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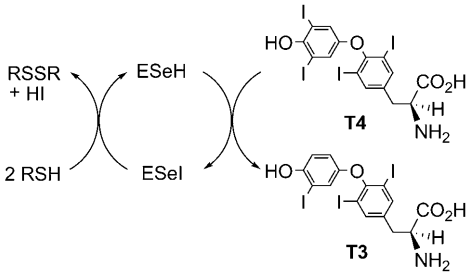
Enzyme Models

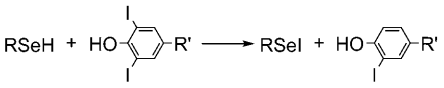
Modeling of the 5′-Deiodination of Thyroxine by Iodothyronine Deiodinase: Chemical Corroboration of a Selenenyl Iodide Intermediate**

Kei Goto, Daiju Sonoda, Keiichi Shimada, Shohei Sase, and Takayuki Kawashima**

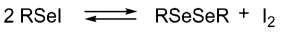
Type I iodothyronine deiodinase (ID-1) is an enzyme that catalyzes the conversion of a human thyroid prohormone (thyroxine; **T4**) into a biologically active hormone (3,5,3′-triiodothyronine; **T3**) through 5′-deiodination (Scheme 1), and contains a selenocysteine residue at its active site.[1] The mechanism proposed for the catalytic cycle of ID-1 involves a ping-pong bisubstrate reaction in which the selenol form of the enzyme (ESeH) reacts with **T4** to form a selenenyl iodide intermediate (ESeI) with release of the deiodinated compound **T3**, and a subsequent reaction between the selenenyl iodide intermediate and possibly a thiol cofactor regenerates the selenol form.[1,2] The involvement of a selenenyl iodide as an intermediate in this deiodination process has been widely accepted, and many model studies on the catalytic cycle of ID-1 have been performed assuming the formation of such intermediates.[3] Investigations of antithyroid drugs have been based on the proposed mechanism depicted in Scheme 1.[4] However, the chemical evidence for the formation of a selenenyl iodide in the deiodination reaction of 2,6-diiodophenol derivatives by an organoselenol [Eq. (1)] has been entirely circumstantial. Although there have been several reports of the reaction of 2,6-diiodophenol derivatives with organoselenols,[3a,b] the formation of the corresponding selenenyl iodide intermediates has never been detected. The verification of the process in Equation (1) has been difficult not only because of the instability of selenenyl iodides,[5] but also as a result of the high reactivity of selenenyl iodides toward selenols.[3c] Herein, we report experimental evidence for the formation of a selenenyl iodide in 5′-deiodination of a thyroxine derivative by an organoselenol, through the use of a nanosized molecular cavity to stabilize the selenenyl iodide intermediate.



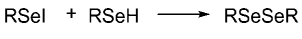
Scheme 1. Proposed mechanism for the deiodination of thyroxine **T4** by ID-1. E = enzyme.



(1)

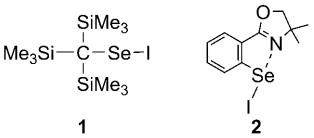


(2)



(3)

Selenenyl iodides usually undergo facile disproportionation to the corresponding diselenide and iodine [Eq. (2)].[5] To demonstrate the process in Equation (1), it is essential that the selenenyl iodide formed in the reaction not undergo such disproportionation or react with the parent selenol to produce the diselenide [Eq. (3)]. There have been several reports of the isolation of selenenyl iodides stabilized against disproportionation through the introduction of a sterically demanding alkyl substituent (**1**)[6] or an intramolecular coordinating group (e.g. **2**).[7] However, it has been reported that even **2** reacts with the corresponding selenol to produce the symmetrical diselenide within several minutes at room temperature.[3c] In the course of our study on the nanosized molecular cavities,[8] we previously developed a novel steric protection group, a Bpq group (cavity-shaped substituent; Scheme 2),[9a] and showed that it very effectively stabilizes organoselenium species, such as a *Se*-nitrososelenol (RSeNO), which are



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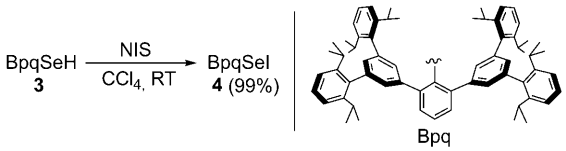


Scheme 2. Synthesis of a stable selenenyl iodide bearing a cavity-shaped steric protection group (Bpq).

otherwise extremely labile.[9b,c] Selenenyl iodide **4** bearing the Bpq group was synthesized by the reaction of selenol **3**[9b] with *N*-iodosuccinimide (NIS) and gave a quantitative yield (Scheme 2). Its structure was established by X-ray crystallography (Figure 1).[10] Selenenyl iodide **4** showed remarkable stability; no decomposition was observed in [D_8]toluene after heating at 100 °C in a sealed tube for seven days. In ^{1}H and ^{77}Se NMR spectra and a UV/Vis spectrum measured at room temperature, an equilibrium between selenenyl iodide **4** and the corresponding diselenide was not observed. Treatment of selenenyl iodide **4** with selenol **3** in $CDCl_3$ at room temperature for seven days resulted in no change. Very slow formation of the corresponding diselenide, BpqSeSeBpq (**5**),[9c] was observed in the presence of triethylamine, but the yield was only 22% after seven days. These results indicate that selenenyl iodide **4** has high compatibility with selenol **3**, which is required to demonstrate the process described by Equation (1). As shown in Figure 1, the SeI functionality of **4** was incorporated in the cavity, and the bimolecular processes shown in Equations (2) and (3) are considered to be effectively prevented by steric repulsion between the periphery of the molecules.

Deiodination of a thyroxine derivative, *N*-butyrylthyroxine methyl ester (**6**),[3b] by selenol **3** was investigated (Scheme 3). Deiodination of **6** proceeded slowly when it was treated with an equimolar amount of **3** in the presence of triethylamine, and after seven days 65% had been converted into the monodeiodinated product **7** with the concomitant formation of selenenyl iodide **4** (55% from **3**). This is the first experimental demonstration of the chemical transformation shown in Equation (1). Notably, only the iodine atom on the outer phenol ring of **6** was removed; no deiodination at the inner ring was detected. This result is similar to the conversion of **T4** into **T3** in the ID-1 catalytic cycle (Scheme 1).

Control experiments were carried out using 2,6-diiodo-4-phenoxyphenol (**8**)[3b] as a model compound of thyroxine.

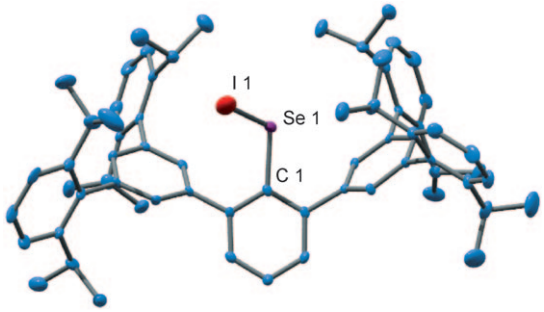
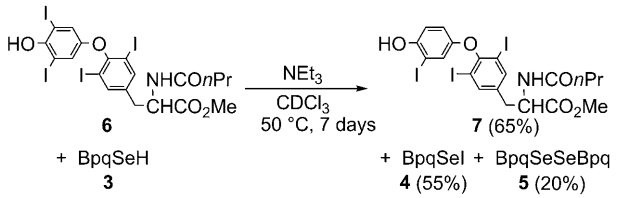


Figure 1. The thermal ellipsoids are drawn at the 50% probability level for **4**. Selected bond lengths [Å] and bond angle [°]: I1–Se1 2.5203(11), Se1–C1 1.946(5); I1-Se1-C1 102.14(15).

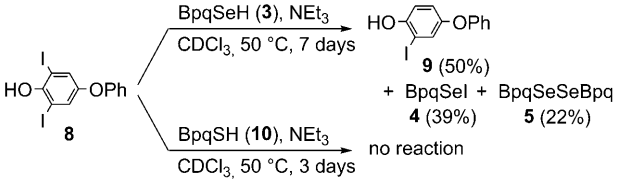


Scheme 3. Deiodination of the thyroxine derivative **6** by selenol **3**. The yields were estimated by ^{1}H NMR spectroscopy.

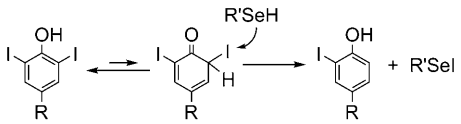
Similar to the reaction of **6**, the reaction of **8** with selenol **3** in the presence of triethylamine produced monoiodophenol **9** and selenenyl iodide **4** (Scheme 4). However, in the absence of amine, no reaction took place under similar conditions. In the active site of ID-1 there are histidine residues near the selenocysteine catalytic center, and it has been proposed that the imidazole unit deprotonates the selenol to increase its reactivity.[11] The present results indicate that the efficiency of the deiodination process largely depends on the nucleophilicity of the selenol functionality.

It has been reported that replacement of the selenocysteine residue of ID-1 with cysteine significantly reduces the catalytic activity of the enzyme.[2a] When thiol **10** bearing a Bpq group was used instead of selenol **3**, no reaction with **8** was observed (Scheme 4). This outcome suggests that the selenol functionality, with greater nucleophilicity, is essential for this deiodination reaction.

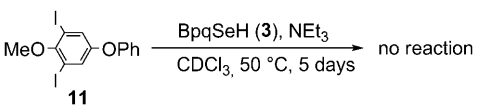
As a mechanism for the deiodination process shown in Equation (1), nucleophilic attack of a selenol (or selenolate) on the iodine center of the keto form of the diiodophenols has been proposed (Scheme 5).[3b] Consistent with this mechanism, methyl ether **11**, which cannot undergo tautomerization to the keto form, gave no reaction with **3** under these conditions (Scheme 6). Selective deiodination at the outer ring of the thyroxine derivative **6** and the inertness of its inner ring (Scheme 3) are also consistent with the mechanism involving tautomerization.



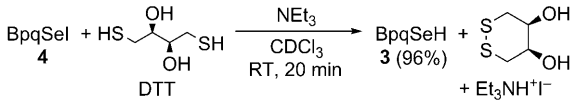
Scheme 4. Reactions of diiodophenol **8** with selenol **3** or thiol **10**. The yields were estimated by ^{1}H NMR spectroscopy.



Scheme 5. Proposed mechanism for the deiodination process via enol/keto tautomerization.



Scheme 6. Reaction of methyl ether **11** with selenol **3**.



Scheme 7. Reduction of selenenyl iodide **4** to selenol **3** by DTT.

Although the physiological cofactor in the reduction of the selenenyl iodide intermediate to the selenol in the ID-1 catalytic cycle (Scheme 1) has not been identified, the use of dithiols, including dithiothreitol (DTT), as the second substrate is common for in vitro experiments with the enzyme.[1b] Treatment of selenenyl iodide **4** with DTT in the molar ratio of 1:2.4 in the presence of triethylamine produced selenol **3** quantitatively within 20 minutes (Scheme 7).[12]

In summary, the formation of a selenenyl iodide in the deiodination of a thyroxine derivative by an organoselenol was demonstrated for the first time by using cavity-shaped molecules. In conjunction with reduction of the selenenyl iodide to the parent selenol by a dithiol, all the chemical transformations included in the ID-1 catalytic cycle were experimentally established, thus corroborating the involvement of a selenenyl iodide as an intermediate in the enzymatic reaction. Further investigations on the mechanistic details of the processes are currently underway.

Experimental Section

Synthesis of selenenyl iodide **4**: CCl_4 (10 mL) was added to a mixture of selenol **3** (1.10 g, 1.15 mmol) and *N*-iodosuccinimide (561 mg, 2.49 mmol) at room temperature. The reaction mixture was stirred for 2 h, filtered through Celite, and the solvent was evaporated. Recrystallization from *n*-hexane gave selenenyl iodide **4** (1.23 g, 1.14 mmol, 99%) as purple crystals. **4**: m.p. 278.0–281.0°C (decomp); ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 1.07$ (d, $J = 6.8$ Hz, 24H), 1.13 (d, $J = 6.8$ Hz, 24H), 2.91 (sept, $J = 6.8$ Hz, 8H), 7.02 (t, $J = 1.5$ Hz, 2H), 7.17 (d, $J = 7.7$ Hz, 8H), 7.24 (d, $J = 1.5$ Hz, 4H), 7.30 (t, $J = 7.7$ Hz, 4H), 7.39–7.49 ppm (m, 3H); ^{13}C NMR (126 MHz, [D_6]benzene; s, d, and q are the multiplicities of the signals in the non-decoupled spectrum): $\delta = 24.1$ (q), 24.5 (q), 30.6 (d), 122.7 (d) × 2, 128.1 (d), 128.3 (d), 129.2 (d), 129.7 (d), 139.2 (s), 140.5 (s), 142.6 (s), 146.8 (s) × 2, 149.8 ppm (s); ^{77}Se NMR (95 MHz, $CDCl_3$): $\delta = 465$ ppm; UV/Vis (benzene) λ_{max} 553 nm (ε 280); elemental analysis calcd (%) for $C_{66}H_{77}ISe$: C 73.66, H 7.21; found: C 73.48, H 7.32. For details of the reaction of **3** with iodophenols, see the Supporting Information.

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[10] **4**·2$CHCl_3$: $C_{68}H_{79}Cl_6ISe$, $M_r = 1314.87$, triclinic, space group $P\bar{1}$, $a = 14.499(7)$, $b = 15.452(7)$, $c = 16.293(6)$ Å, $\alpha = 69.028(12)$, $\beta = 73.775(11)$, $\gamma = 88.047(15)$°, $V = 3264(2)$ Å^3, $Z = 2$, $\rho_{calcd} = 1.338$ g cm^{-3}, $T = 120$ K, $\mu(Mo_{K\alpha}) = 1.332$ mm^{-1}, 21498 measured reflections, 11269 independent, 729 parameters, $R1 = 0.0635$ ($I > 2\sigma(I)$), $wR2 = 0.1781$ (all data). The intensity data were collected on a Rigaku/MSC Mercury CCD diffractometer with graphite-monochromated $Mo_{K\alpha}$ radiation ($\lambda = 0.71070$ Å). The structures were solved by the direct method and refined by full-matrix least squares on F^2 using SHELXL 97 (G. M. Sheldrick, Program for Crystal Structure Refinement, University of Göttingen, **1997**). The non-hydrogen atoms were refined anisotropically. The hydrogen atoms were idealized by using the riding models. CCDC 750141 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

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[12] The reduction of selenenyl iodide **2** has been investigated by du Mont and co-workers.[3c] It was found that the reaction of **2** with monothiols proceeds readily to afford the corresponding selenenyl sulfides (ArSeSR) although further reduction to the selenol is very slow.

