Role of sulfation in thyroid hormone metabolism

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Abstract

The type 1 iodothyronine deiodinase (ID-1) in liver and kidney converts the prohormone thyroxine (T4) by outer ring deiodination (ORD) to bioactive 3,3′,5-triiodothyronine (T3) or by inner ring deiodination (IRD) to inactive 3,3′,5′-triiodothyronine (rT3), while it also catalyzes the IRD of T3 and the ORD of rT3, with the latter as the preferred substrate. Sulfation of the phenolic hydroxyl group blocks the ORD of T4, while it strongly stimulates the IRD of both T4 and T3, 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, T4) is the predominant product secreted by the thyroid gland. T4 is converted in peripheral tissues by outer ring deiodination (ORD) to the bioactive form of the thyroid hormone 3,3′,5-triiodothyronine (T3) or by inner ring deiodination (IRD) to the inactive metabolite 3,3′,5′-triiodothyronine (reverse T3, rT3) (Fig. 1). Both T3 and rT3 are further deiodinated to inactive products, especially 3,3′-diiodothyronine (3,3′-T2), which is generated by IRD of T3 and by ORD of rT3 (Fig. 1). Thus, the bioactivity of the thyroid hormone is controlled by competing ORD (activation) and IRD (inactivation) pathways [1,2].
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 [35S]PAPS and 50 μM substrate. AST I and AST IV showed similar sulfation rates with T3 but varying activities with other iodothyronines. The substrate preferences of these enzymes overlapped for some compounds (e.g. 3,3′-T2 > T3 > rT3 > T4, with T4 sulfation being undetectable in both cases), but differed markedly for others (e.g. 3-T1 and 3′-T1). K_m and V_max values were not determined in this study [6].

With a standard assay utilizing 0.4 μM [35S]PAPS and 150 μM T3, 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, T3 sulfation in human liver was inhibited by low concentrations of 2,6-dichloro-4-nitrophenol (DCNP; IC50 ≈ 5 μM), a selective inhibitor of this isoenzyme [7]. Sulfate conjugation of T3 was also inhibited by T4 (IC50 ≈ 150 μM), but sulfation of T4 was not tested. Reported K_m values amounted to 100–200 μM for T3 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 T3 ST activity in different species with a method similar to that of Sekura et al. [6], employing 50 μM substrate and 200 μM [35S]PAPS. A marked sex difference was found in rats, where T3 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 T3 sulfation rates were 5-fold higher in female than in male animals, while no sex-related difference was found in human liver T3 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 T3 > rT3 > T4; sulfation of 3,3′-T2 was not tested [8]. The involvement of an enzyme homologous to human TS PST was suggested by the finding that T3 sulfation in rat liver cytosol is inhibited by low concentrations of DCNP (IC50 5.5 μM) and pentachlorophenol (IC50 0.065 μM) [8].

Hurd et al. [9] reported recently, in abstract form, on T3 ST activities in different rat tissues using unspecified concentrations of unlabeled PAPS and T3, measuring the production of T3S by specific radioimmunoassay. T3 sulfation rates were highest in liver followed by brain and kidney. Hepatic T3 ST activity was relatively heat stable, showing a K_m value for T3 of 114 μM.

In conclusion, sulfation of T3 and other iodothyronine derivatives is catalyzed by multiple PST isoenzymes in liver and other tissues in various species. Both in human and rat liver, T3 ST activity is associated largely with thermostable forms of PST which are sensitive to inhibition by DCNP. The higher rate of T3 sulfation in male vs. female rats suggests that AST IV is a major T3 ST in rat liver. However, the contribution of different isoenzymes to the sulfation of T3 and analogs both in humans and in rats remains to be established. It is remarkable that T4 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 rT3 as the substrate, its most important physiological purpose is the peripheral production of T3 from T4. 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 T3 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 T4 and T3.

Although T4 itself may be deiodinated by ID-I either in the outer ring to T3 or in the inner ring to rT3, this is changed dramatically after sulfate conjugation [11]. The IRD of T4S 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 T4S is undetectable, which excludes that T4 is converted to T3 by an alternative route via their sulfoconjugates.

ID-I deiodinates primarily the inner ring of T3, a reaction which is also markedly stimulated by sulfoconjugation [12]. The facilitated IRD of T3S 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 T3 by the type I deiodinase in human liver [13].

As mentioned above, rT3 is the preferred ID-I substrate; its ORD to 3,3'-T2 is catalyzed >100-fold more effectively than that of any other known non-sulfated

<table>
<thead>
<tr>
<th>Substrate</th>
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<th>V_max</th>
<th>V_max/K_m</th>
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<tr>
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</tr>
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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 T₄, which is blocked by sulfation, deiodination of rT₃ is not affected [11], while ORD of 3,3′-T₂ 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 T₄ and T₃, while ORD is either inhibited (T₄), unaffected (rT₃) or clearly stimulated (3,3′-T₂) 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 αNH₂ 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. T₃S

Studies with isolated rat hepatocytes have indicated that T₃ is metabolized to roughly similar extents by three pathways [19]: (a) glucuronidation (T₃ → T₃G); (b) IRD, rapidly followed by successive sulfation and ORD (T₃ → 3,3′-T₂ → 3,3′-T₂S → I⁻); and (c) sulfation, rapidly followed by successive IRD and ORD (T₃ → T₃S 3,3′-T₂S → I⁻). Under normal conditions, therefore, T₃G and iodide are the main labeled products produced from [3′-¹²⁵I]T₃. If the sulfotransferase capacity of the cells is inhibited by SO₄²⁻ depletion or by addition of ST inhibitors, I⁻ production is largely inhibited, and 3,3′-T₂ (and 3,3′-T₂G) 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 T₃S and 3,3′-T₂S [15,19,22].

After an intravenous (i.v.) injection of [¹²⁵I]T₃ to bile duct-cannulated rats, we found that [¹²⁵I]T₃G 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 T₃G, but excretion of T₃S and 3,3′-T₂S was strongly increased [23]. While treatment of rats with DCNP had little effect on the biliary excretion of T₃ metabolites compared with untreated rats, DCNP greatly diminished the PTU-induced excretion of T₃S and 3,3′-T₂S [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 T₃S/T₃ 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 T₃S 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, T₃S levels accumulate to high levels, if deiodination of the conjugate by the type I deiodinase is inhibited.

3.2. T₄S

Although in vitro tests of T₄ as the substrate for tissue ST activities have been largely negative, evidence has been reported that T₄ undergoes significant sulfation in liver. Sato et al. [31] noted that T₄ was metabolized in primary cultures of rat hepatocytes by deiodination and by conjugation. Under normal conditions, ORD and IRD were the predominant pathways of T₄ metabolism. However, if deiodination was saturated at high substrate concentrations, conjugates were found to accumulate, including T₄S [31]. We have investigated the biliary excretion products of T₄ after an i.v. injection of [¹²⁵I]T₄ to rats [32]. In control rats, T₄G was the predominant radioactive compound in bile together with smaller amounts of T₃G, rT₃G and T₄S. Pretreatment with PTU resulted in an increase in biliary T₄G parallel with the increased plasma T₄ retention, a marked decrease in T₃G due to inhibited T₄ to T₃ conversion, a marked increase in rT₃G due to inhibited degradation of rT₃, and a large increase in T₄S excretion [32]. Despite the negligible tissue T₄ ST activities detected in vitro, these findings demonstrate that T₄ undergoes significant sulfation in vivo, and that T₄S is predominantly cleared by type I deiodination.

Although T₄S is a significant biliary T₄ metabolite in rats, we have not been able to detect this conjugate in serum after an i.v. injection of [¹²⁵I]T₄ even in PTU-treated rats [32]. Using very sensitive and specific RIAs for T₄S, Wu et al. [33] and Chopra et al. [34] have recently reported on the measurement of low concentrations of T₄S in human serum. However, the mean T₄S levels in normal human subjects differed markedly between the two studies, i.e. 100 and 19 pM, respectively (vs. ≈100 nM for T₄). Compared with serum from normal adults, increased T₄S 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 T₄S after administration of IOP to hyperthyroid patients [33,34]. Remarkably high levels of T₄S were also detected in serum, bile and meconium of fetal sheep and in the allantoic fluid [30]. Together, these finding indicate that sulfation is a significant pathway for metabolism of T₄ 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. rT₃S

We have found minor amounts of rT₃S (and 3,3'-T₃S) after incubation of rT₃
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 rT₃ metabolism in these cells.
Although this converts rT₃ to 3,3'-T₂, little of the latter is normally observed,
because it is rapidly further metabolized by successive sulfation and deiodination,
as described above for hepatic T₃ metabolism [35].

Recently, Wu et al. [36] have also reported on the measurement of rT₃S in
human serum with a newly developed RIA for this sulfoconjugate. rT₃S concentrations
were low (in normal human subjects, i.e. mean 40 pM vs. 0.3 nM for rT₃, 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 rT₃S were observed in fetal cord
serum relative to serum rT₃S in adults [36]. As mentioned above for T₄S and T₃S,
high rT₃S levels were also detected in serum, bile and meconium of fetal sheep and
in the allantoic fluid [37]. Therefore, rT₃ appears to be sulfated to some extent in
humans and sheep, although this may be significant only if ID-I, the principal site
of rT₃ metabolism, is inhibited.

4. Possible role of iodothyronine sulfation

Sulfation is a way to inactivate thyroid hormone: T₃S does not bind to the T₃
receptor and is devoid of thyromimetic activity in several cell systems [38,39]. Fur-
thermore, not only T₃S but also T₄S is rapidly degraded by ID-I. It has been
postulated that sulfation of iodothyronines has an important function when ID-I ac-
tivity is impaired, such as in non-thyroidal illness and during fetal development
[20,21]. Under these conditions, T₃S 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 T₃ may then be recovered from T₃S 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 T₃ following biliary excretion of T₃S [40–42]. Furthermore,
significant hydrolysis of T₃S has been detected in liver and brain microsomes in hu-
mans and rats [43]. Santini et al. [44] have recently observed thyromimetic effects
after administration of T₃S to hypothyroid rats, with a potency of ≈ 20% of that of
T₃, which appeared to be mediated by the liberation of T₃ from the injected T₃S.
An interesting aspect of these findings is that the biological effects of administered
T₃S in hypothyroid subjects are self-limited, since the initially impaired ID-I activ-
ity in hypothyroidism is restored during T₃S treatment, as is the deiodinative
clearance of the conjugate [44]. However, the exact biological function of the dif-
f erent 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 T₃ 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.

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6. References


