Journal of Inclusion Phenomena and Macrocyclic Chemistry (2006) 56: 179–182
DOI 10.1007/s10847-006-9080-7


# A Glutathione Peroxidase Mimic 6,6′-Ditellurobis (6-Deoxy-β-Cyclodextrin) with High Substrate Specificity

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*Key words:* cyclodextrins, glutathione peroxidase, mimic, specificity, tellurium

**Abstract**

Glutathione peroxidase (GPx) is one of the most important antioxidative selenoenzymes in living organisms. The novel GPx mimic 6,6′-ditellurobis(6-deoxy-β-cyclodextrin) (6-TeCD) was prepared and evaluated for its capacity to catalyze the reduction of $H_2O_2$, *tert*-butyl hydroperoxide (*t*-BuOOH), and cumene hydroperoxide (CuOOH) by glutathione (GSH) or 3-carboxy-4-nitrobenzenethiol (ArSH). Compared the ArSH assay with the coupled reductase assay, we found that 6-TeCD exhibited strong substrate specificity for aromatic thiol substrate. The specificity led to efficient peroxidase activity almost 100,000-fold than that for a well-known GPx mimic diphenyl diselenide (PhSeSePh). Furthermore, reduction of lipophilic CuOOH was proceeded ca. 30 times faster than the more hydrophilic $H_2O_2$, which cannot bind into the hydrophobic cavity of β-cyclodextrin. Thus, it seemed that catalytic activity of cyclodextrin-derived GPx models strongly depends on the structurally different both substrates hydroperoxides (ROOH) and thiols.

## Introduction

Glutathione peroxidase (GPx, EC 1.11.1.9) is an important mammalian selenoenzyme that function in cellular redox reactions and plays an essential role in the detoxification of hydroperoxides (ROOH) *in vivo*, thereby protecting lipid membranes from oxidative damage [1]. The enzyme catalytic site contains a selenocysteine residue which undergoes redox cycle shown in Scheme 1 [2]. In contrast to the cytosolic GPx, which uses glutathione (GSH) exclusively as cosubstrate, other enzymes such as plasma GPx or phospholipid hydroperoxide GPx readily accept many thiols as substrates [3]. In recent years, considerable efforts have been made to find synthetic selenium/tellurium compounds that could replicate the properties of the GPx. Ebselen (2-phenyl-1,2-benzisoselenazol-3(2*H*)-one), ebselen homologues, selenenamides, diselenides, R-phenylselenoketones, and selenium-containing enzymes, antibodies, dendrimers, and cyclodextrins and their tellurium analogues have all been demonstrated to catalyze the reduction of ROOH in the presence of thiols [4].

Cyclodextrins have been extensively exploited in the past as enzyme models and molecular receptors because of their capacity to accommodate various organic molecules in their hydrophobic cavities through host–guest chemistry [5]. In our group, 2,2′-diselenobis(2-deoxy-β-cyclodextrin) [6], its tellurium analogue [7], and 6,6′-diselenobis(6-deoxy-β-cyclodextrin) [8] were recently shown to act as GPx mimics. In order to extend the model system, it is necessary to prepare 6,6′-ditellurobis(6-deoxy-β-cyclodextrin) (6-TeCD) for investigating the relationships on substrate specificity and catalytic activity.

In present paper, 6-TeCD catalyzed the reduction of ROOH by GSH or 3-carboxy-4-nitrobenzenethiol (ArSH) was studied in detail. These results clearly indicated that 6-TeCD which has strong substrate specificity produced large rate accelerations when aromatic thiol ArSH acted as a thiol substrate. The large difference in the activities of 6-TeCD with thiol ArSH was ascribed to the role of the binding ability as compared with thiol GSH, and the recognition of substrates in the enzyme model could be delineated from catalytic efficiency.

## Experimental

### *General Procedures*

β-cyclodextrin was purchased from Tianjin Chemical Plant, recrystallized three times from distilled water, and dried for 12 h at 120 °C in vacuo. *p*-Toluene sulfonylchloride was also purchased from Tianjin Chemical

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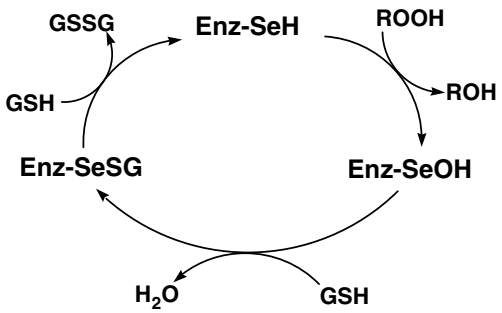



*Scheme 1.* Catalytic cycle for GPx.

Plant. *Tert*-butyl hydroperoxide (*t*-BuOOH) and reduced glutathione (GSH) were obtained from Merck. Cumene hydroperoxide (CuOOH) was purchased from Fluka. Diphenyl ditelluride (PhTeTePh) was obtained from Aldrich. Sodium hydroborate, tellurium powder, diphenyl diselenide (PhSeSePh), 5,5′-dithiolbis(2-nitrobenzoic acid), β-nicotinamide adenine dinucleatide phosphate reduced form (NADPH), and glutathione reductase were purchased from Sigma. Sephadex G-25 was purchased from Amersham Pharmacia Biotech, Uppsala, Sweden. All other chemicals were of the highest purity commercially available and were used without further purification. IR spectra were recorded on a Bruker IFS-FT66V infrared spectrometer. $^1$H NMR and $^{13}$C NMR were measured on a Bruker AM-500 spectrometer. Elemental Analyses were determined on a Perkin-Elmer 240 DS elemental analyzer. Molecular weight was obtained from a LDI-1700 MALDI-TOF-MS (Linear Scientific Inc., USA). The spectrometric measurements were carried out with a Shimadzu 3100 UV–Vis–near-IR Recording Spectrophotometer interfaced with a personal computer. Data were acquired and analyzed by using ultraviolet spectroscopy software. The temperature for UV time course studies was controlled within (±)0.5 °C by use of a LAUDA compact low-temperature thermostat RC6 CP. Phosphate buffer (PBS) was used in the all experiments unless otherwise noted. The buffer pH values were determined with a METTLER TOLEDO 320 pH Meter. The concentrations of the hydroperoxide stock solutions were determined by titration with potassium permanganate.

*Synthesis of 6,6′-ditellurobis(6-deoxy-β-cyclodextrin) (6-TeCD)*

The synthesis route of 6-TeCD was shown in Scheme 2. The regiospecific monotosylation of 6-position hydroxyl of β-cyclodextrin was carried out according to Ref. [9] to synthesize mono-6-tosyl-β-cyclodextrin. Sodium hydrogen telluride (NaTeH) was prepared according to the previous report [7]. Mono-6-tosyl-β-cyclodextrin was dissolved in potassium phosphate buffer (50 mM, pH 7.0) and DMF (cosolvent) and then excess NaTeH was added to the above solution. The mixture was kept under nitrogen for 72 h at 60 °C then oxidized in air and finally purified by centrifugation and Sephadex G-25 column chromatography with distilled water as the eluent. The resultant solution was freeze-dried and the lyophilized powder provided the yellow product in 41% yield. $^1$H NMR (500 MHz, $D_2O$): δ3.23–3.66 (m, 2-H, 4-H), 3.66–4.08 (m, 3-H, 5-H, 6-H), 4.94–5.27 (m, 1-H); $^{13}$C NMR (500 MHz, $D_2O$): δ60.5, 72.3, 72.5, 73.6, 81.5, 102.4; IR ($cm^{-1}$, KBr): υ = 3340 (OH), 2928 (CH, $CH_2$), 1625, 1140, 1080, 1030 (–O–); MALDI-MS: calcd 2491.2 found 2489.6; Anal. Calcd for $C_{84}H_{138}O_{68}Te_2{\cdot}11H_2O$: C, 36.91; H, 5.95. Found: C, 36.75; H, 5.68.

*Coupled reductase assay*

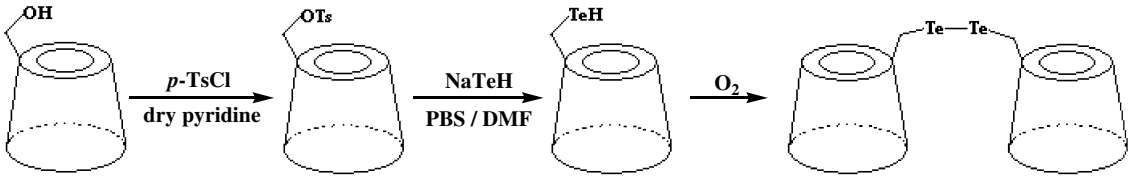
The GPx-like activity of 6-TeCD was measured using the Wilson's method [10] with minor modification. The assay mixture contained 50 mM PBS, pH 7.0, 1 mM EDTA, 100 μM GSH, 250 μM ROOH, 0.25 mM NADPH, 1 unit of glutathione reductase, and a moderate amount of test compound at 25 °C. Reaction was initiated by the subsequent addition of ROOH and the absorbance at 340 nm ($\varepsilon_{NADPH}$ = 6220 $M^{-1}$ $cm^{-1}$) [11] was recorded for a few minutes to calculate the rate of NADPH consumption (eqs. 1 and 2).

$$2GSH + H_2O_2 \xrightarrow{\text{Catalyst}} GSSG + 2H_2O \qquad (1)$$

$$GSSG \xrightarrow[\text{NADPH}]{\text{glutathione reductase}} 2GSH \qquad (2)$$

*Direct assay*

The catalytic activity of 6-TeCD was also studied according to a modified method reported by Wu and Hilvert [12] using 3-carboxy-4-nitrobenzenethiol (ArSH) as a glutathione (GSH) alternative. The initial rates ($\upsilon_0$) for the reduction of ROOH (250 μM) by ArSH (100 μM) in the presence of various catalysts (eq. 3) were determined at 25 °C and pH 7.0 (50 mM PBS,




*Scheme 2.*

*Table 1.* The initial rate ($\upsilon_0$)[a] and activity for the reduction of ROOH (250 $\mu$M) by thiol GSH or ArSH (100 $\mu$M) in the presence of various catalysts at pH 7.0 (50 mM PBS, 1 mM EDTA) and 25 °C

| Catalysts | Hydroperoxide | $\upsilon_0$[b] (M min$^{-1}$) | Activity | |
|---|---|---|---|---|
| | | ArSH | ArSH[c] | GSH[d] |
| PbSeScPh[e] | $H_2O_2$ | $(0.12 \pm 0.01) \times 10^{-7}$ | 1 | 1 |
| PhTeTePh[e] | $H_2O_2$ | $(8.03 \pm 0.17) \times 10^{-7}$ | 67 | 0.90 |
| $\beta$-cyclodextrin | $H_2O_2$ | 0 | 0 | 0.00052 |
| 6-TeCD | $H_2O_2$ | $(0.49 \pm 0.03) \times 10^{-6}$ | 4083 | 2.03 |
| | *t*-BuOOH | $(3.66 \pm 0.12) \times 10^{-6}$ | 30,500 | 4.12 |
| | CuOOH | $(1.50 \pm 0.04) \times 10^{-5}$ | 125,000 | 8.97 |

[a]The initial rate of reaction was corrected for the spontaneous oxidation in the absence of catalyst; [b]All values are means of at least five times and calculated from the first 5–10% of the reaction, and $\upsilon_0$ value = means ± SD; [c]The concentration of catalyst: [PhSeSePh], [PhTeTePh], [$\beta$-cyclodextrin] = 100 $\mu$M and [6-TeCD] = 1 $\mu$M in ArSH assay system. Calculated based upon GPx activity of PhSeSePh equal to 1, assuming the rate has a first-order dependence on the concentration of catalyst; [d]The concentration of catalysts: [PhSeSePh], [PhTeTePh] = 10 $\mu$M, [$\beta$-cyclodextrin] = 100 $\mu$M and [6-TeCD] = 1 $\mu$M in coupled reductase assay system. Calculated based upon GPx activity of PhSeSePh equal to 1; [e]The reaction solution contains 10% methanol (v/v), and methanol has no effect on the activity.

1 mM EDTA) by monitoring the UV absorption at 410 nm due to the disappearance of the thiolate absorption. The initial concentration of ArSH was measured from the 410 nm absorbance ($\varepsilon$ = 13,600 M$^{-1}$ cm$^{-1}$, pH 7.0).

$$\mathrm{ROOH} + 2\mathrm{ArSH} \xrightarrow{\text{Catalyst}} \mathrm{ROH} + \mathrm{ArSSAr} + \mathrm{H_2O} \quad (3)$$

## Results and discussion

The thiol peroxidase activities of 6-TeCD had been assessed under identical experimental conditions in two assay systems: coupled reductase assay and ArSH assay, and were summarized in Table 1. For the peroxidase activity, the enzymatic rates were corrected for the background (nonenzymic) reaction between hydroperoxide and thiol. When GSH was used as a thiol substrate in a classical coupled reductase assay system, the thiol peroxidase activity of 6-TeCD was only 9 times than that of diphenyl diselenide (PhSeSePh), a well-known GPx mimic. However, in ArSH assay system, as shown in Table 1, it was apparent that 6-TeCD reduces $H_2O_2$, *t*-BuOOH and CuOOH efficiently and the thiol peroxidase activity was almost 100,000-fold than that of PhSeSePh. Thus, 6-TeCD with hydrophobic cavity of $\beta$-cyclodextrin was much better catalyst than diphenyl ditelluride (PhTeTePh) carrying no binding group for substrates. This rate enhancement was very remarkable, and reflected the recognition action for thiol substrate in 6-TeCD catalysis. Natural GPx exhibits a strong specificity for its thiol substrate GSH, with small structural changes in the thiol leading to large reductions in catalytic efficiency [3]. Similarly, 6-TeCD seemed to be a preferential scaffold for the compound ArSH (the aromatic group in ArSH) rather than the hydrophilic compound GSH. Inoue *et al.* [13] had recently shown that the size-fit relation between a host cavity and a guest molecule plays an important role in molecular recognition by cyclodextrins, indicating that the hydrogen bonding, van der Waals forces, and hydrophobic interactions should depend upon how the size and/or shape of a guest molecule fit into the host cavity. Very recently, we had shown that inclusion complexation of $\beta$-cyclodextrin and ArSH with a binding constant of above $10^3$ M$^{-1}$ plays an essential role in enzymatic catalytic process [14]. To further prove the inclusion complexation in 6-TeCD catalysis, an inhibitor 1-adamantaneethanol was added to the ArSH assay system and, as expected, a large decrease of catalytic activity of 6-TeCD was observed (data no shown). The indication clearly showed the inclusion complexation of 6-TeCD and thiol substrate ArSH in ArSH assay system. The most notable result from the two assay systems was the specificity for the reduction of CuOOH. 6-TeCD was found to reduce CuOOH as well as *t*-BuOOH much faster (ca. 10–30 times) than $H_2O_2$. A similarly modest specificity for CuOOH was observed for cyclodextrin-based GPx mimics [14, 15]. These results suggested that the thiol peroxidase activity of 6-TeCD depended upon the nature of ROOH.

In conclusion, 6-TeCD is an excellent GPx mimic with strong substrate specificity and its catalytic activity seriously depends on the structurally different both substrates ROOH and thiols. This is a successful example for demonstrating that substrate binding can remarkably enhance the catalytic capacity of enzymatic models.

## Acknowledgement

We are grateful to the financial support of the Natural Science Foundation of China (20534030, 20471023), Innovative Research Team in University (IRT0422), and the Ministry of Education of China.

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