The cDNA for the Type I Iodothyronine 5'-Deiodinase Encodes an Enzyme Manifesting Both High $K_m$ and Low $K_m$ Activity

EVIDENCE THAT RAT LIVER AND KIDNEY CONTAIN A SINGLE ENZYME WHICH CONVERTS THYROXINE TO 3,5,3'-TRIODOTHYRONINE

(Received for publication, December 2, 1991)

Jahangir Sharifi and Donald L. St. Germain

From the Departments of Medicine and Physiology, Dartmouth Medical School, Lebanon, New Hampshire 03756

The enzymatic conversion of thyroxine (T₄) to 3,5,3'-triiodothyronine (T₃) by iodothyronine 5'-deiodinases is an obligate step in the physiologic action of thyroid hormones in most extrathyroidal tissues. In the rat liver and kidney, 5'-deiodinase processes having either high $K_m$ (micromolar range) or low $K_m$ (nanomolar range) values for thyroid hormone substrates have been described. The number of enzymes mediating these reactions, however, remains uncertain and controversial. To examine this question we have compared the 5'-deiodinase activity expressed in membrane preparations of Xenopus laevis oocytes after the injection of either rat liver poly(A)⁺ RNA or in vitro prepared RNA transcribed using the G21 full-length type I 5'-deiodinase cDNA. In oocytes injected with rat liver poly(A)⁺ RNA, high $K_m$ (i.e. type I) activity was observed when 20 mM dithiothreitol was used as the thiol cofactor, whereas $K_m$ values in the nanomolar range were noted with 0.5 mM dithiothreitol, glutathione, or a reconstituted thioredoxin cofactor system. This complex pattern of 5'-deiodinase activity, which mimics that found in homogenates and subcellular fractions of rat liver and kidney, was reproduced exactly in oocytes by the microinjection of G21-derived RNA transcripts. Furthermore, hybrid arrest of translation in oocytes using a partial type I 5'-deiodinase cDNA completely inhibited the expression of both high and low $K_m$ activity after the injection of rat liver poly(A)⁺ RNA. These findings demonstrate that rat liver and kidney contain only a single 5'-deiodinase which manifests either high or low $K_m$ activity depending on the reduced thiol cofactor utilized in the reaction.

Thyroxine (T₄), the principal secretory product of the thyroid gland, functions primarily as a prohormone and undergoes a single deiodination of the phenolic ring in extra-thyroidal tissues to form the metabolically more active hormone 3,5,3'-triiodothyronine (T₃) (1). The biochemical characterization of the iodothyronine 5'-deiodinases which catalyze this reaction has been hampered by the inability to purify these proteins to homogeneity in an active form. Thus, considerable uncertainty and controversy exists as to the number of enzymes which mediate 5'-deiodination (2, 3). Based on kinetic studies, tissue distribution, and susceptibility to inhibition by 6-n-propyl-2-thiouracil (PTU), two principle types of 5'-deiodinase activity have been defined in various tissue homogenates when assayed using high concentrations of dithiothreitol (DTT, e.g. 20 mM) as cofactor (1). Type I 5'-deiodinase is found primarily in the liver, kidney, and thyroid gland, manifests a high $K_m$ for thyroid hormone substrates, and is sensitive to inhibition by PTU. In contrast, type II activity is relatively resistant to inhibition by PTU, manifests much lower substrate $K_m$ values, and is present almost exclusively in brown adipose tissue, the pituitary gland, and the central nervous system.

In addition to the type I activity in the liver and kidney, "low $K_m$" 5'-deiodinase processes have also been described in these tissues when thiol cofactors such as glutathione (4), thioredoxin (5, 6), or low concentrations of DTT (0.5 mM) (7) have been used to support deiodination. Whether or not such low $K_m$ activity is secondary to the type I enzyme or mediated by other 5'-deiodinases is uncertain, but is of considerable importance; the high $K_m$ values of 4 and 0.1 μM of the type I enzyme for $T_3$ and 3,3',5'-triiodothyronine (reverse $T_3$, rT₃), respectively, far exceed their intracellular concentrations. Thus, the low $K_m$ renal and hepatic 5'-deiodinase processes, which manifest $K_m$ values for these same substrates in the nanomolar range and can potentially utilize naturally occurring thiols as cofactors to support deiodination, are likely to be of more physiologic relevance.

The recent isolation of cDNAs for the type I 5'-deiodinase by ourselves (8) and Berry et al. (9) has provided a means of studying the patterns of deiodination in the rat liver and kidney in greater detail and in a more definitive fashion. In particular, the availability of a full-length cDNA for this enzyme (9) affords the opportunity to express the type I 5'-deiodinase in an in vivo model system and to study its properties under conditions of varying substrate and cofactors. For such studies, we chose to use the Xenopus laevis oocyte expression system so that the properties of the enzyme encoded by the recombinant type I 5'-deiodinase cDNA could be compared directly with the activity induced by the injection of native rat liver poly(A)⁺ RNA. Our findings provide strong evidence that rat liver and kidney contain only a single iodothyronine 5'-deiodinase which manifests both type I and low $K_m$ activity.

EXPERIMENTAL PROCEDURES

General Methods—[¹²⁵I]rT₃ (specific activity ∼1200 μCi/μg) or [¹³¹I] $T_4$ (specific activity ∼150 μCi/μg) were obtained from Du Pont-New
Expression of High and Low $K_m$ 5'-Deiodinase Activity

England Nuclear and purified by chromatography using Sephadex LH-20 (Sigma) prior to use. Recombinant *Escherichia coli* thioredoxin was purchased from Calbiochem (San Diego, CA) in lyophilized form and dissolved in 0.1 M potassium phosphate, pH 7.0, 1.0 mM EDTA. Recombinant *E. coli* thioredoxin reductase was generously provided by Dr. James A. Fuchs (University of Minnesota, St. Paul, MN). Male Sprague-Dawley rats (200 g, Charles River Laboratories, Wilmington, MA) were rendered hyperthyroid by the daily injection of T$_3$ (12 $\mu$g/100 g body weight, subcutaneously), and poly(A)$^+$ RNA was prepared from liver tissue as previously described (10). Capped RNA transcripts were synthesized in *in vitro* using T$_3$ RNA polymerase according to instructions provided by the supplier (Stratagene, La Jolla, CA). The transcripts were purified by phenol-chloroform extraction and ethanol precipitation prior to injection into oocytes.

**Description of cDNAs**—The full-length type I 5'-deiodinase G21 cDNA in the Bluescript vector was kindly provided by Drs. Marla J. Berry and P. Reed Larsen (Harvard Medical School, Boston, MA). The partial length B2-21 cDNA, previously isolated in this laboratory (8) and encompassing nucleotides 32-256 of the coding region of the G21 clone, was used in the hybrid arrest experiment.

**Oocyte Injection and Harvesting**—Stage V-VI *X. laevis* oocytes were isolated and microinjected as previously described (10) with 10-25 ng of rat liver poly(A)$^+$ RNA or 0.5 ng of *in vitro* prepared G21 RNA transcripts. Control oocytes were either not injected or were injected with an equal volume (50 nl) of water. After injection oocytes were incubated in L-15 medium at 18°C for 4-8 days prior to harvest.

Poly(A)$^+$ RNA of approximately 60 injected oocytes was homogenized and subjected to differential centrifugation for the preparation of crude nuclei (1,000 $\times$ g pellet), mitochondria (16,000 $\times$ g pellet), microsomes (100,000 $\times$ g pellet), and cytosolic (100,000 $\times$ g supernatant) fractions as previously described (10). In most experiments, the infranatant from the 1,000 $\times$ g initial centrifugation was used to prepare a combined mitochondrial/microsomal ("membrane") subcellular fraction. For this preparation the centrifugate was centrifuged at 100,000 $\times$ g for 1 h in an Airfuge ultracentrifuge (Beckman Instruments, Palo Alto, CA), the pellet rinsed twice in buffer, then resuspended in buffer and centrifuged again at 100,000 $\times$ g for 1 h, followed by another rinse and final resuspension in buffer using a Dounce homogenizer. Several buffers were used in different experiments including: (a) 0.1 M potassium phosphate, pH 7.0, 1.0 mM EDTA (KPhos buffer); (b) 0.25 mM sucrose, 0.02 M Tris-HCl, pH 7.0, 1 mM EDTA (Tris-sucrose buffer); and (c) 0.01 M sodium phosphate, pH 7.4, 0.15 M NaCl (PBS buffer). Thiol reagents were included only in the final assay reaction mixtures and were not present in the buffers during the preparation of the subcellular fractions.

**5'-Deiodinase Assay**—5'-Deiodinase activity was assayed in a total reaction volume of 50 nl as the amount of 125I released from [125I]rT$_3$ or [125I]3,3'-diiodothyronine (7,8), as previously described (10). Reaction mixtures typically contained 20-40 ng of membrane protein, and incubation times ranged from 10 min to 3 h. Thiol cofactors used in the assay included DTT (20 or 0.5 mM final concentration), GSH (5 mM), or a reconstituted thioredoxin system which included thioredoxin (42 $\mu$M), thioredoxin reductase (42 $\mu$M), and NADPH (0.5 mM). Due to the limited amount of oocyte material available, most experiments utilized single data points for each determination. When sufficient material was available to perform duplicate incubations the coefficient of variation of such determinations was <11%. In some experiments ascending paper chromatography using a tertiary amyl alcohol/20-40% aqueous methanol containing 20-40 pg of membrane protein, and incubation times as cofactor) in a subcellular distribution similar to that noted in rat liver and kidney. Highest specific activity is found in the microsomal fraction (100,000 $\times$ g pellet) with lesser, but significant amounts of activity in the mitochondria (16,000 $\times$ g pellet) (10). In an initial experiment, we sought to determine if GSH could support 5'-deiodination in oocytes injected with rat liver poly(A)$^+$ RNA. As shown in Fig. 1, GSH stimulated 5'-deiodinase activity in subcellular fractions from oocytes injected with poly(A)$^+$ RNA from hyperthyroid rat liver, whereas no activity was found in non-injected or water-injected oocytes (data not shown). The distribution of specific activity using GSH as cofactor was identical to that of type I (10), being highest in the microsomal fraction. However, the $K_m$ for rT$_3$ was considerably lower (0.2-0.3 nM) than the value of approximately 0.2 $\mu$M previously determined for the type I process (10) and was thus consistent with values reported in rat liver and kidney microsomes using GSH as a cofactor (4, 13). These results demonstrated that a low $K_m$ 5'-deiodinase process could be induced in *X. laevis* oocytes by the injection of rat liver poly(A)$^+$ RNA. Because significant amounts of low $K_m$ activity were present in the crude mitochondrial fraction (16,000 $\times$ g pellet) we utilized a combined membrane preparation containing both crude mitochondria and microsomes for all subsequent experiments (see "Experimental Procedures").

The time course of *in vitro* deiodination of rT$_3$ in membrane preparations from control (non-injected or water-injected) oocytes or oocytes injected with hyperthyroid rat liver poly(A)$^+$ RNA or *in vitro* prepared G21 transcripts is shown in Fig. 2. In control oocytes (and buffer only reaction mixtures (data not shown)), little or no deiodination was detected during the 130-min incubation period in the absence or presence of thiol cofactors. Significant deiodination was noted, however, in the membrane preparation from hyperthyroid rat liver poly(A)$^+$-injected oocytes (Fig. 2A) in the absence of added cofactors (13% substrate deiodinated after 130 min).

In the presence of GSH or the thioredoxin cofactor system, 5'-deiodinase activity increased approximately 2-fold during the incubation period from 40 to 130 min. Using 0.5 mM DTT as cofactor, activity was increased 9-fold.

In oocytes injected with G21 transcripts, considerable activity (20-30%) was also noted in the absence of cofactors, and this activity was stimulated with GSH, DTT, and the thioredoxin system from 2.5- to 7-fold in both Tris-sucrose (Fig. 2B) and KPhos (Fig. 2C) buffers. No stimulation of activity was noted when thioredoxin and NADPH, in the absence of thioredoxin reductase, were added to the reaction mixture (data not shown). Under all of the conditions utilized above, 125I and [125I]3,3'-diodothyronine production were

Fig. 1. Expression of GSH-stimulated 5'-deiodinase activity in subcellular fractions of *X. laevis* oocytes. Approximately 60 oocytes were each injected with 25 ng of hyperthyroid rat liver poly(A)$^+$RNA, incubated for 4 days, then homogenized in KPhos buffer and subcellular fractions prepared as described under "Experimental Procedures." Kinetic analysis was performed in each fraction using rT$_3$ as substrate and 5 mM GSH as cofactor. Specific activity in terms of $V_{max}$ values for each fraction are shown. $K_m$ values for the mitochondrial, microsomal, and cytosolic fractions are shown in the inset of each bar.
without and with a variety of cofactors and buffers (Fig. and Table I). In the absence of cofactors, 5'-deiodinase activity induced in oocytes by the injection of hyperthyroid rat liver poly(A)+ RNA was abolished deiodinating activity. Continued to 85°C for 30 min completely abolished deiodinating activity.

PTU is a potent inhibitor of type I 5'-deiodinating processes in liver and kidney microsomes (1-7). Using 0.5 mM DTT and 20 mM DTT, the V_max values in Table I were dependent on the cofactor concentration. Using 0.5 mM DTT, V_max values were essentially identical to those previously determined by others in rat liver microsomes using these cofactor concentrations (7).

Experiments were also performed with various cofactors using T3 as substrate (Fig. 6). In un.injected oocytes deiodination of T3 was minimal (±1.2%). In oocytes injected with hyperthyroid rat liver poly(A)+ RNA or G21 transcripts, T3 deiodination was clearly demonstrated in the absence of cofactors and was stimulated by DTT, GSH, and the thioredoxin system. Under all conditions, however, the extent of deiodi-

**Expression of High and Low K_m 5'-Deiodinase Activity**

**FIG. 2.** Time course of 5'-deiodination in membrane preparations of injected oocytes using various thiol cofactors in the reaction assay. **A**, membrane preparations from oocytes injected with water or hyperthyroid rat liver (HRL) poly(A)+ RNA prepared in KPhos buffer and assayed for 5'-deiodinase activity using 3 nM rT3. No cofactors were present during the first 40 min of incubation, and then buffer, GSH (5 mM final concentration), a reconstituted thioredoxin system (as described under "Experimental Procedures," or DTT (0.5 mM final concentration) was added and the incubation continued to 130 min. In membrane preparations from water-injected oocytes, little or no activity was noted in the absence or presence of T3 and cofactors. **B**, results of similar experiments in oocytes injected with water or G21 RNA transcripts with membrane preparations prepared, and activity determined, in Tris-sucrose buffer (panel B) or KPhos buffer (panel C). rT3 and cofactor concentrations are the same as in panel A.

**FIG. 3.** Inhibitory effects of PTU on expressed 5'-deiodinase activity in membrane fractions of oocytes injected with hyperthyroid rat liver poly(A)+ RNA (HRL) or G21 RNA transcripts. Activity was assayed using 1 nM rT3 with 5 mM GSH or a reconstituted thioredoxin system used as cofactor in the absence (−) of presence (+) of 100 μM PTU in the reaction mixture. Values represent the mean of duplicate determinations (coefficient of variation = 9.6%).

**FIG. 4.** Representative Lineweaver-Burk plots of expressed 5'-deiodinase activity in membrane fractions of oocytes injected with hyperthyroid rat liver (HRL) poly(A)+ RNA or G21 RNA transcripts. Kinetic data were generated using rT3 as substrate in a competitive fashion with a membrane preparation from G21 transcript preparations used during the course of these studies, and (c) inherent batch-to-batch variation in oocyte expression. For these reasons comparison of V_max values between different cofactor groups in Table I is difficult.
Expression of High and Low $K_m$ 5'-Deiodinase Activity

Summary of kinetic data of expressed 5'-deiodinase activity in membrane preparations from injected oocytes

| Cofactor  | Buffer  | HRL poly(A)+ RNA | G21 RNA transcripts |
|----------|---------|-----------------|---------------------|
|          |         | $K_m$ (nM) | $V_{max}$ pmol/min mg protein | $K_m$ (nM) | $V_{max}$ pmol/min mg protein |
| None     | Tris-sucrose | 1.5 | 1.8 | 1.0 | 23 |
|          | KPhos    | 1.6 | 41 | 1.6 | 41 |
| Thioredoxin | Tris-sucrose | 1.1 | 14.5 | 2.0 | 199 |
|          | KPhos    | 1.5 | 122 | 1.5 | 122 |
| GSH (5 mM) | KPhos   | 0.5 ± 0.2   | 4.4 ± 1.8 | 0.8 ± 0.5 | 12.9 ± 6.4 |
|          | PBS     | 15 ± 5      | 15 ± 5     | 15 ± 5     |
| DDT (0.5 mM) | PBS   | 21 ± 5      | 59 ± 22    | (n=3)      | (n=4) |
|          | Tris-sucrose | 100, 100 | 0.6, 1.2 | 6.4, 12.9   | 2.0, 4.0   |

* Comparison of $V_{max}$ values between different cofactor groups may not be valid due to differences in potencies of the various preparations of HRL poly(A)+ RNA and G21 transcripts used in the experiments and due to the inherent batch-to-batch variation in oocyte expression.

** Mean ± S.E.

FIG. 5. Competitive inhibitory effects of iopanoic acid (IOP) on expressed 5'-deiodinase activity in membrane fractions of oocytes injected with hyperthyroid rat liver (HRL) poly(A)+ RNA (A) or G21 RNA transcripts (B). Kinetic data were generated using rT$_3$ as substrate and 0.5 mM DTT as cofactor in the absence or presence of IOP in the reaction mixture. A $K_i$ value, as determined by a replot of the slope of the Lineweaver-Burk plot versus the IOP concentration, of 0.5 mM was noted in oocytes injected with either RNA preparation. Similar findings were noted in a second experiment.

FIG. 6. Expressed 5'-deiodinase activity in membrane fractions of noninjected oocytes or oocytes injected with hyperthyroid rat liver (HRL) poly(A)+ RNA or G21 RNA transcripts using 5 nM T$_3$ as substrate and the various cofactors indicated. Values represent percent $^{125}$I production from $^{125}$I$^T_3$. In replicate samples subjected to ascending paper chromatography, production of equivalent amounts of $^{125}$I$^T_3$ and $^{125}$I$^T_4$, were demonstrated. Assay incubation time was 3 h. Similar findings were noted in two additional experiments. Values represent the mean of duplicate determinations (coefficient of variation = 10.8%).

nation was considerably less than when rT$_3$ was used as substrate. This low level of 5'-deiodination precluded accurate kinetic analysis using T$_3$ as substrate. In one experiment the efficiency of 5'-deiodination of T$_3$ compared to rT$_3$ was determined in the same membrane preparation from G21 transcript-injected oocytes using equal concentrations of both substrates (4 nM) and the thioredoxin system as cofactor. The reaction velocity with rT$_3$ was at least 60-fold greater than the velocity using T$_3$, a value similar to that noted in a control experiment using rat kidney microsomes with the same substrate and cofactor system (data not shown). The marked substrate preference for rT$_3$ demonstrated in these studies has been observed previously by other investigators in liver microsomes (5-7, 14). In other experiments using membrane preparations from oocytes injected with hyperthyroid rat liver poly(A)+ RNA or G21 transcripts, PTU (100 PM) inhibited 5'-deiodinase activity by 32–73% when assayed using T$_3$ as substrate and 0.5 mM DTT, 5 mM GSH, or the thioredoxin system as cofactor.

We have previously demonstrated in hybrid arrest experiments that the B2-21 type I 5'-deiodinase cDNA prevents expression of type I activity in oocytes injected with hyperthyroid rat liver poly(A)+ RNA (8). Thus, a similar experiment was conducted to determine if this cDNA could also impair the expression of low $K_m$ activity. As shown in Fig. 7, expression of both type I and low $K_m$ (i.e. GSH-stimulated) 5'-deiodinase activity was inhibited >95% by hybrid arrest with the B2-21 cDNA, whereas no inhibition was noted in oocytes injected with a hybridization mixture containing no cDNA (mock) or cDNA from an irrelevant clone (B2-14).

DISCUSSION

In rat liver and kidney homogenates, a complex pattern of 5'-deiodination is noted, with the kinetic properties of the reaction dependent on the substrates and thiol cofactors employed (1, 2). These markedly different kinetic patterns of deiodinase activity have given rise to considerable uncertainty concerning the number of enzymes capable of, and responsible for, catalyzing thyroid hormone deiodination in these tissues (2, 3). The seminal finding of the present study is that the injection of rat liver poly(A)+ RNA into X. laevis oocytes results in a pattern of 5'-deiodinase expression that exactly mimics that found in homogenates and subcellular fractions...
of rat liver and kidney, and that this complex pattern of 5'-deiodination can be reproduced in its entirety by the injection of RNA transcripts prepared in vitro using a full-length type I 5'-deiodinase cDNA template.

Following the injection of rat liver poly(A)+ RNA, both high and low Km 5'-deiodinase processes were readily detected in membrane preparations from oocyte homogenates. Using high concentrations of DTT as cofactor, typical type I reaction kinetics were noted with Km values for rT3 of approximately 0.1 μM. When lower DTT concentrations (0.5 mM) were utilized, Km values of approximately 20 mM were demonstrated, similar to that previously described by Boada and Chopra (7) in rat liver microsomes. Using GSH or a thioredoxin cofactor system, Km values for rT3 of approximately 1 mM were noted in injected oocytes. Such values are in complete agreement with both our own experience and that of others using these cofactors to support 5'-deiodination in liver and kidney subcellular fractions (4-6, 13). Furthermore, the marked sensitivity of these low Km 5'-deiodinase processes to inhibition by PTU as well as the Km values of 0.5 μM for competitive inhibition by IOI are entirely analogous to the published experience of others (4-7). Of importance, T3 can also serve as a substrate for the induced low Km processes. Thus, the pattern of 5'-deiodinase activity seen in native rat liver and kidney are mimicked in their entirety in X. laevis oocytes by the injection of rat liver poly(A)+ RNA. The finding that the injection of G21 transcripts reproduces this pattern strongly suggests that the type I enzyme encoded by this cDNA is the sole mediator of 5'-deiodination in these organs.

This conclusion is strengthened further by the results of the hybrid arrest experiment. Solution hybridization of rat liver poly(A)+ RNA with the B2-21 cDNA prior to injection into oocytes results in essentially complete inhibition of expression of both high and low Km 5'-deiodination processes, indicating that the type I mRNA is solely responsible for these expressed patterns of activity. Thus, the disparate reaction kinetics noted in liver and kidney homogenates under different reaction conditions appear to be attributable to variances in the interaction of the type I enzyme with specific thiol cofactors. The biochemical basis for such differences remains uncertain. Of note, however, is that in the presence of any of these cofactors, several properties of the reaction are the same (1-7); (a) rT3 is a more efficient substrate than T3, as determined by the higher Vmax and lower Km values for the former, (b) the reaction is sensitive to inhibition by PTU, (c) compounds such as iopanoic acid act as competitive inhibitors, and (d) activity decreases in tissue homogenates from experimental animals rendered hypothyroid and increases in the hyperthyroid state. Such physiologic alterations in activity are paralleled by changes in type I 5'-deiodinase mRNA levels in both tissues (8, 9). Taken together, these similarities further underscore the basic thesis of this report.

In studies reported previously, Goswami and Rosenberg (5) noted that a reconstituted thioredoxin cofactor system would support the 5'-deiodination of rT3, but not of T3, in liver microsomes. This led these investigators to suggest that a separate 5'-deiodinase enzyme may be present in the liver and kidney which specifically deiodinates T3 to T3. The present studies do not support this speculation; using the thioredoxin system the 5'-deiodination of T3 was observed in oocytes injected with rat liver poly(A)+ RNA or G21 RNA transcripts as well as in kidney microsomes (data not shown). These latter results in kidney microsomes, although different from those of Goswami and Rosenberg (5), are in agreement with the findings of others (6).

Of note in the present studies is our observation that the wash of mitochondrial-membrane subcellular fractions of rat liver poly(A)+ RNA or G21 RNA transcripts manifested considerable low Km 5' deiodinating capability in the absence of added cofactors. This finding may be of considerable physiologic importance as it suggests that it is present in the native state, the enzyme functions in a low Km mode. Deiodination in the absence of exogenous cofactors has been noted previously in crude, washed mitochondrial preparations from rat liver (15), although the kinetics of such a process has not been previously reported. Whether this activity is attributable to an endogenous cofactor present in our membrane preparation is uncertain, but is suggested by the finding that product formation continued (albeit nonlinearly) throughout the 130-min incubation period of our time course experiments. Dihydrolipoamide, which is present in mitochondria and has been shown to support 5'-deiodination, represents such a potential endogenous cofactor (15, 16). Alternatively, a cytosolic cofactor could have been present in our membrane preparations in spite of the extensive wash protocol that we utilized, or the activity may be secondary to enzyme present in a reduced, active form at the time of membrane isolation and which subsequently undergoes a single round of catalysis during the in vitro assay.

Recent advances have provided important insights into the structure and function of the type I 5'-deiodinase. The molecular cloning of a full-length cDNA (9), as well as affinity labeling studies followed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (17, 18), have demonstrated that this enzyme (or at least its catalytic subunit) is a selenoprotein of approximately 27 kDa in size. Others, however, have recently reported the purification of an approximately 56-kDa protein(s) from rat liver microsomes which is purported to have 5'-deiodinase activity (19, 20). Of note is that the specific activity of these preparations is quite low despite purification to apparent homogeneity as assessed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and the 5'-deiodinase activity has not yet been demonstrated to reside in the 56-kDa protein band. Thus, the significance of these reports remains unclear.

In summary, the results of the present studies serve to clarify the uncertainty concerning the number of enzymes responsible for the 5'-deiodination of thyroid hormones. In the rat liver and kidney, a single enzyme, the type I 5'-deiodinase, appears to be responsible for all of the activity...
observed in homogenates and subcellular fractions from these tissues. There appears to be little doubt, however, that the type I and II 5'-deiodinases are different proteins based on their kinetic behavior, PTU sensitivity, tissue distribution, differences in size and patterns of proteolytic digestion as determined by affinity labeling followed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and finally the recent evidence that the type II enzyme is probably not a selenoprotein (1, 21-24). The structural and functional relationship between these two proteins awaits their purification and/or the isolation of a cDNA for the type II enzyme.

Acknowledgments—We express our sincere appreciation to Drs. Marla J. Berry and P. Reed Larsen for providing the G21 cDNA and to Dr. James A. Fuchs for providing the recombinant thioredoxin reductase. We also wish to thank Drs. Valerie Anne Galton, William Kinlaw, and Lee Witters for their helpful discussions and Walburga Dittrich for her technical assistance.

REFERENCES

1. Leonard, J. L., and Visser, T. J. (1986) in Thyroid Hormone Metabolism (Hennemann, G., ed) pp. 189-229, Marcel Dekker, New York
2. Rosenberg, I. N. (1991) in Thyroid Hormone Metabolism: Regulation and Clinical Aspects (Wu, S.-Y., ed) pp. 29-39, Blackwell Scientific, New York
3. Chopra, I. N. (1991) in Thyroid Hormone Metabolism: Regulation and Clinical Aspects (Wu, S.-Y., ed) pp. 41-54, Blackwell Scientific, New York
4. Goswami, A., and Rosenberg, I. N. (1988) Endocrinology 123, 192-202
5. Goswami, A., and Rosenberg, I. N. (1987) Endocrinology 121, 1937-1945
6. Bhat, G. B., lwase, K., Hummel, B. C. W., and Walfish, P. G. (1989) Biochem. J. 258, 785-792
7. Boado, R. J., and Chopra, I. J. (1989) Endocrinology 124, 2245-2251
8. St. Germain, D. L., Dittrich, W., Morganelli, C. M., and Cryns, V. (1990) J. Biol. Chem. 265, 20087-20090
9. Berry, M. J., Banu, L., and Larsen, P. R. (1991) Nature 349, 438-440
10. St. Germain, D. L., and Morganelli, C. M. (1989) J. Biol. Chem. 264, 3054-3066
11. Galton, V. A., and Hiebert, A. (1987) Endocrinology 120, 2604-2610
12. Bradford, M. M. (1976) Anal. Biochem. 72, 248-254
13. St. Germain, D. L., and Croteau, W. (1989) Endocrinology 125, 2735-2744
14. Goswami, A., and Rosenberg, I. N. (1984) J. Clin. Invest. 74, 2097-2106
15. Goswami, A., and Rosenberg, I. N. (1983) Endocrinology 112, 1180-1187
16. Sawada, K., Hummel, B. C. W., and Walfish, P. G. (1985) Endocrinology 117, 1259-1263
17. Köhrle, J., Rasmussen, U. B., Ekenbarger, D. M., Alex, S., Rokos, H., Hesch, R. D., and Leonard, J. L. (1990) J. Biol. Chem. 265, 6155-6163
18. Behne, D., Kyriakopoulos, A., Meinhold, H., and Köhrle, J. (1990) Biochem. Biophys. Res. Commun. 173, 1143-1149
19. Goswami, A., and Rosenberg, I. N. (1990) Biochem. Biophys. Res. Commun. 17, 6-12
20. Sakane, S., and Chopra, I. J. (1990) Endocrinology 121, 2709-2715
21. Silva, J. E., Mellen, S., and Larsen, P. R. (1987) Endocrinology 121, 650-656
22. Safran, M., and Leonard, J. L. (1991) J. Biol. Chem. 266, 3233-3238
23. Berry, M. J., Kieffer, J. D., and Larsen, P. R. (1991) Endocrinology 129, 550-552
24. Safran, M., Farwell, A. P., and Leonard, J. L. (1991) J. Biol. Chem. 266, 13477-13480