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Role of sulfation in thyroid hormone metabolism

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Abstract

The type I iodothyronine deiodinase (ID-I) in liver and kidney converts the prohormone thyroxine (T_4) by outer ring deiodination (ORD) to bioactive 3,3',5-triiodothyronine (T_3) or by inner ring deiodination (IRD) to inactive 3,3',5-triiodothyronine (rT_3), while it also catalyzes the IRD of T_3 and the ORD of rT_3 , with the latter as the preferred substrate. Sulfation of the phenolic hydroxyl group blocks the ORD of T_4 , while it strongly stimulates the IRD of both T_4 and T_3 , indicating that sulfation is an important step in the irreversible inactivation of thyroid hormone. This review summarizes recent studies concerning this interaction between sulfation and deiodination of iodothyronines, the characterization of iodothyronine sulfotransferase activities, the measurement of iodothyronine sulfates in humans and animals, and the possible physiological importance of iodothyronine sulfation.

Keywords: Thyroid hormone; Iodothyronines; Sulfation; Deiodination

Under normal conditions the prohormone thyroxine (3,3',5,5'-tetraiodothyronine, T_4) is the predominant product secreted by the thyroid gland. T_4 is converted in peripheral tissues by outer ring deiodination (ORD) to the bioactive form of the thyroid hormone 3,3',5-triiodothyronine (T_3) or by inner ring deiodination (IRD) to the inactive metabolite 3,3',5'-triiodothyronine (reverse T_3 , rT_3) (Fig. 1). Both T_3 and rT_3 are further deiodinated to inactive products, especially 3,3'-diiodothyronine (3,3'- T_2), which is generated by IRD of T_3 and by ORD of rT_3 (Fig. 1). Thus, the bioactivity of the thyroid hormone is controlled by competing ORD (activation) and IRD (inactivation) pathways [1,2].

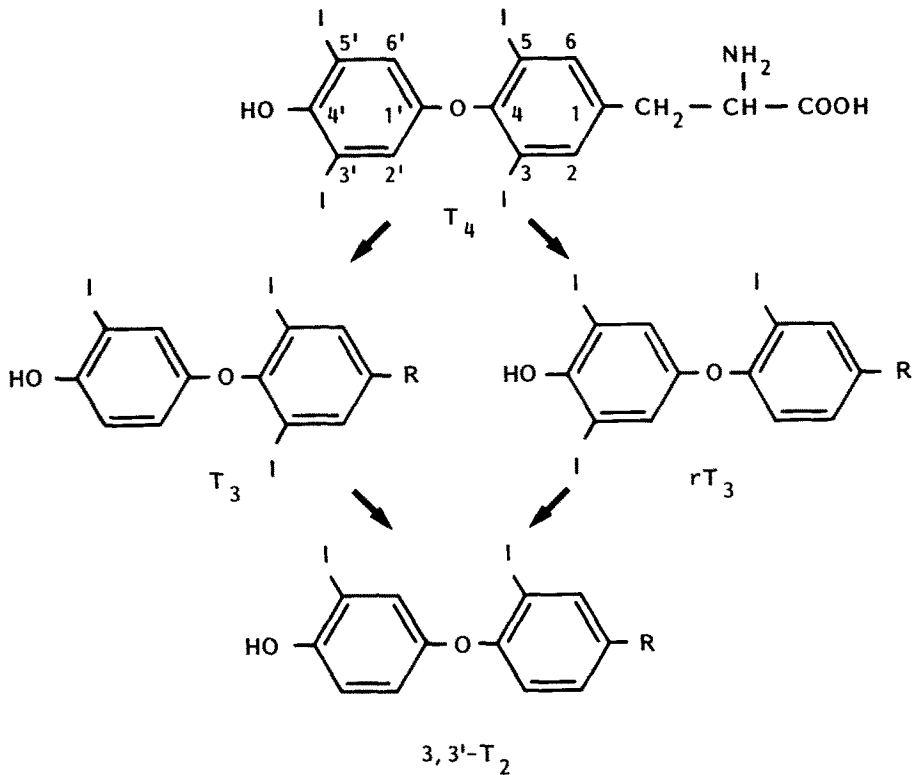


Fig. 1. Stepwise deiodination of T₄ by ORD to T₃ and by IRD to rT₃, and further by IRD of T₃ and ORD of rT₃ to 3,3'-T₂.

Besides deiodination, glucuronidation and sulfation of the phenolic hydroxyl group are other important pathways of iodothyronine metabolism [2,3]. The glucuronides are stable conjugates which are rapidly excreted in the bile [3]. However, the sulfates are rapidly deiodinated in the liver, and little of these conjugates is normally excreted intact in the bile or appears in the serum [3,4]. Sulfation strongly facilitates the IRD of T₄ and T₃, whereas the ORD of T₄ sulfate (T₄S) is inhibited, suggesting that sulfate conjugation is a primary step leading to the irreversible inactivation of thyroid hormone.

1. Sulfation of iodothyronines

Although sulfation of T₃ and other iodothyronines in rats has been known for a long time, little work has been done to characterize the sulfotransferases (STs) involved until recently (for a review, see [5]). Sekura et al. [6] have tested the sulfation

of a variety of iodothyronine derivatives by purified rat liver aryl sulfotransferase (AST) I and AST IV with an assay utilizing 100 μM [^{35}S]PAPS and 50 μM substrate. AST I and AST IV showed similar sulfation rates with T_3 but varying activities with other iodothyronines. The substrate preferences of these enzymes overlapped for some compounds (e.g. $3,3'\text{-T}_2 > \text{T}_3 > \text{rT}_3 > \text{T}_4$, with T_4 sulfation being undetectable in both cases), but differed markedly for others (e.g. 3-T_1 and $3'\text{-T}_1$). K_m and V_{max} values were not determined in this study [6].

With a standard assay utilizing 0.4 μM [^{35}S]PAPS and 150 μM T_3 , Young et al. [7] have demonstrated that the hormone is a substrate for at least three phenol sulfotransferases (PSTs) in human tissues: two forms of thermostable (TS) PST and a thermolabile (TL) PST. Of these, TS PST is especially abundant in the liver, while TL PST is more prevalent in the intestine. In keeping with the prominent role of TS PST, T_3 sulfation in human liver was inhibited by low concentrations of 2,6-dichloro-4-nitrophenol (DCNP; $\text{IC}_{50} \approx 5 \mu\text{M}$), a selective inhibitor of this isoenzyme [7]. Sulfate conjugation of T_3 was also inhibited by T_4 ($\text{IC}_{50} \approx 150 \mu\text{M}$), but sulfation of T_4 was not tested. Reported K_m values amounted to 100–200 μM for T_3 and 0.1–0.4 μM for PAPS with the different isolated isoenzymes as well as with crude human liver cytosol [7].

Gong et al. [8] have recently reported on the measurement of hepatic T_3 ST activity in different species with a method similar to that of Sekura et al. [6], employing 50 μM substrate and 200 μM [^{35}S]PAPS. A marked sex difference was found in rats, where T_3 ST activities were 2–5-fold higher in male than in female animals, which was related to the different growth hormone secretion patterns in males and females. The opposite was found in mice, in that T_3 sulfation rates were 5-fold higher in female than in male animals, while no sex-related difference was found in human liver T_3 ST activity [8]. In agreement with the results of Sekura et al. [6], using AST I and AST IV, iodothyronine sulfation rates in rat liver cytosol were in the order $\text{T}_3 > \text{rT}_3 > \text{T}_4$; sulfation of $3,3'\text{-T}_2$ was not tested [8]. The involvement of an enzyme homologous to human TS PST was suggested by the finding that T_3 sulfation in rat liver cytosol is inhibited by low concentrations of DCNP (IC_{50} 5.5 μM) and pentachlorophenol (IC_{50} 0.065 μM) [8].

Hurd et al. [9] reported recently, in abstract form, on T_3 ST activities in different rat tissues using unspecified concentrations of unlabeled PAPS and T_3 , measuring the production of T_3S by specific radioimmunoassay. T_3 sulfation rates were highest in liver followed by brain and kidney. Hepatic T_3 ST activity was relatively heat stable, showing a K_m value for T_3 of 114 μM .

In conclusion, sulfation of T_3 and other iodothyronine derivatives is catalyzed by multiple PST isoenzymes in liver and other tissues in various species. Both in human and rat liver, T_3 ST activity is associated largely with thermostable forms of PST which are sensitive to inhibition by DCNP. The higher rate of T_3 sulfation in male vs. female rats suggests that AST IV is a major T_3 ST in rat liver. However, the contribution of different isoenzymes to the sulfation of T_3 and analogs both in humans and in rats remains to be established. It is remarkable that T_4 is a poor substrate for all ST activities studied so far.

2. Deiodination of iodothyronine sulfates

Three thyroid hormone-deiodinating enzymes have been identified, of which the type I iodothyronine deiodinase (ID-I) is present in liver, kidney and thyroid [1,2]. It is a transmembrane selenoenzyme located in the endoplasmic reticulum of liver cells, which requires thiols such as dithiothreitol (DTT) as the cofactor, and has both ORD and IRD activity [1,2,10]. Although ID-I shows preference for rT_3 as the substrate, its most important physiological purpose is the peripheral production of T_3 from T_4 . The type II iodothyronine deiodinase (ID-II) is present predominantly in the brain, pituitary and brown adipose tissue [1,2]. ID-II has only ORD activity, and is an important enzyme for local intracellular T_3 production in these tissues. The type III iodothyronine deiodinase (ID-III) is also found in brain but also in skin and in the placenta [1,2]. It has only IRD activity and seems a major site for the degradation of T_4 and T_3 .

Although T_4 itself may be deiodinated by ID-I either in the outer ring to T_3 or in the inner ring to rT_3 , this is changed dramatically after sulfate conjugation [11]. The IRD of T_4S is accelerated about 200 times due to a decrease in apparent K_m value as well as an increase in V_{max} (Table 1). However, ORD of T_4S is undetectable, which excludes that T_4 is converted to T_3 by an alternative route via their sulfoconjugates.

ID-I deiodinates primarily the inner ring of T_3 , a reaction which is also markedly stimulated by sulfoconjugation [12]. The facilitated IRD of T_3S is characterized by a ~30-fold increase in V_{max} with little effect on the apparent K_m value (Table 1). We have observed similar effects of sulfation on the deiodination of T_3 by the type I deiodinase in human liver [13].

As mentioned above, rT_3 is the preferred ID-I substrate; its ORD to 3,3'- T_2 is catalyzed >100-fold more effectively than that of any other known non-sulfated

Table 1
Effects of sulfation on deiodination of iodothyronines by rat liver type I deiodinase

Substrate	Deiodination	Product	K_m	V_{max}	V_{max}/K_m
T_4	ORD	T_3	2.3	30	13
T_4S	ORD	T_3S		undetectable	
T_4	IRD	rT_3	1.9	18	9
T_4S	IRD	rT_3S	0.3	527	1817
T_3	IRD	3,3'- T_2	6.2	36	6
T_3S	IRD	3,3'- T_2S	4.6	1050	230
rT_3	ORD	3,3'- T_2	0.06	559	8730
rT_3S	ORD	3,3'- T_2S	0.06	516	8600
3,3'- T_2	ORD	3- T_1	8.9	188	21
3,3'- T_2S	ORD	3- T_1S	0.3	353	1040

Kinetic parameters were determined using liver microsomes from euthyroid rats in 0.1 M phosphate (pH 7.2), 2 mM EDTA and 3–5 mM DTT. K_m is expressed in μM and V_{max} in pmol/min per mg protein. Data are derived from Refs. [11–15].

iodothyronine [14]. It is remarkable that in contrast to the ORD of T_4 , which is blocked by sulfation, deiodination of rT_3 is not affected [11], while ORD of $3,3'$ - T_2 is even markedly stimulated by sulfate conjugation [15]. In the latter case this is associated with a major decrease in the apparent K_m value and a minor increase in V_{max} (Table 1).

In conclusion, the effect of sulfation of iodothyronines on their deiodination by ID-I strongly depends on the structure of the substrate. Sulfation markedly facilitates the IRD of T_4 and T_3 , while ORD is either inhibited (T_4), unaffected (rT_3) or clearly stimulated ($3,3'$ - T_2) by sulfoconjugation. The mechanism by which sulfation stimulates the type I deiodination of the various substrates remains unclear. In some cases sulfation primarily effects an increase in V_{max} , in others there is a predominant decrease in the apparent K_m value. Since rat and human ID-I are basic proteins [16,17], the facilitated deiodination of sulfated iodothyronines may be due to beneficial ionic interaction of the negatively charged sulfate group with protonated residues in the enzyme active center [4]. The effect of conjugation on iodothyronine deiodination depends both on the site and the nature of the conjugating group. Type I deiodination of different iodothyronines is moderately stimulated by sulfonation of the α NH2 group of these compounds, yielding non-naturally occurring sulfamates, but this effect is much less pronounced than that of sulfation [18]. We have also found that ID-I does not catalyze the deiodination of glucuronidated iodothyronines [19]. Finally, the effects of sulfation appears specific for ID-I, since deiodination of sulfated substrates is not observed with either ID-II (unpublished work) or ID-III [20,21].

3. Production of iodothyronine sulfates in hepatocytes and in vivo

3.1. T_3 S

Studies with isolated rat hepatocytes have indicated that T_3 is metabolized to roughly similar extents by three pathways [19]: (a) glucuronidation ($T_3 \rightarrow T_3G$); (b) IRD, rapidly followed by successive sulfation and ORD ($T_3 \rightarrow 3,3'$ - $T_2 \rightarrow 3,3'$ - $T_2S \rightarrow I^-$); and (c) sulfation, rapidly followed by successive IRD and ORD ($T_3 \rightarrow T_3S \rightarrow 3,3'$ - $T_2S \rightarrow I^-$). Under normal conditions, therefore, T_3G and iodide are the main labeled products produced from [$3'$ - ^{125}I] T_3 . If the sulfotransferase capacity of the cells is inhibited by SO_4^{2-} depletion or by addition of ST inhibitors, I^- production is largely inhibited, and $3,3'$ - T_2 (and $3,3'$ - T_2G) accumulates. If on the other hand ID-I activity is inhibited with 6-propyl-2-thiouracil (PTU) or with iopanoic acid (IOP), I^- production is also decreased, but this is now accompanied by the accumulation of T_3S and $3,3'$ - T_2S [15,19,22].

After an intravenous (i.v.) injection of [^{125}I] T_3 to bile duct-cannulated rats, we found that [^{125}I] T_3G was the predominant radioactive product excreted in the bile [23]. Pretreatment of rats with PTU was found to have little effect on the biliary excretion of T_3G , but excretion of T_3S and $3,3'$ - T_2S was strongly increased [23]. While treatment of rats with DCNP had little effect on the biliary excretion of T_3 metabolites compared with untreated rats, DCNP greatly diminished the PTU-induced excretion of T_3S and $3,3'$ - T_2S [23]. This is in agreement with the above-

mentioned *in vitro* findings, suggesting an important contribution of thermostable, DCNP-sensitive PSTs to hepatic T₃ sulfation.

T₃S is not only excreted in bile but, depending on the conditions, it may also be detected in blood. After *i.v.* administration of [¹²⁵I]T₃ to untreated rats, iodide is the predominant radioactive metabolite detected in plasma. However, if labeled T₃ is injected in PTU-treated rats, the appearance of iodide in plasma is strongly decreased, while T₃S and 3,3'-T₂S accumulate to levels that readily exceed serum T₃ levels [24]. However, PTU does not decrease plasma T₃ clearance. Following an *i.v.* injection of [¹²⁵I]T₃S to control rats, the conjugate is cleared from plasma more rapidly than T₃. Iodide is again the main metabolite observed in plasma, and 20% of the dose is excreted as intact T₃S in the bile [24]. Plasma T₃S clearance is strongly inhibited by PTU, which is associated with a marked decrease in plasma iodide appearance as well as with an increase in the biliary excretion of intact T₃S to 80% of the dose [24]. Therefore, the PTU-induced increase in plasma levels and biliary excretion of T₃S after T₃ injection is explained by the inhibition of T₃S deiodination rather than an increase in T₃ sulfation.

We have developed a radioimmunoassay (RIA) for the measurement of T₃S in serum [25]. Although the antiserum employed in this RIA showed high affinity and relatively high specificity for T₃S, its significant cross-reactivity with T₃ and T₄ in combination with the high serum T₄ levels necessitated the isolation of the serum iodothyronine conjugate fraction prior to the assay. In agreement with the above-mentioned findings, RIA of serum T₃S showed low but detectable levels in normal rats, with a mean of 0.09 nM vs. 1.3 nM for T₃ [26]. Serum T₃S markedly increased after treatment with PTU but not with methimazole, a thyrostatic drug that does not inhibit ID-I [26]. Results similar to those with PTU were obtained in animals treated with the X-ray contrast agent IOP that also inhibits ID-I [22]. The increase in serum T₃S by PTU or IOP occurred even though serum T₃ was decreased, and was associated with a similar increase in serum rT₃ [22,26].

Analysis of human serum with this RIA demonstrated that T₃S levels in normal subjects were mostly undetectable, *i.e.* <0.1 nM vs. 2 nM for T₃ [27]. Serum T₃S became barely detectable in healthy persons treated with T₃ (1 μg/kg body wt./day), and showed an additional slight increase after administration of PTU, while serum T₃S levels increased markedly after administration of IOP [27]. The increases in serum T₃S induced by these regimens were strongly correlated with the increases in serum rT₃, which is also largely cleared by type I deiodination, as serum rT₃ also showed a much larger increase after IOP than after PTU treatment [27]. Data reported by LoPresti *et al.* [28] indicate that in normal humans T₃S is cleared extremely rapidly from the circulation, and that this clearance is inhibited moderately by PTU administration but markedly by IOP administration as well as by fasting, another condition that may be associated with a decrease in hepatic ID-I activity [28].

The groups of Wu and Chopra have recently developed improved RIAs for T₃S, the much greater specificities of which obviate the need to prepurify the samples, so that they can be applied directly to ethanol extracts of serum [29]. Using this RIA, Chopra *et al.* [29] also noted that serum T₃S levels are low in normal subjects (<0.1

nM), and that they increase significantly after treatment of patients with IOP [29]. They also found that the serum T_3S/T_3 ratio is decreased in hyperthyroid patients and strongly increased in hypothyroid patients, in patients with non-thyroidal illness and in newborns, as determined in fetal cord serum [29]. Hepatic ID-I activity is known to be impaired in hypothyroidism, non-thyroidal illness, and in the fetal stage, while enzyme activity is increased in hyperthyroidism [1,2]. Furthermore, extremely high T_3S levels have been detected by Wu et al. [30] in plasma, bile and meconium of fetal sheep (and in the allantoic fluid) at the time hepatic ID-I activity is still very low. Therefore, these recent data demonstrate that also in humans and sheep, T_3S levels accumulate to high levels, if deiodination of the conjugate by the type I deiodinase is inhibited.

3.2. T_4S

Although *in vitro* tests of T_4 as the substrate for tissue ST activities have been largely negative, evidence has been reported that T_4 undergoes significant sulfation in liver. Sato et al. [31] noted that T_4 was metabolized in primary cultures of rat hepatocytes by deiodination and by conjugation. Under normal conditions, ORD and IRD were the predominant pathways of T_4 metabolism. However, if deiodination was saturated at high substrate concentrations, conjugates were found to accumulate, including T_4S [31]. We have investigated the biliary excretion products of T_4 after an *i.v.* injection of [^{125}I] T_4 to rats [32]. In control rats, T_4G was the predominant radioactive compound in bile together with smaller amounts of T_3G , rT_3G and T_4S . Pretreatment with PTU resulted in an increase in biliary T_4G parallel with the increased plasma T_4 retention, a marked decrease in T_3G due to inhibited T_4 to T_3 conversion, a marked increase in rT_3G due to inhibited degradation of rT_3 , and a large increase in T_4S excretion [32]. Despite the negligible tissue T_4 ST activities detected *in vitro*, these findings demonstrate that T_4 undergoes significant sulfation *in vivo*, and that T_4S is predominantly cleared by type I deiodination.

Although T_4S is a significant biliary T_4 metabolite in rats, we have not been able to detect this conjugate in serum after an *i.v.* injection of [^{125}I] T_4 even in PTU-treated rats [32]. Using very sensitive and specific RIAs for T_4S , Wu et al. [33] and Chopra et al. [34] have recently reported on the measurement of low concentrations of T_4S in human serum. However, the mean T_4S levels in normal human subjects differed markedly between the two studies, *i.e.* 100 and 19 pM, respectively (*vs.* \approx 100 nM for T_4). Compared with serum from normal adults, increased T_4S levels were observed in human amniotic fluid and in fetal cord serum obtained at the time of delivery [33,34]. Both groups also reported on an increase in serum T_4S after administration of IOP to hyperthyroid patients [33,34]. Remarkably high levels of T_4S were also detected in serum, bile and meconium of fetal sheep and in the allantoic fluid [30]. Together, these findings indicate that sulfation is a significant pathway for metabolism of T_4 in humans, rats and sheep, and that the concentration of this conjugate in body fluids builds up in conditions with low ID-I activity.

3.3. rT_3S

We have found minor amounts of rT_3S (and 3,3'- T_2S) after incubation of rT_3

with isolated rat hepatocytes, and only if this is done in the presence of PTU [35]. Normally, type I ORD is the foremost pathway of rT_3 metabolism in these cells. Although this converts rT_3 to 3,3'- T_2 , little of the latter is normally observed, because it is rapidly further metabolized by successive sulfation and deiodination, as described above for hepatic T_3 metabolism [35].

Recently, Wu et al. [36] have also reported on the measurement of rT_3S in human serum with a newly developed RIA for this sulfoconjugate. rT_3S concentrations were low (in normal human subjects, i.e. mean 40 pM vs. 0.3 nM for rT_3 , and no significant changes were noted in hypothyroid, hyperthyroid or pregnant subjects [36]. However, marked elevations were produced after administration of IOP to hyperthyroid patients [36]. Dramatic increases of rT_3S were observed in fetal cord serum relative to serum rT_3S in adults [36]. As mentioned above for T_4S and T_3S , high rT_3S levels were also detected in serum, bile and meconium of fetal sheep and in the allantoic fluid [37]. Therefore, rT_3 appears to be sulfated to some extent in humans and sheep, although this may be significant only if ID-I, the principal site of rT_3 metabolism, is inhibited.

4. Possible role of iodothyronine sulfation

Sulfation is a way to inactivate thyroid hormone: T_3S does not bind to the T_3 receptor and is devoid of thyromimetic activity in several cell systems [38,39]. Furthermore, not only T_3S but also T_4S is rapidly degraded by ID-I. It has been postulated that sulfation of iodothyronines has an important function when ID-I activity is impaired, such as in non-thyroidal illness and during fetal development [20,21]. Under these conditions, T_3S is not degraded in tissues with normally high ID-I activity, while the hormone is also protected by sulfation against degradation by ID-III [20,21]. Active T_3 may then be recovered from T_3S through the action of sulfatases in tissues where hormone action is required or by bacterial sulfatases in the intestine. We have indeed demonstrated that bacterial sulfatases are important for the resorption of T_3 following biliary excretion of T_3S [40–42]. Furthermore, significant hydrolysis of T_3S has been detected in liver and brain microsomes in humans and rats [43]. Santini et al. [44] have recently observed thyromimetic effects after administration of T_3S to hypothyroid rats, with a potency of $\approx 20\%$ of that of T_3 , which appeared to be mediated by the liberation of T_3 from the injected T_3S . An interesting aspect of these findings is that the biological effects of administered T_3S in hypothyroid subjects are self-limited, since the initially impaired ID-I activity in hypothyroidism is restored during T_3S treatment, as is the deiodinative clearance of the conjugate [44]. However, the exact biological function of the different iodothyronine sulfates in particular during fetal development remains to be fully explored.

4. Conclusions

Different ST isoenzymes appear to be involved in humans and in rats with the sulfation of T_3 and other iodothyronines. Under normal conditions, sulfation ac-

celerates the deiodination of the inner ring of T₄ and T₃ by the type I deiodinase in liver and probably also in kidney, which results in the irreversible inactivation of the hormone. However, under conditions of low ID-I activity, significant amounts of T₃ may be recovered from T₃S by sulfatases expressed in different tissues as well as by bacteria in the intestine. The former may be important for the generation of local T₃ in tissues where active hormone is required, while the bacterial sulfatases are essential in the supply of systemic T₃. Much further work remains to be done, however, to explore the potential role of T₃S as a reservoir from which active T₃ is released when and where the active thyroid hormone is required.

5. Acknowledgement

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