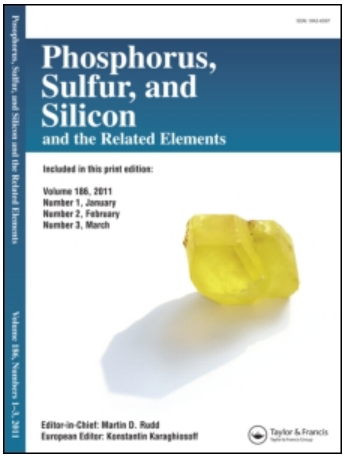

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Applications of Water-Soluble Selenides and Selenoxides to Protein Chemistry

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Two water-soluble organic selenides, 3,3′-selenodipropanonic acid and 2-selenanecarboxylic acid, were synthesized and reversibly converted to the corresponding selenoxides in deuterium oxide by the sequential treatment with stoichiometric amounts of hydrogen peroxide and then dithiothreitol. The antioxidative catalytic activity of 3,3'-selenodipropanonic acid was subsequently investigated by NMR spectroscopy in the reaction of hydrogen peroxide with dithiothreitol, cysteamine, and benzyl mercaptan in deuterated methanol. For all thiol substrates, the selenide exhibited the distinct catalytic activity. However, the activity was remarkably higher for the dithiol substrate (i.e., dithiothreitol). These results suggested that water-soluble selenides are good glutathione peroxidase mimics, in particular when polythiols are employed as the substrate. It was also suggested that water-soluble selenoxides are efficient oxidizing reagents for protein disulfide-bond formation.

Keywords Dithiothreitol; glutathione peroxidase; oxidative protein folding; polythiol; selenide; selenoxide

INTRODUCTION

Interconversion reactions between thiols and disulfides are important chemical processes involved in many biological phenomena, such as antioxidant functions of cells against reactive oxygen species[1] and the oxidative folding of proteins having several disulfide (SS) bonds.[2] Modeling these processes by using small organic molecules is important for elucidation of the phenomena, which in reality are regulated by a complex system comprising a number of enzymes and substrates, at the atomic and molecular resolutions.

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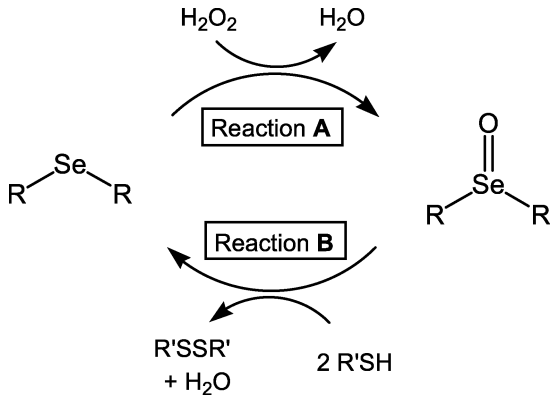

FIGURE 1 Inter-conversion between selenides and selenoxides.

On the other hand, organic selenides and selenoxides can be easily inter-converted by using hydrogen peroxide (H_2O_2) and thiol (R'SH) as the oxidizing and reducing reagents, respectively (Figure 1). The redox reactions normally proceed rapidly and quantitatively, hence the selenide–selenoxide chemistry would be applied to regulation of the redox states in various biological as well as the mimic systems that include thiol and disulfide species.[3] In this paper, we demonstrate the utility of water-soluble selenides as antioxidant enzyme models of glutathione peroxidase (GPx),[4] which is a representative selenoenzyme catalyzing the reduction of hydrogen peroxide to water by using glutathione as the reducing substrate. We also discuss here the possibility of the corresponding selenoxides to be applied as strong oxidizing reagents for the oxidation of a reduced form of a protein, which has several SS bonds in the native state.

RESULTS AND DISCUSSION

Two water-soluble selenides (**1** and **2**) that have one and two carboxylic group(s) were synthesized from 3-bromopropanoic acid and 2,6-dibromohexanoic acid, respectively, by the reaction with sodium hydrogen selenide (NaHSe). The selenides were then treated with H_2O_2 in deuterium oxide (D_2O) to produce the corresponding selenoxides (**3** and **4**) quantitatively (see Reaction **A** in Figure 1). Although the ^{1}H NMR spectra indicated the presence of some conformers, the ^{13}C and ^{77}Se NMR spectra clearly revealed that only one species, which has characteristics of a selenoxide structure ($\delta_{Se} = 865$ ppm for **3** and 757 ppm for **4**), was formed in D_2O. It was previously reported that stable spirocyclic selenurane **5** is produced by oxidation of **1**.[5] However, the species that

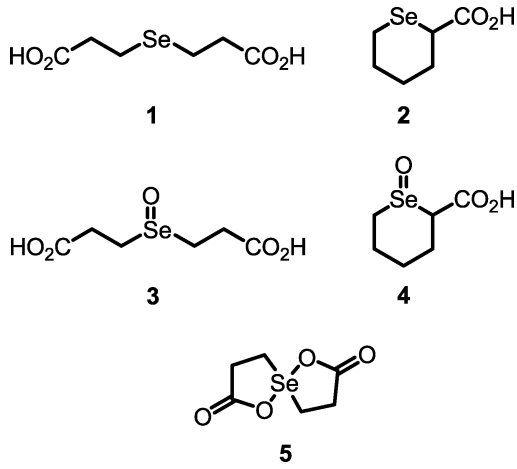

CHART 1

we observed in the oxidation of **1** should be different from **5** because the species was transiently formed in D_2O and started to decompose after 1 hour to a complex mixture of structurally unknown compounds. The both selenoxides (**3** and **4**) generated in situ were reacted with dithiothreitol (DTT^{red}) (**6**) to quantitatively give the selenides (**1** and **2**) and oxidized DTT (DTT^{ox}) (**7**) (see Reaction **B** in Figure 1). It should be noted that both **3** and **4** decomposed instantly when they were isolated in air.

The reversible transformation reactions described above (Reactions **A** and **B**) suggested the possibility that the water-soluble selenides can be catalysts for the reaction between H_2O_2 and DTT^{red} (**6**) (Scheme 1), which is conceivable as a simple model reaction of GPx catalytic cycle.[6] Indeed, it was found that the reaction proceeds significantly faster in the presence of a catalytic amount of **1** than it does in the absence of the selenium catalyst. Since the reaction took place too fast in D_2O to be monitored by ^1H NMR, deuterated methanol (CD_3OD) was selected as a solvent for the assay of the antioxidative catalytic activity.

SCHEME 1

In a series of the ^1H NMR spectra (Figure 2), it was observed that the signals of thiol substrate **6**, which appeared at around 2.64 and

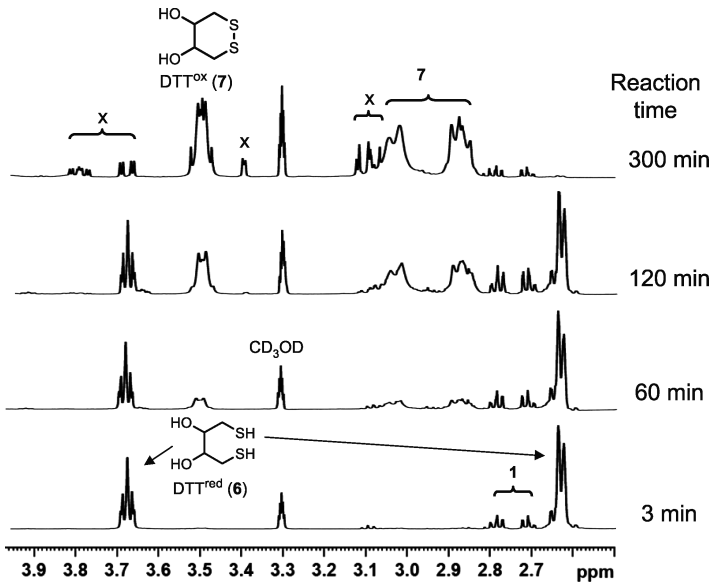

FIGURE 2 A series of 500 MHz ^{1}H NMR spectra obtained in the oxidation of DTT^{red} (**6**) (0.15 mmol) with H_2O_2 (0.15 mmol) in the presence of a catalytic amount of selenide **1** (0.015 mmol) in CD_3OD (1.1 mL) at 298 K. Unknown byproduct (x) was formed after 300 min.

3.68 ppm, decreased with increasing the reaction time, while those of the oxidized product (DTT^{ox}) (**7**), which appeared at around 2.88, 3.04, and 3.52 ppm, increased. After 300 min, the signals of **6** completely disappeared on the ^{1}H NMR spectrum, leaving large signals of **7** and small ones of **1** and an unknown byproduct, which may be derived from **6**. Figure 3 shows remaining ratios of 6 in the reaction mixture as a function of the reaction time. The plots clearly showed that the reaction was efficiently accelerated by addition of a catalytic amount (0.1 molar eq.) of **1**, suggesting that the selenide–selenoxide system (Figure 1) is useful for modeling the GPx catalytic cycle.

The antioxidative catalytic activity of **1** against H_2O_2 was also examined by using monothiols, e.g., cysteamine ($H_2NCH_2CH_2SH$, **8**) and benzyl mercaptan ($PhCH_2SH$, **9**), instead of dithiol **6**. In these cases, however, the catalytic activity of **1** significantly decreased as shown in Figures 4 and 5. The observed reduction of the catalytic activity with the monothiol substrates suggested that the catalytic function of the water-soluble selenide is understood by considering the following mechanism (Figure 6).

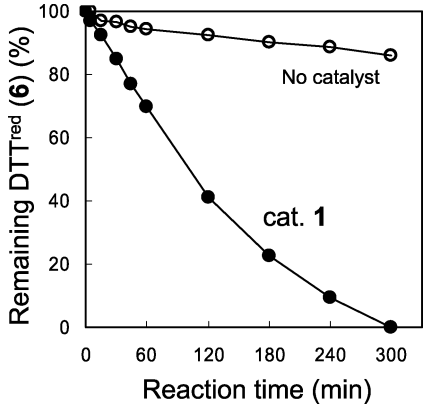

FIGURE 3 Remaining ratios of DTT^{red} (**6**) as a function of the reaction time in the reaction of Scheme 1. See Figure 2 for reaction conditions.

In the first step, a selenide reacts with H_2O_2 to produce a selenoxide and H_2O. In the next step, the selenoxide, which must be highly reactive, would be rapidly captured by a thiol substrate to form a cross-linked intermediate (**10**) with a Se-S bond. In the final step, the intermediate reacts with the second thiol substrate to regenerate the selenide accompanied with formation of a disulfide and H_2O. When the substrate contains two thiol groups in a molecule like DTT^{red} (**6**), the final step (i.e., the step from **10** to the selenide in Figure 6) becomes an intramolecular process, which would make the whole catalytic cycle proceed more

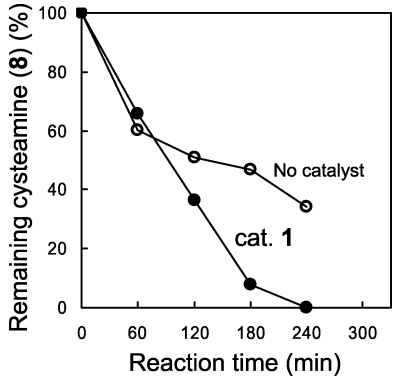

FIGURE 4 Remaining ratios of cysteamine ($NH_2CH_2CH_2SH$, **8**) as a function of the reaction time in the oxidation of **8** (0.12 mmol) with H_2O_2 (0.06 mmol) in the presence of selenide **1** (0.012 mmol) in CD_3OD (1.1 mL) at 298 K.

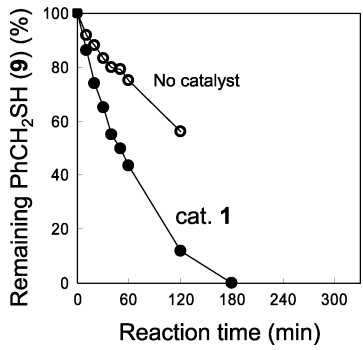

FIGURE 5 Remaining ratios of benzyl mercaptan ($PhCH_2SH$, **9**) as a function of the reaction time in the oxidation of **9** (0.40 mmol) with H_2O_2 (0.20 mmol) in the presence of selenide **1** (0.040 mmol) in CD_3OD (1.3 mL) at 298 K.

smoothly. The catalytic cycle proposed in Figure 6 is an analog to that of GPx:[4] the selenide corresponds to a selenol intermediate (E–SeH), and the selenoxide does to a selenenic acid intermediate (E-SeOH). At the GPx active site, despite being employed a monothiol glutathione (GSH) substrate, the selenenyl sulfide intermediate (E-SeSG) can react with the second GSH molecule very smoothly due to the presence of specific interactions between the enzyme and GSH.[6] Such interactions are of course absent in our model reaction.

Advantages of the use of polythiol substrates in the reactions of selenium reagents were previously pointed out by several research groups.

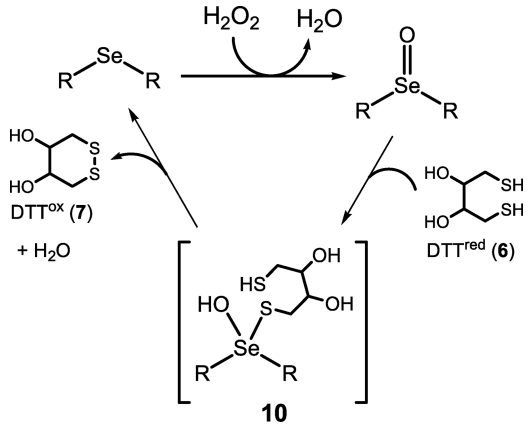

FIGURE 6 A plausible catalytic cycle of selenide **1** in the reduction of H_2O_2 with DTT^{red} (**6**).

For example, Günther reported that aliphatic diselenides can be quantitatively transformed to the selenols by DTT^{red} (**6**) in aqueous solution.[7] It was also reported that the peroxidase activity of ebselen is increased by using a dithiol substrate instead of GSH.[8] Related to this issue, Mugesh recently pointed out that the specificity of the second SH attack at the S atom, not at the Se atom, of the selenenyl sulfide intermediate is important to improve the GPx-like catalytic activity.[9] Thus, the use of polythiol substrates in the selenide–selenoxide system would also be rationalized.

On the other hand, Iwaoka and Tomoda synthesized another water-soluble selenide, called DHS^{red},[10] and applied the corresponding selenoxide, DHS^{ox}, to the oxidative folding study of ribonuclease A,[11] which have four disulfide bonds in the native folded state. The completely reduced protein and the partially oxidized folding intermediates can be considered as polythiol substrates to the selenoxide. Therefore, the selenoxide allowed rapid and quantitative formation of the SS bonds without the detection of the cross-linked intermediates.[11] The results strongly suggested that the selenide–selenoxide chemistry is also applicable for oxidation of protein thiol groups. Such applications are now undertaken elaborately in our laboratory.

In summary, we synthesized water-soluble selenides **1** and **2** and investigated the redox chemistry by using H_2O_2 and DTT^{red} (**6**) as oxidizing and reducing reagents, respectively. When selenide **1** was applied as a catalyst for the GPx model reaction, it was found that the antioxidative catalytic activity was significantly enhanced by using a dithiol substrate, i.e., DTT^{red} (**6**), suggesting the importance of the use of polythiol substrates. The features of water-soluble selenides and selenoxides revealed here would also be useful when water-soluble selenoxides are applied as an oxidizing reagent to oxidative folding study of proteins.

EXPERIMENTAL

3,3′-selenodipropanonic acid (**1**) were synthesized from 3-bromopropanoic acid by the reaction with sodium hydrogen selenide (NaHSe) according to the modified literature method.[12]

2-Selenanecarboxylic Acid (2)

2,6-dibromohexanoic acid[13] (0.10 g, 0.37 mmol) was dissolved in 10 mL of aqueous $NaHCO_3$ solution, and the solution was slowly added to a freshly prepared aqueous NaHSe solution at 1°C under nitrogen atmosphere. After 5 h, the reaction mixture was warmed up at room temperature and stirred overnight under air. Precipitated selenium was

filtered off, and the filtrate was acidified with concentrated HCl solution and extracted with ether. After evaporation, the resulting crude product was purified by recrystallization from water to give selenide **2** in 56% yield (0.040 g) as colorless crystals. mp. 69–70°C. Spectral data for **2**: 500 MHz ^{1}H NMR (in D_2O) δ 1.25–1.35 (1H, m), 1.55–2.20 (1H, m), 1.60–1.80 (2H, m), 1.80–1.90 (1H, m), 2.00–2.10 (1H, m), 2.60–2.75 (2H, m), 3.67 (1H, dd, $J = 3.3$ and 6.0 Hz); 125.8 MHz ^{13}C NMR δ 21.0, 24.6, 26.4, 29.3, 34.4, 177.5; 95.4 MHz ^{77}Se NMR δ 250.0; LRMS *m/z* 194 (M^+, Se×1). Anal. Calcd for $C_6H_{10}O_2Se$: C, 37.32; H, 5.22. Found: C, 37.03; H 5.29.

Oxidation of Selenides 1 and 2

10–30 mg of the selenide was dissolved in 1.0 mL of D_2O, and the solution was added with an equimolar amount of H_2O_2.

3,3′-Seleninyldipropanonic Acid (3)

Spectral data for **3**: ^{1}H NMR (in D_2O) δ 2.88 (4H, m), 3.67–3.84 (2H, m), 3.84–4.04 (2H, m). ^{13}C NMR δ 27.2, 29.7, 176.6; ^{77}Se NMR δ 865.4.

2-Selenanecarboxylic Acid 1-Oxide (4)

Spectral data for **4**: ^{1}H NMR (in D_2O) δ 1.32–1.50 (1H, m), 1.60–1.98 (4H, m), 2.08–2.19 (1H, m), 2.77–30.4 (2H, m), 3.67 (1H, dd, $J = 3.0$ and 6.0 Hz); ^{13}C NMR δ 24.1, 24.3, 25.6, 28.6, 38.5, 176.5; ^{77}Se NMR δ 756.6.

Assay of the Catalytic Activity

DTT^{red} (**6**) (0.15 mmol) and selenide **1** (0.015 mmol) were dissolved in CD_3OD (1.1 mL), and the solution was added with H_2O_2 (0.15 mmol) to start the reaction. ^{1}H NMR spectra were measured at the reaction time of 3, 15, 30, 45, 60, 120, 180, 240, and 300 min at 298 K. The relative populations of the thiol and the disulfide were determined by integrals of the ^{1}H NMR absoptions that were well isolated on the spectra. The similar reactions were carried out in the absence of the selenide catalyst. For the reactions using cysteamine (**8**) and benzyl mercaptan (**9**) instead of DTT^{red} (**6**), 0.5 and 0.1 molar equivalents of H_2O_2 selenide **1**, respectively, were added to the solution.

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