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Remarkable Effect of Chalcogen Substitution on an Enzyme Mimetic for Deiodination of Thyroid Hormones**

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Abstract: *Iodothyronine deiodinases are selenoenzymes which regulate the thyroid hormone homeostasis by catalyzing the regioselective deiodination of thyroxine (T4). Synthetic deiodinase mimetics are important not only to understand the mechanism of enzyme catalysis, but also to develop therapeutic agents as abnormal thyroid hormone levels have implications in different diseases, such as hypoxia, myocardial infarction, critical illness, neuronal ischemia, tissue injury, and cancer. Described herein is that the replacement of sulfur/selenium atoms in a series of deiodinase mimetics by tellurium remarkably alters the reactivity as well as regioselectivity toward T4. The tellurium compounds reported in this paper represent the first examples of deiodinase mimetics which mediate sequential deiodination of T4 to produce all the hormone derivatives including T0 under physiologically relevant conditions.*

Thyroid hormones play an important role in growth, development, and regulation of energy metabolism.[1] Thyroxine (T4), the main secretory hormone produced in the thyroid gland, undergoes peripheral deiodination reactions in various tissues to form biologically more active 3,5,3′-triiodothyronine (T3; by 5′-deiodination) and an inactive 3,3′,5′-triiodothyronine (rT3; reverse T3, by 5-deiodination).[2] The regioselective deiodination reactions are catalyzed by three isoforms of the selenocysteine-containing iodothyronine deiodinases (ID-1, ID-2 and ID-3; Figure 1).[3] Although ID-1 can mediate both 5- and 5′-deiodinations of T4, it is primarily responsible for the production of plasma T3 by 5′-deiodination. ID-2 catalyzes only the 5′-deiodination of T4 and is essential for the local production of intracellular T3. In contrast, ID-3 mediates only the 5-deiodination and is important for the inactivation of T4 (Figure 1). The three isoforms of the enzymes collectively regulate thyroid hormone homeostasis in the body.

Given the importance of thyroid hormone deiodination in human metabolism and lack of information about the mechanism of such deiodination,[4] the development of simple selenium compounds as deiodinase mimetics has attracted significant interest.[5–7] Goto et al. reported a synthetic selenol that mediates the 5′-deiodination or outer-ring deiodination (ORD) of *N*-butyrylthyroxine methyl ester to give the corresponding triiodo derivative.[5b] We reported that the compound **1**, having two thiol moieties, and the compound **2**, having a thiol-selenol pair in the peri-positions, convert T4 and T3 into rT3 and 3,3′-T2, respectively, by 5-deiodination or inner-ring deiodination (IRD).[6a] Recently, we reported that the compound **3**, bearing two selenol moieties, is more active than **1** and **2** in the 5-deiodination of T4 and the regioselectivity of deiodination is not altered upon the substitution of sulfur with selenium.[6b,c] The introduction of an amino group to **3** increases the nucleophilicity of one of the selenol moieties, upon deprotonation, but does not alter the regioselectivity as the amino-substituted compounds **4**–**10** which also mediate the 5-deiodination.[6b,c] Interestingly, **1**–**6** were unable to remove additional iodine atoms from either rT3 or 3,3′-T2, even at higher concentrations, and is in contrast to the ID-3 enzyme, which can remove the second iodine atom from rT3 and 3,3′-T2 to produce 3′,5′-T2 and 3′-T1, respectively. Herein we show, for the first time, that the replacement of either one of the sulfur atoms in **1** or the two selenium atoms

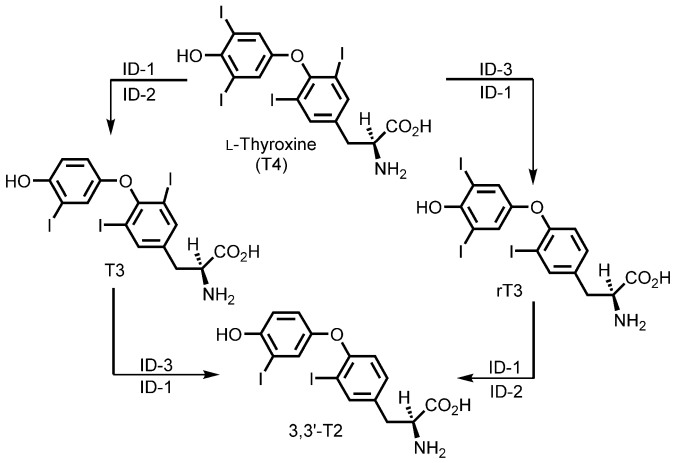
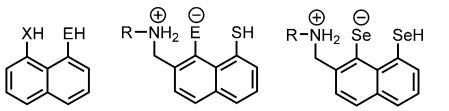


Figure 1. The regioselective deiodination reactions of thyroid hormones catalyzed by three isoforms of iodothyronine deiodinases.



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in **3** by tellurium atoms remarkably alters the regioselectivity of deiodination under physiological conditions.

The dichalcogenides **11** and **12**, which were required for this study, were synthesized by following a procedure reported for the corresponding sulfur and selenium analogues (see the Supporting Information, SI). The reduction of **11** and **12** by $NaBH_4$ afforded the compounds **13** and **14**, respectively (Figure 2A). When **13** (0.75 μM) was treated with T4 (300 μM) in phosphate buffer (pH 7.5) at 37 °C the immediate formation of rT3 was observed. In contrast, a stoichiometric amount of **3** (300 μM) was required for the 5-deiodination of T4 under identical experimental conditions (Figure 2C). Interestingly, when **13** was treated with T4 in 1:1 molar ratio, the HPLC chromatograms showed the formation of six different products. A comparison of the HPLC chromatograms with that of the authentic samples indicated that the reaction generated most of the deiodination products of T4, that is, rT3, 3,3′-T2, 3′,5′-T2, 3-T1, 3′-T1, and T0 (Figure 2B). Similar products were obtained when **14** was treated with T4 in 1:1 molar ratio. These observations indicate that the replacement of sulfur in **1** by a selenium atom (**2**) increases the reaction rate for the conversion of T4 into rT3 without altering the regioselectivity of deiodination. Similarly, no change in the regioselectivity was observed when both the sulfur atoms in **1** were replaced by selenium atoms (**3**). In contrast, the replacement of the selenium atom in **2** by a tellurium atom, dramatically increased the rate of deiodination with a remarkable change in the regioselectivity.

To confirm the sequential deiodination of T4 (Figure 3), we treated T4 and its derivatives independently with the test compounds. The initial rate observed for the IRD of T4 by **13** [(10.6 ± 0.1) μM min^{-1}] at a concentration of 0.75 μM was much higher than that of **3** [(0.9 ± 0.1) μM min^{-1}] at a concentration of 300 μM (Figure 2C), thus indicating that the activity of **13** is thousands of times higher than that of **3**. The rate of IRD of T3 or 3,5-T2 by **13** was also found to be much higher than that of **3**. When rT3 and 3,3′-T2 were used, a 100-fold increase in the concentration of **13** was required to observe an appreciable deiodination. The compound **14**, having two tellurol groups, was found to be less active [(4.4 ± 0.1) μM min^{-1}] for T4 than **13** in the deiodination of all five substrates, although a higher concentration of **14** was required for the IRD of rT3 and 3,3′-T2. This result is surprising because **3**, having two selenol moieties, was found to be significantly more active than **2** which has a thiol-selenol pair. The deiodination reactions of T4 by an increasing the concentration of either **13** or **14** showed the formation of rT3 and a subsequent deiodination, thus leading to the formation of 3′,5′-T2 by IRD and 3,3′-T2 by ORD (Figure 3).

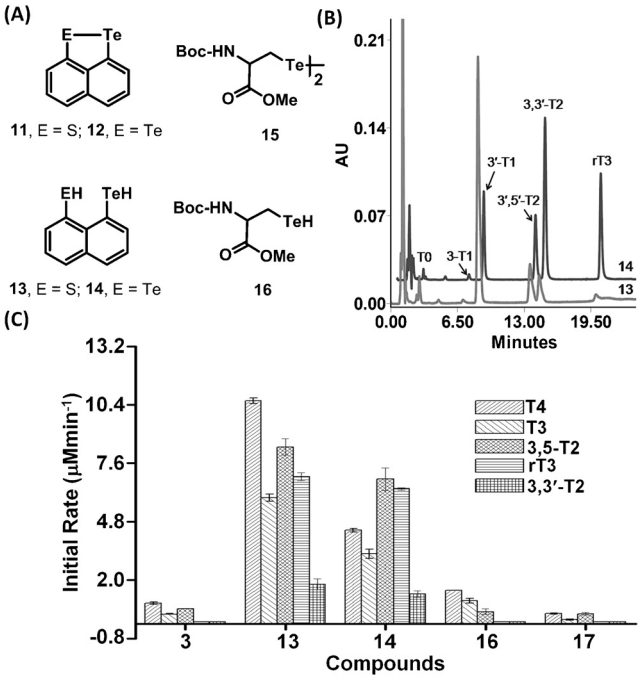

Figure 2. A) Chemical structures of compounds **11**–**16**. B) HPLC chromatograms for the reactions of T4 with **13** and **14**. C) Initial rates for the deiodination of T4 and its derivatives by selenium/tellurium compounds. Assay conditions for **13** and **14**: T4, T3, or 3,5-T2 (300 μM), DTT (2.5 mM), **13** or **14** (0.75 μM), and $NaBH_4$ (13 mM). The reaction mixture was incubated for 10 min in phosphate buffer (pH 7.5) at 37 °C. For the deiodination of rT3 and 3,3′-T2, 150 μM of either rT3 or 3,3′-T2, 75 μM of either **13** or **14**, 2.5 mM DTT, and 26 mM of $NaBH_4$ were used. For **3**, **16**, and PhTeH (**17**): T4, T3 or 3,5-T2 (300 μM), DTT (2.5 mM), test compounds (300 μM), and $NaBH_4$ (26 mM) were incubated for 30 min in phosphate buffer (pH 7.5) at 37 °C and the reactions were followed by HPLC.

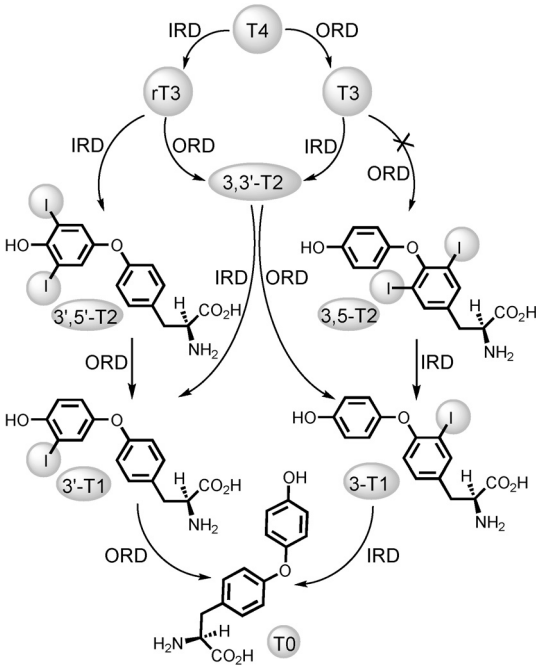

Figure 3. Sequential deiodination of T4 by **13** and **14**. IRD = inner-ring deiodination, ORD = outer-ring deiodination, T4 = L-thyroxine, T3 = 3,5,3′-triiodothyronine, rT3 = 3,3′,5′-triiodothyronine, 3,3′-T2 = 3,3′-diiodothyronine. Stoichiometric amounts of **13** and **14** were required to observe the formation of T0 from T4.

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Although T3 can be converted into 3,3′-T2 by IRD, the formation of 3,3′-T2 occurs mainly via rT3 as almost all the T4 in the reaction mixture is rapidly converted into rT3 by **13** and **14** (see Figures S14 and S15 in SI). Attempts to detect the formation of T3 directly from T4 were unsuccessful, as T3 undergoes a rapid deiodination by IRD to produce 3,3′-T2. However, **13** and **14** do mediate the conversion of T3 into 3,3′-T2 by IRD when T3 was used as the starting material (Figure 3). Interestingly, **13** and **14** do not mediate the ORD of T3 to produce 3,5-T2, but these compounds can remove iodine from 3,3′-T2 by ORD to produce 3-T1, thus indicating that the ORD is not a favored process when two iodines are present in the inner-ring. Similarly, the ORD of 3′-T1 was not observed at lower concentrations of **13** and **14**, whereas the IRD of 3-T1 produced T0 as the final product (Figure 3). Therefore, T4 is converted into the fully deiodinated derivative T0 through the rT3→3,3′-T2→3-T1 pathway, involving two IRD reactions and two ORD reactions. When **13** and **14** were used in large excess (50 equiv), a complete conversion of T4 into T0 was observed, an indication that 3′-T1 also undergoes deiodination to give T0. The diiodo derivative 3,3′-T2 serves as a common intermediate for both 3-T1 and 3′-T1. These observations reveal that the reactivity of C–I bonds in T4 is remarkably altered upon deiodination.

The facile deiodination of T4 and its derivatives by **13** and **14** prompted us to check whether compounds having one tellurol moiety without any additional thiol group can mediate such deiodination. We treated T4 with the tellurocysteine derivative **16** (obtained from the corresponding ditelluride **15** by reduction with $NaBH_4$; Figure 2) in 1:1 molar ratio and monitored the reaction by HPLC. Interestingly, **16** was able to deiodinate T4, T3, and 3,5-T2, although the rate of the reaction was much lower than that of **13** and **14** (Figure 2C). Almost no deiodination was observed when either rT3 or 3,3′-T2 was used as a substrate. However, the initial rate for the deiodination of T4 and T3 by **16** was found to be significantly higher than that of **3** under identical reaction conditions. Similarly, tellurophenol (PhTeH; **17**; obtained in situ by treatment of diphenyl ditelluride with $NaBH_4$) was able to deiodinate T4 and T3 and the activity was lower than that of the other compounds (Figure 2C). In contrast, no deiodination was observed when either T4 or T3 was treated with selenocysteine or selenophenol (PhSeH) under similar experimental conditions.

A previous study on the deiodination of a T4 derivative, the *N*-butyrylthyroxine methyl ester **18**, revealed that tellurium reagents such as NaHTe can remove iodine from the outer-ring (Figure 4, Path A).[8] When **18** was treated with 4 equivalents of NaHTe in ethanol at 50 °C for 4 hours, the T3 derivative **19** (R = Me, R′ = CO*n*Pr) and 3,5-T2 derivative **20** (R = Me, R′ = CO*n*Pr) were obtained in 45 and 5% yield, respectively, along with unreacted starting material (50%).[8] The reason for the formation of **19** and **20** has been ascribed to higher reactivity of the two outer-ring iodines as compared to the inner-ring ones. It has been postulated that one of the possible mechanistic pathways may involve an enol–keto tautomerism of **18** into **21**, and allows the tellurium nucleophile to selectively attack at the outer-ring iodines to produce **19** and **20** (Figure 4, Path A).[8] This assumption was further

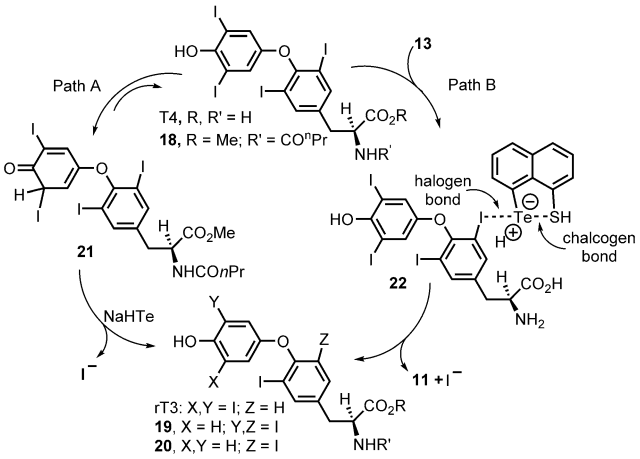

Figure 4. Path A: ORD of **18** involving a nucleophilic attack of the tellurium reagent at iodine atom in the keto form **21**. Path B: Conversion of T4 into rT3 by **13** involving a cooperative halogen and chalcogen bonding. A similar mechanism is applicable for ORD.

supported by another study involving a selective conversion of **18** into **19** by a sterically protected selenol.[5b] However, the sequential removal of all the iodine atoms in T4 by **13** and **14** suggests that the deiodination of T4 may proceed by a cooperative halogen and chalcogen bonding mechanism as proposed earlier for **1**–**3** (Figure 4, Path B).[6c,d]

Recent evidence suggests that another mammalian deiodinase enzyme, iodotyrosine deiodinase (IYD), plays an important role in thyroid gland.[9] This enzyme is responsible for recovering iodide for subsequent reuse in T4 biosynthesis and its mutation can lead to iodide deficiency and ultimately hypothyroidism.[9b] IYD catalyzes the reductive deiodination of 3,5-diiodo-L-tyrosine (DIT; Figure 5), which is produced as

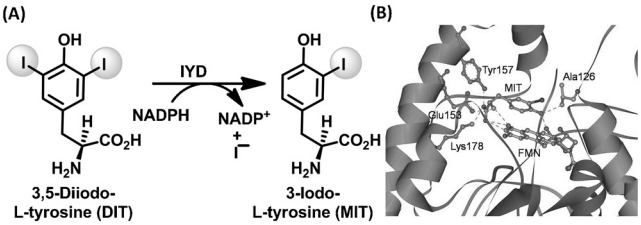

Figure 5. A) Deiodination of 3,5-diiodo-L-tyrosine (DIT) to 3-iodo-L-tyrosine (MIT) by iodotyrosine deiodinase (IYD) using NADPH as cofactor. The enzyme also catalyzes the deiodination of MIT to L-tyrosine. B) Active site of IYD indicating the binding of MIT near the flavin (FMN; PDB ID 3GFD).

a byproduct of T4 biosynthesis. In contrast to the iodothyronine deiodinases, IYD does not use selenocysteine for the deiodination. This enzyme belongs to the group of flavoproteins of the NADH oxidase/flavin reductase family. The $NADPH/FMNH_2$-dependent deiodination of DIT produces 3-iodo-L-tyrosine (MIT), which can be further deiodinated by the same enzyme to produce L-tyrosine.[10] To understand whether the selenium and tellurium compounds that deiodinate T4 and its derivatives can mimic the function of IYD, we

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treated **1**–**3**, PhSeH, **16**, and **17** with DIT and MIT in 1:1 molar ratio. However, no deiodination of DIT and MIT by these compounds was observed. Particularly, **3**, **16**, and **17**, which were able to remove an iodine from 3,5-T2 (Figure 2C), could not mediate the deiodination of DIT, thus indicating that the reactivity of the C–I bond in DIT is significantly different from that of 3,5-T2 because of the presence of phenolic group.

Interestingly, when DIT was treated either **13** or **14** in 1:1 molar ratio, a significant monodeiodination of DIT was observed. The initial rate for the deiodination of DIT by **14** [(0.37 ± 0.03) μM min^{-1}] was found to be higher than that observed for **13** [(0.24 ± 0.03) μM min^{-1}]. This difference is in contrast to the deiodination of T4, in which **13** was found to be more active than **14** (Figure 2C). While *N*-butyryl-3,5-diiodotyrosine methyl ester has been shown to be monodeiodinated by NaHTe in ethanol at higher temperatures,[8] **13** and **14** represent the first examples of compounds that mediate the monodeiodination of DIT under physiological conditions. The mechanism of deiodination of DIT by **13** and **14** appears to be similar to that of T4 and its derivatives. However, **13** and **14** do not mediate the deiodination of MIT under identical reaction conditions, although these compounds are able to remove iodine from 3-T1 (Figure 3).

To understand the reason for the differences in the deiodination of iodothyronines and iodotyrosines, we performed DFT calculations using simplified models for the selenol (MeSeH) and tellurol (MeTeH). The geometries were fully optimized in the gas phase at the B3LYP level of theory[11,12] by using the LANL2DZdp ECP basis set[13] for Se, Te, and I, and 6-31 + G* for other atoms. The natural bond orbital (NBO) analyses[14] were performed with LANL2DZdp ECP for Se, Te, and I, and 6-311 + G** basis set for other atoms. As can be seen from Table 1, the strength

Table 1: The Se···I and Te···I halogen bonding energy for various iodinated compounds with MeSeH and MeTeH, respectively.[a]

Compound	$E_{Se\cdots I}$ [kcal mol^{-1}]	$E_{Te\cdots I}$ [kcal mol^{-1}]
3,5-T2	3.50	4.31
3-T1	2.62	3.39
3,5-DIT	2.60	3.37
3-MIT	2.15	2.78

[a] See the SI for experimental details.

of Se/Te···I interactions (halogen bonding) depends not only on the number of iodine atoms, but also on the number of phenyl rings. While it is clear that the Te···I interactions are significantly stronger than the Se···I interactions in all the mono- and diiodo compounds (Table 1), these interactions are much weaker for the tyrosine derivatives (3,5-DIT and 3-MIT) as compared to that of thyronine-based compounds (3,5-T2 and 3-T1), thus indicating that the presence of an additional phenyl ring in 3,5-T2 and 3-T1 increases the strength of halogen bonding. Therefore, the deiodination of 3,5-T2 by **13** and **14** is more favored than that of 3,5-DIT. The initial rates observed for the deiodination of 3,5-T2 by **13** [(8.37 ± 0.39) μM min^{-1}] and **14** [(6.84 ± 0.54) μM min^{-1}] are much higher than that observed for 3,5-DIT [(0.24 ± 0.03) μM min^{-1} and (0.37 ± 0.03) μM min^{-1}, for **13** and **14**, respectively]. Similarly, the inability of **13** and **14** to remove iodine from 3-MIT is due to much weaker Te···I interactions of 3-MIT with tellurium reagent as compared to that of 3-T1.

In summary, we showed that the introduction of tellurium atoms in place of either sulfur or selenium in deiodinase mimetics alters not only the reactivity but also the regioselectivity of the deiodination. We also showed for the first time that compounds having two tellurol moieties or a thiol-tellurol pair can mediate sequential deiodination of T4 to produce all the possible thyroid hormone derivatives under physiologically relevant conditions. This study provides the first experimental evidence that the regioselectivity of thyroid hormone deiodination is controlled by the nucleophilicity and the strength of halogen bond between the iodine and chalcogen atoms.

Keywords: enzymes · halogens · hormones · iodine · tellurium

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