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Selenoprotein L-inspired nano-vesicular peroxidase mimics based on amphiphilic diselenides

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ABSTRACT

In this study, we developed selenoprotein L-inspired nano-vesicular peroxidase mimics based on amphiphilic diselenides. Selenocystine (SeCyst) was used as the starting material for the synthesis of four liposomal membrane-compatible diselenide derivatives (R–Se–Se–R') with two hydrophobic tails and a polar part. The diselenide derivatives were successfully incorporated into the phosphatidylcholine (PC)-based nano-vesicular scaffold. The results of the particle diameter and zeta-potential measurements suggested that the functional diselenide moiety was placed around the outer surface, not in the hydrophobic interior, of the liposomal membrane structures. The GPx-like catalytic activity of the diselenide/PC liposomes was determined by the conventional NADPH method using glutathione as the reducing substrate. For three peroxide substrates, i.e., hydrogen peroxide, organic *tert*-butyl hydroperoxide and cummen hydroperoxide, the cationic property-possessing diselenide derivatives in the PC-based liposomes resulted in a higher catalytic activity in comparison to electrically neutral and anionic derivatives. Overall, the diselenide derivatives at the surface of a liposomal colloidal scaffold could exert a GPx-like catalytic activity in physiological aqueous media.

1. Introduction

The element selenium belongs to chalcogens in the periodic table, together with oxygen, sulfur and tellurium. After its discovery in the early 19th century, selenium was long considered as a solely poison until it was recognized as a nutrient for bacteria, birds and mammals in the mid 20th century [1]. Nutritional selenium is known to be utilized with diverse chemical forms by biomolecules. The representative chemical form of selenium, called selenocysteine (SeCys), is the 21st amino acid in the natural proteinogenic repertoire. As SeCys is encoded by the UGA termination codon that normally serves as a stop signal [2], its insertion sequence elements (SECIS) are a fundamental aspect of the mRNA encoding selenoproteins in eukaryotes [3]. The SECIS mechanism is utilized for the biosynthesis of 25 selenoproteins in humans; glutathione peroxidases (GPxs), thioredoxin reductase, iodothyronine deiodinases, selenophosphate synthase, selenoprotein P, etc. [4]. In nature, this SeCys insertion process is highly regulated, however, it is quite difficult to prepare selenoproteins using the recombinant expression systems [5].

The human selenoproteins, except for selenoprotein P, have a single SeCys residue (Sec) in their polypeptide chains. Sec is a functional element of the active site of the antioxidative enzyme GPxs. GPxs utilize catalytically-active selenol (—SeH) of Sec to convert hydrogen peroxide and lipid peroxides into water and the corresponding alcohols using glutathione (GSH) as the reducing substrate ($R-OOH + 2GSH \rightarrow R-OH + GSSG + H_2O$). Several other selenoproteins are also oxidoreductases containing the

Abbreviations: Cys, L-cysteine; DAN, 2,3-diaminonaphtalene; GPx, glutathione peroxidase; GSH, glutathione in the reduced form; MALDI TOF-MS, matrix-assisted laser desorption ionization time of flight-mass spectrometry; NADPH, nicotinamide adenine dinucleotide phosphate; PC, hydrogenated egg yolk phosphatidylcholine; SeCys, Sec and U seleno-L-cysteine; H-*Se*Cyst-*OH*, seleno-L-cystine; SelL, selenoprotein L; TBTU, 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate; TEA, triethylamine; *p*-TSA, *p*-toluenesulfonic acid; XPS, X-ray photoelectron spectroscopy.

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catalytic redox-active Sec [6]. Such selenoenzymes can be typically a thousand times more effective in catalysis than their cysteine residue (Cys) homologs in place of Sec [7]; for instance, the rate of thioredoxin reduction increases up to $\approx 10^2 - 10^4$-fold by the seleno-glutaredoxin 3 analogues [8]. The kinetic and thermodynamic properties of the selenol group are also distinctly different from those of the thiol group. Sec in many selenoenzymes forms transient selenenylsulfide bonds (—Se—S—) with proximal Cys as part of their catalytic mechanisms. Oxidoreductases containing either selenenylsulfide or diselenide (—Se—Se—) bonds can have physiologically compatible redox potentials and enhanced reduction kinetics in comparison to their sulfide counterparts.

Marine animals have more selenoproteins than terrestrial animals. The selenoproteomes of fish are greater in number than those of mammals; with 30–37 selenoproteins, the selenoproteomes of fish are among the highest known [9]. The same core selenoprotein families are found in mammals and fish. In addition, fish have several species-specific selenoproteins [fish 15 kDa selenoprotein-like protein, selenoprotein J and selenoprotein L (SelL)] that are missing in mammals [9]. Among the fish-specific selenoproteins, SelL is found in diverse aquatic organisms, including fish, invertebrates, and marine bacteria [10]. This selenoprotein uniquely contains two Sec separated by two other residues to form a −Sec−Xaa−Xaa-Sec− motif (two Sec residues separated by two Xaa ones, Fig. 1a) similar to the catalytic −Cys−Xaa−Xaa−Cys− motif (two Cys residues separated by two Xaa ones) in the thiol/disulfide oxidoreductase of thioredoxin (Trx), which suggests the redox function of this selenoprotein.

Low-molecular-mass organoselenium compounds have been reported as GPx mimics. They mostly do not exert a catalytic activity in physiological aqueous media because of their poor water-compatibility. Therefore, it is very difficult to apply them to medicinal applications. Most selenium-containing small molecules exhibit a low GPx-like catalytic activity because the catalytically-active selenol is chemically unstable, and there is not good way of generating the selenol. We have reported a nano-structured GPx mimic using the diselenide-containing SeCyst derivatives conjugated with the polysaccharide pullulan, which improves the chemical stability of selenium atoms, and hence, increases the GPx-like catalytic activity in comparison to the free SeCyst [11]. We also reported that another GPx mimic using a membrane-compatible selenenylsulfide derivative, which shows the GPx-like catalytic activity comparable to the SeCyst-pullulan conjugate [12]. In this study, we developed a diselenide-containing peroxidase nano-mimic based on a molecular assembly of which the membrane-compatible amphiphilic diselenide derivatives were reconstituted into a liposomal membrane.

2. Materials and methods

2.1. Materials

Seleno-L-cystine (H-SeCyst-OH) was obtained from Sigma Co., Ltd. (St. Louis, MO). *n*-Hexadecanoic acid and 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoro- borate (TBTU) were obtained from Nacalai Tesque, Inc. (Kyoto, Japan). 2,3-Diaminonaphthalene (DAN) and glutathione in the reduced form (GSH) were obtained from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). Hydrogenated egg yolk phosphatidylcholine (PC) was obtained from the NOF 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). Hydrogen peroxide, cumene hydroperoxide and *tert*-butyl hydroperoxide used as the substrates for the GPx-like catalytic activity measurements were purchased from Nacalai Tesque, Inc. A Milli-Q Biocel system (Millipore Corp., Billerica, MA) was utilized to generate water (>18 MΩ cm), which was used throughout this study. All other chemicals were of commercial reagent or special grades and used as received.

2.2. Synthesis of diselenide derivatives

H-SeCyst-OD: A mixture of H-SeCyst-OH (133.6 mg, 0.4 mmol), *p*-toluenesulfonic acid (*p*-TSA) (189 mg, 1 mmol) and *n*-dodecanol (178 μL, 0.8 mmol) was suspended in toluene (30 mL), then refluxed for 40 h. After removal of the toluene using a rotary evaporator, the resulting precipitate was washed several times with saturated $NaHCO_3$ and/or NaCl, then extracted into $CHCl_3$. The solvent was removed under reduced pressure (yield 66.7%). ^{1}H NMR [a Varian Gemini 300 MHz spectrometer (Agilent Technologies Inc., Santa Clara, CA), $CDCl_3$]: δ 0.86−0.90 (t, 6H, J = 6.6 Hz), 1.21−1.31 (m, 32H), 1.52−1.68 (m, 8H), 3.16−3.38 (m, 4H), 3.76−3.78 (dd, 2H, J = 7.65, 12.6 Hz), 4.11−4.16 (t, 4H, J = 6.9 Hz), MALDI TOF-MS [an Ultraflex III (Bruker Daltonics, Bremen, Germany), positive ion mode]: calcd for $C_{30}H_{60}N_2O_4{}^{80}Se_2$ 672.82, found m/z 672.80.

Ac-SeCyst-OD: H-SeCyst-OH (120 mg, 0.18 mmol) was dissolved in CH_2Cl_2 containing $N(CH_3)_3$, then CH_3COCl (28 μL, 0.4 mmol) was added to the mixture in an ice-cold bath. The mixture was stirred overnight at room temperature. After removal of the solvent, the resulting precipitate was washed with saturated $NaHCO_3$, then dissolved in an appropriate volume of $CHCl_3$ containing anhydrous Na_2SO_4 (yield 66.7%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.82−0.90 (t, 6H, J = 15.2 Hz), 1.41−1.18 (m, 32H), 2.26 (s, 6H), 4.07−4.03 (t, 4H, J = 6.8 Hz), 4.15−4.10 (m, 2H), ESI TOF-MS [a JMS-T100LC (JEOL, Tokyo, Japan), positive ion mode]: calcd for $C_{34}H_{64}N_2O_4{}^{80}Se_2$ 756.89, found m/z 779.15.

D-SeCyst-OMe: A mixture of H-SeCyst-OH (334 mg, 1 mmol) and *p*-TSA (3 g, 15.7 mmol) dissolved in 10 mL of absolute CH_3OH was refluxed for 40 h. After removal of the alcohol, the resulting precipitate was left for 24 h in $C_2H_5OC_2H_5$. The obtained yellow solid material was washed several times with $C_2H_5OC_2H_5$, then dried under reduced pressure (yield 90.5%). The obtained H-SeCyst-OMe (36.2 mg, 0.1 mmol) and *n*-dodecanoic acid (44 mg, 0.22 mmol) were dissolved in CH_3CN (10 mL), then TBTU (64.2 mg, 0.2 mmol) and $N(C_2H_5)_3$ (60 μL, 0.43 mmol) were added in this solution. The mixture was stirred overnight at room temperature. After removal of the solvent, the resulting precipitate was washed with saturated $NaHCO_3$, then dissolved in an appropriate volume of $CHCl_3$ containing anhydrous Na_2SO_4. The obtained yellow oil material was washed several times with $CHCl_3$, then dried under reduced pressure (yield 74.3%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.86 (t, 6H, J = 6.6 Hz), 1.26−1.30 (m, 32H), 1.53 (m, 4H), 1.80−2.10 (m, 4H), 3.66 (s, 6H), 4.40 (m, 2H), MALDI TOF-MS (an Ultraflex III, positive ion mode): calcd for $C_{32}H_{64}N_2O_6{}^{80}Se_2$ 728.84, found m/z 728.89.

D-SeCyst-OH: H-SeCyst-OH (334 mg, 1 mmol) was suspended in *tert*-butyl acetate (50 mL) and perchloric acid (0.8 mL). After stirring overnight at room temperature, the mixture was subsequently added to saturated $NaHCO_3$ and extracted into $CH_3COOC_2H_5$ containing anhydrous Na_2SO_4. The resulting H-SeCyst-O*t*Bu (56 mg, 0.125 mmol) and *n*-dodecanoic acid (55 mg, 0.275 mmol) were dissolved in $(CH_3)_2NCHO$, then stirred overnight at room temperature. After removal of the $(CH_3)_2NCHO$ under reduced pressure, the crude material was sequentially washed several times with 10% citric acid and saturated NaCl, and dried with Na_2SO_4. The resulting material was dissolved in CH_2Cl_2 with CF_3COOH and stirred overnight at room temperature, then dried under reduced pressure (yield 70.2%). ^{1}H NMR (300 MHz, $CDCl_3$): δ: 0.86−0.92 (t, 6H, J = 12 Hz), 1.27−1.31 (m, 32H), 1.51−1.58 (m, 4H), 1.80−2.09 (m, 4H), 4.52 (m, 2H), ESI TOF-MS (a JMS-T100LC, positive ion mode): calcd for $C_{30}H_{60}N_2O_6{}^{80}Se_2$ 700.79, found m/z 701.16.