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Deiodination of Thyroid Hormones by Iodothyronine Deiodinase Mimics: Does an Increase in the Reactivity Alter the Regioselectivity?

Debasish Manna and Govindasamy Mugesh*

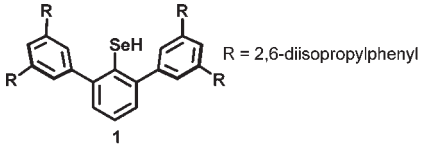
Department of Inorganic and Physical Chemistry, Indian Institute of Science, Bangalore 560 012, India

Supporting Information

ABSTRACT: Organoselenium compounds as functional mimics of iodothyronine deiodinase are described. The naphthyl-based compounds having two selenol groups are remarkably efficient in the inner-ring deiodination of thyroxine. The introduction of a basic amino group in close proximity to one of the selenol moieties enhances the deiodination. This study suggests that an increase in the nucleophilic reactivity of the conserved Cys residue at the active site of deiodinases is very important for effective deiodination.

Iodothyronine deiodinases (IDs) are mammalian selenoenzymes that catalyze the regioselective deiodination of thyroid hormones.[1] Outer-ring (5′) deiodination of 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 convert 3,3′,5′-triiodothyronine (reverse T3 or rT3) to 3,3′-diiodothyronine (3,3′-T2) by outer-ring deiodination (Scheme 1).[2] The type-3 deiodinase (ID-3) catalyzes the convertion of T4 to rT3 by an inner-ring deiodination pathway.[2,3] Furthermore, ID-3 plays an important role in the maintenance of serum T3 concentration by catalyzing the conversion of T3 to 3,3′-T2, which has been shown to be a key step in the protection of tissues from excess thyroid hormone.[2−4]

Initial attempts to develop simple selenium compounds as mimics of the deiodinases have led to only limited success.[5] Recently, Goto et al.[6] reported that selenol **1** bearing sterically hindered substitutents can convert *N*-butyrylthyroxine methyl ester to the corresponding triiodo derivative by outer-ring deiodination. In this case, nucleophilic attack of selenol **1** at one of the outer-ring iodines has been shown to produce the corresponding selenenyl iodide. We have reported that selenol **2** bearing a thiol group in close proximity to selenium mimics the ID-3 activity by converting T4 and T3 to rT3 and 3,3′-T2, respectively, under physiologically relevant conditions.[7] We have also shown that replacement of the selenol moiety in **2** by a thiol group (compound **3**) significantly reduces the deiodinase activity. Similarly, compound **4** bearing a basic *N*-ethylamino group exhibits much lower deiodinase activity in comparison with **2**,



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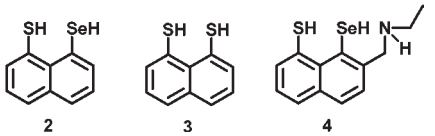
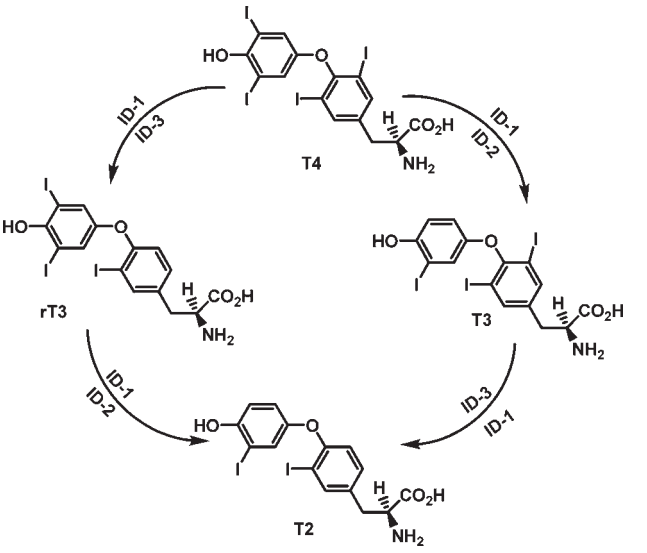


2 3 4

Scheme 1. Regioselective Deiodinations of Thyroid Hormones Catalyzed by Three Iodothyronine Deiodinases (ID-1, ID-2, and ID-3)



indicating that activation of the selenol moiety by the amino group is probably not important for inner-ring deiodination.[7]

The decrease in the deiodinase activity upon introduction of a basic amino group in close proximity to the selenol was unexpected, as one or more histidine residues (His) appear to play crucial roles in the deiodination reactions. Köhrle and others have shown that one of the His residues at the active site of ID-1 forms an imidazolium−selenolate ion pair, and therefore, the Sec residue is strongly activated toward nucleophilic attack.[8] In fact, the His residues corresponding to positions 158 and 174 in human ID-1 are conserved in ID-2 and ID-3.[2a] Goto et al.[6] reported the interesting observation that outer-ring deiodination of *N*-butyrylthyroxine methyl ester by compound **1** takes place only in the presence of triethylamine. Herein we report that the introduction of an additional selenol moiety remarkably enhances the deiodinase activity. We also show for the first time that cooperative effects of a thiol−selenol pair or two selenol groups play a key role in the deiodination.

To understand whether the replacement of the thiol group in compound **2** by a selenol can alter the regioselectivity of the

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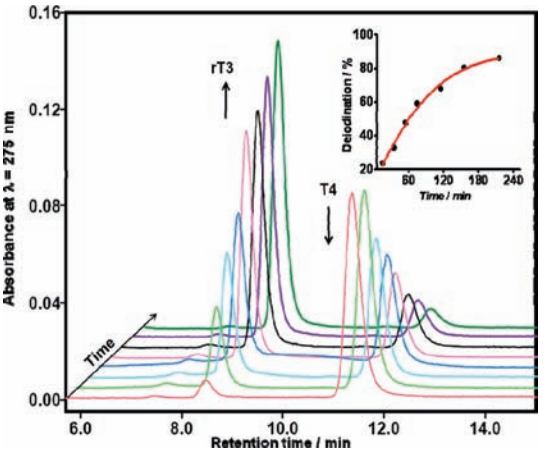
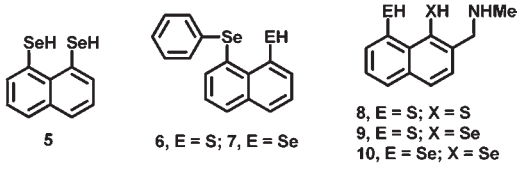


Figure 1. HPLC chromatograms for the deiodination of T4 by compound **5**. Inset: deiodination (%) as a function of time obtained by determining the amount of T4 at various time intervals.

deiodination, we studied the deiodination of T4, rT3, and T3 by compound **5** in the presence of dithiothreitol (DTT). When T4 was treated with **5**, a rapid decrease in the peak due to T4 was observed, with a new peak appearing at 8.5 min. A comparison of the HPLC chromatograms obtained at various time intervals with those of authentic rT3, T3, and 3,3′-T2 along with mass spectral analyses indicated that compound **5** removes iodine exclusively from the inner ring of T4 to produce rT3 as the only deiodinated product (Figure 1). When pure rT3 was treated with **5**, no 3,3′-T2 formation was observed, indicating that compound **5** does not remove iodine from the outer ring of rT3. In contrast, treatment of T3 with compound **5** readily produced 3,3′-T2 (Figure S15 in the Supporting Information), suggesting that compound **5** is selective toward the inner-ring iodines of T4 and T3. Although the deiodination of T4 or T3 by compound **5** occurred even in the absence of DTT, the concentration of DTT appeared to have a significant effect on the reaction rate. While the deiodination was enhanced initially with an increase in the concentration of DTT, the activity was reduced at higher concentrations of DTT (Figure S16). Furthermore, DTT did not affect the ratio of 5′ versus 5 deiodination, as the formation of T3 was not observed during the deiodination of T4 even at very high concentrations of DTT. This is in agreement with the effect of DTT on ID-3-mediated deiodination reactions.[9]



It has been shown that the regioselectivity of the deiodination of T4 and T3 by ID-1 is pH-dependent. While ID-1 catalyzes the outer-ring deiodination of T4 to produce T3 at physiological pH, this enzyme can remove iodine from the inner ring of T3 to produce 3,3′-T2 at alkaline pH. In contrast, ID-3 and its Cys mutant do not catalyze the outer-ring deiodination of T4, T3, or rT3 over a wide pH range. Furthermore, the activity of ID-3 is significantly enhanced under alkaline conditions. To understand whether the pH profile for the inner-ring deiodination of T3 by compound **5** is comparable to that of ID-3, the deiodination experiments were carried out in buffers having

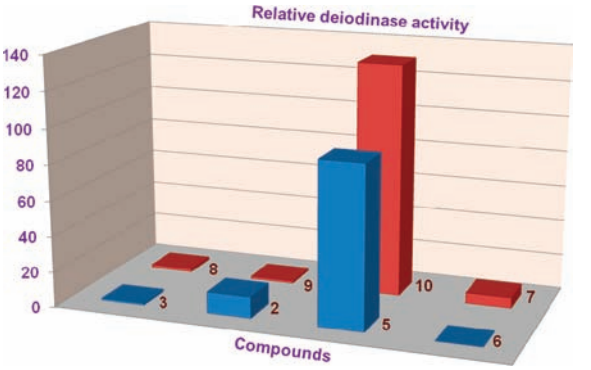


Figure 2. Comparison of the rates of deiodination of T4 by ID mimics **2**, **3**, and **5**−**10**. The reactions were carried out in phosphate buffer (pH 7.5) at 37 °C. The final assay mixture contained 600 μM ID mimic, 300 μM T4, and 12 mM DTT.

different pH values (Figure S17). These experiments indicated that compound **5** exhibits greater activity at alkaline pH, which is consistent with the pH profile of the enzyme. Interestingly, a rapid increase in the activity was observed at pH >7.0, probably as a result of stepwise deprotonation of the two thiol moieties present in DTT, leading to the formation of reactive thiolate.[10]

The facile deiodination of T4 and T3 by compound **5** led us to investigate the deiodinase activity of compounds **6**−**10** having thiol and/or selenol moieties. A comparison of the rates of deiodination for various compounds (Figure 2) indicated that compound **5** having two selenol moieties is more active than compounds **2** and **3**, which contain a thiol−selenol pair and two thiol moieties, respectively. While the replacement of one of the thiol groups in compound **3** by a selenol moiety led to only 12-fold enhancement in the activity, a remarkable 91-fold increase in the deiodinase activity was observed when both thiol groups were replaced by selenol moieties. Interestingly, compound **7**, which has a SePh substituent instead of the SeH group, exhibited very weak deiodinase activity. In contrast, compound **6**, which contains a thiol moiety, has been shown to be inactive in both T4 and T3 assays even at higher concentrations.[7] Kuiper et al.[9] showed that the selenocysteine (Sec) residue in the catalytic center of ID-3 is crucial for inner-ring deiodination, as the substitution of Sec by a cysteine (Cys) significantly reduces the catalytic efficiency. They showed that the substrate turnover numbers for the deiodinations of T4 and T3 by the Cys mutant were 6- and 2-fold lower, respectively, than for the wild-type enzyme. However, it is not known whether the replacement of the conserved Cys residue by a Sec residue can enhance the deiodinase activity of the ID-3 enzyme. The present study suggests that such a modification may lead to the generation of enzymes with higher deiodinase activities.

The investigations of the deiodinase activities of the amino-substituted compounds **8**−**10** led to some interesting observations. While the introduction of an *N*-methylamino group into compound **2** significantly reduced the deiodinase activity, compound **10** having an *N*-methylamino group was found to be more active than the corresponding unsubstituted compound, **5**. A comparison of the deiodinase activities indicated that compound **10** is almost 100 times more active than **9** in the conversion of T4 to rT3 (Figure 2). Similarly, the introduction of an *N*-methylamino group into compound **3** significantly increased the deiodinase activity, whereas dithiol **8** having an *N*-methylamino

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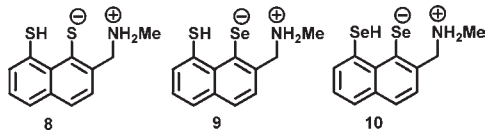


Figure 3. Abstraction of a proton from the thiol or selenol by an *N*-methylamino group in compounds **8**−**10**.

Table 1. Catalytic Parameters for the Monodeiodinations of T4 by Compounds 2, 3, 5, and 8−10[a]

compd	V_{max} (μM min^{-1})	K_m (mM)	$10^{-3}k_{cat}$ (min^{-1})	η (M^{-1} min^{-1})
2	0.78 ± 0.01	0.13 ± 0.01	1.57 ± 0.02	11.64 ± 0.51
3	0.76 ± 0.01	0.17 ± 0.01	1.52 ± 0.03	8.96 ± 0.57
5	1.68 ± 0.07[b]	2.58 ± 0.22	32.20 ± 0.56	12.49 ± 0.83
8	0.74 ± 0.02	0.65 ± 0.04	1.47 ± 0.04	2.28 ± 0.06
9	0.65 ± 0.01	0.09 ± 0.01	1.30 ± 0.01	15.34 ± 1.28
10	0.66 ± 0.05[b]	0.67 ± 0.11	13.10 ± 0.99	19.71 ± 1.85

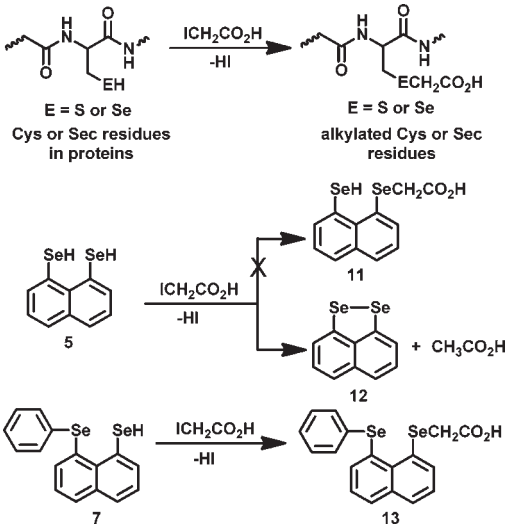
[a] The parameter values were obtained from Lineweaver−Burk plots. The reactions were carried out in phosphate buffer (pH 7.5) at 37 °C using 0.5−1.0 mM of T4, 0.5 mM of DTT, and 500 μM **2**, **3**, **8**, or **9** or 50 μM **5** or **10**. [b] A relatively lower concentration of the catalyst (50 μM) was employed for the assays, as no saturation kinetics was observed at a concentration of 500 μM under identical experimental conditions.

substituent was found to be ∼1.6 times more active than **3**, indicating that the basic amino group may deprotonate one of the thiol groups to produce a more reactive thiolate. Interestingly, the increase in the activity of dithiol **3** upon introduction of an amino group was almost identical to that of diselenol **5**. These observations strongly suggest that the relative nucleophilicity of the two functional groups (thiol−thiol, thiol−selenol, and selenol−selenol) plays an important role in the deiodination.[11]

In the ^{77}Se NMR spectrum of compound **9**, the signal for this compound (δ = 107 ppm) was shifted upfield relative to that for **2** (δ = 162 ppm). Similarly, the ^{77}Se NMR signal for one of the selenol groups in compound **10** (δ = 76 ppm) was shifted upfield significantly relative to that of the other selenol in the same compound (δ = 214 ppm) or that in compound **5** (δ = 156 ppm). These data suggest that the secondary amino group in compounds **8**−**10** can increase the nucleophilic reactivity by deprotonating the adjacent thiol or selenol moiety (Figure 3). When two thiols (compound **8**) or two selenols (compound **10**) are present, the increase in the reactivity of one of the thiol or selenol groups may enhance the cooperative binding of these groups to T4. In compound **9**, the drastic increase in the reactivity of the selenol relative to that of the thiol in the same compound may disfavor the cooperative interactions of the thiol−selenol pair with the substrate. This may account for the differences observed in the deiodinase activity upon introduction a basic amino group in compounds **2**, **3**, and **5**.

To further understand the effect of the T4 concentration on the rate of deiodination, we carried out detailed kinetic experiments. The catalytic parameters [maximum velocity (V_{max}), Michaelis constant (K_m), turnover number (k_{cat}), and catalytic efficiency (η)] for the deiodinations of T4 in the presence of compounds **2**, **3**, **5**, and **8**−**10** were obtained from the corresponding Lineweaver−Burk plots (Table 1). These data indicate that the introduction of a basic amino group enhances the binding of the thiol−selenol moiety to the substrate. The K_m value obtained for compound **10** (0.67 ± 0.11 mM) is much lower than that of **5** (2.58 ± 0.22 mM), indicating that the deprotonation of the selenol moiety in **10** by the secondary amino group facilitates the interaction of this compound with T4. It has been shown that one of the histidine residues (His174) at the active site of ID-1 is critically involved in substrate binding.[8] In this particular case, the His174Asn mutant exhibited a much higher K_m (20.9 ± 2.85 μM) than the wild-type enzyme (0.22 ± 0.04 μM).[8b] A comparison of the catalytic efficiencies (η) indicated that compound **10** having two selenol moieties is almost 9 times more active than the corresponding thiol **8**, although a relatively lower concentration of **10** was used for the determination of the V_{max} and K_m values. This is in agreement with the observations of Kuiper et al.[9] that the relative substrate turnover number for wild-type ID-3 is 6-fold higher than that of the Cys mutant.

Scheme 2. Reactivities of Thiol and Selenol Moieties in Proteins and Compounds 5 and 7 toward Iodoacetic Acid (IAA)



It has been shown that ID-1 activity is irreversibly inhibited by gold thioglucose (GTG), 6-*n*-propyl-2-thiouracil (PTU), and iodoacetic acid (IAA).[1,2] While PTU reacts with the selenenyl iodide (ID-1-SeI) intermediate to form a selenenyl sulfide as a dead-end product, GTG and IAA may react with the selenol group of the enzyme. Interestingly, ID-2 and ID-3 are very less sensitive to the effect of these inhibitors.[1,2] Although all three deiodinases contain a Sec residue at the catalytic center, the reason for the difference in their sensitivities to these inhibitors is still unclear. It is known that IAA is an irreversible inhibitor of several Cys peptidases,[12] the Sec-containing glutathione peroxidase (GPx),[13] and thioredoxin reductase (TrxR).[14] The catalytic Cys and Sec residues in these enzymes react with IAA to produce the corresponding alkylated derivatives (Scheme 2). When compound **5** was treated with IAA, the reaction did not produce the expected Se-carboxymethylated compound **11** but instead produced diselenide **12** with the elimination of acetic acid (Scheme 2). On the other hand, compound **7** having a SePh substitutent instead of the selenol group underwent facile carboxymethylation by IAA to produce compound **13** as the major product. The deiodination of IAA by compound **5** was found to be much faster than that of T4 or T3.[15] These observations suggest that IAA may undergo rapid

deiodination in the presence of ID-3, which may account for the insensitivity of this enzyme toward IAA.

In conclusion, we have shown that naphthyl-based compounds having two selenol groups are remarkably more efficient mimics of iodothyronine deiodinase than ones having two thiol groups or a thiol−selenol pair. An increase in the reactivity of the selenol by introducing a basic amino group or increasing the pH of the reaction medium does not change the regioselectivity of deiodination. The cooperative effects of nucleophilic thiol and selenol groups play an important role in the inner-ring deiodination of thyroxine. This study suggests that an increase in the nucleophilic reactivity of the conserved Cys residue at the active site of ID-3 is very important for effective deiodination, as an activated Cys may cooperate well with the Sec residue in polarizing the C−I bonds in T4 and T3. The insensitivity of ID-3 toward the ID-1 inhibitor iodoacetic acid (IAA) can be ascribed to the facile inactivation of IAA by ID-3-mediated deiodination.

■ ASSOCIATED CONTENT

Supporting Information. Details of experimental procedures, chemical syntheses, and assay conditions. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author
mugesh@ipc.iisc.ernet.in

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(15) The deiodination of IAA could be conveniently followed by ^{1}H NMR spectroscopy. When IAA was added to compound **5**, rapid and quantitative conversion of IAA to acetic acid was observed. Similarly, iodoacetamide, bromoacetic acid, and bromoacetamide were dehalogenated by **5**, although the debromination was found to be much slower than the deiodination.