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Activation and inactivation of thyroid hormone by type I iodothyronine deiodinase

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Abstract

The prohormone thyroxine (T4) is activated by outer ring deiodination (ORD) to 3,3′,5-triiodothyronine (T3) and both hormones are degraded by inner ring deiodination (IRD) to 3,3′,5′-triiodothyronine (rT3) and 3,3′-diiodothyronine, respectively. Indirect evidence suggests that the type I iodothyronine deiodinase (ID-I) in liver has both ORD and IRD activities, with preference for rT3 and sulfated iodothyronines as substrates. To establish this, we have compared the ORD of rT3 and IRD of T3 and T3 sulfate by homogenates of cells transfected with rat ID-I cDNA and by rat liver microsomes. In both preparations rT3 is the preferred substrate, while deiodination of T3 is markedly accelerated by its sulfation. Kinetic analysis provided similar K_m and V_{max} values in cell homogenates and liver microsomes. These data demonstrate unequivocally that ID-I is capable of both activating and inactivating thyroid hormone by ORD and IRD, respectively.

Key words: Thyroid hormone; Iodothyronine; Sulfate; Deiodination; Type I iodothyronine deiodinase; Rat liver; Transfection; Human embryonic kidney (HEK) 293 cell

1. Introduction

In humans, as well as in rats, the major product secreted by the thyroid follicular cells is the prohormone thyroxine (T4), which is activated in peripheral tissues by outer ring deiodination (ORD) to 3,3′,5-triiodothyronine (T3) or inactivated by inner ring deiodination (IRD) to 3,3′,5′-triiodothyronine (rT3) [1–3]. T3 is also degraded by IRD to 3,3′-diiodothyronine (3,3′-T2), a metabolite which is also generated by ORD of rT3 [1–3]. The type II deiodinase in brain, pituitary and brown adipose tissue specifically catalyzes ORD of iodothyronines, while the type III deiodinase in brain, placenta, skin and several fetal tissues has only IRD activity [1–3]. However, circumstantial evidence suggests that the type I iodothyronine deiodinase (ID-I) is capable of both ORD and IRD [1]: (i) both activities have been localized in the microsomal fraction and show the same enrichment during > 10^3-fold enzyme purification [4,5]; (ii) they are affected in identical ways by a variety of substrate analogs and inhibitors [6,7]; (iii) they show identical kinetic mechanisms with dithiothreitol (DTT) as cofactor and propylthiouracil (PTU) as inhibitor [7]; and (iv) both activities are affected dramatically by sulfation of the substrate [8]. Thus, ID-I catalyzes the IRD of T4 sulfate and T3 sulfate (T3S) as well as the ORD of 3,3′-T2 sulfate much better than the reactions with the non-sulfated substrates [8]. Although ID-I is thought to be very important for peripheral T3 production by ORD of T4, the enzyme is by far more effective in the ORD of the inactive metabolite rT3 [1–3].

Recently, the cDNAs of human and rat ID-I have been cloned and sequenced, showing that they represent homologous proteins with molecular weights of ≈ 29 kDa; both contain a selenocysteine (Sec) residue as the catalytic center [9,10]. Deiodinase activity expressed by transfection of cell lines with the rat ID-I cDNA is very similar to ID-I activity in rat liver microsomes, including the substrate preference for rT3 over T4, the potent inhibition by PTU and gold compounds, and the ready labeling of a ≈ 29 kDa enzyme protein with *N*-bromoacetyl-[^{125}I]T3 [9–14]. In this study we have analyzed the ORD of rT3 and IRD of T3 and T3S by homogenates of cells transfected with rat ID-I cDNA in comparison with rat liver microsomes. The results demonstrate that, indeed, ID-I is capable of both activation and inactivation of thyroid hormone.

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2. Materials and methods

2.1. Materials

[3′,5′-^{125}I]rT3 (≈ 1200 μCi/μg) was obtained from Amersham (Amersham, UK); [3,5-^{125}I]T3 (≈ 50 μCi/μg) and unlabeled T3, rT3 and T3S were supplied by MMDRI, Henning Berlin R&D (Berlin, Germany); [3,5-^{125}I]T3S was produced by sulfation of [3,5-^{125}I]T3 as described before [15]; DTT was obtained from Sigma (St. Louis, MO, USA); and Sephadex LH-20 from Pharmacia (Woerden, The Netherlands). [^{125}I]T3 and [^{125}I]T3S could be used without further purification, but [^{125}I]rT3 was purified on Sephadex LH-20 before each assay [16]. Male Sprague–Dawley rat liver microsomes were obtained as previously described [7].

2.2. Expression of rat ID-I

Vectors pUHD10–3 (D10) and pUHD15–1 (D15) were obtained from Dr. Manfred Gossen [17,18]. The full length rat ID-I cDNA, G21 [9], was cloned into D10 between the *Eco*RI and *Xba*I sites. The resulting deiodinase expression vector, G21-D10, was cotransfected with the transactivation plasmid, D15, into human embryonic kidney (HEK) 293 cells by CaPO4-DNA precipitation as described previously [9]. Controls were transfected with D10 vector plus D15 transactivator. Cultures were maintained in DMEM + 10% fetal calf serum in the absence of tetracycline. Cells were harvested 48 h after transfection, sonicated in 0.25 M sucrose, 100 mM sodium phosphate (pH 6.9), 1 mM EDTA and 10 mM DTT, and snap-frozen in liquid nitrogen.

2.3. Deiodinase assays

The principle of these assays was the production of radioiodide by ORD of [3′,5′-^{125}I]rT3 and IRD of [3,5-^{125}I]T3 or [3,5-^{125}I]T3S. Incubation mixtures contained ≈ 60,000 cpm labeled rT3 (≈ 0.2 nM), T3 (≈ 10 nM) or T3S (≈ 10 nM), varying concentrations of unlabeled substrate, and varying amounts of cell homogenate or rat liver microsomes in 200 μl 0.1 M phosphate (pH 7.2), 2 mM EDTA and 10 mM DTT. Mixtures were incubated in triplicate for 5–60 min at 37°C, and the reactions were stopped by the addition of 100 μl 5% bovine serum albumin at 0°C. Protein-bound iodothyronines were precipitated by the addition of 500 μl 10% trichloroacetic acid, and the radioiodide in the supernatant was further isolated on mini Sephadex LH-20 columns as previously described [16]. Enzymatic deiodination was corrected for non-enzymatic $^{125}I^-$ production as determined in blank incubations without enzyme and multiplied by 2 to account for the random labeling and deiodination of the 3′ and 5′ positions in labeled rT3 or the 3 and 5 positions in labeled T3 and T3S.

In some experiments, IRD of T3 was also determined by radioimmunoassay (RIA). For this purpose, incubations were carried out as above using only unlabeled T3 as the substrate. These incubations were stopped by the addition of 800 μl 0.1 N NaOH, and 3,3′-T2 was measured directly in the resulting mixtures by specific RIA relative to standards prepared in the same vehicle as previously described [7].

3. Results

Homogenates of cells transfected with vector without insert were devoid of deiodinase activity towards the different substrates, but cells transfected with ID-I cDNA-containing vector (G21 cells) expressed deiodinase activities similar to those in rat liver microsomes. Fig. 1 shows the IRD of T3 and T3S as well as the ORD of rT3 at 1 μM substrate concentration by G21 cell homogenates as a function of incubation time and cellular protein concentration. Significant deiodination was observed with all substrates tested, rT3 being deiodinated most rapidly, followed by T3S, while T3 was clearly the least active substrate. From the findings depicted in Fig. 1, proper conditions were chosen to analyze initial deiodination rates of the different substrates, and reaction conditions were defined similarly for rat liver microsomes (not shown).

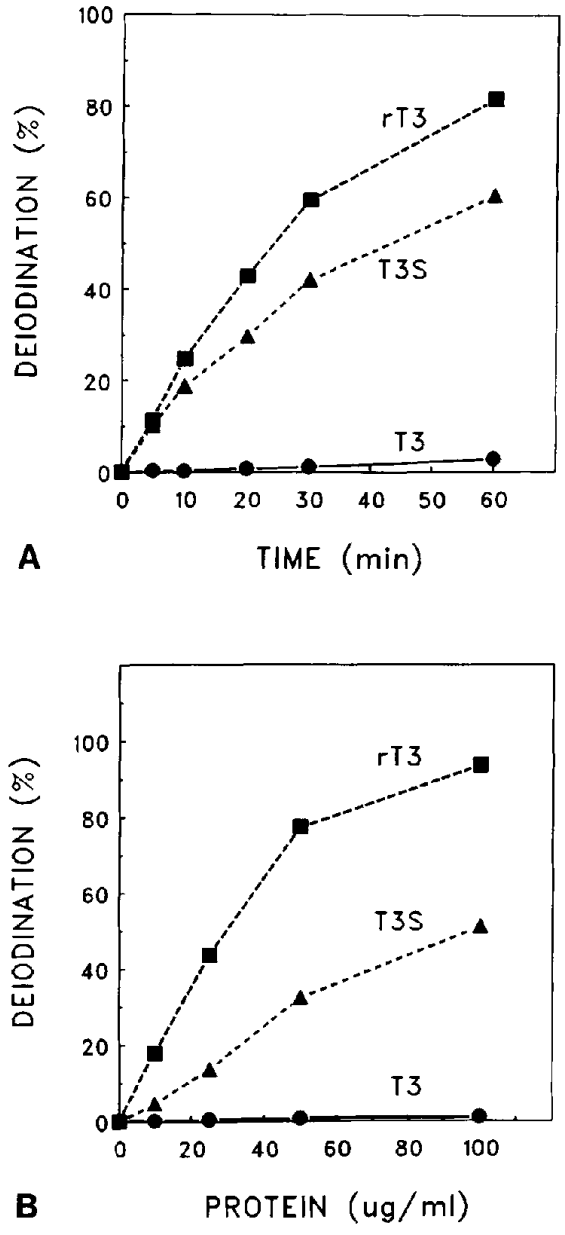

Fig. 1. Deiodination of 1 μM T3, T3S or rT3 by G21 cell homogenate as a function of incubation time using 100 (T3), 50 (T3S) or 25 (rT3) μg cell protein/ml (A) or as a function of cell protein concentration during incubation for 30 min (B). Results are the means of triplicate incubations in a representative experiment.

Table 1 gives the rates of the IRD of T3 and T3S and the ORD of rT3 by G21 cell homogenates as well as rat liver microsomes determined at 1 μM substrate concentration. Since the percentage deiodination of T3 was relatively small, IRD of this substrate was measured in these experiments by RIA of the product 3,3′-T2. Deiodination rates were somewhat higher with G21 cell homogenates than with rat liver microsomes, i.e. 2.2 and 2.3

times for IRD of T3 and T3S, respectively, and 1.3 times for ORD of rT3.

Fig. 2 shows the Lineweaver–Burk plots for the IRD of T3S and the ORD of rT3 by G21 cell homogenates as well as rat liver microsomes, and Table 2 gives the apparent K_m and V_{max} values derived from these plots. While V_{max} values for both T3S and rT3 were very similar with both enzyme preparations, mean apparent K_m values were 2.7-fold lower for T3S and 1.4-fold lower for rT3 when G21 cell homogenates were compared with rat liver microsomes.

4. Discussion

Since ID-I is thought to be very important for peripheral production of T3 from T4 and since it shows optimal catalysis in the ORD of rT3 [1–3], the enzyme is usually referred to as an ORDase or 5′-deiodinase. However, much circumstantial evidence has been presented suggesting that ID-I has also IRDase or 5-deiodinase activity [1,2], and this is proven unequivocally by the present results. Although IRD of T3 by ID-I is orders of magnitude slower than the ORD of rT3, so is the rate by which T4 is converted to T3 by the same enzyme [1–3]. Since ID-I has both ORD and IRD activities, our present findings support the hypothesis that this enzyme is capable of the activation as well as the inactivation of thyroid hormone.

Besides deiodination thyroid hormone is also metabolized by conjugation of the 4′-OH group with glucuronic acid or sulfate [19]. Whereas the glucuronides are rapidly excreted in the bile, only a small proportion of the sulfates is normally excreted intact as they are rapidly deiodinated in the liver [8,19]. In particular, IRD of both T4 and T3 is strongly facilitated by the sulfation of these compounds, suggesting that sulfation is a rate-limiting step preceding the deiodinative clearance of thyroid hormone [8,19]. The present findings clearly confirm previous suggestions that the efficient deiodination of iodothyronine sulfates is catalyzed by ID-I. Since rat and human ID-I are basic proteins [9,10], this may be due to beneficial ionic interaction of the negatively charged 4′-sulfate group of the substrate with protonated residues in the enzyme active center [20]. However, it remains to be established if this is indeed the mechanism for the facilitated deiodination of sulfated substrates by ID-I.

Our observations raise questions about, among other things, how ID-I can deiodinate both the outer and inner ring of different iodothyronines, and if structural modifications of the protein may differentially affect these two activities. It can be envisioned that the enzyme has a single, relatively wide binding pocket to which substrate can bind with a considerable degree of freedom, so that the iodine atoms of both rings may be in close proximity to the catalytic center, i.e. the Sec residue [14]. However,

Table 1
Deiodinase activities of G21 cell homogenate and rat liver microsomes

Reaction	G21 cells	Rat liver
	(pmol/min/mg protein)	
T3 IRD	3.17 ± 0.45	1.38 ± 0.33
T3S IRD	246 ± 18	113 ± 13
rT3 ORD	461 ± 51	348 ± 35

Results represent the means ± S.E.M. of 3–5 experiments each performed in triplicate at 1 μM substrate concentration. IRD of T3 was determined by RIA of 3,3′-T2.

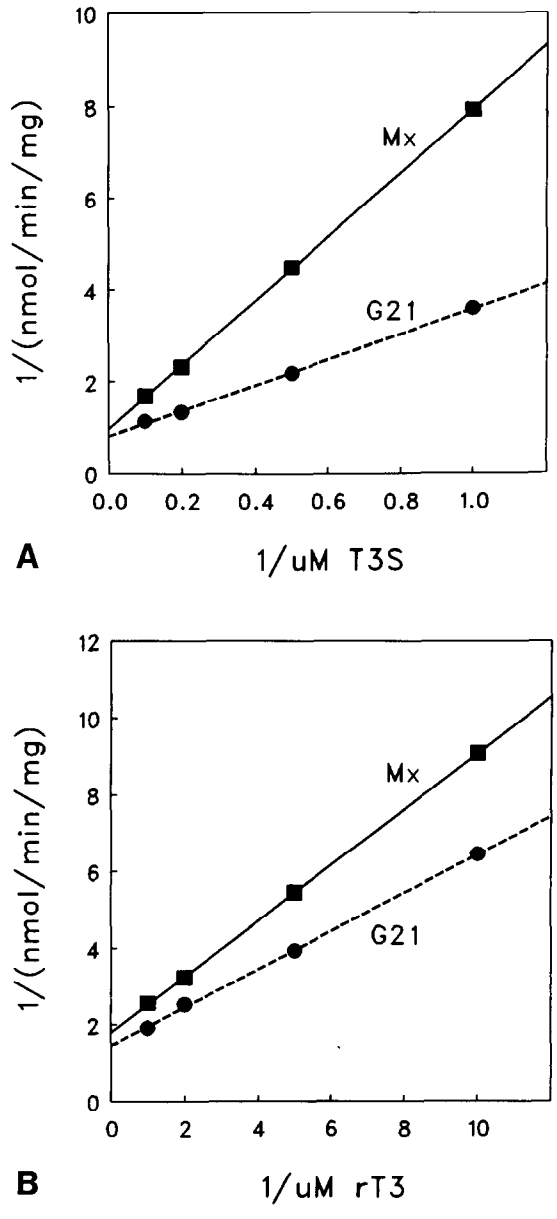

Fig. 2. Lineweaver–Burk plots of the IRD of T3S (A) or ORD of rT3 (B) by G21 cell homogenates (G21) or rat liver microsomes (Mx). Results are the means of triplicate incubations in a representative experiment.

Table 2
Kinetics of T3S and rT3 deiodination by G21 cell homogenate and rat liver microsomes

Reaction	Enzyme	K_m (μM)	V_{max} (pmol/min/mg)
T3S IRD	G21 cells	4.7 ± 1.1	1,396 ± 150
	Rat liver	12.5 ± 3.8	1,377 ± 197
rT3 ORD	G21 cells	0.49 ± 0.10	646 ± 112
	Rat liver	0.68 ± 0.19	552 ± 3

Results represent the means ± S.E.M. of 2–3 experiments such as shown in Fig. 2.

there may also be two distinct binding modalities, so that binding of substrate in one orientation would favour interaction of Sec with the outer ring iodines, while in the alternative orientation the inner ring iodines would be preferentially exposed. There is no evidence that ID-I is composed of different subunits, although the enzyme may be a homodimer [3]. However, based on substrate competition experiments, it is unlikely that ID-I has separate ORD and IRD substrate-binding sites [6,7].

Regulation of thyroid hormone bioactivity could involve changes in the relative ORD and IRD activities by post-translational modification of ID-I. Although a consensus glycosylation site has been identified in the amino acid sequences of human and rat ID-I, it is not known if the enzyme is indeed glycosylated in vivo [9,10]. Recent studies with several ID-I mutants have demonstrated the essential nature of Sec and two His residues for the ORD activity of the enzyme [9,11–13]. Preliminary evidence suggests that modifications of these residues results in parallel decreases in IRD activity (M. Moreno, M.J. Berry, P.R. Larsen and T.J. Visser, unpublished observations). It is of great interest to further investigate if modifications of other residues may be associated with selective changes in either ORD or IRD activity of the enzyme. The enzyme expressed in HEK 293 cells shows somewhat lower K_m values than native ID-I, more so for the IRD substrate, T3S, than for the ORD substrate, rT3. Although it is not excluded that this is due to a difference in post-translational modification, it may very well reflect differences in environment of the enzyme in the two preparations.

In conclusion, our findings demonstrate that the type I deiodinase is capable of outer and inner ring deiodination of iodothyronines, and, thus, can both activate and inactivate thyroid hormone. It is not known if the regulation of thyroid hormone bioactivity involves differential alterations of these two activities.

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