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Sulfation of Thyroid Hormone by Estrogen Sulfotransferase

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Abstract. Sulfation is one of the pathways by which thyroid hormone is inactivated. Iodothyronine sulfate concentrations are very high in human fetal blood and amniotic fluid, suggesting important production of these conjugates *in utero*. Human estrogen sulfotransferase (SULT1E1) is expressed among other tissues in the uterus. Here we demonstrate for the first time that SULT1E1 catalyzes the facile sulfation of the prohormone T_4, the active hormone T_3 and the metabolites rT_3 and 3,3′-diiodothyronine (3,3′-T_2) with preference for $rT_3 \approx$ 3,3′-$T_2 > T_3 \approx T_4$. Thus, a single enzyme is capable of sulfating two such different hormones as the female sex hormone and thyroid hormone. The potential role of SULT1E1 in fetal thyroid hormone metabolism needs to be considered.

Thyroid hormone is essential for the development of different tissues, in particular the brain, and requires the binding of the active hormone T_3 to nuclear receptors (1). Sulfation is one of the pathways by which T_3 and other iodothyronines, including the prohormone T_4, are metabolized (2). This is an inactivating pathway since T_3 sulfate (T_3S) has lost its affinity for the T_3 receptors (3). Moreover, sulfation of T_3 and its prohormone T_4 strongly facilitates their degradation through inner ring deiodination by the type I iodothyronine deiodinase in liver (2). Iodothyronine sulfate concentrations are very high in the human fetal circulation and in the amniotic fluid (4), suggesting important production of these conjugates *in utero*. Human estrogen sulfotransferase (SULT1E1) is known to be expressed, among others, in the endometrium (5,6). In this study we tested the possible sulfation of T_4, T_3 and the metabolites rT_3 and 3,3′-diiodothyronine (3,3′-T_2) by recombinant human SULT1E1 in comparison with the sulfation of estrone (E_1) and 17ß-estradiol (E_2).

Materials and Methods

Materials. [3′,5′-125I]T_4, [3′-125I]T_3, [3H]E_1 and [^{3}H]E_2 were obtained from Amersham (Amersham, UK); T_3, E_2 and 3′-phosphoadenosine-5′-phosphosulfate (PAPS) from Sigma (St. Louis, MO); T_4, rT_3 and 3,3′-T_2 from Henning Berlin GmbH (Berlin, Germany); and E_1 from Ikapharm (Ramat, Israel). 3,[3′-^{125}I]T_2 and [3′,5′-^{125}I]rT_3 were prepared as previously described (7). Human SULT1E1 was expressed in *S. typhimurium* as previously described (8) and used without further purification. Expression in *E. coli* and purification of human SULT1A1, SULT1A3 and SULT1E1 have also been described previously (9,10). Cloning, expression and purification of human SULT1B1 (11) will be described in detail elsewhere. Briefly, the clone was isolated from human liver cDNA by PCR, cloned into the vector pET11a and expressed in *E. coli*. Protein was purified as described (9,10).

Sulfotransferase assays. Iodothyronine sulfotransferase activities were analyzed by incubation of usually 0.1 μM T_4, T_3, rT_3 or 3,3′-T_2 and 10^5 cpm of the ^{126}I-labeled compound for 30 min at 37 C with the indicated amounts of recombinant sulfotransferase in the absence (blank) or presence of 50 μM PAPS in 0.2 ml 0.1 M phosphate (pH 7.2) and 2 mM EDTA. The reactions were stopped by addition of 0.8 ml 0.1 M HCl, and the mixtures were analyzed for sulfate formation as previously described (7). Estrogen sulfotransferase activity was analyzed by incubation of 1-3 nM ^{3}H-labeled E_1 or E_2 for 30 min at 37 C with the indicated amount of recombinant SULT1E1 in the absence (blank) or presence 50 μM PAPS in 0.2 ml phosphate-EDTA buffer. The reactions were stopped by addition of 2 ml ice-cold water, and the mixtures were extracted with 2 ml dichloromethane. Sulfate formation was quantified by counting 1 ml of the aqueous phase. Enzymatic sulfation was corrected for background radioactivity estimated in the blanks. Kinetic parameters were determined by Lineweaver-Burk analysis of the sulfation of varying substrate concentrations. Apparent K_i values were calculated from the change in slope of the Lineweaver-Burk plot in the presence of a fixed inhibitor concentration.

Results and Discussion

Figure 1 shows the sulfation of E_1, E_2, T_4, T_3, rT_3 and 3,3′-T_2 by recombinant human SULT1E1 as a function of the enzyme concentration. The results show that not only the estrogens but also the different iodothyronines are sulfated by human SULT1E1. Under the conditions used, sulfation of E_1 and E_2 requires the lowest enzyme concentrations. Substantially more enzyme is needed for sulfation of 3,3′-T_2 and rT_3, while sulfation of T_3 and T_4 requires the highest enzyme concentrations.

Significant sulfation of iodothyronines, in particular 3,3′-T_2, has been demonstrated previously in human liver and kidney as well as with recombinant human SULT1A1, SULT1A3 and SULT1B1 (11,12). Figure 2 compares the sulfation of the different iodothyronines by purified recombinant human SULT1A1 (13), SULT1A3 (14), SULT1B1 (11) and SULT1E1 (15). In agreement with previous studies, 3,3′-T_2 is by far the preferred substrate for SULT1A1, SULT1A3 and SULT1B1, its sulfation rates being orders of magnitude higher than those for T_3 and rT_3, whereas sulfation of T_4 is negligible. Although 3,3′-T_2 is a better substrate for SULT1A1 than for SULT1E1 and T_3 is sulfated at similar rates by the different isoenzymes, SULT1E1 is much more effective in catalyzing the sulfation of T_4 and, in particular, rT_3 than any other isoenzyme tested.

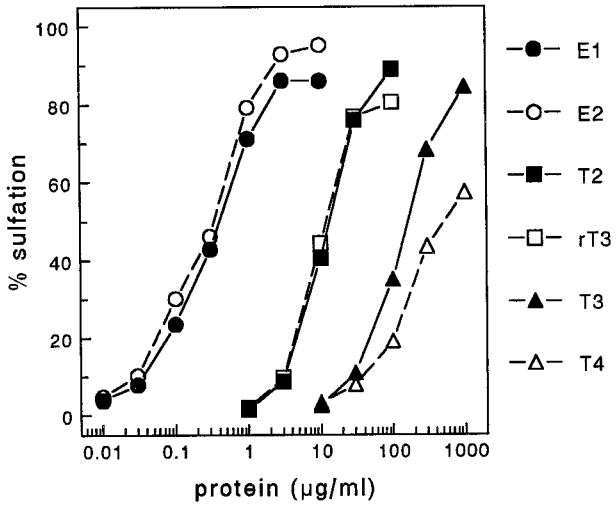

Fig. 1. Sulfation of estrogens and iodothyronines by increasing concentrations of human SULT1E1. Reaction conditions: 3 nM E_1 or E_2, 0.1 μM T_4, T_3, rT_3 or 3,3′-T_2, 50 μM PAPS, and 30 min incubation.

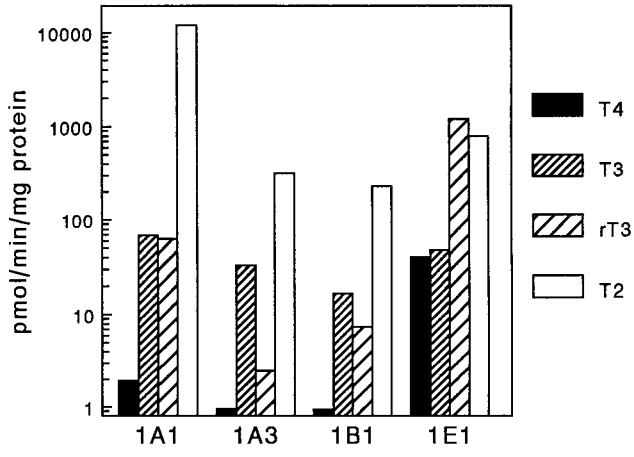

Fig. 2. Sulfation of iodothyronines by purified human sulfotransferases. Reaction conditions: 0.1 μM iodothyronines, appropriate concentrations of enzymes, 50 μM PAPS, and 30 min incubation.

Figure 3A shows the Lineweaver-Burk analysis of the sulfation of the iodothyronines by human SULT1E1, and the values for the kinetic parameters are presented in Table 1. The apparent K_m values for the different iodothyronines are in the micromolar range. They are 5-10 times lower while V_{max} values are 2-8 times higher for rT_3 and 3,3′-T_2 than for T_3 and T_4. Reflecting catalytic efficiency, the V_{max}/K_m ratio decreases in the order $rT_3 \approx$ 3,3′-$T_2 > T_3 \approx T_4$. Lineweaver-Burk analysis of the sulfation of E_2 by SULT1E1 yielded an apparent K_m value of 4 nM (Table 1) in close agreement with reported data (15,16). Similar K_m and V_{max} values were obtained using E_1 as substrate (not shown). Although V_{max} values are lower for E_1 and E_2 than for rT_3 and 3,3′-T_2, their $\approx 10^3$-fold lower apparent K_m values indicate that the estrogens have much higher affinity for SULT1E1 than the iodothyronines.

The different iodothyronines dose-dependently inhibited the sulfation of estrogens by human SULT1E1. The nature of this inhibition was studied by Lineweaver-Burk analysis (Fig. 3B). The results demonstrate that the iodothyronines are mixed-type inhibitors of E_2 sulfation. The apparent K_i values for the iodothyronines are in agreement with their apparent K_m values (Table 1). However, the apparent K_m value for T_4 is higher than its apparent K_i value, which may be due to significant protein binding of T_4 at the higher protein concentrations required for its sulfation than for E_2 sulfation. Conversely, E_1 and E_2 were found to be potent inhibitors of the sulfation of iodothyronines,

using 3,3′-T_2 as the substrate (not shown). That iodothyronines are not pure competitive inhibitors of the sulfation of estrogens by SULT1E1 may be explained by recent findings of two substrate-binding sites on human SULT1E1, the active site as well as an allosteric binding site (16).

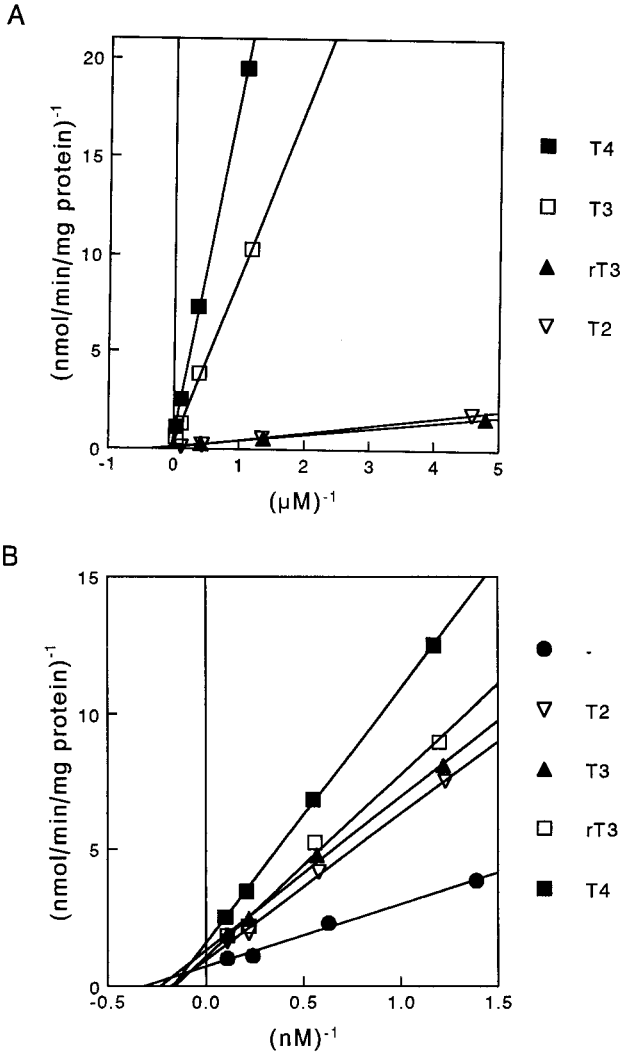

Fig. 3. Kinetics of human SULT1E1. A. Lineweaver-Burk analysis of the sulfation of iodothyronines. Reaction conditions: 0.3-30 μM iodothyronine, 10-100 μg protein/ml, 50 μM PAPS, and 30 min incubation. B. Lineweaver-Burk analysis of the inhibition of the sulfation of E_2 by iodothyronines. Reaction conditions: 1-10 nM E_2, 0.05 μg protein/ml, 50 μM PAPS, and 30 min incubation in the absence or presence of 5-10 μM T_4, 20 μM T_3, 2 μM rT_3, or 5 μM 3,3′-T_2.

Table 1. Kinetic parameters of iodothyronine and estrogen sulfation by human SULT1E1 [a]

Substrate/ competitor	K_i (μM)	K_m (μM)	V_{max} (nmol/min/mg)
E_2	-	0.003 - 0.006	1.1 - 2.8
3,3′-T_2	3.0 - 4.3	3.5 - 6.0	8.9 - 15.3
rT_3	0.6 - 0.9	1.7 - 2.6	4.5 - 8.0
T_3	12.3 - 18.8	15.3 - 36.1	2.2 - 4.4
T_4	2.3 - 2.4	22.6 - 24.6	1.4 - 1.4

[a]) Data are presented as the range of values from 2-3 experiments

These studies indicate that thyroid hormone is sulfated importantly by human SULT1E1. Although the estrogens E_1 and E_2 are clearly the preferred substrates for this isoenzyme, T_4 and especially rT_3 are sulfated much better by human SULT1E1 than by any other known sulfotransferase. Whereas human SULT1A1, SULT1A3 and SULT1B1 show an obvious preference for 3,3′-T_2 as the substrate, rT_3 is sulfated by human SULT1E1 as fast as 3,3′-T_2. The K_m values of the estrogens and iodothyronines for SULT1E1 appear unrelated to their concentrations *e.g.* in amniotic fluid (17). The preference of SULT1E1 for estrogens is reflected in their higher sulfated/free ratios in amniotic fluid compared with iodothyronines (4,17).

The purpose of the rapid sulfation of 3,3′-T_2 and rT_3 by human SULT1E1 is unknown. Both metabolites have little affinity for the nuclear T_3 receptors (1). However, 3,3′-T_2 has been shown to stimulate mitochondrial respiration in different tissues (18) and rT_3 may regulate actin polymerization in brain cells (19), actions which are not mediated by the nuclear T_3 receptors. The possibility that rT_3, 3,3′-T_2 or their sulfates serve a physiological function in the fetus is, therefore, not excluded. It is intriguing in this respect that rT_3 and 3,3′-T_2 are the products of T_4 and T_3 deiodination, respectively, by the type III iodothyronine deiodinase which is abundantly expressed in placenta (20) as well as the pregnant uterus (21).

It is astonishing that a single enzyme is capable of conjugating two such completely different hormones as the female sex hormone and thyroid hormone. E_2 is inactivated by sulfation, which is a reversible process as free E_2 is liberated by hydrolysis of the sulfate by steroid sulfatase expressed in different tissues (5,6). Similarly, in the human fetal circulation, T_4S and in particular T_3S may represent a reservoir of inactive thyroid hormone, from which active hormone may be liberated by action of sulfatases expressed in a tissue-

specific and developmental stage-dependent manner (2). Our results suggest that the iodothyronine sulfates in the human fetal circulation and amniotic fluid may be derived at least in part from sulfation of thyroid hormone by SULT1E1 in the uterus. This may represent another route for the supply of maternal thyroid hormone to the fetus in addition to placental transfer (22). There is one report suggesting that SULT1E1 is also expressed in human placenta (23). SULT1E1 expression in human endometrium is up-regulated by progesterone (5,6). Preliminary findings suggest low levels of SULT1E1 expression in the uterus during the first 13 weeks of pregnancy, but further studies are needed to explore SULT1E1 expression in human endometrium throughout gestation.

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