Synthesis of Nanovesicular Glutathione Peroxidase Mimics with a Selenenylsulfide-Bearing Lipid

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ABSTRACT: In this article, we describe the development of a nanosized-glutathione peroxidase (GPx) mimic based on liposomes of which the amphiphilic selenenylsulfide derivative (R=Se=S−R′) was incorporated into a lipid membrane. A lipid membrane-compatible selenenylsulfide derivative, 1-oxoheadeceyl-seleno-1-cysteine-methyl-Se-y1-S-i- penicillamine methyl ester (OHSeP), was synthesized. X-ray photoelectron spectroscopy revealed that the sulfur and selenium atoms of the OHSeP molecule formed a selenenylsulfide linkage. The use of OHSeP easily allowed the introduction of the seleno-L-cysteine (SeCys) moiety into the liposomal membranes by mixing with the phosphatidylcholines (PCs), which gave rise to the GPx-like catalytic activity because of the selenium atom in the SeCys moiety. The penicillamine moiety of the OHSeP molecule incorporated into the OHSeP/PC liposomes was thought to orient toward the outer water phase. The OHSeP/PC liposomes generated the GPx-like catalytic activity, which was ascribed to the SeCys moiety that was introduced into the PC-based liposomes. Consequently, the lipid/water interface of the liposomal membranes could possibly provide an effective colloidal platform for the development of water-soluble nanosized GPx mimics.

INTRODUCTION

In the periodic table, selenium is a member of the chalcogen group that shares similar chemical properties with oxygen, sulfur, and tellurium. This element is an essential trace nutrient for mammals, birds, and fish. Interest in exploring the biochemical propensity of selenium has increased after the discovery of its biological role in the mid 1950s.1 Selenium is now known in its multifarious chemical forms as a trace element in living systems.2 In particular, it is in the form of the 21st proteinogenic amino acid "selenocysteine" (SeCys, Sec, or U).3 The insertion of SeCys into polypeptide chains is genetically guided by the uracil-guanine-adenine codon that acts as the stop codon in a normal way, and the resulting SeCys-inserted proteins (selenoproteins) broadly occur in the entire body.4,5 In humans, SeCys has catalytic and/or structural roles in the 25 selenoproteins that were deduced from a selenoproteome analysis.6 The best-known selenoproteins are the glutathione peroxidases (GPxs) that control the redox state in the living systems by catalyzing the reduction of H2O2 and organic hydroperoxides (R=O−O−H) to water and the corresponding alcohols (R−OH) using glutathione in the reduced form (GSH) as the reducing substrate (R−OH + 2GSH → R−OH + GSSG + H2O).7 These selenium-dependent GPxs are the major antioxidant defense systems in mammals, birds, and fish. Among the five selenium-dependent GPxs, phospholipid hydroperoxide GPx (GPx-4) plays a unique and vital antioxidant role; it is the only enzyme that can directly catalyze the reduction of phospholipid hydroperoxides.8 A systemic knockout of the entire GPx-4 gene is lethal, because GPx-4 plays critical roles as a component of the mammalian antioxidant defense system.9

A catalytic cycle for natural GPxs was first proposed by Ganther and Kraus, in which a selenol (−SeH) in SeCys serves to reduce the peroxide species.10 Afterward, the resulting selenenic acid (−SeOH) is recycled back to the selenol through a sequential reaction with two molecules of GSH via the selenenylglutathione (−Se−SG)-forming oxidized GSH (GSSG) and water (Figure 1).5,11 The selenenylsulfides (−Se=S−) are the crucial intermediates in the GPx catalytic cycle. A recent structural study of the natural GPxs elucidated a conserved structure of functionally significant amino acid residues (asparagine, glutamine, and tryptophan) in the vicinity of the SeCys (catalytic tetrad).12 The catalytic activity of the natural GPxs requires the supporting roles of several amino acid residues within their polypeptide chains, which hardly seems to be achieved with low-molecular-mass organoselenium compounds. The biological (therapeutic) effect of such organoselenium compounds is mainly attributed to their peroxidase catalytic activity, and the activity appears to depend on the reduction of the selenenic acid to the selenol by thiols. Therefore, macromolecular scaffolds with certain higher-order structures could possibly provide a means for obtaining a better
chemical stability of the selenium atom and thus an improvement of the GPx-like catalytic activity. On the basis of such an idea, we have synthesized polymer and/or polymeric GPx mimics using biocompatible polymers and biopolymers. We have reported a nanostructured GPx mimic using the pullulan conjugated with seleno-γ-l-cystine (SeCyst), which improves the chemical stability of the selenium atoms and hence increases the GPx-like catalytic activity. A polypeptide material with a peculiar three-dimensional structure is one of the potential scaffold candidates for such a purpose. We have also developed albumin-supported GPx mimics with selenylnsulfide as a functional element. Many characteristics of our macromolecular system were found to be different from the low-molecular-mass GPx mimics: (i) the ability to improve the solubility of water and stability of the chemically liable selenium compounds to function as the active site; (ii) the capability of forming a hydrophobic environment around the selenium compound; and (iii) the ability to concentrate the local selenium compound in polymers.

In the present study, we have developed a nanosized GPx mimic based on the molecular assembly of which an amphiphilic selenylnsulfide derivative was incorporated into the liposomal membrane with a certain molecular orientation. Liposomes are closed vesicles consisting of lipid bilayer membranes. The lipid/water interface of the liposomal membranes could provide a unique colloidal platform for various chemical reactions. The lipid bilayer membrane-compatible selenylnsulfide derivative, 1-oxo-hexadecyl-seleno-γ-l-cysteine-methyl-seleylnsulfide methyl ester (OHSεP), was synthesized, and the nanosized structure of the liposomal membrane was used as an alternative potential scaffold.

**EXPERIMENTAL SECTION**

**Materials.** SeCyst and l-penicillamine (Pen) were obtained from Sigma Co., Ltd. (St. Louis, MO) and Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan), respectively. l-Cystine (Cyst), n-hexadecanoic acid, butyric acid, and 2-(1H-benzo-triazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (TBTU) were obtained from Nacalai Tesque, Inc. (Kyoto, Japan). 2,3-Diaminonaphthalene (DAN) and glutathione in reduced form (GSH) were obtained from Tokyo Chemical Corporation (Tokyo, Japan). GSH reductase and nicotinamide adenine dinucleotide phosphate in the reduced form (NADPH) were obtained from Wako Pure Chemical Ind., Ltd. (Osaka, Japan). H$_2$O$_2$ used as the substrate for the GPx-like catalytic activity measurements was purchased from Nacalai Tesque, Inc. A Milli-Q Biocel system (Millipore Corp., Billerica, MA) was utilized to generate water (>18 MΩ cm), which is used throughout this study. All other chemicals were of commercial reagent or special grades and used as received.

**Syntheses of OHSeP and Related Compounds.** The SeCyst methyl ester (H-SeCyst-OMe) was synthesized according to the procedures reported by Bondanszky. Briefly, a mixture of SeCyst (66.7 mg) and p-toluensulfonic acid (190 mg) dissolved in 10 mL of absolute CH$_2$OH was refluxed for 40 h. After the removal of the alcohol, the resulting precipitate was left for 24 h in CH$_2$OC$_2$H$_5$. The obtained yellow solid material was washed several times with CH$_2$OC$_2$H$_5$ and then dried under reduced pressure (yield 91.7%). H-SeCyst-OMe:

$^1$H NMR (300 MHz, CD$_3$OD) $\delta$ 2.37 (3 H, 3H), 3.29–3.31 (m, 2H), 3.85 (3 H, 3H), 4.42 (1 H, J = 12.6 Hz), 7.24 (2 H, J = 8.1 Hz), 7.71 (2 H, J = 8.4 Hz); MALDI TOF-MS ([matrix; 2,5-dihydrobenzoic acid (DHB), positive ion mode] calcd for C$_8$H$_8$N$_2$O$_4$S$_8$O$_2$ m/z 364.2, found 364.2.

A mixture of the H-SeCyst-OMe (70.7 mg) and tert-butoxycarbonyl (Boc)-Pen methyl ester (Boc-Pen-OMe, 52.6 mg) dissolved in 5 mL of CH$_2$CN containing N(C$_2$H$_5$)$_3$ (30 μL) was stirred for 48 h at room temperature. After the removal of the solvent, the resulting solid material was sequentially washed with 10 w/v % citric acid and saturated NaHCO$_3$, and then dissolved in an appropriate volume of CH$_2$OC$_2$H$_5$ with anhydrous Na$_2$SO$_4$. After the addition of n-hexadecanoic acid (15.5 mg), the mixture was dissolved in 5 mL of CH$_2$CN containing N(C$_2$H$_5$)$_3$ (30 μL) and then stirred at room temperature for another 24 h in the presence of TBTU (32.1 mg) (yield 61.8%). After the removal of the solvent, the resulting solid was sequentially washed with 10 w/v % citric acid and saturated NaHCO$_3$. The Boc adduct was further exposed to 2 mL of CF$_3$COOH to remove the Boc group. After the removal of the CF$_3$COOH by evaporation, the resulting mixture was left in a 1:6 mixture of CH$_2$OC$_2$H$_5$ and CH$_2$(CH$_3$)$_2$CH$_3$ for 24 h to precipitate the solid materials and then separated by filtration. The solid materials were dissolved in Milli-Q water, followed by lyophilization, and then a pale yellow powder was obtained. OHSeP: $^1$H NMR (400 MHz, CD$_3$OD) $\delta$ 0.89–0.91 (t, 3 H, J = 6.8 Hz), 1.24–1.40 (m, 24 H), 1.49–1.53 (d, 6 H, J = 13.6 Hz), 1.58–1.65 (m, 2 H), 2.22–2.26 (m, 2 H, J = 7.4 Hz), 3.13–3.18 (dd, 1 H, J = 9.0, 12.7 Hz), 3.39–3.48 (dd, 1 H, J = 5.1 and 12.7 Hz), 3.74 (4 H, 3H), 3.87 (3 H, 3H), 4.11 (s, 3 H), 4.75–4.78 (dd, 1 H, J = 5.1 and 9.1 Hz); FAB-MS (matrix; glycerol) calcd for C$_{26}$H$_{50}$N$_2$O$_{5}$S$_8$O$_2$ m/z 583.3 [M + H]$^+$, found 583.3.

H-SeCyS-OMe-Se-yl-S-Boc-Pen-OMe: $^1$H NMR (300 MHz, CD$_3$OD) $\delta$ 1.34 (s, 6 H), 1.44 (s, 9 H), 3.07–3.26 (m, 2 H), 3.72 (d, 6 H, J = 6.6 Hz), 3.79 (t, 1 H, J = 6.2 Hz); MALDI TOF-MS (matrix; DHB, positive ion mode) calcd for C$_{30}$H$_{30}$N$_2$O$_{5}$S$_{10}$O$_2$ m/z 444.5, found 444.5.

1-Butyryl-SeCyS-methyl-seylnsulfide methyl ester. $^1$H NMR (300 MHz, CD$_3$OD) $\delta$ 0.96 (t, 3 H, J = 7.5 Hz), 1.37 (s, 6 H), 1.59–1.71 (m, 2 H), 2.25 (t, 2 H, J = 7.5 Hz), 3.10 (dd, 1 H, J = 8.9 and 12.8 Hz), 3.56 (s, 1 H), 3.73 (s, 6 H), 4.73 (dd, 1 H, J = 5.3 and 8.9 Hz); MALDI TOF-MS (matrix; DHB, positive ion mode) calcd for C$_{19}$H$_{26}$N$_2$O$_{5}$S$_{10}$Se m/z 414.3, found 414.4.

1-Oxo-hexadecyl-γ-l-cysteine-methyl-seylnsulfide methyl ester. $^1$H NMR (300 MHz, CD$_3$OD) $\delta$ 0.90 (t, 3 H, J = 6.8 Hz), 1.21–1.40 (m, 24 H), 1.44 (s, 3 H), 1.52 (s, 3 H), 1.58–1.65 (m, 2 H), 2.25 (t, 2 H, J = 7.5 Hz), 3.07 (dd, 1 H, J = 9.3...
CHCl₃ was gently removed. After the addition of 50 mL of 10 mM Tris/HCl buffer (pH 7.4). The reaction was initiated by the addition of H₂O₂. The catalytically active selenol reduces peroxides to form a selenenic acid, which further reacts with thiols to regenerate the selenenylsulfides. The selenenylsulfide is the crucial intermediate in the catalytic cycle of the natural GPxs (Figure 1). Generally, the nucleophilic thiol attack at the selenium atom is both kinetically and thermodynamically more favorable than that at the sulfur atom. A nucleophilic attack of thiol at the selenium atom would lead to a thiol exchange that hinders the regeneration of the catalytically active selenol. An analysis of the catalytic site features of the natural GPxs elucidates that the sulfur atom in the selenenylsulfide intermediate is involved in a weak interaction with the amido nitrogen atom of the threonine-S₄ residue, lowering the energy barrier to increase the possibility of a nucleophilic attack of the negatively charged thiolate at the sulfur atom in the selenenylsulfide. The selenenylsulfide intermediate can be converted to the corresponding selenol by high concentrations of glutathione (GSH) in the living cell cytosol. Thus, the thiol exchange that takes place at the selenium atom in the selenenylsulfide is thought to result in poor GPx-like catalytic activity. In low-molecular-mass selenium compounds, both the thiol exchange at the selenium atom and the low reactivity of the selenol toward oxidation may result in poor catalytic activity of the selenenylsulfide derivative. A key factor for achieving a better GPx-like catalytic activity is to promote the desired thiol exchange at the sulfur atom in the selenenylsulfide and to generate the catalytically active selenol and disulfide. For this purpose, we have attempted to use the lipid/water interface of the liposomal membranes for mimicking the GPx-like catalytic activity in water. A selenenylsulfide derivative with a lipid membrane-compatible n-hexadecanoyl chain was designed to orient the outer water phase, for promoting the desired thiol exchange of the thios (Figure 2).

In the present study, seleno-L-cystine (SeCys) was used as the source material for the introduction of SeCys at the lipid/water interface of the liposomes. The α-carboxyl groups of SeCyst were esterified with methanol, and the H-SeCyst-OMe was allowed to react with the Boc-L-penicillamine methyl ester (Boc-Pen-OMe) as a thiol exchange reagent with the diselenium to form a selenenylsulfide between SeCys and Pen. Subsequently, the selenenylsulfide derivative was then coupled with n-hexadecanoic acid to allow coupling with the membrane-compatible moiety through its α-amino groups (OHSERP, Scheme 1). The Boc protection at the α-carboxyl group of Pen was removed by treatment with CF₃COOH, followed by mixing with phospholipid molecules. A selenenylsulfide intermediate in the natural GPx catalytic cycle can be cleaved by the coumarin substrate. The selenenylsulfide linkage is intrinsically less stable than the diselenide ones. However, the substitution of Cys by Pen notably improves the chemical stability of labile selenium species, such as R–S–Se–R', and

\[ \text{GPx-like catalytic activity} = \frac{\text{OD}_{\text{BLK}} - \text{OD}_{\text{SMP}}}{1000 / \epsilon_{\text{mm}} / \text{c}} \]  

where \( \Delta \text{OD}_{\text{SMP}} \) is the decrease in OD at 340 nm of the sample solutions between 10 and 70 s after the addition of \( \text{H}_2\text{O}_2 \), \( \Delta \text{OD}_{\text{BLK}} \) is the decrease in OD at 340 nm per minute of the solutions without the liposome, \( \epsilon_{\text{mm}} \) is the extinction coefficient for 1 mM NADPH solution [6.22/(mM-cm)], and \( c \) is the final selenium concentration in \( \mu \text{M} \).
NO because of steric constraints by the two methyl groups at the β carbon atom.32

To obtain information about the orientation of OHSeP molecules in the liposomal membranes, its analogue, 1-butryryl-SeCys-methyl-Se-yl-S-l-penicillamine methyl ester (BSeP), which possesses the n-butryl chain in place of the n-hexadecanoyl one, was synthesized by similar synthetic procedures using n-butryic acid. Although acetyl chain-substituted analogue of OHSeP was synthesized, it is easily decomposed to produce elemental selenium as the terminal product within a week at room temperature in a desiccator. Meanwhile, OHSeP was fairly stable without developing red even when it was maintained for several months under the same conditions, indicating that the addition of a long acyl chain also resulted in improvement of the chemical stability of selenenylsulfi de derivatives. We expected that the lipid/water interface could further stabilize selenenylsulfi de derivatives through interfering with any intermolecular interaction among OHSeP molecules. As another control material, the cysteine analogue (1-oxo-hexadecyl-l-cysteine-methyl-S-yl-S-l-penicillamine methyl ester, OHSP), which is substituted by sulfur in place of selenium in the OHSeP molecule, was similarly synthesized using Cyst as the starting material.

Selenium has six naturally occurring isotopes and their abundances are 8.73 for 82Se, 49.61 for 80Se, 23.77 for 78Se, 7.63 for 77Se, 9.37 for 76Se, and 0.89 for 74Se in at %. Thus, one selenium atom-containing compound is supposed to show the characteristic mass spectral pattern, as reflected by its natural isotope abundance. When the OHSeP and BSeP samples were subjected to mass spectrometric analyses, the characteristic selenium isotopic pattern involving one selenium atom in a molecular ion was observed, and the 80Se-containing molecular ion peaks of OHSeP and BSeP were at m/z 583.3 [M + H]+ and 414.4, respectively (Figure 3A,B). On the contrary, such a distinctive feature of the isotopic pattern did not appear in the mass spectrum of OHSP bearing a disulfi de (Figure 3C).

To characterize the oxidation and chemical bond state of the sulfur and selenium atoms, OHSeP was analyzed by XPS. The XPS measures the kinetic energy of electrons emitted from sample surfaces under X-ray radiation. Knowing the energy of the incoming X-ray beam and measuring the kinetic energy of electrons leaving the surface determines the binding energy obtained by the spectrometer. The binding energy is specific to both the elements and electronic orbitals from which electrons originate. The obtained absorption spectra and estimated values for the binding energy are listed in Figure 4 and Table 1, respectively. The sulfur 2p electrons from Cyst gave an absorption peak at 161.4 and a shoulder at 162.4 eV (Figure 4A). The absorption peak shape of OHSP (Figure 4C) was almost identical to that of Cyst (Figure 4A). The values for the binding energy of the sulfur 2p electrons in OHSP were 161.9 and 163.0 eV. The selenium 3p electrons from SeCyst gave two absorption peaks at 159.8 and 165.3 eV (Figure 4B). Meanwhile, the sulfur 2p and selenium 3p electrons in OHSeP with the selenenylsulfi de linkage (Figure 4D) provided distinctive absorption peaks at 164−169 and 157−164 eV. The two absorption peaks from OHSeP were separated into the sulfur 2p and selenium 3p components. The peak envelope of the sulfur 2p and selenium 3p components from OHSeP (orange dotted line in Figure 4D) was mostly identical to the observed spectrum (black solid lines in Figure 4D). In addition, the values for the binding energy of the selenium 3p components of OHSeP (160.2 and 166.1 eV) were nearly equal to those of SeCyst (159.8 and 165.3 eV). The values for the binding energy of the sulfur 2p components of OHSeP

**Scheme 1. Synthetic Pathway of OHSeP**

**Figure 2.** Schematic drawing of a liposomal glutathione peroxidase mimic with a selenenylsulfide-bearing lipid and its activation by a thiol exchange. R1−SH, a thiol that can be attacked on the selenenylsulfide; R2−SH, a thiol that can be released from the selenenylsulfide.

**Figure 2.** Schematic drawing of a liposomal glutathione peroxidase mimic with a selenenylsulfide-bearing lipid and its activation by a thiol exchange. R1−SH, a thiol that can be attacked on the selenenylsulfide; R2−SH, a thiol that can be released from the selenenylsulfide.
The particle diameters and zeta potentials of the OHSeP and egg yolk PC (hydrogenated) by the conventional Bangham method. The particle diameters of the OHSeP/PC liposomes were slightly larger than those of the PC (100) and OHSP (20)/PC (80) liposomes. The addition of a certain amount of detergent, such as Triton X-100, to the liposome solutions is known to break up the lipid bilayer structure to form the mixed micelles. During the preparation of the liposomes, formation of the mixed micelles was not observed even at 20 mol % of OHSeP, BSeP, and OHSP in the mixtures with PC. Actually, remarkable decreases in the particle diameter accompanying the addition of OHSeP, BSeP, and OHSP were also not observed for the OHSeP (20)/PC (80) and OHSP (20)/PC (80) liposomes. The value for the zeta potential of the PC (100) was almost zero because of the electrically neutral head groups of PC and the BSeP/PC liposome. The value for the zeta potential of the OHSeP/PC liposomes slightly increased with an increase in the OHSeP content, which was seemingly because of the head groups of the OHSeP embedded in the outer leaflet of the PC bilayer membrane. A similar trend in the zeta potential was also observed from the PC-free OHSeP solution and the OHSP/PC liposome.

The OHSeP and PC composition of the OHSeP/PC liposomes was actually estimated from the determination of the selenium and phosphorus concentrations in the liposome solutions (Table 4). The actual lipid compositions of the OHSeP/PC liposomes were almost in good agreement with those used in the preparation of the respective liposomes, which demonstrated that OHSeP molecules were mostly incorporated into the liposomal membrane structure.

The orientation of OHSeP molecules in the liposomal membranes is a key factor affecting the expression of the GPx-like catalytic activity. When the selenenylsulfide linkage of the OHSeP molecule was buried in the hydrophobic interior of the liposomal membranes, the GPx-like catalytic activity would hardly be found because the functional element of OHSeP could not be accessible to the substrate peroxide species and the coenzyme GSH. Liposomes were prepared from a mixture of BSeP and PC (described as “BSeP/PC liposome” in tables and figures), and the lipid composition of the obtained liposomes was analyzed. If BSeP molecules could penetrate into the PC liposomal membranes, the analytical results of the lipid composition of the obtained liposomes were supposed to be nearly the same as those used in the preparation of the liposomes and in the case of the OHSeP/PC liposomes. When the BSeP/PC liposomes were attempted to prepare at a 20:80 molar ratio of BSeP to PC, the molar ratio of BSeP to PC in the obtained liposome solution was estimated as 87.1 ± 3.8:12.9 ± 1.3 from the results of the selenium and phosphorus analyses. BSeP in the obtained liposome solution was thought to be mostly free, not incorporated into the liposomal membranes, that is, the Pen moiety of BSeP was not thought to penetrate toward the hydrophobic interior of the PC liposomal membranes. The obtained particle diameter of the BSeP/PC liposomes was 80.5 ± 1.2 nm, which was almost identical to that of the PC (100) liposome (Table 2). From these results, BSeP bearing a short acyl chain was thought to be much less preferable to the liposomal membranes than OHSeP. Taken together, the Pen moiety of the OHSeP molecule incorporated into the OHSeP/PC liposomes was thought to orient toward the inner and outer water phases. The OHSP molecules in the OHSE/PC liposomes were also thought to take a similar orientation in the PC-bilayer membrane structure.

The GPx-like catalytic activity of the OHSeP/PC liposomes was further evaluated by the NADPH method using H2O2 as a substrate (Figure 5). Both the PC (100) and the OHSP (20)/
PC (80) liposomes did not show any GPx-like catalytic activity. The BSeP (20)/PC (80) liposomes showed a quite low value for the GPx-like catalytic activity, which is thought to result from the free BSeP molecules in this liposome solution. A PC-free OHSeP solution gave an even lower value. To improve the solubility (dispersity) of OHSeP molecules, Triton X-100 and

![Figure 4](image-url)

**Figure 4.** X-ray photoelectron spectroscopy binding energies of the selenium 3p and sulfur 2p electrons in Cyst (A), SeCyst (B), OHSP (C), and OHSeP (D). Black solid line: found, brown solid line: selenium 3p$_{3/2}$ green solid line: selenium 3p$_{1/2}$ purple solid line: sulfur 2p$_{3/2}$ blue solid line: sulfur 2p$_{1/2}$ orange dotted line: envelope.

### Table 1. X-ray Photoelectron Spectroscopy Binding Energies of Selenium 3p and Sulfur 2p Electrons in L-Cystine, Seleno-L-Cystine, OHSP, and OHSeP

| atom level | binding energy (eV) |
|------------|---------------------|
|            | L-cystine          | SeL-cystine | OHSP | OHSeP |
| selenium 3p$_{3/2}$ | none             | 159.8       | none | 160.2 |
| selenium 3p$_{1/2}$ | none             | 165.3       | none | 166.1 |
| sulfur 2p$_{3/2}$   | 161.4             | none        | 161.9| 162.6 |
| sulfur 2p$_{1/2}$   | 162.4             | none        | 163.0| 163.8 |

**Values in parentheses represent mol % of OHSeP, BSeP, OHSP, and PC used in the preparation of liposomes.**

### Table 2. Particle Diameter and Polydispersity Index of Liposomes

| item$^a$ | particle diameter$^b$ (nm) | polydispersity index |
|----------|----------------------------|----------------------|
| OHSeP (05)/PC (95) | 113.7 ± 3.9               | 0.200                |
| OHSeP (10)/PC (90) | 114.4 ± 3.5               | 0.390                |
| OHSeP (20)/PC (80) | 102.2 ± 1.9               | 0.356                |
| BSeP (20)/PC (80)  | 80.5 ± 1.2                | 0.190                |
| OHSP (20)/PC (80)  | 85.3 ± 3.0                | 0.195                |
| PC (100)          | 80.8 ± 1.0                | 0.205                |
| OHSeP solution    | 173.5 ± 2.5               | 0.433                |

**Values in parentheses represent mol % of OHSeP, BSeP, OHSP, and PC used in the preparation of liposomes.**

### Table 3. Zeta Potential of OHSeP/PC Liposomes

| item$^a$ | zeta potential$^b$ (mV) |
|----------|-------------------------|
| OHSeP (05)/PC (95) | −3.51 ± 1.03           |
| OHSeP (10)/PC (90) | −6.77 ± 2.81           |
| OHSeP (20)/PC (80) | −12.40 ± 0.27          |
| BSeP (20)/PC (80)  | −2.25 ± 0.95           |
| OHSP (20)/PC (80)  | −10.20 ± 0.51          |
| PC (100)          | −1.56 ± 0.18           |
| OHSeP solution    | −12.00 ± 2.76          |

**Values in parentheses represent mol % of OHSeP, BSeP, OHSP, and PC used in the preparation of liposomes.**

### Table 4. Lipid Composition Analysis of OHSeP/PC Liposomes

| item$^a$ | OHSeP or BSeP | PC |
|----------|---------------|----|
| OHSeP (05)/PC (95) | 5.6 ± 1.0     | 94.4 ± 0.8 |
| OHSeP (10)/PC (90) | 10.1 ± 1.2    | 89.9 ± 2.1 |
| OHSeP (20)/PC (80) | 22.1 ± 0.8    | 77.9 ± 3.8 |
| BSeP (20)/PC (80)  | 87.1 ± 3.8    | 12.9 ± 1.3 |
| PC (100)          | 0.0           | 100.0     |

**Values in parentheses represent mol % of OHSeP, BSeP, OHSP, and PC used in the preparation of liposomes.**

$^a$Summation of OHSeP or BSeP and PC contents was defined as 100 mol %.

free OHSeP solution gave an even lower value. To improve the solubility (dispersity) of OHSeP molecules, Triton X-100 and
sodium dodecyl sulfate at above respective critical micelle concentrations and albumin were added to this PC-free OHSeP solution; however, no remarkable changes in the values of its catalytic activity were observed. On the other hand, the OHSeP/PC liposomes generated the GPx-like catalytic activity, which is apparently ascribed to the SeCys moiety that was introduced in PC-based liposomes. Such an activity of the OHSeP/PC liposomes was higher than that of a solution of H-SeCyst-OMe with diselenide as a functional element. The observed activity of the OHSeP/PC liposomes was much higher than that of ebselen, a standard for comparing the GPx-like catalytic activity of selenium compounds, for H2O2 (0.99 μmol Se·min−1)34 and that of H-SeCyst-OMe for H2O2 (0.51 μmol Se·min−1).34 The three OHSeP/PC liposomes with different OHSeP contents (5, 10, and 20 mol %) demonstrated similar values for the GPx-like catalytic activity. The OHSeP molecules in the OHSeP/PC liposomes were thought to disperse favorably in the liposomal membranes up to 20 mol % without forming structurally isolated aggregate. The structures of the liposomal membrane also appear to be one of the factors governing the GPx-like catalytic activity of the OHSeP molecule. Thus, in addition to PC, other phospholipids that possess various polar head groups may be effective for further improvement of the GPx-like catalytic activity.

In conclusion, we synthesized a lipid membrane-compatible selenenylsulphide derivative, OHSeP. SeCyst-bearing diselenide was substituted with Pen to form the selenenylsulphide linkage. Then, the selenenylsulphide (SeCys–Se–S–Pen) was conjugated with the lipid membrane-compatible n-hexadecanoyl chain through the α-amino group of the SeCys in OHSeP. The use of OHSeP easily allowed the introduction of the SeCys moiety into the liposomal membranes by mixing with phospholipids, which gave rise to the GPx-like catalytic activity because of the selenium atom in the SeCys moiety. Overall, the lipid/water interface of the liposomal membranes could possibly provide an effective colloidal platform for the development of water-soluble nanosized GPx mimics. Hence, our procedure using the selenenylsulphide derivative would be extendable to the design of novel nanosized materials with an antioxidant GPx-like catalytic activity. We will apply these vesicular assemblies to medicine as a nanoparticulate antioxidant material for the treatment of oxidative stress-related diseases.
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