Enzyme Mimetics

The Exceptional Glutathione Peroxidase-Like Activity of Di(3-hydroxypropyl) Selenide and the Unexpected Role of a Novel Spirodioxaselenanonane Intermediate in the Catalytic Cycle**

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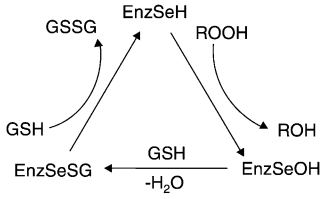
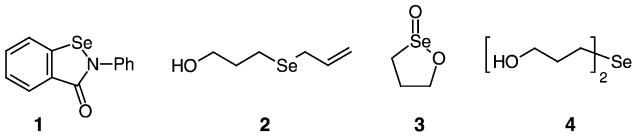
Oxidative stress results from the formation of peroxides and other reactive oxygen species during the course of normal aerobic metabolism.[1] These reactive intermediates and the free radicals they produce cause damage to various biologically important molecules present in cells. Peroxides have been implicated in a number of degenerative processes and disease states, including inflammation, mutagenesis, and cancer, atherosclerosis, Alzheimer's disease, and the aging process.[2] Glutathione peroxidase (GPx) is a selenoenzyme that protects cells by catalyzing the reduction of peroxides with the stoichiometric reductant glutathione.[3] The catalytic cycle of the enzyme is shown in Scheme 1 and involves the



Scheme 1. Catalytic cycle of glutathione peroxidase (EnzSeH). GSH = glutathione.

reduction of a peroxide molecule by the selenol moiety of a selenocysteine residue of the enzyme, followed by reduction of the corresponding selenenic acid with glutathione. The resulting selenenyl sulfide then reacts with a second mole equivalent of glutathione to regenerate the original selenol and the disulfide of glutathione.

In view of the biological importance of GPx as a natural protective agent against oxidative stress, considerable effort has been expended to discover small-molecule selenium compounds that emulate GPx.[4] One such compound, ebselen (**1**),[5] has undergone clinical trials as an antiinflammatory agent. Ebselen and many other GPx mimetics that have been studied contain a covalent Se–N bond or an amino substituent capable of coordinating with the selenium atom at various stages of its redox cycle.[6,7] Aryl selenides generally display poor catalytic activity in the thiol-mediated reduction of peroxides,[7] while alkyl selenides are prone to decomposition by *syn* elimination of their corresponding selenoxides. However, we recently reported that allyl 3-hydroxypropyl selenide (**2**) possesses remarkably high GPx-like activity (ca. one order of magnitude greater than that of ebselen) by producing the corresponding cyclic seleninate **3** in situ by a series of oxidation and [2,3] sigmatropic rearrangement steps.[8] Seleninate **3** then serves as the true catalyst and is recovered at the end of the catalytic cycle. These experiments revealed that O–Se compounds can be even more effective catalysts than the more widely studied N–Se analogues. We now report that di(3-hydroxypropyl) selenide (**4**)[9] serves as a highly effective GPx mimetic and functions by a remarkable mechanism that involves a novel spirodioxaselenanonane as an intermediate.



In the course of our studies on GPx mimetics, we have employed a model system in which excess *tert*-butyl hydroperoxide (*t*BuOOH) is treated with benzyl thiol (BnSH), which serves as the sacrificial reductant, in the presence of 10 mol% of the selenium-containing catalyst at 18 °C.[6g,8] The reaction is easily followed by HPLC with naphthalene as an internal standard, and the time ($t_{1/2}$) required for the oxidation of 50% of the thiol to its disulfide (BnSSBn) provides a convenient means for comparing the effectiveness of various catalysts. Thus, under identical conditions, **4** gave $t_{1/2} = 2.9$ h, compared with 4.8 h for **2**, 2.5 h for **3**, 42 h for **1,** and >300 h for a control reaction conducted in the absence of a catalyst (see Figure 1).[10]

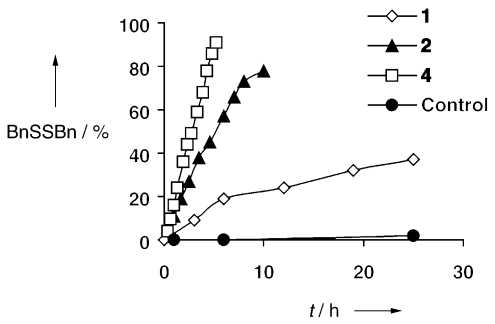


Figure 1. Rates of formation of BnSSBn from oxidation of BnSH (0.031 M) with 90% aqueous *t*BuOOH (0.043 M) in the presence of catalysts **1**, **2**, and **4** (0.0031 M) in dichloromethane/methanol (95:5) at 18 °C.

Further investigation revealed that selenide **4** was, as expected, inert toward BnSH but reacted rapidly (complete reaction in less than 5 min) with excess *t*BuOOH in the absence of the thiol to afford the novel spirodioxaselenanonane **6** quantitatively (see Scheme 2). The latter product could be readily isolated and stored at −5 °C. When **6** was subjected to our assay with excess *t*BuOOH and BnSH, it displayed the same $t_{1/2}$ as the original selenide **4** and was the

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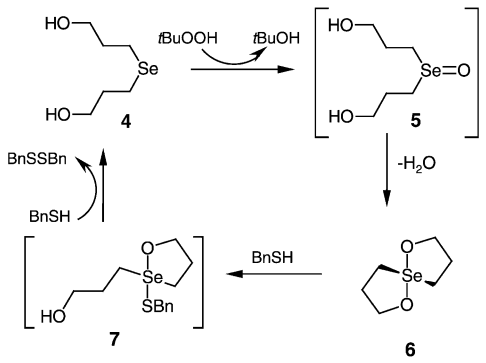
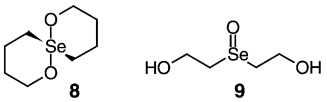


Scheme 2. Catalytic cycle of selenide **4** and spirodioxaselenanonane **6**.

sole selenium-containing product that remained in the mixture upon completion of the reaction. This confirms its catalytic role in the process. Moreover, when **6** was treated with 2 mol BnSH in the absence of *t*BuOOH, it rapidly afforded the disulfide BnSSBn and regenerated selenide **4** quantitatively. Based on these observations, we propose that the catalytic activity of selenide **4** is the result of the mechanism shown in Scheme 2. Evidently, oxidation of **4** with *t*BuOOH produces the transient selenoxide **5**, which spontaneously cyclizes to give the dioxaselenanonane **6**, as confirmed by the control experiment conducted in the absence of the thiol. In the presence of BnSH, however, we propose that **6** undergoes substitution of one alkoxy group to produce intermediate **7**, followed by reductive elimination with a second equivalent of thiol to regenerate selenide **4** along with BnSSBn.[11] In the presence of excess *t*BuOOH, **4** is recycled to **6** and the catalytic process continues.

The homologous di(2-hydroxyethyl) and di(4-hydroxybutyl) selenides were also investigated under similar conditions. The latter afforded the corresponding dioxaselenaundecane **8**, which showed lower catalytic activity ($t_{1/2} = 5.1$ h) than **6**. On the other hand, the 2-hydroxyethyl derivative produced only the corresponding selenoxide **9** when oxidized with *t*BuOOH. Failure of **9** to cyclize under the usual conditions is attributed to the increased strain in the corresponding spiro compound.



The novel dioxaselenanonane **6**[12] was fully characterized by spectroscopic methods and by X-ray crystallography.[13] The X-ray structure revealed that the O-Se-O moiety is roughly linear with a bond angle of 172.99°, while the C-Se-C bond angle is only 102.95° (Figure 2). Thus, the oxygen atoms occupy the apical positions of a distorted trigonal bipyramid centered around the selenium atom, while the carbon substituents and the selenium lone pair occupy the equatorial positions.

These experiments reveal that selenide **4** is a remarkably potent catalyst for the reduction of *t*BuOOH with BnSH, displaying a half-life that is 14.5 times shorter than that of the widely studied compound ebselen (**1**) under the same conditions. This is especially noteworthy because the mechanism for the present process proceeds via the unusual spirodioxaselenanonane **6** and is thus distinct from that employed by GPx itself and by many of its small-molecule mimetics.

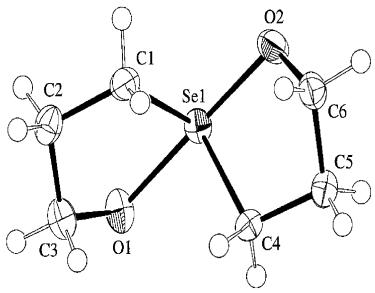


Figure 2. X-ray crystal structure of **6** (ORTEP diagram).

Experimental Section

6: *tert*-Butyl hydroperoxide (0.47 mL, 3.4 mmol, 70 %) was added to a solution of di(3-hydroxypropyl) selenide (300 mg, 1.52 mmol) in 10 mL dichloromethane, and the mixture was stirred at room temperature for 17 h. The solvent was removed in vacuo, and the residue was purified by chromatography (elution with 30 % methanol/ethyl acetate) to afford 260 mg (87 %) of the product as a colorless oil, which solidified upon standing. IR (neat): $\tilde{\nu} = 1052$, 785 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$): $\delta = 4.08$–3.90 (m, 2H), 3.88–3.70 (m, 2H), 3.39–2.98 (m, 4H), 2.17–1.78 ppm (m, 4H); ^{13}C NMR (50 MHz, $CDCl_3$): $\delta = 65.4$, 42.2, 27.3 ppm; ^{77}Se NMR ($CDCl_3$): $\delta = 769.0$ ppm (relative to Me_2Se at $\delta = 0.0$ ppm); MS: *m*/*z* (%): 196 (3, M^+), 195 (6), 138 (62), 107 (100), 58 (65); Exact mass calcd for $C_6H_{12}O_2{}^{80}Se$: 196.0003; found: 196.0007; Anal. calcd for $C_6H_{12}O_2Se$: C 36.93, H 6.20; found: C 36.80, H 6.40.

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[11] We cannot unequivocally rule out the possibility that both oxygen moieties of **6** are first displaced by BnSH in successive reactions, followed by a reductive elimination of the corresponding dithioselena compound.

[12] Although a few aryl dioxaselena compounds have been reported, they contain aromatic moieties attached to the Se atom or acyloxy instead of alkyloxy Se substituents. The parent compounds **6** and **8** are novel. For examples of aryl and acyldioxy derivatives, see: a) H. J. Reich, *J. Am. Chem. Soc.* **1973**, *95*, 964; b) B. Dahlén, B. Lindgren, *Acta Chem. Scand.* **1973**, *27*, 2218; c) S. Claeson, V. Langer, S. Allenmark, *Chirality* **2000**, *12*, 71; d) J. Drabowicz, J. Luczak, M. Mikolajczyk, Y. Yamamoto, S. Matsukawa, K. Akiba, *Tetrahedron: Asymmetry* **2002**, *13*, 2079; e) J. Zhang, S. Takahashi, S. Saito, T. Koizumi, *Tetrahedron: Asymmetry* **1998**, *9*, 3303; f) F. Ohno, T. Kawashima, R. Okazaki, *Chem. Commun.* **1997**, 1671; g) T. Kawashima, F. Ohno, R. Okazaki, *J. Am. Chem. Soc.* **1993**, *115*, 10434.

[13] X-ray crystallographic data for **6**: $C_6H_{12}O_2Se$, $M_w = 195.12$, monoclinic, space group $P2_1/c$, $a = 9.767(5)$, $b = 8.414(5)$, $c = 8.855(7)$ Å, $\beta = 91.928(3)°$, $V = 727.3(8)$ Å^3, $T = 173(2)$ K, 2573 reflections collected. CCDC-222210 (**6**) contains the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge CB21EZ, UK; fax: (+44) 1223-336-033; or deposit@ccdc.cam.ac.uk).

