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SHORT COMMUNICATION

Sulfation of Iodothyronines by Human Sulfotransferase 1C1 (SULT1C1)*

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ABSTRACT. Sulfation is an important component of human thyroid hormone metabolism. The role of the human sulfotransferase 1C1 (SULT1C1) is not known. Because SULT1C1 is present in the adult thyroid, intra-thyroidal sulfation of thyroid hormones and their metabolites might occur. We tested this hypothesis by determining the ability of recombinant human SULT1C1 to catalyze iodothyronine sulfation. Apparent K_m values for 3,3′,5-triiodothyronine (T_3), 3,3′-diiodothyronine (3,3′-T_2), 3′,5′,3-triiodothyronine (rT_3), and 3,3′,5,5′-tetraiodothyronine (T_4) with SULT1C1 were 28.7, 10.3, 10.2, and 59.3 μM, respectively. Thermal stability and responses to inhibitors also were tested with T_3 as the substrate. Enzyme aliquots were measured simultaneously to determine SULT1C1 substrate preferences at optimal iodothyronine concentrations. SULT1C1 activity obtained with T_3 was used as 100%, and the activities with 3,3′-T_2, rT_3, T_4, and 3,5-diiodothyronine (3,5-T_2) were 614, 314, 25, and 4%, respectively. We report for the first time the characterization of human SULT1C1 with T_3 and the preferences of the enzyme for various iodothyronines. The presence of SULT1C1 in the adult thyroid gland raises the possibilities that the enzyme can contribute to intraglandular thyroid hormone processing and iodide reutilization. BIOCHEM PHARMACOL **60**;11:1713–1716, 2000. © 2000 Elsevier Science Inc.

KEY WORDS. SULT1C1; sulfation; triiodothyronine; thyroid hormone metabolism

Sulfation inactivates T_3‡ and other iodothyronines by the addition of a sulfuryl moiety to the 4′-hydroxyl group. Not only does sulfation enhance deiodination, but receptor-inactive sulfated iodothyronines also may serve as a hormone reservoir that can be desulfated in specific tissues when active hormone is required. Up to 55% of the T_3 produced daily in humans is sulfate conjugated to T_3S, a major product of human T_3 metabolism [1, 2]. Presently, it is not clear which enzymes are most responsible for iodothyronine sulfation *in vivo*.

Cytosolic sulfotransferases [3] are now referred to as SULTs, as recommended by an International Sulfotransferase Enzyme Nomenclature Workshop (ISSX North American Meeting, Seattle, WA, August 1995). Human SULTs with documented activity toward thyroid hormone include SULT1A1 (thermostable phenol SULT) [4–6], SULT1A3 (thermolabile phenol SULT) [4, 5], SULT1B1 [7, 8], SULT1E1 (estrogen SULT) [9, 10], and SULT2A1 (DHEA SULT) [9]. SULT1C1 was shown to be expressed in the adult human thyroid gland, stomach, kidney, and fetal liver and kidney by northern blot analysis [11] and to catalyze the sulfation of 4-nitrophenol [12] and *N*-hydroxy-2-acetylaminofluorene [13], but no endogenous substrate was identified. In this report, we demonstrated that expressed human SULT1C1 catalyzes the sulfation of T_3 and other iodothyronines. We also characterized biochemical properties of this enzyme with T_3 as the substrate, identified T_3S as the reaction product, and determined the preference of SULT1C1 for T_3, T_4, rT_3, 3,3′-T_2, and 3,5-T_2.

MATERIALS AND METHODS

Materials

COS-1 cells were obtained from the American Type Culture Collection (ATCC). Human recombinant SULT1C1 cloned into vector p91023(B) was expressed in COS-1 cells by transfection using lipofectamine (15 μg/mL), and high-speed supernatants were prepared as described previously [9]. The resulting high-speed supernatants were mixed 1:1 (v/v) with a 5 mM potassium phosphate buffer, pH 7.5, that contained 5 mg/mL of BSA and then were stored at −75° until used. We found that the presence or absence of BSA in the assay did not change the apparent K_m value for T_3, but did increase by 2-fold the activity measured with T_3 as the substrate. [^{35}S]PAPS

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‡ *Abbreviations*: T_3, 3,3′,5-triiodothyronine; 3,3′-T_2, 3,3′-diiodothyronine; rT_3, 3′,5′,3-triiodothyronine; T_4, 3,3′,5,5′-tetraiodothyronine; 3,5-T_2, 3,5-diiodothyronine; T_3S, T_3 sulfate; DCNP, 2,6-dichloro-4-nitrophenol; DHEA, dehydroepiandrosterone; DTT, dithiothreitol; [^{35}S]PAPS, [^{35}S]3′-phosphoadenosine-5′-phosphosulfate; and SULT, sulfotransferase.

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TABLE 1. SULT1C1 kinetic data and iodothyronine preferences*

Substrate	Concentration ranges (μM)	K_m (μM)	V_{max} (Units/mg protein)	Optimal Concentration (μM)	Activity (%, †)	Activity (Units/mg protein)
T_3	0.001–300	28.7	0.047	100	100	0.028
T_4	1–100	59.3	0.016	100	25	0.007
rT_3	0.01–30	10.2	0.280	10	314	0.088
3,3′-T_2	0.03–50	10.3	0.327	25	614	0.172
3,5-T_2	1–200	ND	ND	1	4	0.001

* Each value represents the mean of 3 determinations, except for the K_m and V_{max} data with T_3, which represent the mean of 12 determinations. ND = not detected.
† Results are expressed using activity obtained with T_3 as 100%.

(specific activity from 1.52 to 2.50 Ci/mmol) was purchased from New England Nuclear (NEN) Dupont. 3,3′-T_2 was a gift from Dr. S-Y. Wu. T_3, rT_3, T_4, 3,5-T_2, DCNP, and Ecteola cellulose (medium mesh) were purchased from the Sigma Chemical Co. DTT was purchased from Calbiochem. Lipofectamine was purchased from Gibco. The human SULT1C1 cDNA was a gift from Dr. R. M. Weinshilboum [11].

SULT Assays

Iodothyronine SULT activity was measured by the method of Borchardt *et al.* [14] as modified by Young *et al.* [4] and Li and Anderson [9]. The enzyme assay was performed at optimal conditions with T_3 as the substrate and [^{35}S]PAPS (0.4 μM final concentration) as the co-substrate. Iodothyronine substrates were dissolved with 10 N sodium hydroxide and double-distilled water (approximately 1:1000, v/v). The solubilized thyroid hormones were added to the assay reaction mixture after addition of the assay buffer. SULT1C1 preparations at 15–30 μg of high-speed supernatant protein were used in all assays. The sample pH optimum was 6.61 for SULT1C1 activity in the presence of 8.8 mM potassium phosphate buffer. $MgCl_2$ (0.5 mM) was included because it was found to increase activity by 40%. Mock transfected COS-1 cells (no cDNA) served as controls with each assay. Net activities were calculated by subtraction of the mock transfected activities/mg protein from the transfected activities/mg protein to remove the potential effect of endogenous SULT activities. Net SULT activities were expressed as Units/mg protein. One Unit of enzyme activity represented 1 nmol of sulfated product formed/hr at 37°. Thermal stability was tested by the methods of Reiter *et al.* [15] as modified by Anderson *et al.* [16]. Synthesis of T_3S was performed by our modification [9] of the method of Mol and Visser [17]

Enzyme Assay Reaction Product Identification

The final eluant from an Ecteola column was mixed with 0.1 mL of [^{125}I]T_3S as a known standard. A 3-mL volume of this mixture was applied to a Sephadex LH-20 column (1 × 2 cm). The column was eluted with 32 mL of double-distilled water, and 2.2-mL fractions were collected. Aliquots (1 mL) from each fraction were mixed with 5 mL of scintillation fluid (Biosafe II), and counts per minute were determined in a scintillation counter. Equivalent 1-mL aliquots were counted in a gamma counter.

Data Analysis

Apparent K_m and V_{max} values were calculated by the direct linear plot method [18] with the Enzpack 3 program by Williams (Elsevier-Biosoft). The 50% inactivation temperatures and 50% inhibitory concentrations (IC_{50}) for DCNP and NaCl were determined with a curve-fitting program (GraphPad Software).

RESULTS AND DISCUSSION

Expressed SULT1C1 Biochemical Properties

K_m and V_{max}. The apparent K_m value for [^{35}S]PAPS with T_3 as the constant substrate was 0.09 μM with SULT1C1, a value similar to the result obtained with SULT2A1 [9]. SULT1C1 was tested with final T_3 concentrations ranging from 1 nM to 300 μM (Table 1). The apparent K_m value for T_3 with SULT1C1 activity was similar to the values reported with other human recombinant phenol SULTs [5–9]. Apparent K_m and V_{max} values for T_3, T_4, rT_3, 3,3′-T_2, and 3,5-T_2 with SULT1C1 are presented in Table 1.

Thermal Stability and Inhibitor Effects

Further characterization of SULT1C1 was done with T_3 at a final concentration of 100 μM. Thermal stability is an important biochemical characteristic used for the definition of human SULTs [15, 16]. The 50% inactivation temperature of SULT1C1 was 38.3°. This activity was more thermolabile than SULT1E1 and SULT2A1 activities when assayed with T_3 [9].

DCNP and NaCl are known inhibitors of SULTs [4, 9, 19]. SULT1C1 activity in the presence of various concentrations of DCNP yielded an IC_{50} value of 65 μM. It was similar to the value obtained with SULT1E1 and less than the value with SULT2A1 [9]. The IC_{50} value for NaCl was 113.6 mM with SULT1C1, a value similar to those obtained with both SULT1E1 and SULT2A1 [9]. Because SULT1C1 and SULT1E1 belong to the same major phenol SULT family [3, 11], it is not surprising that the character-

istics of SULT1C1 are more similar to those of SULT1E1 than the hydroxysteroid sulfotransferase SULT2A1.

SULT1C1 Preferences for Iodothyronine

The relative ability of SULT1C1 to use other iodothyronines as substrates was estimated by both substrate specificity and enzyme activities (Table 1). Assays were done under the optimal conditions established with T_3 as the substrate. Kinetic data for several iodothyronines with SULT1C1 are presented. The optimal thyroid hormone concentrations identified in the kinetic studies were used to determine relative SULT1C1 activities toward the substrates when equivalent enzyme aliquots (15 μg protein) were analyzed simultaneously. The observed activities confirmed the substrate preferences determined from the kinetic data. SULT1C1 preferred 3,3′-T_2 and rT_3 more than T_3 and T_4, and was less active toward 3,5-T_2. The preferences were the same if estimated from a V_{max}/K_m ratio. This pattern was more similar to the iodothyronine preferences of SULT1E1 than to the preferences of SULT2A1 [9].

Identification of the Reaction Product

The ^{35}S-labeled reaction product of the SULT1C1 assay (97.5% of the eluted ^{35}S-radioactivity) coeluted with ^{125}I-labeled T_3S from a Sephadex LH-20 column. We have shown previously that this method provided data very similar to HPLC analysis [9]. The finding supported the conclusion that recombinant SULT1C1 catalyzed the sulfation of T_3.

CONCLUSIONS

We have demonstrated that recombinant human SULT1C1 catalyzes the sulfation of T_3 and four other iodothyronines. SULT1C1 behaves as a member of the phenol SULT family with regard to its substrate specificities. The sensitivities of SULT1C1 to inhibitors of SULTs were more similar to those of SULT1E1, a member of the phenol SULT family, than to SULT2A1, a member of the hydroxysteroid SULT family.

Although the role of SULT1C1 *in vivo* is unknown, SULT1C1 in the adult thyroid gland could participate in local metabolism of endogenous thyroid hormones in concert with known thyroid gland deiodinases to facilitate the degradation, and thus, the regulation of the available iodothyronines [20]. Based on the kinetic data, the primary role of intrathyroid SULT1C1 appears to be sulfation of T_2 and rT_3 to facilitate their deiodination for efficient iodide recycling and new hormone formation. A defect in the SULT1C1 system might lead to loss of iodide from the gland, decreased efficiency of hormone production, and goiter development. After thyroglobulin proteolysis and release of thyroid hormone within the thyrocyte, some intra-thyroidal T_4 to T_3 conversion does occur. Any excess intracellular thyroid hormone after release from thyroglobulin may be sulfoconjugated and further deiodinated to enhance iodide reutilization and to prevent increased effects within the thyroid. It is not known whether SULT1C1 activity is affected by thyroid status or thyrotropin as is the case for deiodinases. SULT1C1 preferentially sulfoconjugates 3,3′-T_2. There is evidence that 3,3′-T_2 acts through non-nuclear pathways to enhance mitochondrial respiration [21]. Whether sulfation of 3,3′-T_2 affects this non-nuclear action is not clear.

There are now at least six known human cytosolic SULTs that contribute to the sulfation of thyroid hormones. Our observations point out the necessity for further studies to detail the potential roles of SULT1C1 within the thyroid and in overall endogenous and exogenous thyroid hormone metabolism.

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