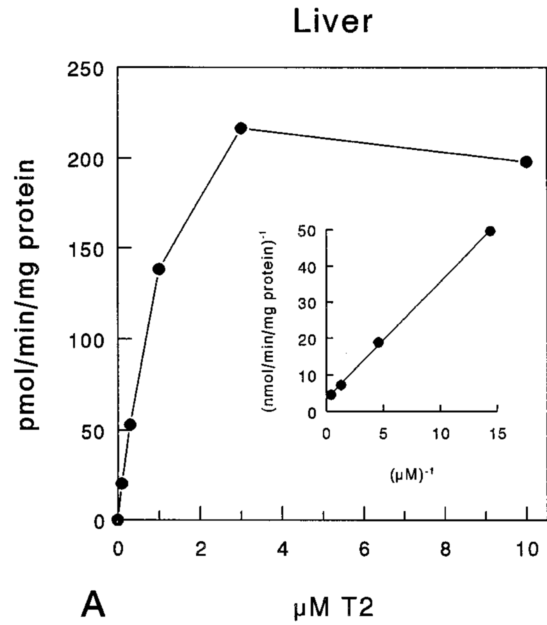


FIG. 2. Effects of incubation time (A) and protein concentration (B) on the sulfation of 3,3′-T_2 by human liver cytosol. Reaction conditions were 1 μmol/L 3,[3′-^{125}I]T_2, 50 μg protein/mL (A), 50 μmol/L PAPS, and 20-min incubation (B). Results are the means of triplicate determinations from a representative experiment.



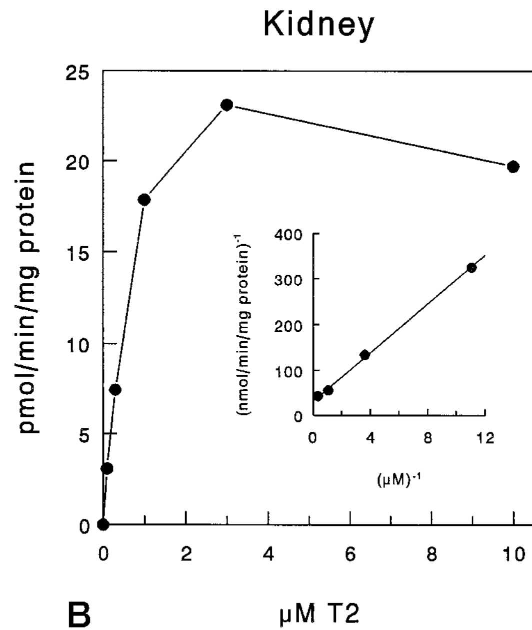


FIG. 3. Effects of substrate concentration on the sulfation of 3,3′-T_2 by human liver (A) or kidney cytosol (B). The *insets* show the double reciprocal plot. Reaction conditions were 0.1–3 μmol/L 3,[3′-^{125}I]T_2, 50 (A) or 100 (B) μg protein/mL, 50 μmol/L PAPS, and 60-min incubation.

concentration from 25 to 50 μg/mL resulted in a 3-fold increase in 3,3′-T_2S formation. Further increases in protein concentration resulted in roughly linear increases in 3,3′-T_2 sulfation until significant substrate depletion occurred. Similar results were obtained when 3,3′-T_2 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′-T_2 by human liver (A) and kidney (B) cytosol as a function of the substrate concentration. In both tissues, saturation kinetics were observed in the range of the 3,3′-T_2 concentrations tested, with maximum sulfation rates obtained at 3 μmol/L 3,3′-T_2. At higher 3,3′-T_2 concentrations, a decrease in the sulfation rate was observed, suggesting substrate inhibition. The double reciprocal plots of sulfation rate *vs.* 3,3′-T_2 concentration were linear, allowing the calculation of apparent K_m values for 3,3′-T_2 and maximal velocity (V_{max}) values. Table 1 presents the kinetic parameters for 3,3′-T_2 sulfation by human liver and kidney cytosol at 50 μmol/L PAPS determined in dif-

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TABLE 1. Kinetic parameters of human iodothyronine sulfotransferases

Enzyme source	K_m (μmol/L)	V_{max} (pmol/min · mg protein)
Variable substrate: 3,3′-T_2 (50 μmol/L PAPS)		
Liver cytosol	1.02 ± 0.11	337 ± 88
Kidney cytosol	0.64 ± 0.17	38.5 ± 18.1
SULT1A1 (*Salmonella*)	0.12 ± 0.05	465 ± 184
SULT1A3 (*Salmonella*)	31.2 ± 2.0	782 ± 239
SULT1A3 (V79 cells)	34.7 ± 6.1	2097 ± 474
Variable substrate: T_3 (50 μmol/L PAPS)		
Liver cytosol	54.9 ± 2.6	22.7 ± 8.6
Kidney cytosol	27.8 ± 2.6	2.7 ± 0.1
SULT1A1 (*Salmonella*)	29.1 ± 12.3	239 ± 82
SULT1A3 (*Salmonella*)	112 ± 23	158 ± 94
Variable substrate: PAPS (0.1 μmol/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 (*Salmonella*)	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′-T_2 was 1.02 μmol/L in liver and 0.64 μmol/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 T_3 determined under similar conditions. Compared with 3,3′-T_2, apparent K_m values for T_3 were approximately 50-fold higher, *i.e.* 54.9 μmol/L in liver and 27.8 μmol/L kidney, whereas apparent V_{max} values were roughly 10-fold lower.

Figure 4 depicts the sulfation of 3,3′-T_2 by SULT1A1 (A) and SULT1A3 (B) as a function of the substrate concentration. Maximum sulfation rates were obtained at lower 3,3′-T_2 concentrations for SULT1A1 than for SULT1A3, with SULT1A1 showing clear substrate inhibition at 3,3′-T_2 levels above 1 μmol/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 μmol/L for 3,3′-T_2 sulfation by SULT1A1 and SULT1A3, respectively. Apparent K_m values were identical for SULT1A3 expressed in either *Salmonella* or V79 cells. Again, T_3 sulfation was characterized by much higher apparent K_m values, *i.e.* 29.1 μmol/L for SULT1A1 and 112 μmol/L for SULT1A3, and lower apparent V_{max} values compared with those for 3,3′-T_2 (Table 1).

Figure 5 shows the effects of increasing PAPS concentration on the sulfation of 0.1 μmol/L 3,3′-T_2 by human liver (A) and kidney (B) cytosol. Sulfation approached maximum rates at PAPS concentrations of 30 μmol/L or more. The double reciprocal plots of these data were linear, from which K_m and V_{max} values (at 0.1 μmol/L 3,3′-T_2) were calculated. Table 1

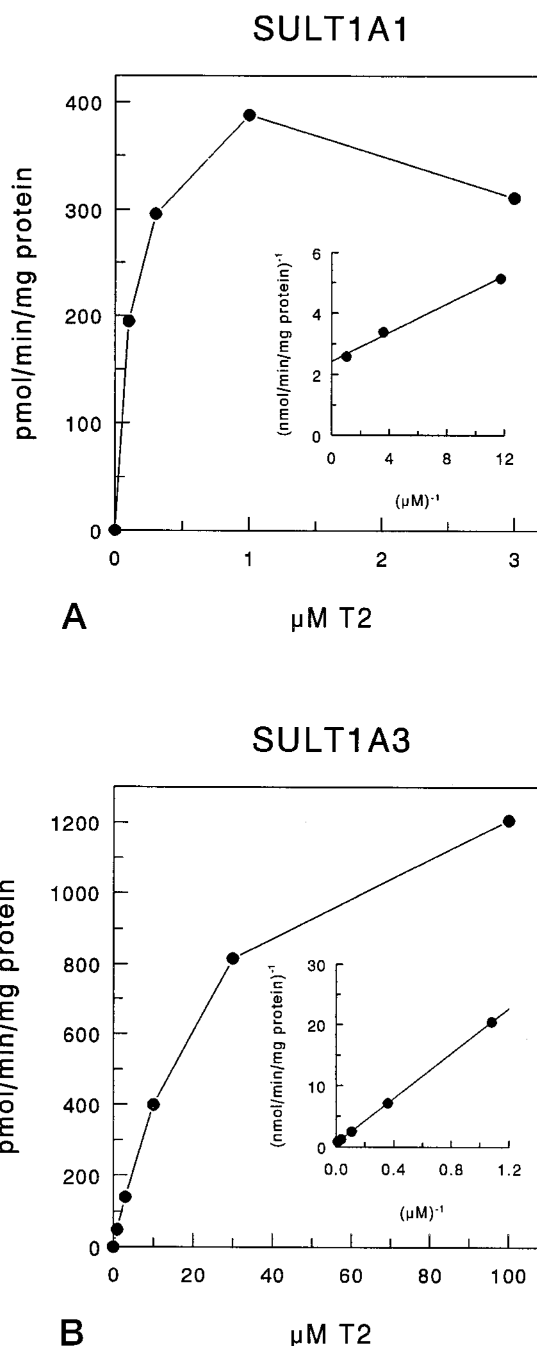

FIG. 4. Effects of substrate concentration on the sulfation of 3,3′-T_2 by SULT1A1 (A) and SULT1A3 (B). The *insets* show the double reciprocal plot. Reaction conditions were 0.1–100 μmol/L 3,[3′-^{125}I]T_2, 5 (A) or 100 (B) μg protein/mL, 50 μmol/L PAPS, and 30-min incubation.

summarizes the results from different experiments. The mean apparent K_m value for PAPS was 6 μmol/L in liver and 9 μmol/L in kidney cytosol.

Figure 6 presents the sulfation of 0.1 μmol/L 3,3′-T_2 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 μmol/L

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

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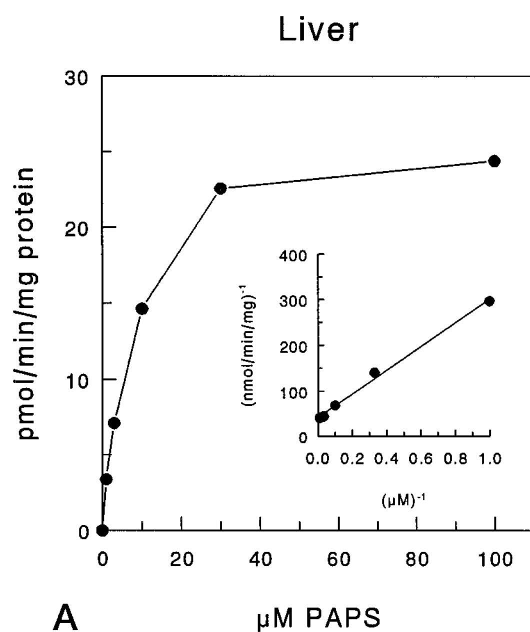


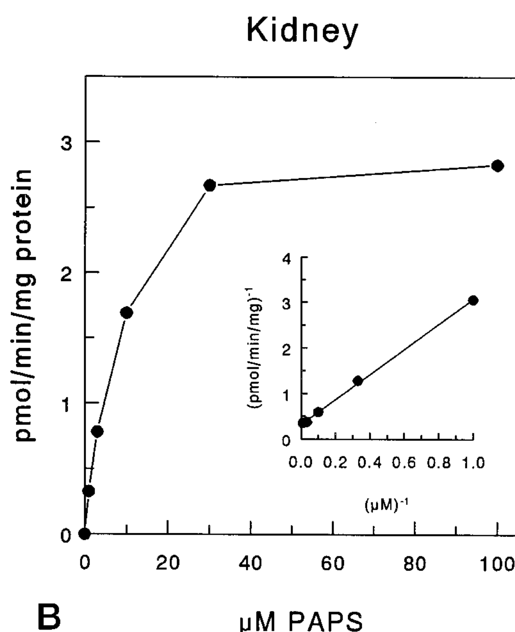
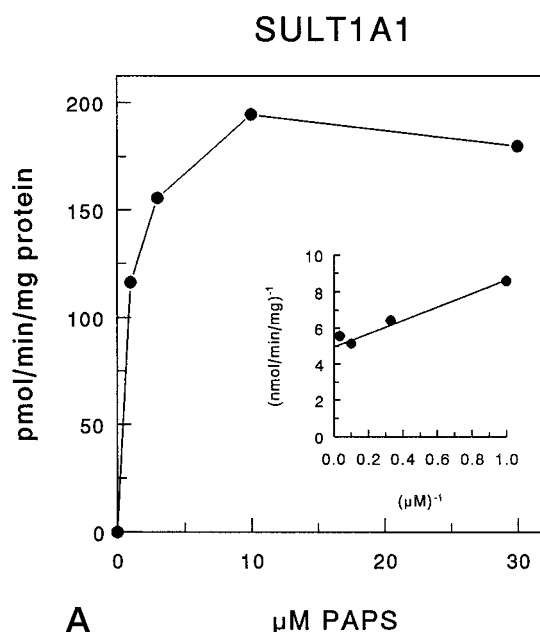


FIG. 5. Effects of cofactor concentration on the sulfation of $3,3'$-T_2 by human liver (A) or kidney (B) cytosol. The *insets* show the double reciprocal plot. Reaction conditions were 0.1 μmol/L $3,[3'$-$^{125}I]T_2$, 50 (A) or 100 (B) μg protein/mL, 1–100 μmol/L PAPS, and 30-min incubation.

$3,3'$-T_2) 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 $3,[3'$-$^{125}I]T_2$ (1 μmol/L) by human liver cytosol. T_0 had no effect, whereas 3-T_1 and 3,5-T_2 produced only 10–20% inhibition at the highest concentration tested. All other iodothyronines inhibited the sulfation of labeled $3,3'$-T_2



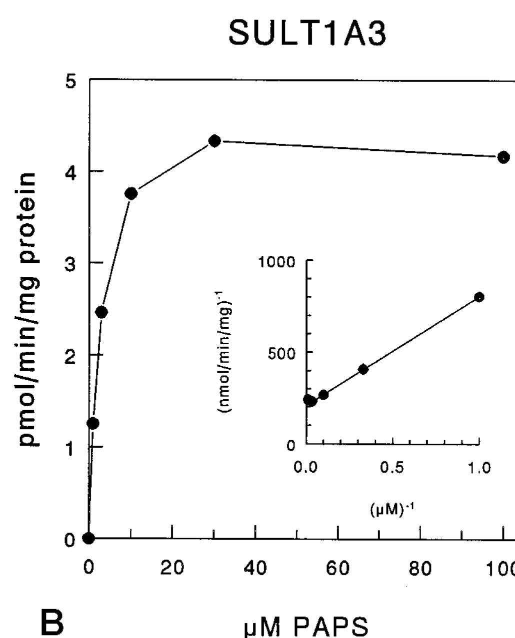


FIG. 6. Effects of cofactor concentration on the sulfation of $3,3'$-T_2 by SULT1A1 (A) and SULT1A3 (B). The *insets* show the double reciprocal plot. Reaction conditions were 0.1 μmol/L $3,[3'$-$^{125}I]T_2$, 5 (A) or 100 (B) μg protein/mL, 1–100 μmol/L PAPS, and 30-min incubation.

dose dependently, with potencies decreasing in the order $3,3'$-$T_2 > rT_3 > 3',5'$-$T_2 > 3'$-$T_1 > T_3 \approx T_4$.

Table 2 compares the effects of unlabeled iodothyronines (10 μmol/L) on the sulfation of $3,[3'$-$^{125}I]T_2$ (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

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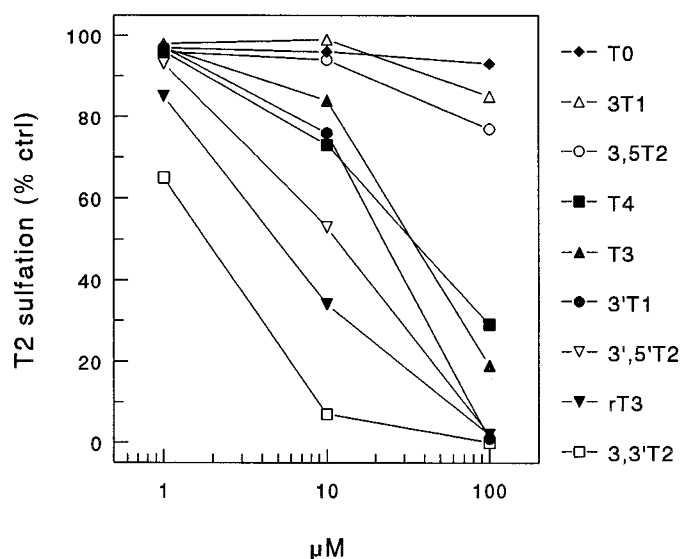

FIG. 7. Effects of 1–100 μmol/L unlabeled iodothyronines on the sulfation of 3,[3′-^{125}I]T_2 by human liver cytosol. Reaction conditions were 1 μmol/L 3,[3′-^{125}I]T_2, 50 μg protein/mL, 50 μmol/L PAPS, and 30-min incubation. Results are the means of triplicate determinations from a representative 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′-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
rT_3	43 ± 4	42 ± 10	86 ± 1	1 ± 1
3′,5′-T_2	47 ± 7	41 ± 14	86 ± 2	6 ± 1
3,3′-T_2	87 ± 2	88 ± 2	95 ± 1	19 ± 2
3,5-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′-^{125}I]T_2 (1 μmol/L) was incubated for 30 min at 37 C with appropriate amounts of enzyme protein in the absence or presence of 10 μmol/L unlabeled iodothyronine. The percent inhibition is calculated from the 3,[3′-^{125}I]T_2 sulfation rate in the presence *vs.* that in the absence of analog. Data are presented as the mean ± 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′-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′-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′-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′-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.

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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′-T_2 as the substrate. Both SULT1A1 and SULT1A3 are much less efficient in catalyzing the sulfation of T_3, which does not imply that these isoenzymes are not important for T_3 sulfation *in vivo*. This is supported by the significant sulfation of T_3 in both human liver and kidney cytosol. Sulfation of T_4 is almost undetectable, not only with recombinant SULT1A1 and SULT1A3 but also in human liver and kidney. Nevertheless, high serum T_4S levels have been detected in human newborns (14, 15), suggesting sulfation of T_4 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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