Enzyme Mimics

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A Chemical Model for the Inner-Ring Deiodination of Thyroxine by Iodothyronine Deiodinase**

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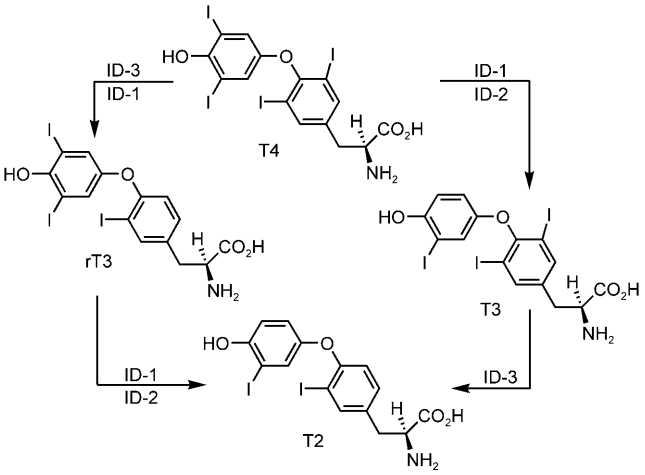
Iodothyronine deiodinases (IDs) are mammalian selenoenzymes that play an important role in the activation and inactivation of thyroid hormones.[1] The outer-ring (5′) deiodination of the prohormone thyroxine (T4) by the type 1 and 2 deiodinases (ID-1 and ID-2) produces the biologically active hormone, 3,5,3′-triiodothyronine (T3). These two enzymes also catalyze the outer-ring deiodination of 3,3′,5′-triiodothyronine (reverse T3, rT3) to produce 3,3′-diiodothyronine (T2) (Scheme 1).[2] The conversion of T4 into



Scheme 1. Inner- and outer-ring deiodinations of T4 catalyzed by three iodothyronine deiodinases.

rT3 is an inner-ring deiodination process, which is catalyzed by the type 3 enzyme (ID-3).[2,3] Importantly, ID-3 catalyzes the inactivation of T3 by inner-ring deiodination to produce T2, which is a key step in the maintenance of serum T3 concentration. This enzyme has been shown to contribute to thyroid hormone homeostasis by protecting tissues from an excess of thyroid hormone.[2–4]

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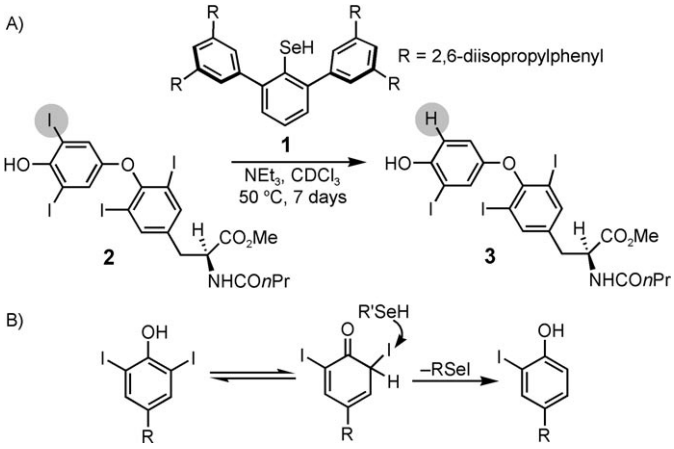
As the enzymatic deiodination of thyroxine is very complicated, there have been attempts to develop simple selenium compounds that can functionally mimic the deiodinases. It has been reported that nucleophilic selenium and tellurium reagents such as PhSeH, PhTeH, and NaHTe can remove iodine from a variety of activated 2,6-diiodophenol derivatives.[5] Recently, Goto et al. reported that the sterically hindered selenol **1** converts the thyroxine derivative **2** (*N*-butyrylthyroxine methyl ester) into the corresponding triiodo derivative **3** by an outer-ring deiodination (Scheme 2 A).[6]

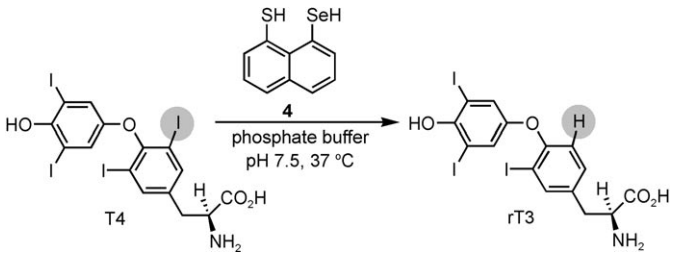


Scheme 2. A) Deiodination of thyroxine derivative **2** by selenol **1** and B) mechanism for the deiodination by enol–keto tautomerism as reported by Goto et al.[6]

Although the reaction was carried out in organic solvent ($CDCl_3$) and a relatively higher temperature (50 °C) and longer reaction time (7 days) were required for about 65 % conversion, this study provided an experimental evidence for the formation of a selenenyl iodide (RSeI) in the deiodination of a thyroxine derivative by an organoselenol.

All the above model studies lead to a conclusion that the two outer-ring iodine atoms of T4 are more reactive than the inner-ring ones and an enol–keto tautomerism is required for the deiodination (Scheme 2 B). Therefore, it is not clear whether the type 3 enzyme uses any additional functional groups for the selective removal of iodine from the inner ring of T4 (5-deiodination). Herein, we report on the first chemical model for the inner-ring deiodination of T4 and T3 by ID-3, the naphthyl-based selenol **4** (Scheme 3) bearing a thiol group in the proximity to the selenium atom that selectively produces rT3 and T2, respectively, under physiologically relevant conditions.

The deiodination reactions were carried out in 100 mM phosphate buffer (pH 7.5) with 1 mM dithiothreitol (DTT) and they were monitored by reverse-phase HPLC. When T4 was treated with compound **4** (Scheme 3), a decrease in the



Scheme 3. Highly selective inner-ring monodeiodination of T4 by compound **4**.

peak due to T4 was observed with a new peak appearing at 29.8 min. Mass spectral analysis of this peak and comparison of the HPLC chromatogram with that of authentic T3, rT3, and T2 revealed that T4 undergoes an exclusive inner-ring deiodination by **4** to produce rT3 as the only deiodinated product. When an excess amount of **4** (2 equiv) was employed in the assay, almost quantitative conversion of T4 into rT3 was observed within 30 h, and there was no indication for the formation of T3 or T2 (Figure 1). Furthermore, the deiodination of T4 by compound **4** is highly pH-dependent (Figure S17, Supporting Information), which is in agreement with the enzymatic reaction. When pure rT3 was used for the assay instead of T4, no deiodination was observed, indicating that compound **4** does not mimic the activity of ID-1 or ID-2.

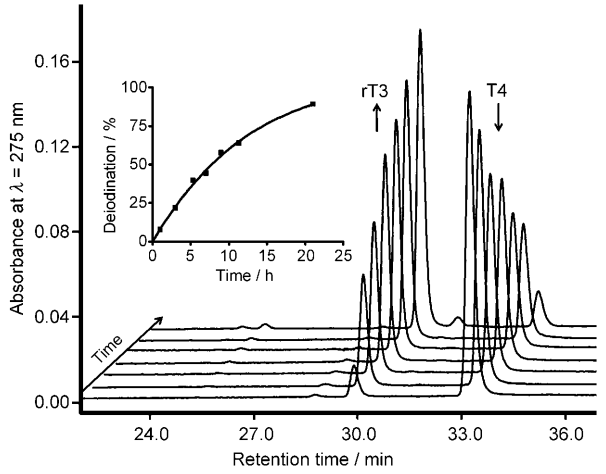
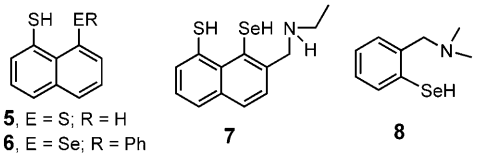


Figure 1. HPLC chromatograms for the conversion of T4 into rT3 by compound **4**. Inset: Deiodination as a function of time obtained by determining the amount of T4 at various time intervals.

As removal of the inner-ring iodine in the active hormone T3 by ID-3 is the major inactivation pathway, we have treated T3 with compound **4** in phosphate buffer. Interestingly, facile conversion of T3 into T2 by **4** was observed, and no further deiodination was detected by HPLC even in the presence of an excess amount (5 equiv) of compound **4** (Figure S18, Supporting Information).[7] The formation of T2 as the major deiodination product was further confirmed by treating an authentic sample of T2 with **4** under identical experimental conditions. These observations clearly indicate that compound **4** acts as an exclusive inner-ring deiodinating agent, and therefore it mimics the activity of ID-3 with both T4 and T3 as substrates. When the reactions were carried out in methanol instead of buffer, similar results were obtained for the deiodinations of T4 and T3.

The replacement of the selenol moiety in compound **4** by a thiol group (compound **5**) significantly reduced the deiodinase activity. When T4 was used as a substrate, an almost



twofold decrease in the activity for **5** relative to that for **4** was observed (Figure 2). This is in agreement with the report of Kuiper et al. that the selenocysteine (Sec) residue in the

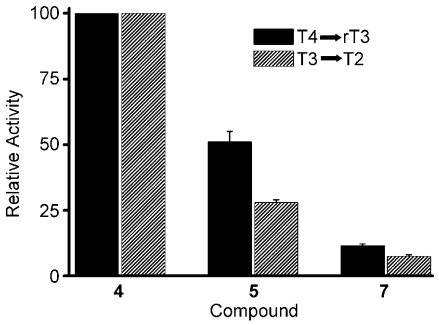


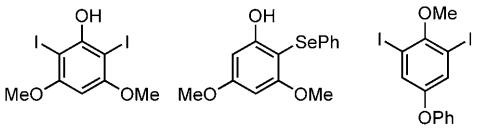
Figure 2. Relative activity for the deiodination of T4 and T3 by compounds **4**, **5**, and **7**. The reaction rates were calculated from the initial 5–10% of the conversion. The reactions were carried out in phosphate buffer (pH 7.5) at 37 °C. The final assay mixture contained 100 μM of **4**, **5**, or **7** and 500 μM of T4 or 500 μM of T3.

catalytic center of ID-3 is essential for efficient inner-ring deiodination as the substitution of Sec by a cysteine (Cys) reduces the catalytic efficiency.[8] The substrate turnover numbers for the deiodination of T4 and T3 by the Cys mutant have been shown to be six- and twofold, respectively, lower than that of the wild-type enzyme. Interestingly, replacement of the selenol moiety in compound **4** by a phenyl selenenyl group led to a complete loss of deiodinase activity: Compound **6**, which has an SePh substituent instead of the SeH group, was found to be inactive in both T4 and T3 assays even at higher concentrations. This result indicates that the presence of a selenol–thiol pair or of two thiol groups is crucial for the deiodination. Sun et al. demonstrated that a highly conserved Cys residue (Cys-124) in the active site of rat

ID-1 plays an important role in enhancing catalytic efficiency for both outer- and inner-ring deiodination.[9] In contrast, Croteau et al. reported that the conserved Cys residues in ID-1 are not essential for catalytic activity.[10] However, the deiodinase activity of compound **4** suggests that the Cys residue may play a crucial role in the inner-ring deiodination catalyzed by ID-3.

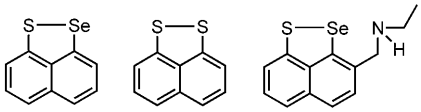
In addition to the conserved Cys residue, the presence of histidine (His) residues has been shown to be important for the deiodinase activity of ID-1. Köhrle and others have shown that one of the His residues at the active site of ID-1 activates the selenol by forming an imidazolium–selenolate ion pair.[11] Goto et al. also reported that the 5′-deiodination of **2** by the selenol **1** takes place only in the presence of triethylamine.[6] However, it is not known whether the His residue present at the active site of ID-3 plays any key role in the enzymatic reaction. To understand the importance of an amino group, we carried out the deiodination experiments in the presence of compound **7**, which contains a secondary amino group adjacent to the selenol moiety. The ^{77}Se NMR spectrum of compound **7** shows that the signal for this compound is significantly shifted upfield ($\delta = 109$ ppm) relative to that of compound **4** ($\delta = 162$ ppm), indicating that the amino group abstracts the proton from the selenol moiety to generate a more nucleophilic selenolate. Unexpectedly, compound **7** was found to be much less active than **4** in the deiodination of T4 and T3 (Figure 2). Similarly, addition of triethylamine to the reaction mixture did not enhance the deiodinase activity of **4**. Furthermore, no deiodination was observed when compound **8**, which has a *tert*-amino group capable of deprotonating the selenol moiety, was employed. It should be noted that compound **8** has been shown to be an efficient mimic of glutathione peroxidase (GPx), a Sec-containing enzyme that acts as an antioxidant.[12] A large upfield shift in the ^{77}Se NMR signal for compound **8** (3 ppm) suggests that the selenol moiety in **8** is more nucleophilic than that in compounds **4** and **7**. These observations indicate that the presence of an additional thiol group in proximity to the selenium atom is more important for the inner-ring deiodination than basic amino groups.

Vasil′ev and Engman have shown that the reaction of PhSeH with the activated diiodophenol **9** affords the deiodination/substitution product **10**, indicating that the substituents in the phenolic ring modulate the reactivity of the selenium reagents.[5b] In contrast, Goto et al. reported that compound **11**, which cannot generate the corresponding keto form, does not undergo any deiodination by selenol **1**.[6] Therefore, the mechanism for the inner-ring deiodination mediated by compound **4** appears to be different from the one proposed for the outer-ring deiodination. It should be noted that ID-1, which deiodinates T4 in the outer-ring to produce T3, can also remove iodine from the inner-ring of T4 (Scheme 1). It is not clear whether this enzyme uses two different mechanisms for the deiodination reactions. Although the imidazolium–selenolate ion pair in ID-1 may be sufficient for the conversion of T4 into T3, the conserved Cys residue appears to be important for the inner-ring deiodination.



9 **10** **11**

Interestingly, the deiodination of T4 by compounds **4**, **5**, and **7** occurred even in the absence of DTT. When T4 was treated with an excess amount of compound **4** (5 equiv), complete conversion of T4 into rT3 was observed. The deiodination of T4 was also observed in the presence of compounds **5** and **6**, although the activity of these compounds was found to be much lower than that of **4**. During the deiodination, compounds **4, 5**, and **7** were oxidized to **12**, **13**,



12 **13** **14**

and **14**, respectively. As the formation of **12**–**14** was observed even under nitrogen atmosphere, the thiol group may act as an in-built cofactor for the deiodination reaction. It should be noted that compounds **12**–**14** are remarkably stable in aqueous solutions. These compounds can be recovered quantitatively from the reaction mixture, converted into compounds **4**, **5**, and **7**, respectively, and then employed for further deiodination reactions without any noticeable decomposition.[13] Sun et al. previously suggested that one of the Cys residues in the active site of ID-1 may interact with the selenium atom to produce a selenenyl sulfide (−Se−S−) species.[9] The formation of such a bond has been described for the selenium-dependent thioredoxin reductase.[14]

The mechanism of deiodination of T4 by compound **4** may involve the formation of a halogen bond between selenol group and iodine atom.[15] It should be noted that halogen bonds play an important role in the recognition of thyroid hormones. It has been shown that T4 forms short I···O contacts with its transport protein transthyretin and T4 can bind to RNA sequences through halogen bonds.[16] The flavoprotein iodotyrosine deiodinase (IYD),[17] which salvages iodide from mono- and diiodotyrosine formed during the biosynthesis of T4, may utilize halogen bonds for deiodination reactions. As S···I interactions are generally weaker than Se···I interactions,[15] the deiodinase activity of **5** is lower than that of compound **4**. Furthermore, the decrease in the positive charge on the second iodine atom upon removal of the first may weaken the halogen bond. This decreased charge may account for the inability of compound **4** to remove iodine from rT3 to produce T2.[7]

In conclusion, the first chemical model for the inner-ring deiodination of thyroxine (T4) and 3,5,3′-triiodothyronine (T3) by iodothyronine deiodinase ID-3 is demonstrated. This study suggests that the nature of substituents around the

selenol functionality may modulate its reactivity towards outer- or inner-ring iodine atoms. The presence of an in-built thiol group in proximity to the selenium group is important, as this group not only acts as a thiol cofactor in the deiodination reactions, but may also assist the selenol atom in polarizing the C–I bond. The effective removal of iodine from the inner ring of T4 indicates that enol–keto tautomerism is not required for the deiodination. Although the activation of selenocysteine by histidine is believed to be important for the ID-1-mediated deiodination, such activation is probably not essential for the ID-3-catalyzed reaction. Further work is in progress to elucidate the mechanism of the selenium-mediated deiodination reactions.

Experimental Section

Deiodination assay: The deiodination reactions were carried out in 100 mM phosphate buffer (pH 7.5) with 1 mM dithiothreitol (DTT) at 37 °C. Selenols and thiols were freshly prepared by reducing the corresponding selenenyl sulfides or disulfides by $NaBH_4$ prior to use. The assay was performed in 1 mL sample vials and an autosampler was used for sample injection. The reaction products were analyzed by reverse-phase HPLC (Princeton C18 column, 4.6 × 150 mm, 5 μm) with gradient elution using acetonitrile/ammonium acetate (pH 4.0) as the mobile phase. The formation of rT3 or T2 was monitored at $\lambda =$ 275 nm, and the amounts of deiodinated products formed in the reactions were calculated by comparing the peak areas.

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