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The Role of Selenium in Thyroid Hormone Action*

MARLA J. BERRY AND P. REED LARSEN

Thyroid Division and Howard Hughes Medical Institute, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, Massachusetts 02115

I. Introduction

RECENT progress in the field of thyroid hormone deiodination has led to a greater understanding, not only of this critical process, but also of mechanisms more basic to protein synthesis in general. The cloning of the type I deiodinase, demonstration that this enzyme is a selenoprotein, and subsequent identification of selenocysteine insertion sequences in the noncoding portion of the gene have provided considerable insight into the mechanism of incorporation of this unusual amino acid into proteins. The purpose of this review is to discuss some of these advances and their implications. We begin by providing a brief background of the deiodination process and the discovery of the enzymes involved, followed by a discussion of some of the extensive characterization studies that have been performed on these enzymes over the years. Comprehensive reviews of these earlier studies have been published previously (1, 2). We next describe the expression cloning of the type I deiodinase and its identification as a selenoenzyme, evidence of which was provided from nutritional, biochemical, and molecular biological techniques. The latter permitted biochemical analyses of the wild type selenocysteine-containing deiodinase and a mutant enzyme containing cysteine, providing insight into the importance of this rare amino acid in the active site of the enzyme. Evidence is reviewed which indicated that the type II deiodinase is not a selenoprotein. The discovery that selenocysteine is encoded by a UGA codon, normally a stop codon, led to the question: How is UGA encoding selenocysteine distinguished from UGA specifying termination? Studies addressing this recognition process are presented. Finally, the presence of selenium in the type I deiodinase has important clinical implications for treatment of combined selenium and iodine deficiency, and these are addressed.

Thyroid hormone is essential for many biological processes, including normal development, growth, and metabolism. It has been recognized for a number of years that most, if not all of the biological effects of thyroid hormone can be attributed to T_3. The thyroid gland is the sole source of thyroid hormones, and when iodine is sufficient, its main secretory product is T_4. Since the identification of T_3 in human plasma by Gross and Pitt-Rivers in 1952 (3) and the demonstration of its greater biological potency compared to T_4, the importance of iodothyronine deiodination in thyroid hormone action has received increasing attention. The subsequent discovery that thioureylene drugs impaired both the physiological effects of T_4 and its metabolism was a major advance toward systematic evaluation of the role of deiodination in thyroid hormone function (4). Propylthiouracil (PTU) was shown to block T_4 to T_3 conversion and decrease T_4 stimulation of hepatic α-glycerophosphate dehydrogenase (GPD) (5), lending support to the thinking, generally accepted at that time, that T_3 was the biologically active thyroid hormone. However, the situation was complicated by the subsequent demonstration that PTU impaired certain T_4-mediated processes, while having minimal impact on others. In agreement with the studies above, PTU reduced T_4 stimulation of GPD to about one-third of the level in untreated animals,

Address requests for reprints to: Marla J Berry, Ph.D., Thyroid Division, Brigham and Women's Hospital, Harvard Medical School, 75 Francis Street, Boston, Massachusetts 02115.
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and serum T_3 levels were decreased to a similar extent. However, in the same animals, PTU had little or no effect on either T_4 stimulation of growth or its suppression of TSH secretion (6), both of which are pituitary-specific processes.

These results suggested one of two possible explanations. Either T_4 was biologically potent in pituitary but not in other tissues, or T_3 was the active hormone, and T_4 to T_3 conversion in the pituitary was not blocked by PTU. Several lines of evidence eventually proved the latter explanation to be correct. The first of these was the demonstration that suppression of TSH secretion correlated both quantitatively and temporally with T_3 binding to pituitary nuclear receptors, not with serum T_3 (7). After injection of labeled T_4, virtually all of the labeled iodothyronine bound to nuclear receptors was T_3 (7, 8). Silva and Larsen (7) also showed that, after administration of either T_4 or T_3 to hypothyroid rats, the quantities of nuclear T_3 were identical, and TSH was suppressed to a similar extent. At about the same time, it was shown that in different tissues, the contributions of serum T_4 and T_3 to nuclear T_3 differed (8, 9). This approach used [^{125}I]T_4 and [^{131}I]T_3 to follow the fate of the two iodothyronines *in vivo*. These studies showed that in pituitary a significant fraction of nuclear T_3 was derived from serum T_4 by local or intracellular deiodination, the remainder being contributed by serum T_3, whereas in liver, most of the nuclear T_3 derived from serum T_3. The nuclear thyroid hormone receptors in pituitary were shown to be approximately 80% saturated, whereas in liver, receptors were only approximately 50% saturated, the difference being accounted for by intracellular T_4 to T_3 deiodination in the pituitary (8). The next major advance was the finding that iopanoic acid inhibition of T_4 to T_3 conversion in pituitary blocked T_4-mediated suppression of TSH secretion and induction of GH synthesis. Iopanoic acid had no effect on the ability of T_3 to induce these responses, confirming that conversion to T_3 is required for these biological effects (10, 11). And finally, demonstration of PTU-insensitive T_4 to T_3 conversion in pituitary fragments, homogenates, and microsomes clearly showed that the deiodination process in this tissue differed from that in liver and kidney (12–14). PTU-insensitive deiodination was subsequently demonstrated in cerebral cortex (15, 16), brown adipose tissue (17, 18), and placenta (19), and the ensuing years produced numerous studies elucidating the differences between the PTU-sensitive (type I) and -insensitive (type II) deiodinase pathways and enzymes (20, 21).

II. Biochemical Properties and Physiological Roles of Type I and II Deiodinases

The properties of type I and II deiodinases are summarized in Table 1. In addition to the critical differences in PTU sensitivity and tissue distribution discussed above, these enzymes are distinguishable by their enzyme kinetics and substrate specificity, and more importantly by their physiological roles and responses to thyroid status (13, 14, 16, 20, 22). Both enzymes catalyze 5′-deiodination and can use either T_4 or reverse T_3 (rT_3) as substrate, and both require reduced thiol as cofactor. However, type I deiodinase exhibits a strong preference for rT_3 as substrate over T_4 and follows a ping-pong reaction mechanism with thiol as second substrate, whereas the type II deiodinase displays a slight preference for T_4 over rT_3, following a sequential reaction mechanism. The identity of physiologically important thiol cofactors are not known, but *in vitro* studies have shown that naturally occurring thiols, including glutathione, dihydrolipoamide, glutaredoxin, and thioredoxin, and the synthetic dithiol, dithiothreitol (DTT), can function in this capacity (23–27). The inhibition of type I deiodinase by PTU is competitive with thiol cosubstrate and uncompetitive with iodothyronine, due to the reaction of this inhibitor with enzyme-substrate complex, but not with free enzyme.

The physiological role of type I deiodination is to provide a circulating source of T_3 to the peripheral tissues, derived from T_4 produced by the thyroid gland. Type I deiodinase activity in liver and kidney increases in hyperthyroid and decreases in hypothyroid animals (28). In the thyroid cell, both TSH and the circulating thyroid-stimulating immunoglobulin present in patients with Graves' disease increase type I deiodinase activity (29–35). The former is particularly prominent in iodine-deficient rats, in which enhanced thyroidal T_4 to T_3 conversion may even contribute to an enhanced efficiency of total body T_4 to T_3 conversion (36). Furthermore, the elevated serum T_3 may itself stimulate the conversion of T_4 to T_3 in hyperthyroidism due to Graves' disease and thus contribute to the markedly greater sensitivity of T_4 to T_3 conversion in hyperthyroid than euthyroid humans, to inhibition by PTU (37).

Type II deiodinase functions to regulate intracellular T_3 levels in those tissues where T_3 is most critical, the brain, pituitary, brown fat, and placenta. The type II enzyme allows the pituitary to monitor circulating T_4, thereby regulating TSH secretion, and thus thyroidal T_4 synthesis. In brown fat, type II deiodinase is regulated by α-1-adrenergic stimulation (17, 38). During cold stress, increased type II activity produces the elevated intracellular T_3 required for thermogenesis, which is mediated by T_3-induced synthesis of the uncoupling protein, thermogenin (39, 40). The response of type II deiodinase to thyroid status is opposite that of type I (15, 21). When T_4 is limiting, its rate of deiodination to T_3 in type II tissues is increased, allowing the intracellular environment in these tissues to remain relatively euthyroid when

TABLE 1. Properties of rat type I and II microsomal iodothyronine deiodinases

	Type I	Type II
Substrate preference	$rT_3 \gg T_4 > T_3$	$T_4 > rT_3$
Deiodination site	Outer and inner ring	Outer ring
K_m for rT_3	1×10^{-7} [a]	3×10^{-9} [b]
K_m for T_4	2×10^{-6} [a]	1×10^{-9} [b]
K_i for PTU[rT_3]	1×10^{-6} [c]	$\sim 10^{-3}$ [c]
K_i for PTU[DTT]	5×10^{-7} [c]	$\sim 10^{-3}$ [c]
K_i for GTG	3×10^{-9} [d]	3×10^{-7} [d]
Monomer size	29 kDa	?
Active site	Se (selenocysteine)	S (cysteine)?
Effect of T_4 administration	Increase	Decrease
Physiological role	Extracellular T_3 production	Intracellular T_3 production

K_i values are for inhibtion of rT_3 deiodination for type I and T_4 deodination for type II assays, at the DTT concentrations indicated below.
[a] Liver microsomes assayed at 3–5 mM DTT (2).
[b] Brain microsomes assayed at 20 mM DTT (16).
[c] Kidney microsomes assayed at 5 mM DTT, BAT, and brain microsomes assayed at 0.5, 1, and 2 mM DTT (20).
[d] Liver microsomes assayed at 1 mM DTT, BAT microsomes assayed at 40 mM DTT (65).

the rest of the body is hypothyroid. Under these circumstances, however, the type II deiodinase may also contribute significantly to the pool of circulating T_3, as type II enzyme levels are elevated and type I levels depressed (41). *In vivo*, hyperthyroidism decreases levels of type II deiodinase activity. *In vitro* administration of T_4 to cultured astrocytes produces rapid changes in the actin cytoskeleton and a parallel decrease in type II activity, presumably mediated by alterations in the subcellular distribution of the type II deiodinase protein (42, 43). A third deiodinating enzyme catalyzes inactivation of T_3 by removal of a tyrosyl ring iodide, a process termed 5 deiodination (15). This enzyme, designated type III, is present in brain and placenta. The response of type III deiodinase to thyroid status parallels type I, resulting in increased T_3 degradation when hormone is abundant and slower degradation in the hypothyroid state, conserving active hormone in the critical tissues when it is limiting.

In addition to the tissue differences in iodothyronine metabolism, thyroid hormone transport also varies among tissues. In particular, exchange of T_3 between serum and brain is significantly slower than exchange with other tissues, thus intracellular T_4 to T_3 conversion is critical for thyroid hormone action in brain (44).

III. Purification and Characterization of Type I Deiodinase

During the past decade, a number of laboratories have devoted considerable efforts toward the purification and biochemical characterization of the type I deiodinase.

Mol *et al.* (45, 46) succeeded in purifying rat liver type I deiodinase approximately 2400-fold by affinity chromatography on PTU-Sepharose. This resulted in a preparation of approximately 50% purity exhibiting a subunit molecular mass of approximately 25 kilodaltons (kDa). Subsequent affinity labeling studies with the substrate analog, bromoacetyl-T_4, showed that the amounts of deiodinase activity in liver and kidney microsomes after various treatments (*e.g.* hypo- and hyperthyroidism) correlated with the amount of labeling of a 27-kDa protein (47). Kohrle *et al.* (48, 49) further established that the potency of competitive inhibitors of type I deiodination paralleled their ability to inhibit labeling of the 27-kDa protein. All of these results pointed to the 27-kDa protein as a component of the type I enzyme. The native molecular mass of the type I deiodinase was subsequently determined by sedimentation analysis to be approximately 55 kDa (50). Despite the progress in purification of this enzyme and the ability to label it covalently, sufficient sequence information to identify a complementary DNA (cDNA) for this protein was not forthcoming. Another approach to this problem, the use of antibodies against deiodinase preparations to screen cDNA libraries, resulted in the identification of a cDNA-encoding protein disulfide isomerase (PDI), presumably due to contaminating anti-PDI antibodies (51). PDI was subsequently shown to be unrelated to type I deiodinase (52, 53).

IV. Cloning of the cDNA for the Type I Deiodinase

A quite different strategy, expression cloning, has proven successful in isolating clones for a number of proteins that possess potent bioactivity but which, due to properties such as low abundance, instability, or membrane localization, are difficult to purify. When these problems were encountered with the type I deiodinase, the specificity, sensitivity, and simplicity of the deiodinase assay suggested the use of this strategy. In this approach, a cDNA encoding a particular protein is iden-

tified in a pool, or library, on the basis of the activity it expresses. The pool is subdivided and the assay for activity repeated until a single clone is obtained. The assays can be performed after expression by transient transfection of cells in culture, or more commonly, by injection of RNA transcripts of the cDNA into *Xenopus* oocytes. The oocyte system has the advantage of being highly efficient at synthesizing proteins from microinjected RNAs but the disadvantage of being quite tedious compared to transfection, as each cell must be injected individually. The first step in this approach is to demonstrate expression of activity from injected messenger RNA (mRNA). We and others showed that type I deiodinase activity was easily detectable in oocytes after injection of rat liver and kidney mRNA (53, 54).

Before construction of a cDNA library, we wished to identify the best tissue source of RNA for this enzyme. It had been shown some time ago that levels of renal and hepatic type I deiodinase activity were elevated in hyperthyroid and depressed in hypothyroid animals. Using oocyte expression of deiodinase activity to quantitate levels of deiodinase mRNA, we established that these changes in activity reflected changes in the amount of type I deiodinase mRNA (53). The high levels of deiodinase mRNA in hyperthyroid liver led us to choose this source for cDNA synthesis. Another requirement for successful expression cloning is that a functional protein must be encoded by a single mRNA, as the mRNAs for multimeric proteins would be separated in the subdivision process. And, because expression screening relies on expression of a functional protein, a full-length cDNA must be present to detect activity. One way to circumvent these requirements is to use hybrid arrest of translation. This approach measures the ability of a cDNA to inhibit expression from a positive mRNA pool, by virtue of the cDNA hybridizing to the functional mRNA and blocking its ability to be translated. The cDNA does not have to be full length to inhibit translation, and inhibition of expression of one subunit in a multisubunit protein will block activity. Hybrid arrest screening was, in fact, used by St. Germain *et al.* (55) to identify a partial cDNA for the type I deiodinase.

We elected to use the direct expression approach, because it ensures that if a positive clone is obtained, it will be full length and functional. We size-fractionated hyperthyroid rat liver mRNA before injection, which established a fairly discrete size for the active mRNA species, approximately 2 kilobases (kb) (53). Although the subunit composition of the type I enzyme was unknown at that time, this result pointed to a single mRNA species, providing us the encouragement needed to attempt direct screening. Construction of a cDNA library was the next step. Traditionally, cDNAs are inserted into cloning vectors after ligation of linkers that produce symmetrical ends, resulting in insertion in random orientation. Transcription from either side of the cloning site will thus result in approximately 50% of the products being antisense, which would in turn decrease the efficiency of expression by half. In practice, the impact may be greater, due to hybridization of antisense with sense transcripts, resulting in hybrid arrest. For these reasons, we used an orientation-specific strategy to construct our cDNA libraries (Fig. 1). The choice of cloning vector is also crucial. Vectors derived from bacteriophage-λ allow highly efficient cDNA insertion and replication. However, a cDNA of this size would be only 3–5% the size of the vector, and large amounts of phage DNA would be required to produce small quantities of RNA transcripts. Lambda *Zap*II has been engineered to allow simple *in vivo* conversion to a small plasmid vector, Bluescript, whereby the cDNA insert then represents 30–50% of the total DNA, and transcription from the plasmid is quite efficient. We prepared several λ*Zap* libraries using cDNA size-fractionated to the range of approximately 2 kb. Size fractionation reduced the total amount of cDNA by about 10-fold, thus increasing the screening efficiency by this amount. Libraries were subdivided into pools, converted

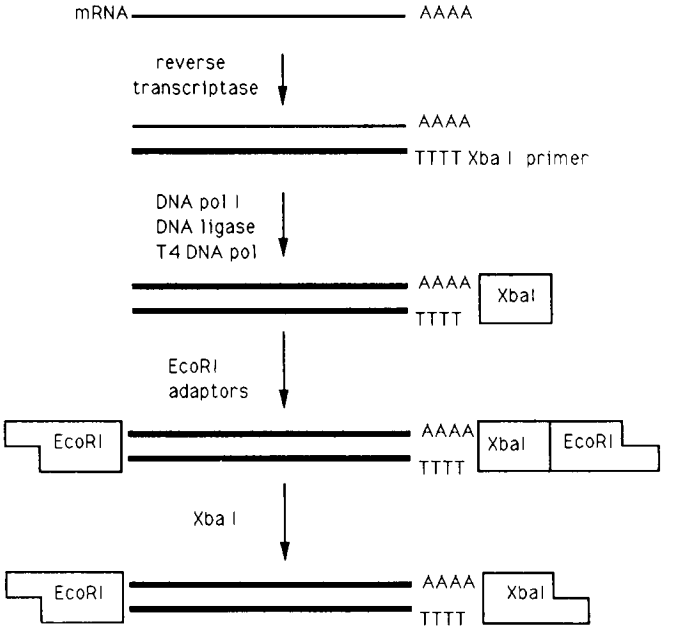


FIG. 1. Type I iodothyronine 5′-deiodinase cloning strategy. Isolate RNA from hyperthyroid rat liver. Oligo dT select poly(A)$^+$ RNA. Synthesize orientation-specific cDNA. Size-fractionate on agarose to obtain 1.9–2.5 kb cDNA. Ligate into *Eco*RI + *Xba*I-digested λZAP DNA. *In vitro* package DNA into λ-phage particles. Titer library and subdivide into 10 pools. Convert to plasmid by coinfection with phagemid. Prepare plasmid DNA. Transcribe RNA *in vitro*. Inject into *Xenopus* oocytes. Incubate oocytes 2–3 days, then homogenize. Assay for [^{125}I]rT_3 deiodination. Subdivide active pool, prepare DNA, transcribe, inject, and assay. Repeat screening until a small pool is obtained. Plate to obtain individual colonies and repeat screening until a single positive clone is identified.

to plasmid, and tested for expression of deiodinase in oocytes. Finally, a signal of activity was detected, only slightly above background, but reproducible. When this positive pool was further subdivided and screened, a higher signal was obtained, and repeating this process eventually resulted in the identification of a single positive clone containing a 2.1-kb insert (56). The kinetic properties, substrate specificity, and PTU sensitivity of the protein encoded by this cDNA confirmed its identity as type I deiodinase. Using a labeled RNA probe generated from the clone, we examined the tissue distribution of deiodinase RNA in rat. Hybridizing RNA of about 2.1 kb was present in liver, kidney, thyroid, and pituitary, tissues which express type I deiodinase. No hybridization was observed in RNA from brown adipose, lung, spleen, or small intestine, tissues in which the enzyme is not detectable. Northern analysis of RNA from hypothyroid, euthyroid, and hyperthyroid rats showed that levels of hybridizing RNA paralleled thyroid status, confirming the expression studies in oocytes (Fig. 2).

V. Type I Deiodinase Contains Selenocysteine, Encoded by a TGA Codon

The sequence of the type I deiodinase cDNA showed an initiator methionine (ATG) codon at nucleotides (nt) 7–9 and an open reading frame of 125 codons, terminating with a TGA (UGA) codon at nt 382–384. This open reading frame predicted a protein of 125 amino acids, or approximately 14 kDa, a surprising result, since affinity-labeling studies indicated an approximately 27-kDa subunit size. The close agreement between the size of the cDNA (2106 nt) and the hybridizing mRNA (2.1 kb) made it likely that little if any 5′-untranslated sequence was missing. If this open reading frame represented the entire coding sequence, then deletion of sequences beyond the TGA codon should have no effect on expression in oocytes. In fact, this deletion was inactive, establishing that sequences downstream of the UGA are necessary for activity (Fig. 3a). To determine the function of these sequences, we inserted two nucleotides at a position just beyond the UGA codon, generating a frame shift of the sequences downstream of the insertion. The frame shift mutant was also inactive, indicating that sequences downstream of the UGA must be in the same reading frame as the upstream sequence, and therefore are likely to be coding. Deletion of nt 745–1363 produced a protein with full activity, confirming that sequences downstream of the UGA are essential.

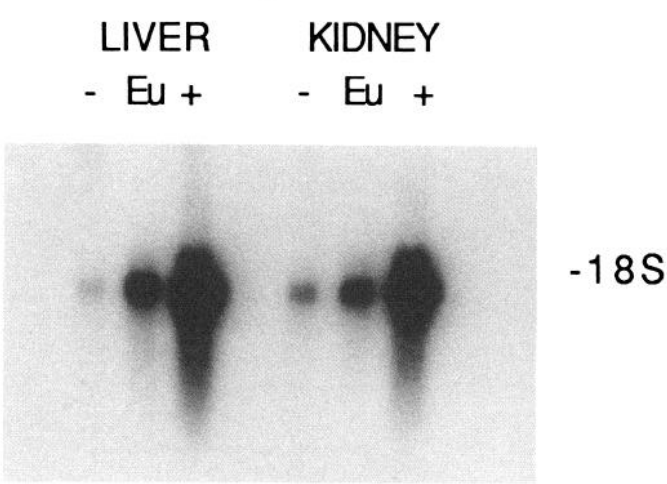
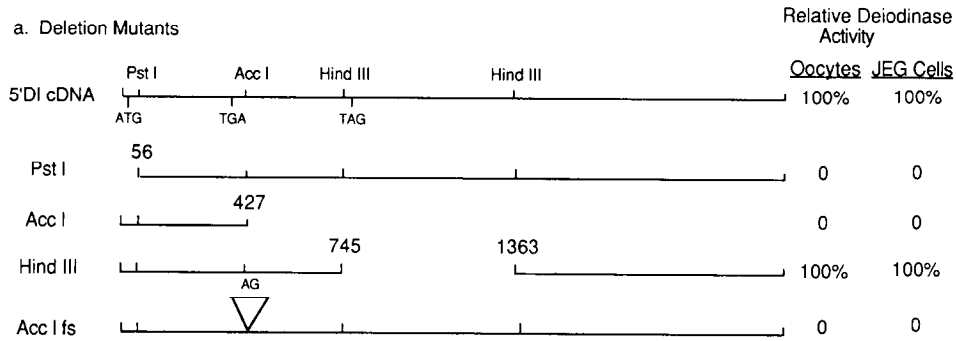


FIG. 2. Effect of thyroid status on Type I 5′-deiodinase mRNA levels. Rats were made hypothyroid by treatment for three weeks with 0.02% methimazole in drinking water. Hyperthyroidism was induced by ip injection of 50 μg T_3 daily for 3 days. Liver and kidney poly(A)$^+$ RNA (5 μg) from hypothyroid (−), euthyroid (Eu), and hyperthyroid (+) rats was probed with type I deiodinase cRNA. The migration position of 18S RNA is shown. [Reprinted with permission from Berry *et al.*: *Nature* 353:273, 1991 (84).]

An explanation for these puzzling results was suggested from studies on mammalian glutathione peroxidase (57) and the bacterial enzymes formate dehydrogenase (58) and glycine reductase (59). In each of these enzymes, UGA encodes the amino acid selenocysteine, an analog of cysteine in which sulfur is replaced by selenium. If the UGA codon in the type I deiodinase were also translated as selenocysteine, the protein would terminate at a UAG stop codon at nt 778–780, and its predicted molecular mass would be approximately 29 kDa, in close agreement with the size of the affinity-labeled protein. To confirm that this was the case, we used site-directed mutagenesis to change the UGA codon to a UAA stop codon. This mutation resulted in loss of deiodinase expression in oocytes, confirming that UGA was not read as a stop signal (Fig. 3b). We next changed UGA to UUA, encoding leucine, and this mutant was also inactive. Thus, leucine cannot substitute for selenocysteine in producing a functional enzyme. Finally, and of greatest interest, mutation of UGA to UGU, a cysteine codon, produced a functional deiodinase, albeit with altered properties compared to the wild type protein. Analysis of the protein products of the wild type and mutant cDNAs by *in vitro* translation in reticulocyte lysates confirmed the results of the expression studies in oocytes. *In vitro* synthesized wild type deiodinase RNA produced a small amount of full-length approximately 29-kDa protein, with the majority of the translated product being approximately 14 kDa, consistent with termination at the UGA codon. We interpreted this as being due to inefficient translation of UGA as selenocysteine in the reticulocyte lysate. Translation of the UAA stop codon mutant resulted in loss of the 29-kDa protein without affecting production of the 14-kDa protein. Both the leucine and cysteine codon mutants produced a 29-kDa protein with high efficiency. These results confirmed the translation of UGA in type I deiodinase as



b. UGA Codon Mutants

cDNA	mRNA	Translation	
TGA	UGA	opal or Secys	100%
TAA	UAA	ochre	0
TTA	UUA	leucine	0
TGT	UGU	cysteine	10%

FIG. 3. Expression of type I 5′-deiodinase from wild type and mutant constructs. a, Deletions were constructed by restriction digestion at the indicated sites, followed by agarose gel purification of the desired fragments, religation, and mapping of the resulting constructs. RNAs were transcribed *in vitro* and 0.1–20 ng injected per oocyte. DNA transfections and deiodinase assays are as described previously (37). Oocyte activity of 100% is defined as deiodination of 30–40% 2 nM [^{125}I]rT_3/h with a homogenate of four oocytes injected with 0.1 ng deiodinase RNA per oocyte. JEG-3 cell sonicates were incubated with 25 mM dithiothreitol and 5 nM [^{125}I]rT_3 for 1 h and $^{125}I^-$ was quantitated as described (37). All assays were in duplicate. ND, Not done. b, The TGA codon at nt 382 was replaced by the indicated codons using the P-select *in vitro* mutagenesis system of Promega (Madison, WI). Oligonucleotides corresponding to the desired changes were annealed to single-stranded DNA, and double-stranded DNA was synthesized. The entire coding regions of plasmids thus obtained were sequenced to confirm that these were the only mutations. Injections and assays were as described above. [Reprinted with permission from Berry *et al.*: *Nature* 353:273, 1991 (84).]

selenocysteine, establishing this enzyme as a new member of the UGA-selenocysteine family.

VI. Nutritional Studies Suggest a Role for Selenium in Thyroid Hormone Metabolism

While these cloning studies were in progress, a role for selenium in thyroid hormone metabolism was emerging from nutritional studies. Arthur, Beckett, and their colleagues (60) found that rats maintained on a selenium-deficient diet for 4–6 weeks had elevated levels of serum T_4 and depressed serum T_3 compared to animals maintained on a normal selenium-sufficient diet. These alterations in hormone levels were subsequently shown to be accompanied by a marked decrease in hepatic deiodinase activity. Time course studies showed these changes to be progressive from 0–5 weeks of selenium deficiency (61), leading these investigators to propose that selenium was either a component of the type I deiodinase itself or of a necessary cofactor (62, 63). Plasma TSH levels were elevated nearly 2-fold, and pituitary GH levels decreased by one-third in selenium deficiency, despite high levels of serum T_4 (64). These effects can be explained by the combinatorial effects of lower pituitary type II deiodinase activity due to high T_4, which would counterbalance the elevated levels of T_4, and the lower serum T_3 leading to decreased saturation of nuclear receptors. Selenium deficiency also resulted in depletion of thyroidal iodine and thyroid hormones, presumably due to increased synthesis and secretion of thyroglobulin by the thyroid, stimulated by the elevated TSH levels.

Studies with [^{75}]Se by Behne *et al.* (65) showed that, after severe selenium depletion, the distribution of label in different tissues indicated that the brain and endocrine glands have priority on supplies of this element. This idea was supported by results of Arthur *et al.* (64), showing that repletion of rats with a single injection of 10 μg Se/kg body wt restored pituitary GH, plasma TSH, and thyroidal iodide and hormone levels, but did not affect hepatic deiodinase or serum T_4. A higher dose, 200 μg Se, restored serum hormone levels and hepatic deiodinase activity to normal. Beckett *et al.* (62) also observed a decrease in brain type II deiodinase activity in selenium-deficient animals, and proposed that this protein might be a selenoenzyme. However, as discussed above, changes in subcellular distribution of type II deiodinase as a result of the elevated T_4 concentrations associated with selenium deficiency might also explain the decrease in enzyme activity. Subsequent studies by our group and by others argue that the type II deiodinase is not a selenoprotein (see below) (66, 67).

The initial [^{75}]Se labeling studies described above identified an approximately 27-kDa selenoprotein in rat liver, kidney, and thyroid (68). Behne *et al.* (69) and Arthur *et*

al. (70) subsequently demonstrated that [^{75}]Se and the substrate analog, [^{125}I]BrAcT_3, both label a 27-kDa protein in rat liver, providing compelling evidence that selenium was a component of the type I deiodinase.

VII. Selenocysteine Confers Specific Properties on the Deiodinase Enzyme

Although the oocytes allowed us to assay wild type and mutant type I deiodinase proteins, this system was not amenable to production of large amounts of enzyme on a routine basis. Further characterization of these enzymes was facilitated by first introducing the cDNAs into a plasmid, CDM-8, which can be expressed in mammalian cells, for example COS-7 monkey kidney cells. The plasmid is then introduced into cells by transient transfection, achieved by adding precipitated DNA to the cell monolayer and chemically permeabilizing the membranes. Transient expression of transfected DNAs is efficient, reproducible, and technically easy. Using this system, we were able to perform detailed biochemical analyses of the wild type and cysteine mutant deiodinase proteins (71). As mentioned above, the mutant in which selenocysteine was replaced by cysteine was active, but its properties, summarized in Table 2, were quite different from those of the wild type enzyme. The wild type deiodinase exhibits an approximately 100-fold preference for rT_3 over T_4 as substrate. Substitution of cysteine resulted in a 10-fold decrease in the inhibition constant (K_i) for T_4 and a 10-fold increase in the Michaelis-Menten constant (K_m) for rT_3, thus eliminating the preference for rT_3 characteristic of the wild type enzyme. This increase in K_m was interpreted in our initial studies as a decrease in activity in the mutant, due to the conditions of the assay, which employed tracer concentrations (~2 nM) of rT_3 (Fig. 3). When assayed at higher substrate concentrations, this difference in activity is less apparent. The cysteine mutant exhibits an approximately 300-fold decrease in sensitivity to both competitive inhibition by PTU *vs.* thiol and uncompetitive inhibition *vs.* iodothyronine. Previous studies comparing gold inhibition of the selenoenzyme, glutathione peroxidase, to three thiol active-site enzymes showed that the selenoenzyme was much more sensitive to competitive inhibition by gold (72). This inhibition is thought to be due to direct interaction of gold (Au^+) with selenium (Se^-). We found the cysteine mutant deiodinase to be approximately 100-fold less sensitive to competitive inhibition by gold thioglucose (GTG) than the wild type selenoenzyme. BrAcT_3 affinity labeling of the wild type and cysteine mutant enzymes with ^{125}I-labeled substrate analogs in the presence of these inhibitors correlates closely with deiodinase activity (Fig. 4). Selenocysteine and cysteine are structurally similar, differing solely by the substitution of a selenium atom for a sulfur atom. Selenium is directly below sulfur in the periodic table, thus the two atoms have the same valence. However, the selenol of selenocysteine has a pK of 5.2, whereas the thiol of cysteine has a pK of 8.3, thus cysteine is mostly protonated at physiological pH, whereas selenocysteine is ionized and therefore more reactive. As the selenium to sulfur change is the only difference between the wild type deiodinase and the cysteine mutant, the higher reactivity of selenocysteine must account for the greater sensitivity of the wild type enzyme to PTU and gold. We conclude from these studies that the presence of selenocysteine determines many of the specific biochemical properties of the type I deiodinase.

Based on these results, we propose the scheme for type I iodothyronine deiodination, competitive inhibition by gold, and uncompetitive inhibition by PTU shown in Fig. 5. According to this scheme, the selenolate anion of selenocysteine is the iodide acceptor in the active center of the enzyme. The enzyme reacts with T_4, removes the 5′-iodide, and releases T_3. The selenolyl iodide form of the enzyme can then react with thiol cofactor, release free iodide, and regenerate free enzyme. PTU competes with thiol for the selenolyl iodide form of the enzyme and forms the inactive mixed selenolyl-thiol complex. The competitive inhibitor, gold, reacts with the selenolate anion in the free enzyme to form the inactive selenolyl-gold complex.

TABLE 2. Properties of wild type and cysteine mutant type I deiodinases

	Wild type (Sec)	Cysteine mutant (Cys)
K_m for rT_3 (μM)	0.25	2.7
K_i for T_4 (μM)	11	1
K_i for PTU[rT_3] (μM)	0.2	55
K_i for PTU[DTT] (μM)	0.12	14
K_i for GTG (nM)	6.6	600
Active site	Se (selenocysteine)	S (cysteine)

All reactions contained 10 mM DTT. All values are for inhibition of 5′-monodeiodination of T_3 under conditions in which enzyme is rate limiting (55).

VIII. Evidence That Type II Deiodinase Is Not a Selenoprotein

The properties of the cysteine mutant bear some similarity to those of the type II deiodinase found in brain, pituitary, and brown adipose, suggesting that this enzyme may contain cysteine instead of selenocysteine in the active site, and the reduced sensitivity to gold exhibited by the cysteine mutant provided a way of addressing this question. Rats were administered 5 mg/100 g body wt GTG or thioglucose as a control, and deiodinase activities from various tissues were assayed (67). A single

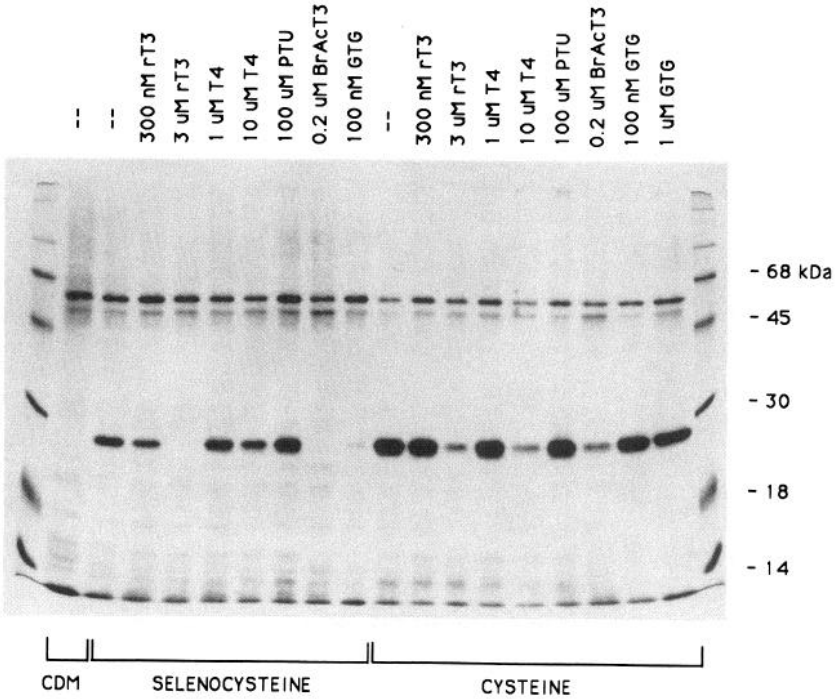


FIG. 4. Affinity labeling of wild type and cysteine mutant deiodinase proteins produced by transient transfection of COS-7 cells. COS-7 cells were transfected with either wild type (selenocysteine) 5′-deiodinase cDNA or cysteine-mutant (cysteine) deiodinase cDNA in the vector, CDM-8, or with vector alone (CDM), as indicated at the *bottom* of the lanes. Labeling with [^{125}I]BrAcT$_3$ was for 10 min with the indicated additions.

injection of GTG inhibited liver type I deiodinase by approximately 50% but had no effect on type II activity in brown adipose tissue (BAT). Injection of the same dose for 2 days completely inhibited types I and II deiodinase, indicating that both enzymes were inhibitable, but their sensitivities differed. Because *in vitro* studies are less susceptible to variations in tissue uptake or metabolism of the inhibitor, we examined the effects of GTG on deiodinase activities in tissue sonicates or microsomal protein. Type I deiodinase from liver was approximately 100-fold more sensitive to GTG than was type II enzyme from BAT. Manipulation of reaction conditions allowed us to assay both deiodinase activities in the same tissue, pituitary. Type I and type II deiodinases from pituitary display an approximately 100-fold difference in sensitivity to GTG, giving strong support to the hypothesis that the type II enzyme does not possess the unique selenocysteine active center. This is not to suggest that the only difference between the type I and II enzymes is the presence or absence of selenocysteine. Type II deiodinase exhibits an approximately 1000-fold lower K_m for T_4 and rT_3 and an approximately 100-fold higher K_i for PTU than does the cysteine mutant of the type I enzyme. Sedimentation studies by Safran and Leonard (50) indicated a native molecular mass for the type II enzyme of approximately 199 kDa, *vs.* approximately 55 kDa for the type I enzyme. Further, Northern analysis under low stringency conditions failed to identify a type I cross-reactive mRNA in cold-stimulated BAT, indicating that the degree of sequence homology between the type I and II sequences was low (56).

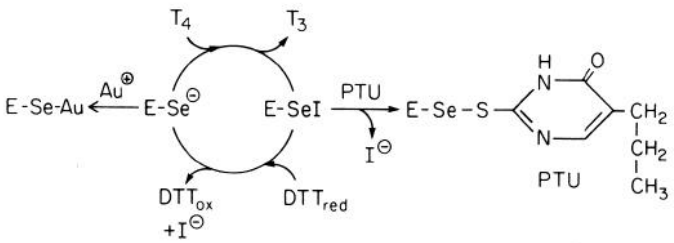


FIG. 5. Proposed mechanism for type I iodothyronine deiodination and inhibition by PTU and gold. [Reprinted with permission from Berry *et al.*: *J Biol Chem* 266:14155, 1991 (71).]

This hypothesis was further strengthened by results of Safran *et al.* (66), which showed that the type II deiodinase in glial cells did not incorporate [^{75}]Se under conditions permitting labeling of the type I deiodinase in porcine kidney cells. Selenium depletion of the culture media did not affect type II activity, whereas both type I deiodinase and glutathione peroxidase activity were inhibited. Furthermore, in more recent studies it was shown that in hypothyroid selenium-deficient rats, brain type II deiodinase activity was not different from selenium-supplemented controls (73). This further supports the above indirect evidence that the type II deiodinase does not contain selenocysteine in the active site.

IX. Why is Selenocysteine Present in Proteins?

The selenoenzymes glutathione peroxidase, formate dehydrogenase, glycine reductase, and 5′-deiodinase are all oxidoreductases which undergo ping-pong reaction mechanisms. It is possible that selenocysteine has been

maintained in these proteins through evolution because of its high efficiency at catalyzing this type of reaction. It has been speculated that early in evolution, TGA was more frequently used as a selenocysteine codon, and that this usage may have been selected against by the emergence of oxygen in the atmosphere, such that it was only maintained in anaerobic environments or in proteins where it is protected from oxidation (74, 75). Evolution of UGA from a sense codon to a stop codon is supported by the fact that UGA is more commonly used than UAG and UAA stop codons (74). The fact that UGA encodes selenocysteine in organisms ranging from *Escherichia coli* to man also supports early usage of UGA as a selenocysteine codon.

A cysteine variant (UGC codon) of formate dehydrogenase occurs in nature in methanobacteria. This protein is otherwise highly homologous to the selenocysteine-containing formate dehydrogenase in *E. coli*, indicating a common origin (76). However, the cysteine form is less active and is present in greater amounts, presumably to compensate for this decrease in activity (76). *In vitro* mutagenesis of the *E. coli* formate dehydrogenase selenocysteine to cysteine produced an enzyme with a 300-fold decrease in turnover (k_{cat}) (77). Our preliminary results indicate that this same substitution in the type I deiodinase results in a 100 to 300-fold decrease in k_{cat} (our unpublished results). Synthetic selenoproteins have also contributed to our understanding of the unique properties of selenocysteine. Using automated peptide synthesis, an analog of metallothionein was produced in which the seven cysteines were replaced by selenocysteines, resulting in altered copper binding (78). Chemical conversion of the active site cysteine in subtilisin to selenocysteine changed the activity of this enzyme from that of a protease to an acyl transferase (79), which also can function as a glutathione peroxidase (80).

X. New Insights into Protein Synthesis in Eukaryotes

Identification of selenocysteine in the active site of the type I deiodinase introduces an interesting physiological question: How does the cell distinguish a UGA codon for selenocysteine from a UGA stop codon? This process has been shown to occur cotranslationally, as transfer RNAs which recognize UGA and insert selenocysteine have been identified in bacteria and animals (81), and an elongation factor specific for this transfer RNA has been identified in *E. coli* (82). However, specificity must be conferred on the recognition process. Studies of bacterial formate dehydrogenase implicated a stem-loop or hairpin structure in the mRNA in the region of the UGA codon as being necessary and sufficient for translation of UGA as selenocysteine (83). We have shown that this is not the case for the type I deiodinase (84). Deletion of the sequences beyond nt 907 of the wild type deiodinase cDNA resulted in complete loss of activity (Fig. 6a). This deletion begins approximately 125 nt beyond the end of the coding region, in the 3′-untranslated (3′ut) portion of the mRNA. Deletion of the same sequences in the cysteine mutant had no effect; this construct produced a fully active product. Sequences in the 3′ut region must therefore be present for selenocysteine, but not for cysteine, incorporation. The deletions shown in Fig. 6a identified the region between nt 1363–1615 as being essential for selenocysteine insertion. These sequences are located greater than 1 kb downstream of the UGA codon in the wild type mRNA but are still functional if the spacing is altered. Inversion of these sequences abolished their function, even though this mutation preserves the secondary structure of the mRNA. The analogies to glutathione peroxidase already discussed led us to test whether the 3′ut region of this mRNA could substitute for the deiodinase 3′ut in supporting selenocysteine insertion. We constructed a hybrid between the coding region of the deiodinase clone and the 3′ut sequences of the rat glutathione peroxidase clone (Fig. 6b). Since it is a seleno-enzyme, glutathione peroxidase should also possess selenocysteine insertion sequences, and, in fact, it does. The 3′ut from glutathione peroxidase could substitute for the deleted sequences in the deiodinase mRNA, restoring its capacity to direct selenocysteine insertion. Deletion analyses of the hybrid construct identified a functional region of approximately 230 nt in the glutathione peroxidase 3′ut. Similarly, hybrids between the coding region of the rat deiodinase and the 3′ut from a human deiodinase clone are functional (Fig. 6b). The essential regions in the deiodinase and glutathione peroxidase 3′uts bear very little primary sequence similarity, suggesting that secondary structures may be involved in regulating selenocysteine insertion.

Computer analyses of the 3′ut sequences of the deiodinases and rat glutathione peroxidase predict the formation of stable RNA stem-loop structures. Support for the existence of such structures was obtained by analyzing small, carefully chosen deletions in the rat deiodinase and glutathione peroxidase 3′ut sequences. Removal of the the loop and the last 20% of the stem in the rat deiodinase clone resulted in complete loss of deiodinase activity (Fig. 6b). Even deletion of the loop alone in either the rat deiodinase (9 nt) or glutathione peroxidase clone (8 nt) abolished activity. Confirmation that these mutations abolished selenocysteine incorporation was obtained by *in vitro* transcription and translation of the wild type and mutant constructs. The deiodinase and hybrid deiodinase-glutathione peroxidase constructs which produced deiodinase activity also program translation of an approximately 29-kDa protein, whereas the

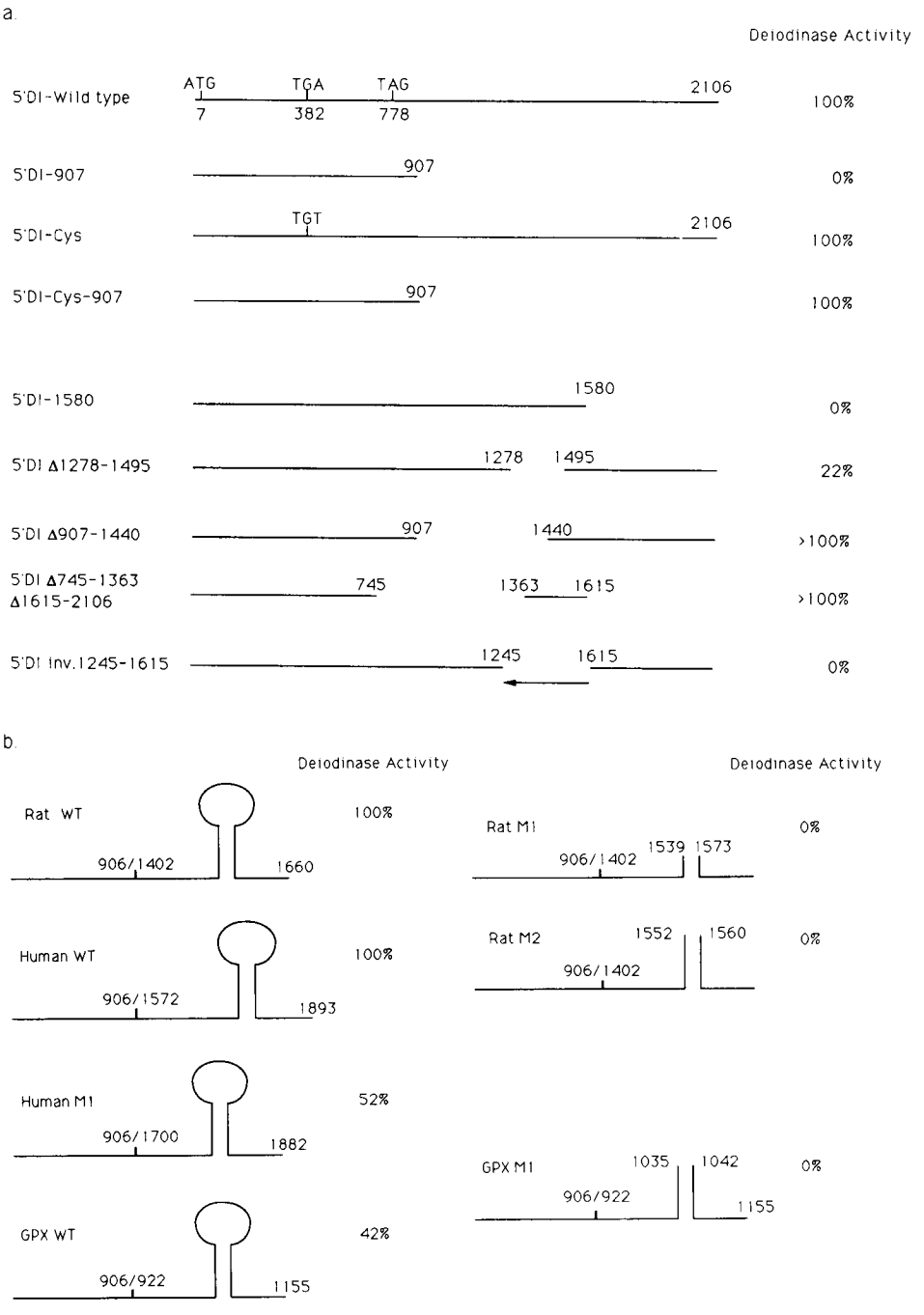


FIG. 6. Expression of 5′-deiodinase activity from wild type and mutant 5′-deiodinase constructs. a, Deletion and inversion mutations of rat 5′-deiodinase cDNA 3′ut region. Constructs were assayed for production of 5′-deiodinase activity after transient transfection in COS-7 cells. Reactions were performed as described for JEG cell sonicates in the legend for Fig. 3, except that rT_3 was present at 300 nM. Deiodinase activity at the level of the wild type rat 5′-deiodinase construct is defined as 100% and was equivalent to 5′-deiodination of 2 pmol rT_3/min·mg protein for TGA-containing constructs and 1 pmol rT_3/min·mg protein for TGT-containing constructs. Reprinted from Berry *et al.* (84). b, Rat 5′-deiodinase constructs containing 3′ut sequences from rat or human 5′-deiodinase or rat glutathione peroxidase cDNAs. Constructs containing either rat or human 5′-deiodinase or rat glutathione peroxidase 3′ut sequences adjacent to rat 5′-deiodinase coding sequences were assayed for production of 5′-deiodinase activity as above. Adapted from Berry *et al.*: *Nature* 353:273, 1991 (84).

inactive loop and stem-loop mutants produce the 14-kDa protein expected from termination at the UGA codon. These results show that sequences located greater than 1 kb downstream of the UGA codon in the deiodinase mRNA are necessary for its translation as selenocysteine, and that these sequences function in an orientation-specific but spacing-independent manner. We have, therefore, termed these essential 3′ut regions selenocysteine-insertion sequence motifs. How these selenocysteine-insertion sequence motifs function remains to be determined.

XI. Human Iodothyronine Deiodinase and Clinical Implications

The sensitivity of iodothyronine deiodination in man to PTU has been a subject of debate. PTU administration inhibits T_4 to T_3 conversion in hyperthyroid humans by approximately 50% (37) but has little effect in euthyroid patients (85–87). This may be due to the enhanced type I deiodinase activity in hyperthyroid liver, kidney, and thyroid (28, 33), together with the specific immunoglobulin stimulation of type I deiodinase through the TSH

receptor (34). The differences in sensitivity to PTU of the wild type and cysteine mutant rat type I deiodinases raised the question of whether selenocysteine was also present in the human type I enzyme. Using the rat type I deiodinase as a probe, we recently isolated a partial cDNA for the human liver type I deiodinase. A portion of this clone was then used as a probe to obtain the remaining coding region from a human kidney cDNA library, and splicing the two together resulted in a functional clone capable of expressing deiodinase activity. Sequencing of the human clone confirmed the presence of an in-frame UGA codon corresponding to the position of the UGA selenocysteine codon in the rat cDNA, and affinity labeling studies indicate that the protein product of the human cDNA is approximately 28 kDa, confirming translation of the UGA (88). Overall, the coding regions of the human and rat clones are about 88% homologous at the amino acid level, and the properties of the two enzymes reflect this homology. The sensitivity of the transiently expressed human enzyme to PTU is about 8-fold greater than that of the rat enzyme, but other kinetic properties of the two enzymes are quite similar. These studies indicate that selenocysteine is also present in the human type I deiodinase, and this finding has important clinical implications for the treatment of hyperthyroidism.

In central Africa, myxoedematous cretinism is prevalent in areas of severe iodine deficiency. These cretins are characterized by hypothyroidism with onset before or shortly after birth, absence of goiter, and progressive thyroid damage and destruction (89, 90). The severity of cretinism was found to be proportional to the degree of hypothyroidism, but also to be correlated with selenium deficiency (90, 91). The hypothesis was put forth that defective glutathione peroxidase due to selenium deficiency resulted in lack of protection against peroxidative damage induced by the high levels of H_2O_2 in the thyroid cell. Glutathione peroxidase activity was found to be decreased in selenium-deficient areas in Zaire and Ubangi, and the enzyme activity in cretins was half the level in normal subjects (91). Selenium supplementation for 2 months corrected glutathione peroxidase levels in both normal subjects and cretins (90, 91). However, this supplementation also produced decreases in serum T_4 and T_3 and an increase in TSH (91). Selenium supplementation in the cretins, already suffering thyroid dysfunction, produced a further decrease in serum T_4. For this reason, the selenium supplementation trial was discontinued, and patients were supplemented with iodine, which resulted in reversal of thyroid parameters. These results suggested that, by decreasing the rate of thyroid hormone metabolism, selenium deficiency could protect against some of the consequences of iodine deficiency. In view of these findings, Contempre *et al.* (91) stressed the importance of supplementation with iodine before administration of selenium in patients deficient in both of these elements.

A previous analysis in one individual (92) in whom type I deiodinase levels appeared to be reduced showed a circulating hormonal profile quite similar to that described above for experimental selenium deficiency in rats. In this patient, serum total and free T_4 values were elevated, whereas serum total and free T_3 results were persistently in the lower half of the normal range. Despite the elevation in serum-free T_4, which would be expected to decrease TSH synthesis and/or release (1), serum TSH was 2.2–2.4 mU/liter and TRH response normal or enhanced. In another family studied by Maxon *et al.* (93), an elevation in total and free T_4 was associated in six affected members with high normal total T_3 levels and normal TSH. Although this pattern might also be seen in mild generalized thyroid hormone resistance, the elevation in serum T_4 (mean 210 nM) was out of proportion to that of serum T_3 (2.7 nM), suggesting an impairment in type I deiodinase activity. The fact that in both selenium-deficient rats and in the patients mentioned, serum T_4 is elevated whereas serum TSH is normal, implies a role for type I deiodinase in the regulation of TSH. With the present data, it is not possible to determine whether this reduced pituitary feedback sensitivity to T_4 can be explained simply on the basis of slightly subnormal circulating T_3 concentrations or of a previously unrecognized role of type I deiodinase as a source of locally produced T_3 in the hypothalamus or pituitary (1).

The convergence of biochemical, molecular biological, and nutritional studies on selenium and thyroid hormone metabolism has yielded considerable advancement in our understanding of iodothyronine deiodination and, in addition, is providing new insight into selenium metabolism and regulation of protein translation, with implications ranging from the clinical to basic cellular processes and evolutionary biology.

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References

1. Larsen PR, Silva JE, Kaplan MM 1981 Relationships between circulating and intracellular thyroid hormones: physiological and clinical implications Endocr Rev 2:87
2. Leonard JL, Visser TJ 1986 Biochemistry of deiodination. In: Hennemann G (ed) Thyroid Hormone Metabolism. W. B. Saunders, Philadelphia, p 189
3. Gross J, Pitt-Rivers R 1952 The identification of 3,5,3′-L-triiodothyronine in human plasma. Lancet 1:439
4. Morreale de Escobar G, Escobar del Rey F 1967 Extrathyroidal effects of some antithyroid drugs and their metabolic consequences. Recent Prog Horm Res 23:87

5. Oppenheimer JH, Schwartz HL, Surks MI 1972 Propylthiouracil inhibits the conversion of L-thyroxine to L-triiodothyronine. An explanation of the antithyroxine effect of propylthiouracil and evidence supporting the concept that triiodothyronine is the active thyroid hormone. J Clin Invest 51:2493
6. Frumess RD, Larsen PR 1975 Correlation of serum triiodothyronine (T_3) and thyroxine (T_4) with biologic effects of thyroid hormone replacement in propylthiouracil-treated rats. Metabolism 24:547
7. Silva JE, Larsen PR 1977 Pituitary nuclear 3,5,3′-triiodothyronine and thyrotropin secretion: an explanation for the effect of thyroxine. Science 198:617
8. Silva JE, Dick TE, Larsen PR 1978 Contribution of local tissue thyroxine monodeiodination to the nuclear 3,5,3′-triiodothyronine in pituitary, liver, and kidney of euthyroid rats. Endocrinology 103:1196
9. Silva JE, Larsen PR 1978 Contributions of plasma triiodothyronine and local thyroxine monodeiodination to triiodothyronine to nuclear triiodothyronine receptor saturation in pituitary, liver, and kidney of hypothyroid rats. J Clin Invest 61:1247
10. Larsen PR, Dick TE, Markovitz BP, Kaplan MM, Gard TG 1979 Inhibition of intrapituitary thyroxine to 3,5,3′-triiodothyronine conversion prevents the acute suppression of thyrotropin release in hypothyroid rats. J Clin Invest 64:117
11. Obregon MJ, Pascual A, Mallol J, Morreale de Escobar G, Escobar Del Rey F 1980 Evidence against a major role of L-thyroxine at the pituitary level: studies in rats treated with iopanoic acid (Telepaque). Endocrinology 106:1827
12. Cheron RG, Kaplan MM, Larsen PR 1979 Physiological and pharmacological influences on thyroxine to 3,5,3′-triiodothyronine conversion and nuclear 3,5,3′-triiodothyronine binding in pituitary. J Clin Invest 64:1402
13. Silva JE, Leonard JL, Crantz FR, Larsen PR 1982 Evidence for two tissue-specific pathways for *in vivo* thyroxine 5′-deiodination in the rat. J Clin Invest 69:1176
14. Visser TJ, Kaplan MM, Leonard JL, Larsen PR 1983 Evidence for two pathways of iodothyronine 5′-deiodination in rat pituitary that differ in kinetics, propylthiouracil sensitivity, and response to hypothyroidism. J Clin Invest 71:992
15. Kaplan MM, Yaskoski KA 1980 Phenolic, tyrosyl ring deiodination of iodothyronines in rat brain homogenates. J Clin Invest 66:551
16. Visser TJ, Leonard JL, Kaplan MM, Larsen PR 1982 Kinetic evidence suggesting two mechanisms for iodothyronine 5′-deiodination in rat cerebral cortex. Proc Natl Acad Sci USA 79:5080
17. Silva JE, Larsen PR 1983 Adrenergic activation of triiodothyronine production in brown adipose tissue. Nature 305:712
18. Leonard JL, Mellen SA, Larsen PR 1982 Thyroxine 5′-deiodinase activity in brown adipose tissue. Endocrinology 112:1153
19. Kaplan MM, Shaw EA 1984 Type II iodothyronine 5′-deiodination by human and rat placenta *in vitro*. J Clin Endocrinol Metab 59:253
20. Silva JE, Mellen SA, Larsen PR 1987 Comparison of kidney and brown adipose tissue iodothyronine 5′-deiodinases. Endocrinology 121:650
21. Leonard JL, Kaplan MM, Visser TJ, Silva JE, Larsen PR 1981 Cerebral cortex responds rapidly to thyroid hormones. Science 214:571
22. Silva JE, Gordon MB, Crantz FR, Leonard JL, Larsen PR 1984 Qualitative and quantitative differences in the pathways of extrathyroidal triiodothyronine generation between euthyroid and hypothyroid rats. J Clin Invest 73:898
23. Goswami A, Rosenberg IN 1983 Stimulation of iodothyronine outer ring monodeiodinase by dihydrolipoamide. Endocrinology 112:1180
24. Goswami A, Rosenberg IN 1985 Purification and characterization of a cytosolic protein enhancing GSH-dependent microsomal iodothyronine 5′-monodeiodination. J Biol Chem 26:6012
25. Goswami A, Rosenberg IN 1987 Thioredoxin stimulates enzymatic outer ring monodeiodination of reverse triiodothyronine. Endocrinology 121:1937
26. Goswami A, Rosenberg IN 1988 Effects of glutathione on iodothyronine 5′-deiodinase activity. Endocrinology 123:192
27. Visser TJ, Van der Does-Tobé I, Docter R, Hennemann G. 1976 Subcellular localization of a rat liver enzyme converting thyroxine into tri-iodothyronine and possible involvement of essential thiol groups. Biochem J 157:479
28. Kaplan MM 1979 Changes in the particulate subcellular component of hepatic thyroxine-5′-monodeiodinase in hyperthyroid and hypothyroid rats. Endocrinology 105:548
29. Laurberg P 1978 Selective inhibition of triiodothyronines from the perfused canine thyroid by propylthiouracil. Endocrinology 103:900
30. Ishii H, Inada M, Tanaka K, Mashio Y, Naito K, Nishikawa M, Imura H 1981 Triiodothyronine generation from thyroxine in human thyroid: enhanced conversion in Graves' thyroid tissue. J Clin Endocrinol Metab 52:1211
31. Erickson VJ, Cavalieri RR, Rosenberg LL 1982 Thyroxine-5′-deiodinase of rat thyroid, but not that of liver, is dependent on thryotropin. Endocrinology 111:434
32. Ishii H, Inada M, Tanaka K, Mashio Y, Naito K, Nishikawa M, Matsuzuka F, Kuma K, Imura H 1983 Induction of outer and inner ring monodeiodinases in human thyroid gland by thyrotropin. J Clin Endocrinol Metab 57:500
33. Toyoda N, Nishikawa M, Horimoto M, Yoshikawa N, Mori Y, Yoshimura M, Masaki H, Tanaka K, Inada M 1990 Synergistic effect of thyroid hormone and thyrotropin on iodothyronine 5′-deiodinase in FRTL-5 rat thyroid cells. Endocrinology 127:1199
34. Toyoda N, Nishikawa M, Horimoto M, Yoshikawa N, Mori Y, Yoshimura M, Masaki H, Tanaka K, Inada M 1990 Graves' immunoglobulin G stimulates iodotyronine 5′-deiodinating activity in FRTL-5 rat thyroid cells. J Clin Endocrinol Metab 70:1506
35. Borges M, Ingbar SH, Silva JE 1990 Iodothyronine deiodinase activities in FRTL-5 cells: predominance of type I 5′-deiodinase. Endocrinology 126:3059
36. Pazo-Moura CC, Moura EG, Dorris ML, Rehnmark S, Melendez L, Silva JE, Taurog A 1991 Effect of iodine deficiency and cold exposure on thyroxine 5′-deiodinase activity in various rat tissues. Am J Physiol 260:E175
37. Abuid J, Larsen PR 1974 Triiodothyronine and thyroxine hyperthyroidism. J Clin Invest 54:201
38. Silva JE, Larsen PR 1986 Hormonal regulation of iodothyronine 5′-deiodinase in rat brown adipose tissue. Am J Physiol 251:E639
39. Bianco AC, Silva JE 1987 Intracellular conversion of thyroxine to triiodothyronine is required for the optimal thermogenic function of brown adipose tissue. J Clin Invest 79:296
40. Bianco AC, Silva JE 1987 Optimal response of key enzymes and uncoupling protein to cold in BAT depends on local T3 generation. Am J Physiol 253:E255
41. Silva JE, Larsen PR 1985 Potential of brown adipose tissue type II thyroxine 5′-deiodinase as a local and systemic source of triiodothyronine in rats. J Clin Invest 76:2296
42. Farwell AP, Leonard JL 1989 Identification of a 27-kDa protein with the properties of type II iodothyronine 5′-deiodinase in dibutyryl cyclic AMP-stimulated glial cells. J Biol Chem 264:20561
43. Farwell AP, Lynch RM, Okulicz WC, Comi AM, Leonard JL 1990 The actin cytoskeleton mediates the hormonally regulated translocation of type II iodothyronine 5′-deiodinase in astrocytes. J Biol Chem 265:18546
44. Silva JE, Matthews PS 1984 Production rates and turnover of triiodothyronine in rat developing cerebral cortex and cerebellum: responses to hypothyroidism. J Clin Invest 74:1035
45. Mol JA, van den Berg TP, Visser TJ 1988 Partial purification of the microsomal rat liver iodothyronine deiodinase I. Solubilization and ion-exchange chromatography. Mol Cell Endocrinol 55:149
46. Mol JA, van den Berg TP, Visser TJ 1988 Partial purification of the microsomal rat liver iodothyronine deiodinase II. Affinity chromatography. Mol Cell Endocrinol 55:159
47. Safran M, Kohrle J, Braverman LE, Leonard JL 1990 Effect of biological alterations of type I 5′-deiodinase activity on affinity-labeled membrane proteins in rat liver and kidney. Endocrinology 126:826
48. Kohrle J, Rasmussen UB, Rokos H, Leonard JL, Hesch RD 1990 Selective affinity labelling of a 27-kDa integral membrane protein in rat liver and kidney with *N*-bromoacetyl derivatives of L-thyroxine and 3,5,3′-triiodo-L-thyronine. J Biol Chem 265:6146
49. Kohrle J, Rasmussen UB, Ekenbarger DM, Alex S, Rokos H, Hesch

RD, Leonard JL 1990 Affinity labelling of rat liver and kidney type I 5′-deiodinase. J Biol Chem 265:6155
50. Safran M, Leonard JL 1991 Comparison of the physicochemical properties of type I and type II iodothyronine 5′-deiodinase. J Biol Chem 266:3233
51. Boado RJ, Campbell DA, Chopra IJ 1988 Nucleotide sequence of rat liver iodothyronine 5′-monodeiodinase (5′MD): its identity with the protein disulfide isomerase. Biochem Biophys Res Commun 155:1297
52. Schoenmakers CHH, Pigmans IGAJ, Hawkins HC, Freedman RB, Visser TJ 1989 Rat liver type I iodothyronine deiodinase is not identical to protein disulfide isomerase. Biochem Biophys Res Commun 162:857
53. Berry MJ, Kates AL, Larsen PR 1990 Thyroid hormone regulates type I deiodinase messenger RNA in rat liver. Mol Endocrinol 4:743
54. St. Germain DL, Morganelli CM 1989 Expression of type I iodothyronine 5′-deiodinase in *Xenopus laevis* oocytes. J Biol Chem 264:3054
55. St. Germain DL, Dittrich W, Morganelli CM, Cryns V 1990 Molecular cloning by hybrid arrest of translation in *Xenopus laevis* oocytes. J Biol Chem 265:20087
56. Berry MJ, Banu L, Larsen PR 1991 Type I iodothyronine deiodinase is a selenocysteine-containing enzyme. Nature 349:438
57. Chambers I, Frampton J, Goldfarb P, Affara N, McBain W, Harrison PR 1986 The structure of the mouse glutathione peroxidase gene: the selenocysteine in the active site is encoded by the "termination" codon, TGA. EMBO J 5:1221
58. Zinoni F, Birkmann A, Stadtman T, Bock A 1986 Nucleotide sequence and expression of the selenocysteine-containing polypeptide of formate dehydrogenase (formate-hydrogenase-lyase-linked) from *Escherichia coli*. Proc Natl Acad Sci USA 83:4650
59. Garcia GE, Stadtman TC 1991 Selenoprotein A component of the glycine reductase complex from Clostridium purinolyticum: nucleotide sequence of the gene shows that selenocysteine is encoded by UGA. J Bacteriol 173:2093
60. Beckett GJ, Beddows SE, Morrice PC, Nicol F, Arthur JR 1987 Inhibition of hepatic deiodination of thyroxine is caused by selenium deficiency in rats Biochem J 248:443
61. Arthur JR, Nicol F, Hutchinson AR, Beckett GJ 1990 The effects of selenium depletion and repletion on the metabolism of thyroid hormones in the rat. J Inorg Biochem 39:101
62. Beckett GJ, MacDougall DA, Nicol F, Arthur JR, 1989 Inhibition of type I and type II iodothyronine deiodinase activity in rat liver, kidney and brain produced by selenium deficiency. Biochem J 259:887
63. Beckett GJ, Nicol F, Proudfoot D, Dyson K, Loucaides G, Arthur JR 1990 The changes in hepatic enzyme expression caused by selenium deficiency and hypothyroidism in rats are produced by independent mechanisms. Biochem J 266:743
64. Arthur JR, Nicol F, Rae PWH, Beckett GJ 1990 Effects of selenium deficiency on the thyroid gland and on plasma and pituitary thyrotrophin and growth hormone concentrations in the rat. Clin Chem Enzymol Commun 3:209
65. Behne D, Hilmert H, Scheid S, Gessner H, Elger W 1988 Evidence for specific selenium target tissues and new biologically important selenoproteins. Biochem Biophys Acta 966:1251
66. Safran M, Farwell AP, Leonard JL 1991 Evidence that type II 5′-deiodinase is not a selenoprotein. J Biol Chem 266:13477
67. Berry MJ, Kieffer JD, Larsen PR 1991 Evidence that cysteine, not selenocysteine, is in the catalytic site of type II iodothyronine deiodinase. Endocrinology 129:550
68. Behne D, Scheid S, Kyriakopoulos A, Hilmert H 1990 Subcellular distribution of selenoproteins in the liver of the rat. Biochem Biophys Acta 1033:219
69. Behne D, Kyriakopoulos A, Meinhold H, Kohrle J 1990 Identification of Type I iodothyronine 5′-deiodinase as a selenoenzyme. Biochem Biophys Res Commun 173:1143
70. Arthur JR, Nicol F, Beckett GJ 1990 Hepatic iodothyronine 5′-deiodinase: the role of selenium. Biochem J 272:537
71. Berry MJ, Kieffer JD, Harney JW, Larsen PR 1991 Selenocysteine confers the biochemical properties of the type I iodothyronine deiodinase. J Biol Chem 266:14155
72. Chaudiere J, Tappel AL 1984 Interaction of gold(I) with the active site of selenium glutathione peroxidase. J Inorg Biochem 20:313
73. Chanoine JP, Safran M, Farwell AP, Arthur JR, Beckett GJ, Leonard JL, Effects of selenium deficiency on brain type II iodothyronine 5′-deiodinase in rats. Program of the 65th Annual Meeting of the American Thyroid Association, Boston, 1991 (Abstract 45)
74. Mizutani T, Kurata H, Yamada K 1991 Study of mammalian selenocysteyl-tRNA synthesis with [^{75}Se]HSe−. Fed Eur Biochem Soc 289:59
75. Bock A, Forchhammer K, Heider J, Leinfelder W, Sawers G, Veprek B, Zinoni F 1991 Selenocysteine: the 21st amino acid. Mol Microbiol 5:515
76. Stadtman TC 1990 Selenium Biochemistry. Annu Rev Biochem 59:111
77. Axley MJ, Bock A, Stadtman TC 1991 Catalytic properties of an *Escherichia coli* formate dehydrogenase mutant in which sulfur replaces selenium. Proc Natl Acad Sci USA 88:8450
78. Oikawa T, Nobuyoshi E, Tanaka H, Soda K 1991 Metalloselenonein, the selenium analogue of metallothionein: synthesis and characterization of its complex with copper ions. Proc Natl Acad Sci USA 88:3057
79. Wu ZP, Hilvert D 1989 Conversion of a protease into an acyl transferase: selenosubtilisin. J Am Chem Soc 111:4513
80. Wu ZP, Hilvert D 1990 Selenosubtilisin as a glutathione peroxidase mimic. J Am Chem Soc 112:5647
81. Lee BJ, Worland PJ, Davis JN, Stadtman TC, Hatfield DL 1989 Identification of a selenocysteyl-tRNA^{Ser} in mammalian cells that recognizes the nonsense codon, UGA. J Biol Chem 264:9724
82. Forchhammer K, Leinfelder W, Bock A 1989 Identification of a novel translation factor necessary for the incorporation of selenocysteine into protein. Nature 342:453
83. Zinoni F, Heider J, Bock A 1990 Features of the formate dehydrogenase mRNA necessary for decoding of the UGA codon as selenocysteine. Proc Natl Acad Sci USA 87:4660
84. Berry MJ, Banu L, Chen Y, Mandel SJ, Kieffer JD, Harney JW, Larsen PR 1991 Recognition of UGA as a selenocysteine codon in type I deiodinase requires sequences in the 3′ untranslated region. Nature 353:273
85. Saberi M, Sterling FH, Utiger RD 1975 Reduction in extrathyroidal triiodothyronine production by propylthiouracil in man. J Clin Invest 55:218
86. Geffner DL, Azukizawa M, Hershman JM 1975 Propylthiouracil blocks extrathyroidal conversion of thyroxine to triiodothyronine and augments thyrotropin secretion in man. J Clin Invest 55:224
87. LoPresti JS, Eigen A, Kaptein E, Anderson KP, Spencer CA, Nicoloff JT 1989 Alteration in 3,3′,5′-triiodothyronine metabolism in response to propylthiouracil, dexamethasone, and thyroxine administration in man. J Clin Invest 84:1650
88. Mandel SJ, Berry MJ, Kieffer JD, Harney JW, Warne RL, Larsen PR, Cloning and *in vitro* expression of human selenoprotein, Type I iodothyronine deiodinase. J Clin Endocrinol Metab, in press
89. Goyens P, Golstein J, Nsombola B, Vis H, Dumont JE 1987 Selenium deficiency as a possible factor in the pathogenesis of myxoedematous endemic cretinism. Acta Endocrinol (Copenh) 114:497
90. Vanderpas JB, Contempre B, Duale NL, Goossens W, Bebe N, Thorpe R, Ntambue K, Dumont J, Thilly CH, Diplock AT 1990 Iodine and selenium deficiency associated with cretinism in northern Zaire. Am J Clin Nutr 52:1087
91. Contempre B, Dumont JE, Bebe N, Thilly CH, Diplock AT, Vanderpas J 1991 Effect of selenium supplementation in hypothyroid subjects of an iodine and selenium deficient area: the possible danger of indiscriminate supplementation of iodine-deficient subjects with selenium. J Clin Endocrinol Metab 73:213
92. Kleinhaus N, Faber J, Kahana L, Schneer J, Scheinfeld M 1988 Euthyroid hyperthyroxinemia due to a generalized 5′-deiodinase defect. J Clin Endocrinol Metab 66:684
93. Maxon HR, Burman KD, Premachandra BN, Chen IW, Burger A, Levy P, Georges LP 1982 Familial elevations of total and free thyroxine in healthy, euthyroid subjects without detectable binding protein abnormalities. Acta Endocrinol (Copenh) 100:224