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Phosphine-mediated Reduction of a Selenocysteine Selenenyl Iodide to a Selenocysteine Selenol

Satoru Kuwano, Ko Fujisaki, Kei Goto*

Department of Chemistry, School of Science, Institute of Science Tokyo

Abstract

Trivalent phosphorus compounds are widely used for the reduction of biologically relevant sulfur- and selenium-containing species due to its strong redox potential, broad pH stability, and ability to minimize unwanted side reactions or competitive interactions with other thiol- or selenol-containing compounds. Selenocysteine selenenyl iodides (Sec–SeIs) have attracted increasing attention as key intermediates in the enzymatic functions of iodothyronine deiodinases. Investigating whether Sec–SeIs can serve as substrates for reduction by trivalent phosphorus compounds could provide valuable insights into the existence and behavior of Sec–SeI in proteins. However, to date, there have been no studies examining the reactivity between trivalent phosphorus compounds and selenenyl iodides. In this study, phosphine-mediated reduction of a selenocysteine selenenyl iodide to a selenocysteine selenol was developed using isolable model compounds stabilized by nanosized molecular cradle. The present study demonstrates that phosphines serve as excellent non-thiol reducing agents for selenenyl iodides, particularly in terms of their high reduction efficiency and lack of interfering thiol or selenol groups.

Keywords: Selenocysteine, Selenenyl iodide, Selenol, Phosphine, Reduction

Statement about COI: The authors declare no conflict of interest associated with this manuscript.

Dedication: Dedicated to Professor Takayuki Kawashima on the occasion of his 77th birthday.

Introduction

Trivalent phosphorus compounds are widely used for the reduction of sulfur- and selenium-containing species in biological systems. A representative example is tris(2-carboxyethyl)phosphine (TCEP), which is often preferred over alternative reducing agents, such as dithiothreitol (DTT), due to its strong redox potential, broad pH stability, and ability to minimize unwanted side reactions or competitive interactions with other thiol- or selenol-containing compounds [1-4]. It has also been reported that trivalent phosphorus compounds can reduce reactive intermediates formed by the oxidative modification of thiols, such as sulfenic acids (R-SOH) [5-12, 13-15]. For instance, the reduction of cysteine sulfenic acid (Cys-SOH) to cysteine thiol (Cys-SH) by TCEP has been considered experimental evidence supporting the presence of Cys-SOH (**Scheme 1**) [16].

Correspondence ;
Kei Goto
2-12-1 Ookayama, Meguro-ku, Tokyo 152-8550, Japan
Tel ; +81-3-5734-3543
e-mail ; goto@chem.titech.ac.jp

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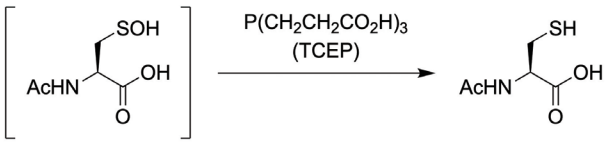
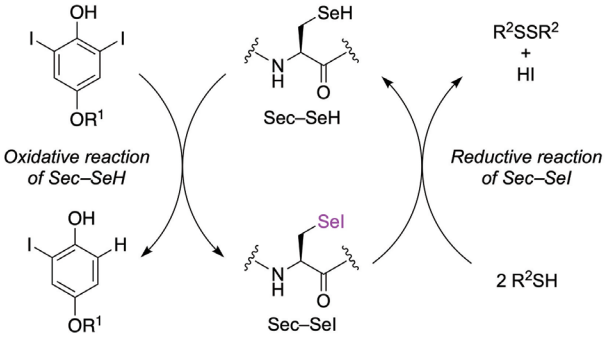


Scheme 1. Reduction of a transiently generated sulfenic acid by TCEP.

Selenocysteine selenenyl iodides (Sec–SeI) have attracted increasing attention as key intermediates in the enzymatic functions of iodothyronine deiodinases (Dios). These enzymes regulate the concentration of active thyroid hormones through the deiodination of iodothyronines, a process mediated by selenocysteine selenols (Sec–SeH) at the catalytic site, which is believed to involve the formation of Sec–SeI intermediates (**Scheme 2**) [17-31]. Investigating whether Sec–SeIs can serve as substrates for reduction by trivalent phosphorus compounds could provide valuable insights into the existence and behavior of Sec–SeI in proteins. However, to date, there have been no studies examining the reactivity between trivalent phosphorus compounds and selenenyl iodides, including non-selenocysteinyl derivatives [32-34]. This is largely due to the inherent instability of selenenyl iodides, which readily undergo disproportionation to diselenides and elemental iodine (**Scheme 3**) [35, 36].



Scheme 2. Proposed catalytic mechanism of iodothyronine deiodinases.

Scheme 3. Disproportionation of selenenyl iodides.

As a protective group to stabilize the biologically relevant reactive intermediates, we have developed a nanosized molecular cradle that can accommodate a reactive amino acid residue [37-42]. By using the molecular cradle as an *N*-terminal protecting group (henceforth denoted as "Bpsc"), we recently succeeded in the synthesis and isolation of selenocysteine-derived selenenyl iodide **1** (**Figure 1**) [38-43]. Here, we report the reduction of the stably isolated Sec–SeI to the corresponding Sec–SeH by phosphines, providing direct experimental evidence of this reaction.

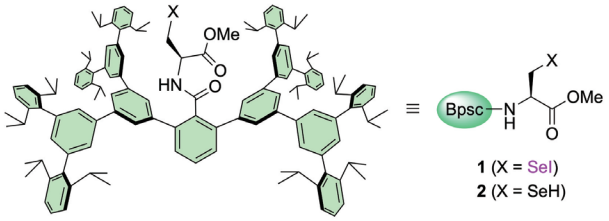


Figure 1. Isolable Sec–SeI (**1**) and Sec–SeH (**2**) stabilized by a molecular cradle.

Materials and methods

General

All synthetic experiments were performed under argon atmosphere. Selenocysteine selenenyl iodide **1** was prepared according to the reported procedure [42]. Anhydrous THF was purchased from Kanto Chemical and passed through a Kayama Oxygen solvent purification system prior to use. Other chemicals were purchased from commercial sources and used as received. ^{1}H NMR spectra were recorded on a JEOL ECS-400, and the chemical shifts of ^{1}H are referenced to the residual proton signal of $CDCl_3$ (δ 7.26). ^{31}P NMR spectra were recorded on JEOL ECX-500 using $CDCl_3$ as a solvent, and the chemical shifts of ^{31}P were referenced to PPh_3 (δ −5.65) as an external standard.

Reductive transformation from selenocysteine selenenyl iodide 1 to selenocysteine selenol 2 promoted by Ph_3P.

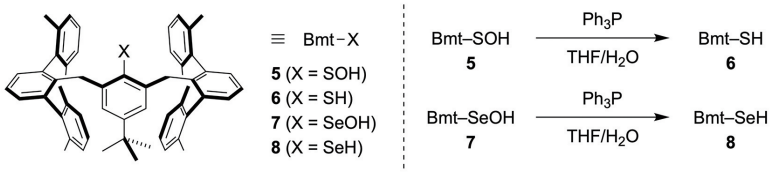
To the mixture of THF (0.8 mL) and H_2O (0.2 mL) in Schlenk flask, solution of Ph_3P in THF (0.111 M, 50.0 µL, 5.58 µmol, 1.20 eq) and solution of selenenyl iodide **1** in THF (0.0186 M, 250 µL, 4.65 µmol) were added. The resulting mixture was stirred for 10 seconds at room temperature. To the Schlenk flask, H_2O (2.0 mL) was added, and the aqueous layer was extracted with Et_2O. Combined organic layers were concentrated to give crude white solids. The crude mixture was washed with MeCN and dried. By ^{1}H NMR spectroscopy, the content of selenocysteine selenol **2**, the corresponding diselenide **3** and the corresponding dehydroalanine **4** was estimated to be 72%, 15% and 3%, respectively. The filtrate was concentrated to yield white solids, in whose ^{31}P NMR spectrum triphenylphosphine oxide was the only detectable Ph_3P-derived product, along with the remaining Ph_3P.

Reductive transformation from selenocysteine selenenyl iodide 1 to selenocysteine selenol 2 promoted by $^nBu_3P$.

To the mixture of THF (0.8 mL) and H_2O (0.2 mL) in Schlenk flask, solution of $^nBu_3P$ in THF (0.100 M, 55.0 µL, 5.50 µmol, 1.19 eq) and solution of selenenyl iodide **1** in THF (0.0186 M, 250 µL, 4.64 µmol) were added. The resulting mixture was stirred for 10 seconds at room temperature. To the Schlenk flask, H_2O (2.0 mL) was added, and the aqueous layer was extracted with Et_2O. Combined organic layers were concentrated to give crude white solids. The crude mixture was washed with MeCN and dried. By ^{1}H NMR spectroscopy, the content of selenocysteine selenol **2**, the corresponding diselenide **3** and the corresponding dehydroalanine **4** was estimated to be 67%, 31% and 2%, respectively.

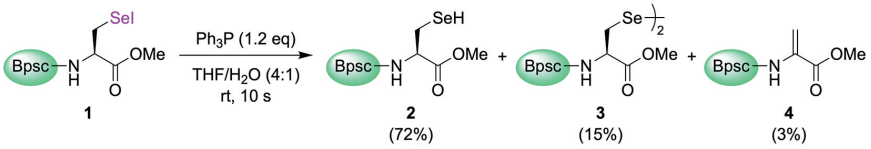
Results and discussion

While there have been several reports on the reductions of sulfenic acids by trivalent phosphorus compounds, these reactions have been limited to transiently generated R–SOHs [13-15], as sulfenic acids are inherently unstable due to their tendency to undergo self-condensation. In contrast, we have synthesized a stable sulfenic acid **5** bearing a cavity-shaped steric protecting group (a Bmt group) and demonstrated its reduction to the corresponding thiol **6** by triphenylphosphine in a mixed solvent of THF and H_2O (**Scheme 4**) [44]. We have also demonstrated the phosphine-mediated reduction of a selenenic acid (R–SeOH) through the reaction of selenenic acid **7** stabilized by a Bmt group with triphenylphosphine, yielding selenol **8**.



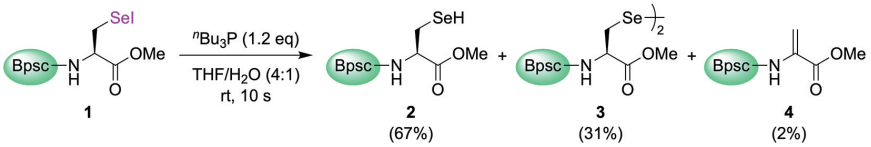
Scheme 4. Phosphine-promoted reduction of a sulfenic acid and a selenenic acid stabilized by a cavity-shaped steric protection group.

Based on these findings, Sec–SeI **1** was treated with 1.2 equivalents of triphenylphosphine in a mixed solvent of THF and H_2O at room temperature (**Scheme 5**). Within 10 seconds, purple color of the Sec–SeI **1** disappeared, and compound **1** was completely consumed. ^{1}H NMR spectroscopic analysis revealed that Sec–SeH **2** was obtained as the major product in 72% yield, indicating that triphenylphosphine reduces selenenyl iodides very efficiently. In addition to the target Sec–SeH **2**, the corresponding diselenide **3** and dehydroalanine **4** were produced in 15% and 3% yields, respectively. The formation of diselenide **3** may be attributed to the reaction between the starting material, Sec–SeI **1**, and the generated product, Sec–SeH **2**. The small amount of dehydroalanine **4** is likely derived from thermal deselenation of Sec–SeOH [38], which is produced by hydrolysis of Sec–SeI **1** in a water-containing solvent [42]. However, because the hydrolysis is very sluggish under neutral conditions, the amounts of the resulting Sec–SeOH and its deselenation product **4** are considered to be very low. ^{31}P NMR spectroscopic analysis revealed that triphenylphosphine oxide, which is considered to be formed by the hydrolysis of an initially generated phosphonium salt, was the only detectable triphenylphosphine-derived product, along with the remaining triphenylphosphine.



Scheme 5. Ph_3P-promoted reductive transformation of Sec–SeI **1** to Sec–SeH **2**.

In addition to the triarylphosphine, we also investigated trialkylphosphine as a reductant for selenenyl iodides. When Sec–SeI **1** was treated with tributylphosphine under the same conditions, Sec–SeH **2** was obtained as the major product in 67% yield, along with diselenide **3** and dehydroalanine **4** (**Scheme 6**). These results demonstrate that trialkylphosphine is also a good reducing agent for selenenyl iodides, and strongly suggests that phosphine reagents such as TCEP will be useful for the efficient reduction of Sec–SeIs generated in proteins.



Scheme 6. $^nBu_3P$-promoted reductive transformation of Sec–SeI **1** to Sec–SeH **2**.

In conclusion, we have demonstrated for the first time the phosphine-mediated reduction of selenocysteine selenenyl iodide to selenocysteine selenol. In the catalytic cycle of Dios, selenenyl iodide intermediates are postulated to be reduced to their parent selenols by thiol cofactors. We previously reported thiol-mediated reductions of selenenyl iodides bearing both selenocysteinyl and nonselenocysteinyl backbones [38-43, 45, 46]. The present study clearly shows that phosphines serve as excellent non-thiol reducing agents for selenenyl iodides, particularly in terms of their high reduction efficiency and lack of interfering thiol or selenol groups.

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