

Different thyroid hormone-deiodinating enzymes in tilapia (*Oreochromis niloticus*) liver and kidney

Koen Mol^a, Ellen Kaptein^b, Veerle M. Darras^a, Wim J. de Greef^c, Eduard R. Kühn^a and Theo J. Visser^b

^aLaboratory of Comparative Endocrinology, Catholic University of Leuven, B-3000 Leuven, Belgium, ^bDepartment of Internal Medicine III and ^cDepartment of Endocrinology and Reproduction, Erasmus University Medical School, 3000 DR Rotterdam, The Netherlands

Received 9 March 1993; revised version received 10 March 1993

Enzymes catalyzing the outer ring deiodination (ORD) of iodothyronines are important for the regulation of thyroid hormone bioactivity. We have studied ORD of thyroxine (T4) and 3,3',5'-triiodothyronine (rT3) in liver and kidney microsomes of fish, i.e. tilapia (*Oreochromis niloticus*). Tilapia kidney contains an enzyme which resembles the mammalian selenoenzyme type I iodothyronine deiodinase (ID-I) with respect to substrate preference (rT3>T4) and high ($\approx \mu\text{M}$) K_m values, but is much less sensitive to selenocysteine (Sec)-targeted inhibitors, including 6-propyl-2-thiouracil (PTU). In contrast, tilapia liver contains an enzyme very similar to mammalian type II deiodinase (ID-II) with respect to substrate preference (T4 > rT3), low ($\approx \text{nM}$) K_m values, and lack of sensitivity to Sec inhibitors.

Thyroid hormone; Iodothyronine; Deiodination; Fish; Rat; Liver; Kidney; Selenocysteine; Propylthiouracil; Iodoacetate; Aurothioglucose

1. INTRODUCTION

The thyroid secretes predominantly the biologically inactive prohormone thyroxine (T4), while the bioactive hormone 3,3',5'-triiodothyronine (T3) is largely produced by enzymatic outer ring iodination (ORD) of T4 in peripheral tissues [1,2]. In mammals, this ORD step is catalyzed by two types of enzymes localized in the microsomes of different tissues [1,2]. ID-I (mammalian type I iodothyronine deiodinase) is a high- K_m ($\approx \mu\text{M}$) selenoenzyme, which is present in liver, kidney and thyroid, and prefers the bioinactive metabolite rT3 over T4 as the substrate [1–8]. Iodoacetate (IAc) and aurothioglucose (ATG) are potent inhibitors of ID-I due to reaction with the reduced Sec residue, while PTU (6-propyl-2-thiouracil) is a specific non-competitive inhibitor, probably because it reacts with a substrate-induced oxidized form of Sec [5–9]. On the other hand, the low- K_m ($\approx \text{nM}$) ID-II, which is found in the pituitary, brain and brown adipose tissue, is not a selenoenzyme [10,11]. This enzyme prefers T4 over rT3 as substrate, is not inhibited by PTU, and is much less sensitive than ID-I to inhibition by IAc or ATG [1,2,10,12]. Both enzymes are stimulated by thiol cofactors such as dithiothreitol (DTT) [1,2]. It is generally believed that ID-I is most important for production of circulating T3, while ID-II serves a major function in the production of local tissue T3 [1,2].

With respect to thyroid hormone deiodination in non-mammals, hepatic ORD activity in birds has similar properties as mammalian ID-I [13–16], while in frogs ORD activity is only found in extrahepatic tissues [17]. Using T4 as the substrate, ORD activity in fish has mainly been found in liver and kidney, which is inhibited only by high ($> \text{mM}$) PTU concentrations [18–20]. The purpose of the present study was to more fully characterize the ORD activities in liver and kidney of tilapia (*Oreochromis niloticus*). This resulted in the identification of two different deiodinases in these tissues. Tilapia kidney ORD activity is similar to mammalian ID-I, although it shows little evidence of a selenoprotein nature, while tilapia liver ORD activity corresponds most closely with mammalian ID-II.

2. MATERIALS AND METHODS

2.1. Animals

Tilapia (*Oreochromis niloticus*) were obtained from CERER-University of Liège (Tihange, Belgium) and were kept in the laboratory in 200-l tanks, supplied with running and aerated water of 25°C under a 12 h light:12 h dark photoperiod. Fish were fed once daily ad libitum with carp pellets (Joosen Luyckx, Turnhout, Belgium). Male Wistar rats were obtained from Harlan Sprague-Dawley (Zeist, The Netherlands). Rats were made hypothyroid by surgical thyroidectomy, followed after 1 week by administration of 0.75 mCi $^{131}\text{I}^-$, and they were sacrificed 2 weeks later.

2.2. Materials

T4, T3 and rT3 were obtained from Henning GmbH (Berlin, Germany); [$3',5'-^{125}\text{I}$]T4 and [$3',5'-^{125}\text{I}$]rT3 ($\approx 1,700 \text{ Ci/mmol}$) from Amersham (Amersham, UK); and DTT, PTU, IAc and ATG from Sigma (St. Louis, MO, USA).

Correspondence address: Th.J. Visser, Department of Internal Medicine III, Erasmus University Medical School, PO Box 1738, 3000 DR Rotterdam, The Netherlands. Fax: (31) (10) 463-5430.

2.3. Subcellular fractionation

Livers and kidneys were isolated from tilapia and homogenized in 5 vols. of buffer A (0.25 M sucrose, 10 mM HEPES, pH 7, 1 mM DTT). The homogenate (H) was centrifuged for 10 min at $25,000 \times g$ to obtain a crude premicrosomal pellet (P1). The supernatant was spun for 60 min at $100,000 \times g$, resulting in a supernatant (S) and a pellet composed of a firm lower layer and a fluffy upper layer. Each layer was resuspended in 5 μ l of buffer B (0.1 M phosphate (pH 7.2), 2 mM EDTA, 1 mM DTT) and recentrifuged for 60 min at $100,000 \times g$, yielding pellets P2 and P3, respectively. Microsomes consisted mainly of P3 in liver and of P2 in kidney. P1, P2 and P3 were resuspended in 5 μ l buffer B, and aliquots thereof and of H and S were stored at -20°C until further analysis. For comparison, microsomes of liver and kidney from normal rats and of brain from thyroidectomized rats were prepared similarly. Protein concentrations were determined with the BCA protein assay reagent (Bio-Rad, Oud Beijerland, The Netherlands).

2.4. Deiodinase assay

ORD activity was determined by analysis of the production of radioiodide from outer ring-labeled T4 and rT3 [21]. This represented true monodeiodination of these substrates as confirmed by parallel HPLC analysis of the formation of T3 from T4 and of 3,3'-T2 from rT3 (not shown). In brief, 100,000 cpm (≈ 0.25 nM) [^{125}I]T4 or [^{125}I]rT3 and varying concentrations unlabeled iodothyronines were incubated in triplicate for 30–60 min at 37°C with varying amounts of tissue protein in 200 μ l 0.1 M phosphate (pH 7.2), 2 mM EDTA and 5–25 mM DTT. Incubations were also done with increasing concentrations of PTU, IAc or ATG to test their possible inhibitory effects. Reactions were started by addition of enzyme, and they were stopped by adding 100 μ l 5% BSA on ice. Subsequently, 500 μ l 10% TCA was added to precipitate protein-bound iodothyronines, and $^{125}\text{I}^-$ release was determined by counting the radioactivity in the supernatant. Results were corrected for non-enzymatic deiodination, which was assessed in incubations without tissue protein and amounted to 2–4% of added substrate. The data presented are from representative experiments, which were repeated at least once with the same results. The coefficients of variation of the triplicates in these experiments were $< 7\%$.

3. RESULTS

Homogenates of tilapia liver showed high ORD activity with low concentrations of T4 as the substrate,

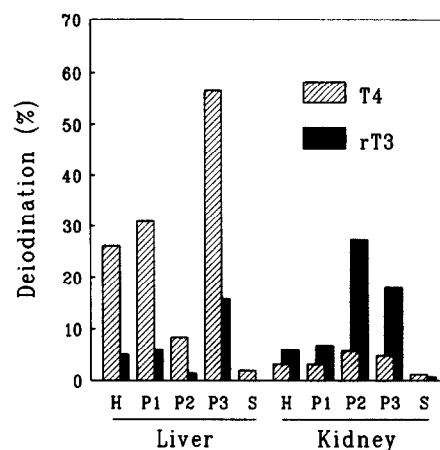


Fig. 1. Deiodination of rT3 or T4 by subcellular fractions of tilapia liver and kidney. Incubations were done in triplicate for 30 min at 37°C using 0.25 nM [^{125}I]rT3 or [^{125}I]T4, 0.1 mg tissue protein/ml, and 25 mM DTT.

which was about 5 times the rate of rT3 deiodination (Fig. 1). Highest specific ORD activity was located in the P3 subfraction of the high-speed pellet, which was used for all further studies. On the other hand, homogenates and subcellular fractions of tilapia kidney showed higher ORD activity with rT3 than with T4 as the substrate. Highest specific ORD activity was observed in the P2 subfraction of the high-speed pellet, which was also used for all further analyses.

The above results, indicating that tilapia liver deiodinase catalyzes ORD of T4 more effectively than that of rT3, are reminiscent of the substrate preference of rat ID-II [1,2]. Therefore, we further compared these deiodinase activities by analysis of the effects of substrate analogs and inhibitors on T4 ORD by tilapia liver or hypothyroid rat brain microsomes. Fig. 2A shows that the ORD of [^{125}I]T4 by these preparations is inhib-

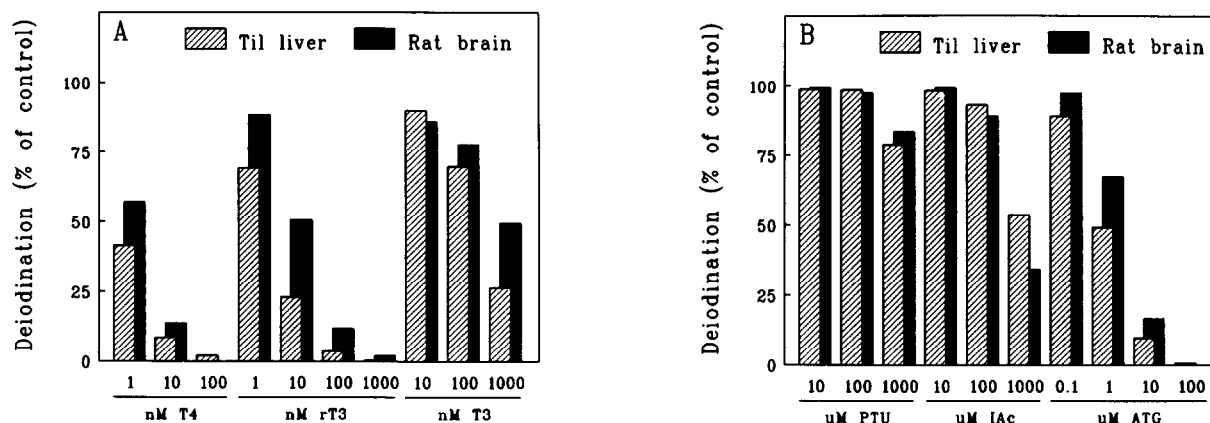


Fig. 2. Effects of iodothyronines (A) and inhibitors (B) on T4 deiodination in tilapia liver and rat brain. Incubations were done in triplicate for 30 min at 37°C using 0.25 nM [^{125}I]T4, 0.1 mg tilapia liver P3 protein/ml or 0.5 mg rat brain microsomal protein/ml, and 25 mM DTT, in the absence (control) or presence of the indicated concentrations of unlabeled iodothyronines or inhibitors.

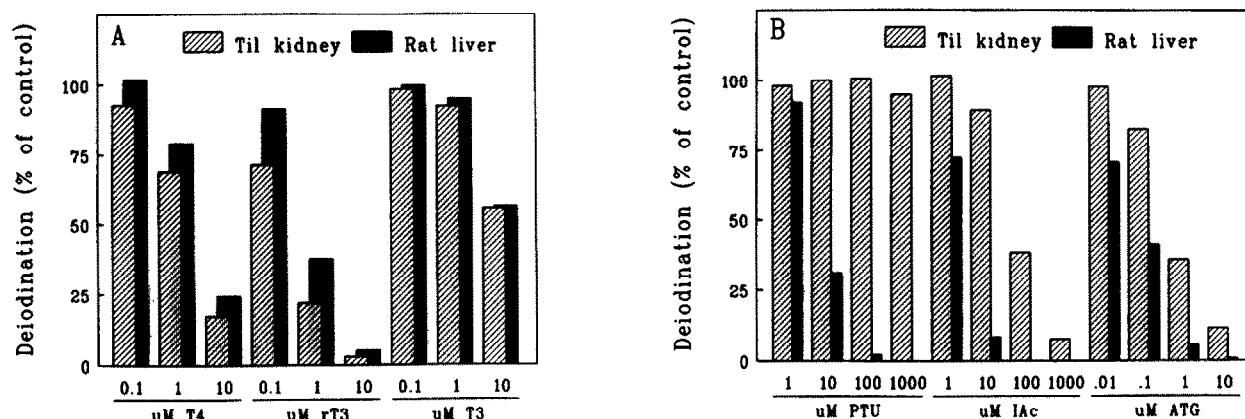


Fig. 3. Effects of iodothyronines (A) and inhibitors (B) on rT3 deiodination in tilapia kidney and rat liver. Incubations were done in triplicate for 30 min at 37°C using 0.25 nM [125 I]rT3, 0.1 mg tilapia kidney P2 protein/ml or 0.01 mg rat liver microsomal protein/ml, and 10 mM DTT, in the absence (control) or presence of the indicated concentrations of unlabeled iodothyronines or inhibitors.

ited dose-dependently by non-radioactive T4, rT3 and T3. The potency of T4, with an IC_{50} value of ≈ 1 nM, is roughly 1 and 3 orders of magnitude higher than that of rT3 and T3, respectively. Fig. 2B shows the effects of increasing concentrations of PTU, IAc and ATG on the ORD of T4 by tilapia liver deiodinase and rat ID-II. The results indicate similar, low sensitivities of these enzymes for the different inhibitors, with IC_{50} values of $\gg 1$ mM PTU, ≈ 1 mM IAc and ≈ 1 μ M ATG.

The preference of tilapia kidney ORD activity for rT3 over T4 as the substrate (Fig. 1) is similar to mammalian ID-I [1,2]. Therefore, we compared these deiodinases in more detail by analysis of the effects of substrate analogs and inhibitors on rT3 ORD by tilapia kidney or rat liver microsomes. Results identical with the latter were obtained using rat kidney microsomes as source of ID-I, and are not presented. Fig. 3A shows that addition of increasing concentrations of unlabeled iodothyronines has similar effects on the ORD of radioactive rT3 by the tilapia kidney deiodinase and rat ID-I. With both enzymes μ M iodothyronine concentrations are required to affect the deiodination of the labeled substrate, with $rT3 > T4 > T3$ in order of potency. Fig. 3B, however, demonstrates widely different susceptibilities of the tilapia kidney deiodinase and rat ID-I to inhibition by PTU, IAc and ATG. Thus, while rT3 deiodination by

rat ID-I is characterized by IC_{50} values of ≈ 5 μ M PTU, ≈ 2 μ M IAc, and ≈ 0.05 μ M ATG, corresponding values for the tilapia kidney deiodinase amount to $\gg 1$ mM PTU, ≈ 50 μ M IAc and ≈ 0.5 μ M ATG.

In addition to the different substrate and inhibitor profiles, the ORD activities in tilapia liver and kidney also showed different DTT requirements. Near-optimal rT3 ORD rates by the tilapia kidney deiodinase were observed with ≈ 10 mM DTT, while in case of T4 ORD by the tilapia liver deiodinase ≈ 25 mM DTT was required (data not shown). At these optimal DTT levels, the kinetic parameters of T4 ORD in tilapia liver and rT3 ORD in tilapia kidney have been determined. The results are presented in Table I and compared with K_m and V_{max} values for rT3 ORD by rat liver and kidney ID-I and T4 ORD by rat brain ID-II. These data indicate similar K_m values for T4 ORD by tilapia liver deiodinase and rat brain ID-II, but V_{max} values are higher in tilapia liver. In addition, K_m values are similar for rT3 ORD by tilapia kidney deiodinase and rat liver or kidney ID-I, but V_{max} values are much higher in rat tissues.

4. DISCUSSION

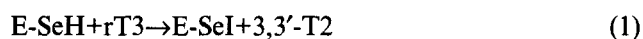
Since T4 is the predominant secretory product of the

Table I
Comparison of the kinetic parameters of ORD activities in tilapia and rat tissues

Tissue	Substrate	DTT	K_m	V_{max}^a
Tilapia liver	T4	25 mM	0.75 nM	213 fmol
Tilapia kidney	rT3	10 mM	0.33 μ M	31 pmol
Rat liver (ID-I)	rT3	10 mM	0.22 μ M	1,101 pmol
Rat kidney (ID-I)	rT3	10 mM	0.29 μ M	1,913 pmol
Rat brain (ID-II)	T4	25 mM	2.2 nM	35 fmol

^aper min per mg protein.

thyroid, while T3 is the ultimate ligand for the thyroid hormone receptor, conversion of T4 to T3 in peripheral tissues is a very important step in the regulation of thyroid hormone bioactivity [1,2]. The enzymes catalyzing this ORD reaction are well-characterized in rats and other mammals [1,2,7,16]. Rat liver and kidney contain the same deiodinase (ID-I), which has recently been identified as a Sec-containing enzyme [3–7]. The following ping-pong type reaction mechanism has been proposed for the conversion of T4 to T3 by ID-I, where E-SeH is the native selenol form and E-SeI the selenenyl iodide form of the enzyme, and RSH is the cofactor [1,2,7,8].



The uncompetitive inhibition of ID-I activity by PTU is thought to be due to reaction with E-SeI under formation of an enzyme-PTU selenosulfide complex. Inhibition of ID-I activity by IAc and ATG is competitive with substrate and is presumably due to modification of E-SeH [1,2,5–9].

In tilapia, hepatic and renal ORD activities have different characteristics. The kidney deiodinase is similar to the ID-I of rat liver and kidney with respect to (a) localization in the microsomal fraction, (b) marked substrate preference for rT3 over T4, (c) K_m values for the substrates in the μM range, and (d) optimal cofactor activity at $\approx 10 \text{ mM}$ DTT [1,2]. However, there is a remarkable difference in susceptibility to the inhibitors tested. Tilapia kidney ORD activity is insensitive to 1 mM PTU, whereas rat ID-I is completely inhibited at 0.1 mM PTU (Fig. 3). In addition, inhibition of tilapia kidney ORD activity requires ≈ 25 -fold higher concentrations of IAc and ≈ 10 -fold higher concentrations of ATG compared with their effects on rat ID-I (Fig. 3). The lower V_{\max} of rT3 deiodination in tilapia kidney than in rat liver or kidney (Table I) may reflect a lower enzyme content and/or a lower turnover number of the tilapia deiodinase.

It has been demonstrated that the mutant protein obtained by replacement of Sec in ID-I with Leu is enzymatically inactive, while replacement of Sec with Cys results in a strong decrease in deiodinase activity [5–7]. The Cys mutant is also much less sensitive to inhibition by PTU or ATG than the wild-type ID-I, indicating that Sec is the residue modified by these inhibitors [5–7]. From our results it is, therefore, tempting to speculate that the deiodinase in tilapia kidney is homologous to mammalian ID-I, but either it does not contain Sec or this residue has a different role in the catalytic process.

Although the primary structure of ID-II has not yet been elucidated, this enzyme is probably not a selenoprotein, since ID-II activity is not impaired by Se defi-

ciency in vivo in brain of hypothyroid rats [22] or in vitro in glial cell cultures [11]. This is supported by observations that ID-II is much less sensitive than ID-I to inhibition by IAc or ATG, while it is also hardly affected by PTU [1,2,10,12]. Perhaps the most intriguing findings of our study are the remarkable similarities between the ORD activity in tilapia liver and mammalian ID-II. Both enzymes show (a) location in the microsomal fraction of the tissues, (b) preference for T4 over rT3 as the substrate, (c) K_m values for the substrates in the nM range, (d) a relatively high cofactor requirement ($\approx 25 \text{ mM}$ DTT), and (e) lack of sensitivity to inhibition by PTU, IAc and ATG. These findings extend recent studies of Leatherland [18] and Eales [19] and coworkers, who also reported on low- K_m , T4 ORD activities in liver of different teleost species with a low sensitivity for PTU.

Together, these observations strongly suggest that the hepatic ORD activity in fish is homologous to ID-II, an important enzyme in mammals for the local production of T3 from T4 in extrahepatic tissues such as the brain, pituitary, and brown adipose tissue [1,2]. ID-II activity in rats is stimulated by hypothyroidism [1,2], but even compared with hypothyroid rat brain ORD activity is more abundant in tilapia liver (Table I). Therefore, the latter may be a useful source for the purification of this type of deiodinase or the cloning of its cDNA.

Acknowledgements. K.M. was supported by a grant from the National Foundation for Scientific Research. We wish to thank Dr. Ch. Mèlard and Prof. J.-C. Phillippart (University of Liège) for supplying the fish, and Dr. N. Byamungu, S. Van der Geyten, M.L. Cannon and W.A.P. Breeman for their assistance in the experiments.

REFERENCES

- [1] Visser, T.J. (1988) in: *Hormones and Their Action, Part I* (B.A. Cooke, R.J.B. King, and H.J. van der Molen Eds.) pp. 81–103, Elsevier, Amsterdam.
- [2] Köhrle, J., Hesch, R.D. and Leonard J.L. (1991) in: *The Thyroid* (L.E. Braverman and R.D. Utiger Eds.) pp. 144–189, Lippincott, Philadelphia.
- [3] Arthur, J.R., Nicol, F. and Beckett, G.J. (1990) *Biochem. J.* 272, 537–540.
- [4] Behne, D., Kyriakopoulos, A., Meinhold, H. and Köhrle, J. (1990) *Biochem. Biophys. Res. Commun.* 173, 1143–1149.
- [5] Berry, M.J., Banu, L. and Larsen, P.R. (1991) *Nature* 349, 438–440.
- [6] Berry, M.J., Kieffer, J.D., Harney, J.W. and Larsen, P.R. (1991) *J. Biol. Chem.* 266, 14155–14158.
- [7] Berry, M.J. and Larsen, P.R. (1992) *Endocr. Rev.* 13, 207–219.
- [8] Visser, T.J. (1991) in: *Progress in Thyroid Research* (A. Gordon, J. Gross and G. Hennemann Eds.) pp. 27–31, Balkema, Rotterdam.
- [9] Leonard, J.L. and Visser, T.J. (1984) *Biochim. Biophys. Acta* 787, 122–130.
- [10] Berry, M.J., Kieffer, J.D. and Larsen, P.R. (1991) *Endocrinology* 129, 550–552.
- [11] Safran, M., Farwell, A.P. and Leonard, J.L. (1991) *J. Biol. Chem.* 266, 13477–13480.
- [12] Visser, T.J., Frank, S. and Leonard, J.L. (1983) *Mol. Cell. Endocrinol.* 33, 321–327.

- [13] Lam, S.K. and Harvey, S. (1986) *J. Endocrinol.* 110, 441–446.
- [14] Rudas, P. (1986) *Gen. Comp. Endocrinol.* 63, 400–407.
- [15] Darras, V.M., Visser, T.J., Berghman, L.R. and Kühn, E.R. (1992) *Comp. Biochem. Physiol.* 103A, 131–136.
- [16] Schoenmakers, C.H.H., Pigmans, I.G.A.J. and Visser, T.J. (1992) *Biochim. Biophys. Acta* 1121, 160–166.
- [17] Galton, V.A. (1988) *Endocrinology* 122, 1746–1750.
- [18] Leatherland, J.F., Reddy, P.K., Yong, A.N., Leatherland, A. and Lam, T.J. (1990) *Fish Physiol. Biochem.* 8, 1–10.
- [19] MacLatchy, D.L. and Eales J.G. (1992) *Gen. Comp. Endocrinol.* 86, 313–322.
- [20] Byamungu, N., Mol, K. and Kühn, E.R. (1992) *Gen. Comp. Endocrinol.* 88, 199–208.
- [21] Mol, J.A., Docter, R., Hennemann, G. and Visser, T.J. (1984) *Biochem. Biophys. Res. Commun.* 120, 28–36.
- [22] Chanoine, J.P., Safran, M., Farwell, A.P., Tranter, P., Ekenbarger, D.M., Arthur, J.R., Beckett, G.J., Braverman, L.E. and Leonard, J.L. (1992) *Endocrinology* 130, 479–484.