**Selenocysteine Confers the Biochemical Properties Characteristic of the Type I Iodothyronine Deiodinase**

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The conversion of thyroxine to 3,5,3'-triiodothyronine (T3) is the first step in thyroid hormone action, and the Type I iodothyronine deiodinase supplies most of this extrathyroidal T3 in the rat. We found that the cDNA coding for this enzyme contains an in-frame UGA encoding the rare amino acid selenocysteine. Using site-directed mutagenesis, we have converted selenocysteine to cysteine and expressed the wild-type and cysteine mutant enzymes in JEG-3 cells by transient transfection. The kinetic properties of the transiently expressed wild-type enzyme are nearly identical to those reported for rat liver Type I deiodinase. Substitution of sulfur for selenium causes a 10-fold increase in the $K_m$ of the enzyme for the favored substrate 3,3',5'-triiodothyronine (rT3), a 100-fold decrease in the sensitivity of rT3 deiodination to competitive inhibition by gold and a 300-fold increase in the apparent $K_i$ for uncompetitive inhibition by 6-n-propylthiouracil. These results demonstrate that selenium is responsible for the biochemical properties which characterize Type I iodothyronine monodeiodination.

Conversion of the prohormone thyroxine (T4) to the biologically active hormone 3,5,3'-triiodothyronine (T3) is required for thyroid hormone action. Type I iodothyronine deiodinase is a microsomal protein present in highest amounts in liver, kidney, and thyroid which, in the iodine-sufficient rat, provides most of the plasma T3 (1, 2). The 5' monodeiodination catalyzed by Type I deiodinase is a ping-pong, bi-substrate reaction in which iodothyronine first reacts with deiodinase to form an enzyme-iodide complex with release of deiodinated iodothyronine (reviewed in Ref. 3). Subsequent reaction of this complex with an unidentified cytoplasmic thiol cofactor (7 GSH) releases I- and regenerates the active enzyme. The thiourea drug, 6-n-propylthiouracil (PTU), reacts with the enzyme-iodide complex, blocking enzyme re-generation. Thus, PTU inhibition of Type I deiodination is uncompetitive with respect to iodothyronine and competitive with respect to thiol cofactor. By analogy with earlier studies of $\beta$-lactoglobulin, it has been postulated that PTU inhibits deiodination by forming a mixed disulfide with an essential thiol group of the enzyme (8-10). In rats, PTU inhibits T4 to T3 conversion and causes a proportional reduction in the biological effect of T3, to induce hepatic $\alpha$-glycerophosphate dehydrogenase (6). It also inhibits T3 to T4 conversion in man (7) and for this reason is often used in preference to methimazole in the acute treatment of the severely hyperthyroid patient (8).

We have recently cloned the rat liver Type I iodothyronine deiodinase using a Xenopus oocyte expression technique (9). A striking and, until recently (10-13), unanticipated finding was that the mRNA contains an in-frame UGA codon which encodes the rare amino acid selenocysteine. Given the important role of selenium in the selenocysteine-containing Escherichia coli enzyme formate dehydrogenase (14), we compared the reaction kinetics of transiently expressed selenocysteine-containing (SeC) and hepatic Type I enzyme (3, 15) with a cysteine-containing mutant (Cys) to assess the specific role of selenium in its function.

**EXPERIMENTAL PROCEDURES**

Materials—Male Sprague-Dawley rats were from Charles River Laboratories. Gold thioglucone was from Sigma; PTU was from U. S. Biochemical Corp. All other chemicals were of reagent grade.

**Plasmid Construction and Site-directed Mutagenesis**—Site-directed mutagenesis of the TGA selenocysteine codon to a TGT cysteine codon in the altered sites in vitro mutagenesis system and cloning into CDM-8 (16) was described previously (9).

**DNA Transfections**—Transfection of JEG-3 cells by calcium phosphate precipitation was as described previously (17, 18). Two days after transfection, cells were trypsinized, scraped into Dulbecco's modified Eagle's medium, rinsed with 0.1 M potassium phosphate, pH 6.9, 1 mM EDTA (PE) containing 5 mM dithiothreitol (DTT), resuspended, and sonicated in 800 $\mu$l of the same buffer containing 25 mM DTT. Transfection efficiencies were monitored by assay of human growth hormone in the media derived by cotransfecting a constitutive thymidine kinase promoter-directed human growth hormone-expressing plasmid, pTKGH (17, 18). Transfection efficiencies varied within a factor of 2 for the two plasmids with no systematic difference between them.

**Deiodinase Assays**—Cell homogenates were assayed for deiodination of $[^{125}]$T4, under varying conditions as described for each experiment. Deiodinase reactions contained 100-250 $\mu$g of homologate protein in PE, varying DTT concentrations, and 2 mM $[^{125}]$T4 plus varying quantities of unlabeled rT3 in a reaction volume of 500 $\mu$l. Incubations were for 60 min at 37°C. $[^{125}]$T4 was quantitated as described (19). The quantity of protein assayed was adjusted to consume less than 30% of substrate. Reaction rates were linear with time from 10 to 60 min and with protein content in the range used. Equal quantities of $[^{125}]$T4 and $[^{131}]$I-3,3'-diiodothyronine are produced by both the SeC and Cys proteins during this reaction (9). T3 to T4 conversion was evaluated by incubation of 700 $\mu$g of SeC or vector-transfected JEG cell homogenates with 25 mM DTT, 100 mM $[^{125}]$T4, and 100 mM rT3 for 16 h at 37°C in 500 $\mu$l of PE with or without 0.5 mM PTU. T3 was quantitated as described (19). All assays were performed in duplicate, and each experiment was repeated several times. Since the transiently expressed enzyme is not pure, all kinetic constants are apparent.

**RESULTS**

Substitution of Sulfur for Selenium Alters the Kinetics and Substrate Preference of Type I Iodothyronine Deiodinase—
Both SeC and Cys deiodinase enzymes are expressed in an active form in JEG-3 cells after transient transfection. There is no endogenous deiodinase present in these cells (9). The apparent $K_a$ of the SeC enzyme for $rT_3$, $0.25 \pm 0.038$ (S.E.) $\mu M$, is similar to that of rat liver deiodinase (kinetic results summarized in Table I). However, the apparent $V_{\text{max}}$ ($3.7 \pm 0.53 \text{ pmol min}^{-1} \text{ mg}^{-1}$) is much lower than in rat liver microsomes, due to the inefficiency of DNA uptake and the transient nature of deiodinase expression. These technical factors do not affect the $K_a$ or other kinetic analyses but do influence the $V_{\text{max}}$. Exchanging sulfur for selenium causes a 10-fold increase in the apparent $K_a$ for $rT_3$ ($2.7 \pm 0.55$ $\mu M$). The $V_{\text{max}}$ is also increased approximately 6-fold ($22 \pm 5.4 \text{ pmol min}^{-1} \text{ mg}^{-1}$) although this may not be due to an intrinsic difference in the enzyme (see "Discussion"). $T_4$ was a competitive inhibitor of $rT_3$ deiodination for both SeC and Cys enzymes (Fig. 1). However, the apparent $K_i$ of $T_4$ for $rT_3$ deiodination was 10-fold lower for the Cys as compared with the SeC enzyme (Table I).

We previously observed a marked decrease in the sensitivity of the Cys mutant to inhibition by gold thioglucose (GTG). Thioglucose had no effect on enzyme activity, indicating that gold is the active species producing this effect (9). Double-reciprocal plots indicate that inhibition of deiodination by gold is competitive with respect to iodothyronine (Fig. 2). The apparent $K_i$ of GTG for inhibition of $rT_3$ deiodination is greatly increased (100-fold) for the Cys as compared with the SeC enzyme (Table I).

Sensitivity of Type I Enzyme to PTU Inhibition Is Markedly Reduced in the Cys Mutant—In vitro studies, DTT substitutes for endogenous thiol as the second substrate for this enzyme. This reagent increases the $K_a$ and $V_{\text{max}}$ for $rT_3$ deiodination by the SeC enzyme (Fig. 3, left). The apparent $K_i$ for DTT was 30 $\mu M$ for the SeC protein. In contrast, the Cys enzyme was not stimulated by DTT at concentrations above 2 $\mu M$ (Fig. 3, right).

Uncompetitive inhibition of SeC deiodinase activity by PTU is indicated by parallel upward displacement of lines in a double-reciprocal plot of reaction rate versus $rT_3$ concentration at increasing concentrations of PTU (Fig. 4A). The Cys enzyme is about 300-fold less sensitive to PTU than is the SeC enzyme, and the mechanism of inhibition is noncompetitive.
The kinetic properties we observed for the transiently expressed SeC enzyme are quite similar to those of rat liver with the exception of the apparent $V_{\text{max}}$ which cannot be compared for the reasons already discussed. The $K_m$ of the SeC enzyme for $rT_3$ is higher than that reported for the rat liver deiodinase (3), due to the higher DTT concentrations (10 mM) used in the present studies (Fig. 3) than with liver microsomes (3–5 mM). The apparent $K_i$ of T$_4$ for inhibition of rT$_3$ deiodination was 10.8 uM, about 40-fold higher than the $K_i$ for T$_3$, this enzyme showing a similar preference for rT$_3$ as is exhibited by the liver enzyme. T$_4$ to T$_3$ conversion is also catalyzed by the SeC enzyme. GTG is a competitive inhibitor (with H$_2$O$_2$) of the selenoenzyme glutathione peroxidase (20). We found that rT$_3$ deiodination by the SeC enzyme is quite sensitive to inhibition by GTG ($K_i$ = 6.6 nM). This $K_i$ is similar to the $K_i$ of 3.2 nM we have recently observed for GTG inhibition of rat liver microsomal rT$_3$ deiodination and about 100-fold lower than the apparent $K_i$ of GTG for T$_3$, 5′-deiodination by brown adipose tissue Type II deiodinase (21). These results establish that the SeC cDNA codes for an enzyme with the typical properties of the Type I deiodinase.

Substitution of cysteine for selenocysteine produced an enzyme with a 10-fold higher apparent $K_m$ for rT$_3$. While the $V_{\text{max}}$ was 6-fold higher for the Cys mutant, this could reflect the less efficient translation of the UGA than UGU-containing mRNA rather than an intrinsic property of the Cys enzyme. A lower translation efficiency for UGA than for UGU-containing deiodinase mRNA was quite striking in reticulocyte lysates (9). Differences in either transfection or translation efficiency between the two cDNAs would not affect the other parameters examined in this study since the reaction rates are proportional to protein concentration. The changes in the apparent $K_m$ values of T$_4$ and especially of GTG for rT$_3$, 5′ deiodination demonstrate that interaction of the first substrate with the enzyme is dependent on selenium. This is consistent with earlier results showing that enzymes with thiol-active centers are much less sensitive to inhibition by gold than is glutathione peroxidase (20). This inhibition is thought to be a consequence of a direct interaction of gold with selenium or sulfur. Although the structure of selenocysteine is similar to that of cysteine, the two amino acids differ in one important respect. The pK$_a$ values of the cysteine sulfhydryl (pK$_a$ = 8.3) and selenocysteine selenol (pK$_a$ = 5.5) predict that the sulfhydryl will be largely protonated at neutral pH whereas the selenol will be largely ionized (22). This is consistent with the results in Fig. 2.

A marked sensitivity of 5′ deiodination to PTU is one important characteristic used to distinguish between the Type I and Type II deiodinase pathways of T$_4$ to T$_3$ conversion (3, 15). The results in Fig. 4 show that sensitivity to this reagent is highly dependent on the selenium atom. Thus, it seems likely that PTU inhibition of Type I deiodinase is due to the formation of an enzyme-Se-S-PTU adduct rather than the mixed disulfide previously postulated (3). Reverse T$_3$ deiodination by the Cys enzyme was 300-fold less sensitive to PTU than wild-type enzyme with respect to iodothyronine and 100-fold less with respect to thiol. Further, PTU inhibition of deiodination by the Cys enzyme was noncompetitive, not competitive, for iodothyronine, suggesting that PTU interacts with this protein either as free enzyme or as enzyme-substrate complex. This behavior is qualitatively and quantitatively characteristic of deiodination by the Type II, rather than the Type I pathway (3, 15).

A suggested scheme for Type I iodothyronine 5′ deiodination incorporating the present results is shown in Fig. 5. With
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Regarding Type II deiodinase, the relative insensitivity of this pathway to inhibition by gold and PTU (3, 15, 21) suggests that this enzyme does not contain selenocysteine.

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REFERENCES