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Aryl Thiol Substrate 3-Carboxy-4-Nitrobenzenethiol Strongly Stimulating Thiol Peroxidase Activity of Glutathione Peroxidase Mimic 2, 2'-Ditellurobis(2-Deoxy-β-Cyclodextrin)

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Abstract: Artificial glutathione peroxidase (GPx) model 2, 2'-ditellurobis(2-deoxy-β-cyclodextrin) (2-TeCD) which has the desirable properties exhibited high substrate specificity and remarkably catalytic efficiency when 3-carboxy-4-nitrobenzenethiol (ArSH) was used as a preferential thiol substrate. The complexation of ArSH with β-cyclodextrin was investigated through UV spectral titrations, fluorescence spectroscopy, ^{1}H NMR and molecular simulation, and these results indicated that ArSH fits well to the size of the cavity of β-cyclodextrin. Furthermore, 2-TeCD was found to catalyze the reduction of cumene peroxide (CuOOH) by ArSH 200 000-fold more efficiently than diphenyl diselenide (PhSeSePh). Its steady-state kinetics was studied and the second rate constant k_{max}/K_{ArSH} was found to be 1.05×10^7 M^{-1} min^{-1} and similar to that of natural GPx. Moreover, the kinetic data revealed that the catalytic efficiency of 2-TeCD depended strongly upon the competitive recognition of both substrates for 2-TeCD. The catalytic mechanism of 2-TeCD catalysis agreed well with a ping-pong mechanism, in analogy with natural GPx, and might exert its thiol peroxidase activity via tellurol, tellurenic acid, and tellurosulfide.

Introduction

It was well established that a variety of human diseases have been generated by oxidative stress of reactive oxygen species (ROS). ROS were generated as byproducts of cellular metabolism and were mainly controlled by antioxidative defense system, especially by antioxidant enzymes.[1] In biological organism the antioxidant enzymes, superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) contribute dominatingly to enhance cellular antioxidative defense against oxidative stress.[2] GPx (EC 1.11.1.9) is a well-known selenoenzyme which catalyzes the reduction of harmful hydrogen peroxides and organic peroxides by glutathione (GSH, **1**) and protects the biomembranes and other cellular components from oxidative damage.[3] The enzyme active site includes a selenocysteine residue which forms a catalytic triad with Trp148 and Gln70 in a depression on the protein's surface, and some charged and hydrophobic amino acid residues (Phe, Trp, Asp) form a hydrophobic cavity for thiol GSH binding.[3d] The selenium undergoes a redox cycle involving the selenol (ESeH) as the active form. The selenol is first oxidized to selenenic acid (ESeOH), which reacts with reduced glutathione (GSH, **1**) to form selenenylsulfide (ESeSG). A second glutathione then regenerates the active form of the enzyme by attacking the ESeSG to form the oxidized glutathione (GSSG) (Scheme 1).[4] In contrast to the cytosolic GPx, which uses GSH exclusively as cosubstrate, other enzymes such as plasma GPx or phospholipid hydroperoxide GPx readily accept many thiols as substrates.[5]

In recent years, there were increasing interests in mimicking the functions of this important antioxidant enzyme not only for elucidating catalytic mechanism but also for potential pharmaceutical application, and several attempts had been made to produce synthetic selenium/tellurium compounds which mimic the properties of glutathione peroxidase.[4,6] Ebselen (2-phenyl-1,2-benzoisoselenazol-3(*2H*)-one), ebselen homologues, selenenamides, diselenides, α-phenylselenoketones, selenium-containing enzymes, antibodies, and cyclodextrins and their

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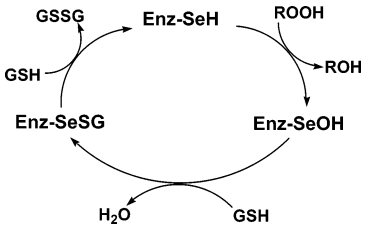
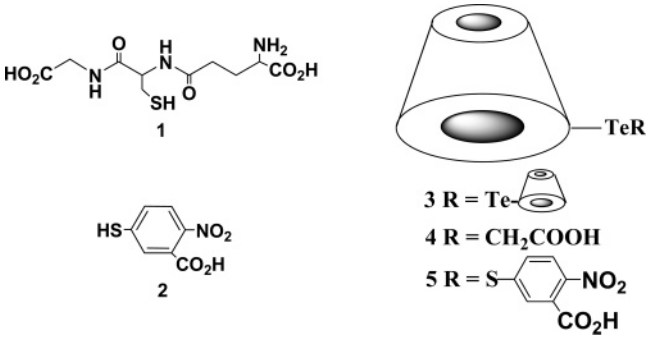
Scheme 1. Catalytic Cycle for GPx



tellurium analogues had all been demonstrated to catalyze the reduction of hydroperoxides (ROOH) in the presence of thiols.[4,6]

In the early effects to design the enzyme model with GPx-like activity, the main ideas were focused on the imitation of Se···N interaction which was demonstrated important in stabilization and activation of reactive selenol moiety in catalytic cycle.[7] Although some GPx mimics had some increase in activity, most of them displayed only limited catalytic enhancement. On the basis of the structural understanding for GPx, the nature of enzyme for molecular recognition and catalysis as well as the early work,[6d] we conceived that the generation of specific binding ability for thiol substrate and correct incorporation of the functional selenium/tellurium groups should be critical approaches for the construction of an effective GPx model. The previous studies from our group in preparing GPx models by monoclonal antibody technique,[8] bioimprinting,[9] and transferring nature enzymes[10] had demonstrated this hypothesis. The remarkable activity enhancements had been obtained when chemical incorporation of catalytic selenocysteine groups into existing or artificially generated thiol binding scaffolds. Hilvert's work[11] in preparing semisynthetic selenoprotein, selenosubtilisin, also supported this speculation. The selenosubtilisin which had evolved to bind specific substrate, was 70 000 times more efficient than diphenyl diselenide (PhSeSePh) in catalyzing the reduction of *tert*-butyl hydroperoxide (*t*-BuOOH) by thiol substrate 3-carboxy-4-nitrobenzenethiol (ArSH, **2**).[11] However, the studies of structure−functional relationship had been blocked by the complicated nature of macromolecular proteins. Construction of small molecular GPx models becomes a good alternative for elucidating the origin of substrate recognition in enzyme catalysis and for potentially therapeutic applications. In this respect, cyclodextrins were an attractive species for enzyme model design. This molecule has the ability to accommodate various substrates to their cavities, and the two rims of hydroxyl groups can be used to introduce prosthetic groups.[12]

By coorperating of both recognition by cyclodextrin and catalysis by ditelluride moiety, cyclodextrin-based enzyme model 2, 2′-ditellurobis(2-deoxy-β-cyclodextrin) (2-TeCD, **3**)



had been reported to act as a GPx mimic by us[13] and its catalytic efficiency was 24-fold than that of PhSeSePh when thiol GSH (**1**) was used as substrate. In our artificial enzyme model 2-TeCD the active site designed had been placed on the secondary side of cyclodextrin since it was established that a bound substrate would have its prosthetic groups at the secondary side of cyclodextrin. Cyclodextrins were known to favor aryl groups in the cavities.[12,14] The aryl thiol ArSH (**2**) may be able to take some advantage of the binding cavities of 2-TeCD.

In present paper, 2-TeCD catalyzed the reduction of ROOH by ArSH (**2**) was studied in detail. The complexation of ArSH (**2**) with β-cyclodextrin was investigated through UV spectral titrations, fluorescence spectroscopy, ^{1}H NMR and molecular simulation. It was clearly shown that 2-TeCD which has strong hydrophobic interaction produced large rate accelerations when ArSH (**2**) acted as a thiol substrate. The large difference in the activities of 2-TeCD with thiol ArSH (**2**) was ascribed to the role of the binding ability as compared with thiol GSH (**1**), and the recognition of substrates in the enzyme model could be delineated from the catalytically kinetic data.

Results

Catalytic Activity. The catalytic activity was studied according to a modified method reported by Hilvert et al.[11,15] using 3-carboxy-4-nitrobenzenethiol (ArSH, **2**) as a glutathione (GSH, **1**) alternative. The initial rates (v_0) for the reduction of ROOH (250 μM) by ArSH (**2**) (100 μM) in the presence of various catalysts (eq 1) were determined at 25 °C and pH 7.0 (50 mM phosphate buffer, 1 mM EDTA) by monitoring the UV absorption at 410 nm due to the disappearance of the thiolate absorption.

$$\mathrm{ROOH} + 2\mathrm{ArSH} \xrightarrow{\text{catalyst}} \mathrm{ROH} + \mathrm{ArSSAr} + \mathrm{H_2O} \quad (1)$$

The relative activities of the compounds were summarized in Table 1. For the peroxidase activity, the enzymatic rates were corrected for the background (nonenzymic) reaction between hydroperoxide and thiol. The initial concentration of ArSH (**2**) was measured from the 410-nm absorbance (ϵ = 13 600 M^{-1} cm^{-1}, pH 7.0). The initial rate of the background (nonenzymic) reaction between H_2O_2 and ArSH (**2**) was very slow (v_0 = 0.507 μM min^{-1}), but a slight enhancement in the rate was observed when PhSeSePh (100 μM) was added (v_0 = 0.012 μM min^{-1}).

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Table 1. Initial Rate (ν_0)[a] and Activity for the Reduction of ROOH (250 μM) by Thiol GSH (1) (100 μM) and ArSH (2) (100 μM) in the Presence of Various Catalysts at pH 7.0 (50 mM PBS, 1 mM EDTA) and 25 °C

		ν_0[b](M·min^{-1})	activity	
catalyst	hydroperoxide	ArSH	ArSH[c]	GSH[d]
PhSeSePh[e]	H_2O_2	$(0.12 \pm 0.01) \times 10^{-7}$	1	1
ebselen	H_2O_2			0.48
PhTeTePh[e]	H_2O_2	$(8.03 \pm 0.17) \times 10^{-7}$	67	0.90
β-cyclodextrin	H_2O_2	0	0	0.00052
2-TeCD	H_2O_2	$(2.55 \pm 0.16) \times 10^{-6}$	21250	23.87
	t-BuOOH	$(5.45 \pm 0.27) \times 10^{-6}$	45417	32.53
	CuOOH	$(2.45 \pm 0.09) \times 10^{-5}$	204167	44.62

[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 ν_0 value = means ± S. D. [c] The concentration of catalyst: [PhSeSePh] = 100 μM, [ebselen] = 10 μM, [PhTeTePh] = 100 μM, [β-cyclodextrin] = 100 μM and [2-TeCD] = 1 μM 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 catalyst: [PhSeSePh] = 10 μM, [ebselen] = 5 μM, [PhTeTePh] = 10 μM, [β-cyclodextrin] = 100 μM and [2-TeCD] = 1 μM 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.

The initial rate in the presence of diphenyl ditelluride (PhTeTePh) (100 μM) was also slow ($\nu_0 = 0.803$ μM min^{-1}). The β-cyclodextrin itself was found to be inactive in this assay system. When ebselen (10 μM) was tested, the initial rate had some extent decrease (ca. 10%) compared to the spontaneous oxidation. Ebselen had a too slow turnover in catalytic process, and the stoichiometric reaction of ebselen and ArSH (**2**) maybe resulted in this decrease. Under the identical conditions the cyclodextrin-based ditelluride (2-TeCD) (1 μM), however, exhibited a remarkable rate enhancement (2.546 μM min^{-1}). Assuming that the rate had a first-order dependence on the concentration of catalyst catalyzed the reduction of ROOH by ArSH (**2**), these data (Table 1) suggested that the 2-TeCD was at least 200 000-fold more efficient than PhSeSePh.

The activities of these compounds were also assessed using GSH (**1**) as a thiol substrate in the classical coupled reductase assay system[16] under identical experimental conditions. The relative activities of the compounds were also listed in Table 1. These data indicated that 2-TeCD was only 24 and 49 times more efficient than PhSeSePh and ebselen, respectively.

These results should not be surprising, since the enzyme model had been designed to bind specific substrates, and this specificity was one of its most desirable properties. For structurally distinct thiol substrates, 2-TeCD exhibited a large difference in thiol peroxidase activity. Cyclodextrins seem to be a preferential scaffold for the compound ArSH (**2**) (the aryl group in ArSH) rather than the hydrophilic compound GSH (**1**).[17] This observation allowed the difference between the activities seen in the two assay systems to be elucidated and suggested that the attempts to enhance the catalytic activity of 2-TeCD should focus on recognition for the thiol substrate. Furthermore, the notable result from this assay system was the

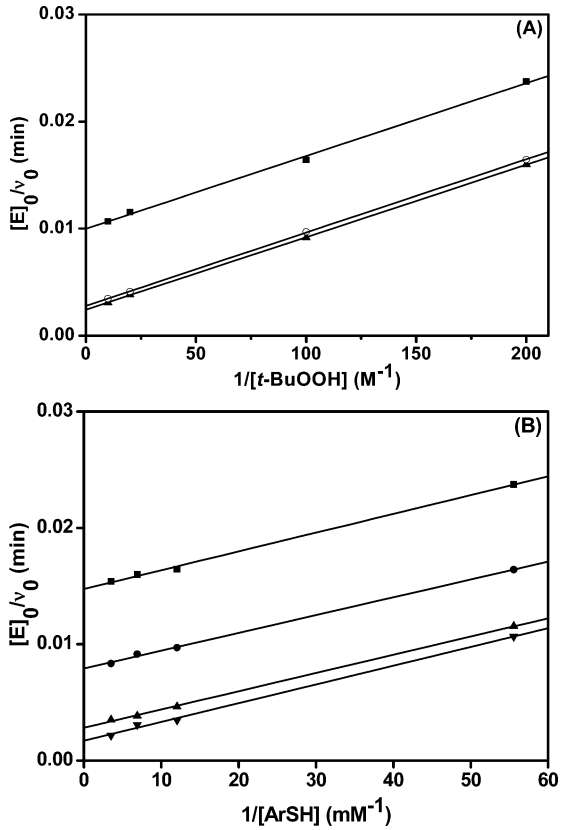

Figure 1. (A) $[E]_0/\nu_0$ (min) vs 1/[*t*-BuOOH] (M^{-1}) for 1 μM 2-TeCD in PBS, pH 5.5 and 25 °C, at [ArSH] = 18 μM (■), 83 μM (○), 145 μM (▲). (B) $[E]_0/\nu_0$ (min) vs 1/[ArSH] (mM^{-1}) for 1 μM 2-TeCD in PBS, pH 5.5 and 25 °C, at [*t*-BuOOH] = 5.1 mM (■), 10.1 mM (●), 50.0 mM (▲), 100.0 mM (▼).

specificity for reduction of aryl cumene hydroperoxide (CuOOH). In the presence of 2-TeCD, CuOOH was reduced by ArSH (**2**) at least 10 times faster than hydrogen peroxide (Table 1), similar result had been reported recently.[6g] It therefore seemed that the hydrophobic cavity provided by the cyclodextrin of 2-TeCD acts as a binding site for the hydroperoxide substrate.

Kinetics. To probe the mechanism of 2-TeCD promoting the peroxidase reaction, detailed kinetic studies were undertaken. 2-TeCD catalyzed the reduction of a variety of structurally distinct hydroperoxides (ROOH), from the hydrophilic H_2O_2 via the *tert*-butyl hydroperoxide (*t*-BuOOH) to the bulky aryl cumene hydroperoxide (CuOOH). Double-reciprocal plots (Figure 1, 2, and see Supporting Information) of initial rate versus substrate concentration at all the individual concentration revealed the characteristic parallel lines of a ping-pong mechanism with at least one covalent intermediate,[18] in analogy with natural GPx.[3a] Saturation kinetics were observed for each of the enzymatic peroxidase reactions at all the individual concentrations of ArSH (**2**) and ROOH investigated. The kinetic parameters for the enzymatic reactions between ArSH (**2**) and the hydroperoxide substrates H_2O_2, *t*-BuOOH, and CuOOH were shown in Table 2. These values were deduced from fitting the experimental data to a ping-pong kinetic mechanism. The data did not fit well to other models, such as sequential or

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Table 2. Kinetic Parameters for the Peroxidase Activity of 2-TeCD[a]

hydroperoxide	k_{max} (min^{-1})	K_{ROOH}	K_{ArSH}(μm)	k_{max}/K_{ROOH} (M^{-1} min^{-1})	k_{max}/K_{ArSH} (M^{-1} min^{-1})
H_2O_2	300 ± 17	48 ± 3	28 ± 3	$(6.00 \pm 0.05) \times 10^3$	$(1.05 \pm 0.06) \times 10^7$
t-BuOOH	900 ± 59	55 ± 7	120 ± 18	$(1.64 \pm 0.14) \times 10^4$	$(7.50 \pm 0.31) \times 10^6$
CuOOH	3710 ± 118	23 ± 1	627 ± 28	$(1.61 \pm 0.11) \times 10^5$	$(5.92 \pm 0.12) \times 10^6$

[a] Values = means ± S. D..

equilibrium-ordered mechanisms. The relevant steady-state equation (eq 2) for the enzymatic peroxidase reaction is

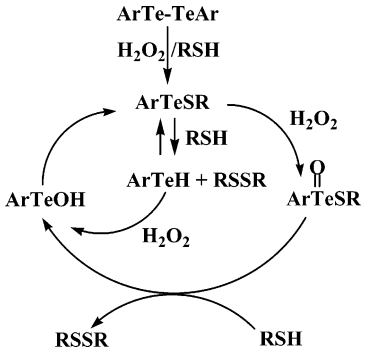
$$\frac{\nu_0}{[E]_0} = \frac{k_{max}[ArSH][ROOH]}{K_{ROOH}[ArSH] + K_{ArSH}[ROOH] + [ArSH][ROOH]} \quad (2)$$

where ν_0 is the initial reaction rate, $[E]_0$ is the initial enzyme mimic concentration, k_{max} is a pseudo-first-order rate constant and K_{ROOH} and K_{ArSH} are the Michaelis−Menten constants for the hydroperoxide and ArSH (**2**), respectively.

The rate constants of the background reaction between ArSH (**2**) and hydroperoxide had been reported to vary in the order $k(H_2O_2) > k(CuOOH) > k(t\text{-BuOOH})$.[15b] In contrast, the analogous rate constants of 2-TeCD and hydroperoxide ($k = k_{max}/K_{ROOH}$) vary as $k(CuOOH) > k(t\text{-BuOOH}) > k(H_2O_2)$. It was possible that the first series reflected the intrinsic rate of reaction between the hydroperoxides and a thiolate in the absence of any significant binding effects, while the latter series indicated that CuOOH and *t*-BuOOH were able to take binding advantage of the cyclodextrin template and hence raised its k_{max}/K_{ROOH} above that of hydrogen peroxide by increasing k_{max} and lowering K_{ROOH}. It was worth noting that the Michaelis−Menten contant (K_{ArSH}) values listed in Table 2 vary in magnitude in the order K_{ArSH} (H_2O_2) < K_{ArSH} (*t*-BuOOH) < K_{ArSH} (CuOOH), for the enzymatic reactions with H_2O_2, *t*-BuOOH, and CuOOH. This clearly indicated that the competitive binding affinity of thiol and hydroperoxide substrates for GPx mimic 2-TeCD did exist in the enzymatic reaction. Furthermore importantly, Table 2 revealed that k_{max}/K_{ROOH} was not identical and increased upon in turn changing from H_2O_2 via *t*-BuOOH to CuOOH. This revealed that the GPx mimic 2-TeCD has substrate specificity for substrate hydroperoxide.

For GPx, acting on ROOH and GSH, at physiological pH and 37 °C, the bimolecular rate constants for the reactions were as follow: $k_{+1}(ROOH) = 10^8$ M^{-1} min^{-1} and $k_{+1}(GSH) = l0^7$ M^{-1} min^{-1} as determined previously.[3a,h,5,19] The equivalent parameters for 2-TeCD, acting on ROOH and ArSH (**2**), at pH 5.5 and 25 °C, were $k_{+1}(CuOOH) = 1.61 \times 10^5$ M^{-1} min^{-1} and $k_{+1}(ArSH) = 5.92 \times l0^6$ M^{-1} min^{-1}; $k_{+1}(t\text{-BuOOH}) = 1.64 \times l0^4$ M^{-1} min^{-1} and $k_{+1}(ArSH) = 7.50 \times l0^6$ M^{-1} min^{-1}; $k_{+1}(H_2O_2) = 6 \times l0^3$ M^{-1} min^{-1} and $k_{+1}(ArSH) = 1.05 \times l0^7$ M^{-1} min^{-1}. However, under the similar conditions, the equivalent parameters for selenosubtilisin, acting on *t*-BuOOH and ArSH (**2**), were $k_{+1}(t\text{-BuOOH}) = 4 \times l0^3$ M^{-1} min^{-1} and $k_{+1}(ArSH) = 1.5 \times l0^7$ M^{-1} min^{-1}.[11] Although these parameters were measured at different pH and temperature for different thiol substrates, and could not be directly equated, they did allow an approximate comparison of these systems. The bimolecular reactions between GPx, selenosubtilisin, and 2-TeCD and their respective thiol substrates were very similar, as evidenced by the values of k_{+1}(RSH) above. It was apparent from this efficiency that the size of ArSH (**2**) fits well to the cavity of β-cyclodextrin to facilitate the binding of the thiol substrate in β-cyclodextrin cavity and produce large rate accelerations. The semisynthetic enzyme selenosubtilisin which had evolved to bind specific substrate was in detail investigated and acted as an excellent GPx mimic.[15] Surprisingly, 2-TeCD catalyzed *t*-BuOOH by ArSH (**2**) with a higher second rate constant ($k_{+1}(t\text{-BuOOH}) = 1.64 \times 10^4$ M^{-1} min^{-1}) compared to selenosubtilisin ($k_{+1}(t\text{-BuOOH}) = 4 \times l0^3$ M^{-1} min^{-1}). For structurally distinct thiol substrates, the second rate constant of 2-TeCD-catalyzed reduction of ROOH by GSH (**1**) ($k_{+1}(GSH) = l0^4$ M^{-1} min^{-1})[13a] was lower as compared with ArSH (**2**) ($k_{+1}(ArSH) = l0^7$ M^{-1} min^{-1}). When GSH (**1**) acted as a thiol substrate 2-TeCD was clearly far less efficient than with ArSH (**2**). It was obviously shown that the capacity to bind the thiol substrate is essential for the enzymatic catalytic efficiency.

Scheme 2. Proposed Mechanism of the Thiol Peroxidase Reaction of Ditellurides



Mechanism. The kinetic data were presented in support of a ping-pong mechanism with at least one covalent intermediate. Further experiments were needed to characterize each of the intermediates in the catalytic cycle. Engman and co-workers had recently reported that diaryl ditelluride which mimic the properties of GPx carried out the reaction mechanism as shown in Scheme 2.[20]

It was known that the rate of the enzymatic reaction depends on the concentration of intermediate ESeSG in Scheme 1. Mugesh and co-workers had confirmed that the catalyst-substrate complex (RSeSPh) does exist during the catalytic cycle through a detailed kinetic studies.[7b] Herein, we did similar kinetic studies to characterize the catalyst-substrate complex. Double-reciprocal plots (Lineweaver−Burk plots) of initial rate versus substrate concentration yielded families of linear lines (Figures 2 and 3). The parallel lines corresponded to different concentrations of the catalyst and indicated that the rates increase linearly with the concentration of 2-TeCD. When the concentration of

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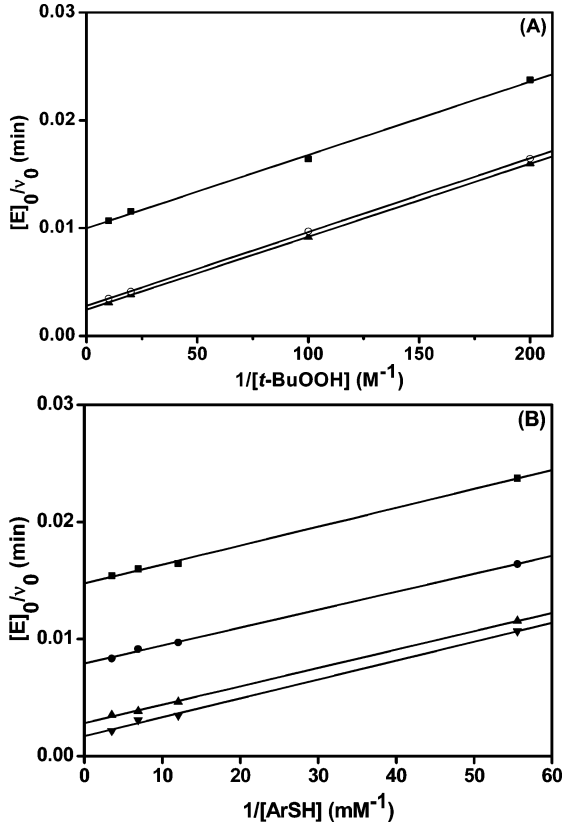


Figure 2. (A) $[E]_0/v_0$ (min) vs 1/[CuOOH] (M^{-1}) for 1 μM 2-TeCD in PBS, pH 5.5 and 25 °C, at [ArSH] = 83 μM (■), 188 μM (○), 260 μM (▲). (B) $[E]_0/v_0$ (min) vs 1/[ArSH] (mM^{-1}) for 1 μM 2-TeCD in PBS, pH 5.5 and 25 °C, at [CuOOH] = 3.1 mM (■), 6.3 mM (●), 20.0 mM (▲), 50.0 mM (▼).

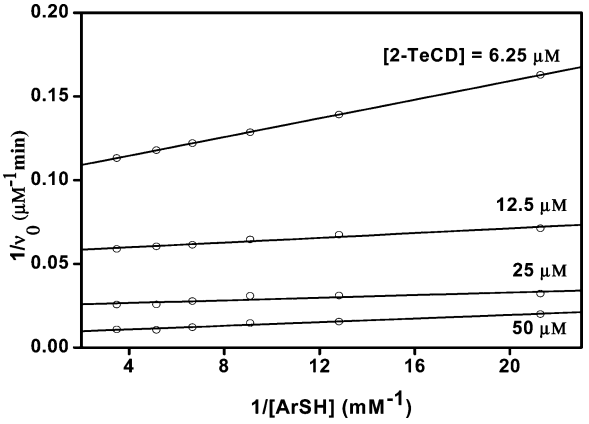


Figure 3. Lineweaver−Burk plots obtained for the model reaction in the presence of different enzyme mimic concentration [2-TeCD] at pH 5.5 (PBS) and 25 °C. The initial H_2O_2 concentration was fixed to 250 μM.

2-TeCD was maintained constant while substrate concentration [ArSH] was increased, a rapid increase of rate was observed in the initial stages; however, when the substrate concentration was increased further, the rate became constant (see Supporting Information). At the same time, when the concentration of 2-TeCD was increased, the rates become very high for higher concentration of ArSH (**2**). From this observation, it was clearly shown that the intermediate (CDTeSAr (**5**)) did exist during the catalytic cycle.

Concerning the reaction mechanism of 2-TeCD catalysis, we had good reasons to believe that CDTeSAr (**5**) is a key intermediate in the catalytic cycle. In the presence of hydroperoxide, diaryl ditelluride reacted with thiols to give tellurosulfide (eq 3).[20] The tellurenyl sulfides derived from reactions

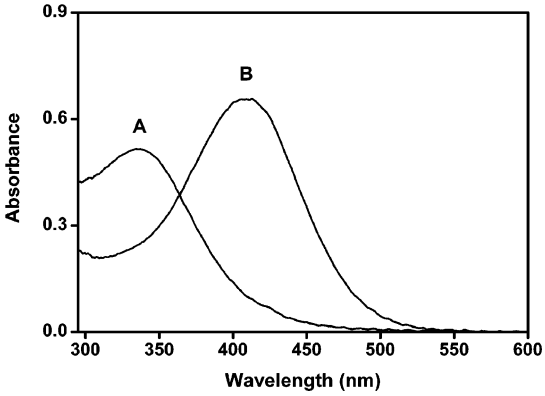
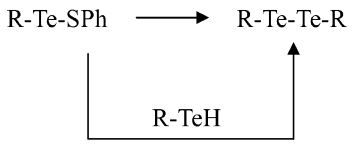


Figure 4. (A) UV spectra of isolated CDTeSAr in phosphate buffer, pH 5.5 ($\epsilon_{336} = 9000\ M^{-1}\ cm^{-1}$). (B) After addition of excess DTT (for ArSH, pH 5.5, $\epsilon_{410} = 12\,600\ M^{-1}\ cm^{-1}$).

Scheme 3



$$ArTe{-}TeAr + 2RSH + H_2O_2 \rightarrow 2ArTeSR + 2H_2O \quad (3)$$

between ditellurides and thiol may disproportionate to the corresponding dichalcogenides.[21] Moreover, the tellurenyl sulfides may react with the corresponding tellurols to produce the dichalcogenides as shown in Scheme 3.[22] However, in the absence of hydroperoxide, the reaction of 2-TeCD with ArSH (**2**) (eq 4) could be readily monitored spectroscopically by the

$$2\text{-TeCD} + ArSH \rightleftharpoons CDTeH + CDTeSAr \quad (4)$$

disappearance of thiolate at 410 nm. The CDTeSAr (**5**) could be isolated via the reaction of 2-TeCD with 2 equiv of ArSH (**2**) under air by gel filtration on Sephadex G-15 and was characterized (vide infra). The treatment of CDTeSAr (**5**) with excess 1, 4-dithio-DL-threitol (DTT) led to the release of ArSH (**2**) (Figure 4). The dependence of the yield of isolated CDTeSAr (**5**) on the thiol concentration suggested to us that the form of CDTeSAr (**5**) was in equilibrium (eq 5) with the corresponding tellurolate:

$$CDTeSAr + ArS^- \rightleftharpoons CDTe^- + ArSSAr \quad (5)$$

The position of equilibrium lay far to the left. In this case, the enzymatic activity was observed to decrease with increasing pH (Figure 5). To study the nature of this variation in enzymatic catalysis, the complexation of β-cyclodextrin and ArSH (**2**) was carried out by UV spectral titrations at various pH. It was found that the complex stability constant of β-cyclodextrin and ArSH (**2**) decreased with increasing pH (see Supporting Information). The deprotonated nature of ArSH (**2**) in different pH was responsible for this decrease, and the polar substituents at high

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(22) Haene, G. R. M. M.; De Rooij, B. M.; Vermeulen, N. P. E.; Bast, A. *Mol. Pharmacol.* **1990**, *37*, 412.

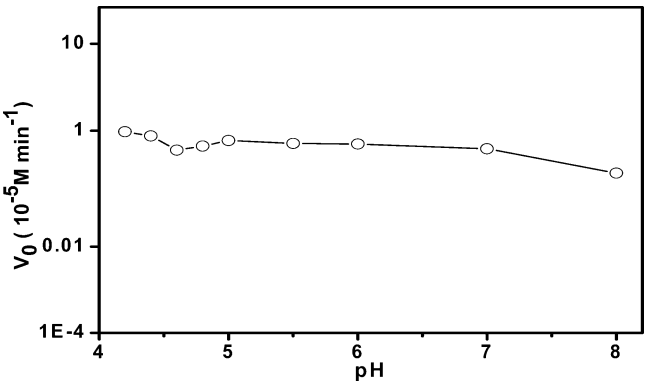
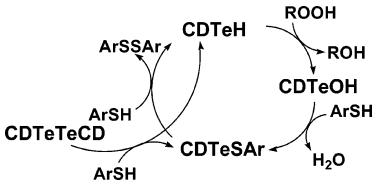


Figure 5. Plots of v_0 vs pH for the reduction of *t*-BuOOH (250 μM) by ArSH (100 μM) in the presence of 2-TeCD (1 μM) at 25 °C in phosphate buffer.

pH (the deprotonated form of ArSH) led to a low binding in the highly hydrophobic interior of β-cyclodextrin. It was interesting to note that the pH value of the undulating range in Figure 5 is remarkably close to pK_a (pK_a = 4.4)[15a] of ArSH (**2**), and the enzymatic activity had some increasing from pH 4.6 to 5.0. Thiols were much less nucleophilic than their corresponding thiolate ions. This would be consistent with a requirement for thiolate, ArS^-, as a nucleophile in the rate-determining step, again suggestive of the conversion of CDTeSAr to $CDTe^-$. The tellurolate would be readily oxidized aerobically to generate 2-TeCD. We anticipated that the tellurol might also be irreversibly trapped by a suitable alkylating agent.[15,23] To test this hypothesis, 2-TeCD was incubated with a large excess of iodoacetic acid at pH 4.0 in the presence of ArSH (**2**). The compound was recovered by gel filtration on G-15 Sephadex after additions of thiol, and was found to lose its peroxidase activity completely. The compound was fully characterized, and the formation of diorganyl telluride (**4**) revealed the tellurol was one of the intermediates of the catalytic cycle. Furthermore, compound **4** could also be obtained from the reaction of 2-TeCD and iodoacetic acid in the presence of sodium hydroborate (vide infra). As a control, 2-TeCD was similarly treated with iodoacetic acid, but in the absence of thiol. This batch of 2-TeCD was recovered with full activity. These results showed that the tellurol group, produced by reduction of 2-TeCD, can be irreversibly modified by iodoacetic acid and that this modifications of the prosthetic group abolishes peroxidase activity. Since these compounds were diselenides[7b] or ditellurides,[20] they may obey the catalytic mechanism that had been proposed for the natural GPx (Scheme 1). In general, organotellurium compounds can be oxidized to overoxidized tellurium species. Cyclodextrin-based telluronic acid was obtained by the reaction of 2-TeCD and an excess H_2O_2, however, the overoxidized tellurium species catalyzed the reduction of H_2O_2 by ArSH (**2**) at least 50 times less efficiently than 2-TeCD in catalytic process.[24] This effectively rules out any significant role of the overoxidized tellurium species in catalytic cycle. Furthermore, a ping-pong mechanism from the kinetic data also supported this conclusion. As discussed above, overoxidized tellurium species may not exist in the primary catalytic cycle

Scheme 4. Proposed Catalytic Mechanism of 2-TeCD.



of enzymatic reaction and the turnover reaction may proceed via the mechanism shown in Scheme 4. 2-TeCD exerted its peroxidase activity via tellurol, tellurenic acid, and tellurosulfide in ArSH assay system.

Complexation of ArSH (2) and β-Cyclodextrin. Since β-cyclodextrins are cavities in the molecular center, they could accommodate various guest molecules in their cavities, forming inclusion complexes. Recently, the studies of cyclodextrin complexes were reviewed by Inoue[25] and Schneider[26] in detail. The aryl thiol ArSH (**2**) may be able to take some advantage of the binding site of 2-TeCD, since the enzymatic system exhibited a significant rate advantage for thiol peroxidase activity. The specific binding for thiol substrate which contributes to high activity of GPx mimic had been demonstrated by catalytic antibody and seleno-glutathione transferase.[6d] We thought that the high catalytic activity of 2-TeCD was also driven by the strong binding between 2-TeCD and ArSH (**2**). To support this hypothesis, the binding constant of β-cyclodextrin with ArSH (**2**) was measured by UV spectral titrations. According to the modified Hildebrand−Benesi equation,[27] the linear plot of the reciprocal of the absorbance difference ΔA and molar concentration of the host molecule [H] indicated a 1:1 complex between the host molecule β-cyclodextrin and the guest molecule ArSH (**2**) with a binding constant of above 10^3 M^{-1} (see Supporting Information). We also characterized the complexation of β-cyclodextrin and ArSH (**2**) by means of fluorescence spectroscopy and found that the fluorescence intensity of ArSH (**2**) obviously increase after addition of β-cyclodextrin (see Supporting Information). This experiment also provided the proof for the complexation between β-cyclodextrin and ArSH (**2**). To further confirm the inclusion complexation of β-cyclodextrin and ArSH (**2**), the catalytic activity of 2-TeCD-catalyzed the reduction of peroxide by ArSH was assessed in the presence of an inhibitor, 1-adamantaneethanol. Since the adamantane group is strongly bound to the cavity of β-cyclodextrin (association constant: $K_a > 10^4$ M^{-1}),[25] the adamantane group could rival with ArSH (**2**) in the inclusion complexation of 2-TeCD and could decrease the catalytic activity. As expected a large decrease of catalytic activity of 2-TeCD was observed (see Supporting Information). At the same time, a ^{1}H NMR study on the binding inhibit was investigated and indicated that in the presence of 1-adamantaneethanol some amount of ArSH (**2**) did not enter into the cavity of β-cyclodextrin, although under identical conditions in absence of any inhibitor all of ArSH (**2**) entered into the cavity of β-cyclodextrin (see Supporting Information). These results strongly supported the inclusion complexation of enzyme mimic and thiol substrate ArSH (**2**) in ArSH assay system. In this case, ^{1}H NMR spectra were expected to provide more

(23) Forstrom, J. W.; Zakowski, J. J.; Tappel, A. L. *Biochemistry* **1978**, *17*, 2639.
(24) The GPx-like activity of cyclodextrin-based telluronic acid catalyzed the reduction of hydrogen peroxide was found to be 420 (calculated based upon GPx-like activity of PhSeSePh equal to 1) in ArSH assay system under identical experimental conditions.
(25) Rekharsky, M. V.; Inoue, Y. *Chem. Rev.* **1998**, *98*, 1875.
(26) Schneider, H. J.; Hacket, F.; Rüdiger, V. *Chem. Rev.* **1998**, *98*, 1755.
(27) (a) Benesi, H. A.; Hildebrand, J. H.; *J. Am. Chem. Soc.* **1949**, *71*, 2703. (b) Rossotti, F. J. C.; Rossotti, H. *The Determination of Stability Constants*; McGraw-Hill Book Co., Inc.: New York, 1961, p 276.

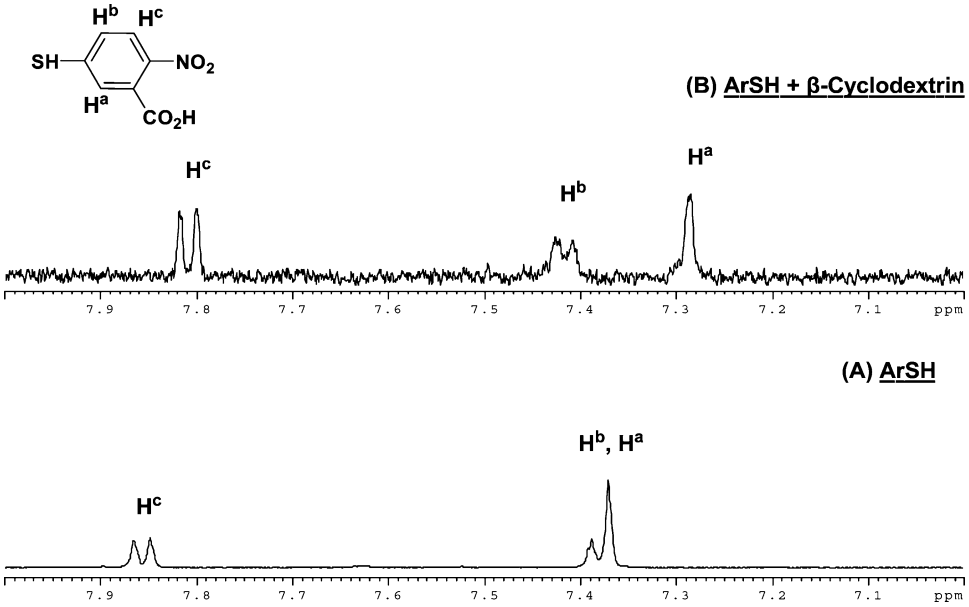


Figure 6. ^{1}H NMR spectrum of ArSH (**2**) in the absence (A) and in the presence (B) of β-cyclodextrin in D_2O at ambient temperature.

information about the cyclodextrin complex state, since analyses of the UV spectral titrations and fluorescence spectrum of the cyclodextrin complex indicated the ArSH (**2**) was located in the cavity. The ^{1}H NMR spectra (Figure 6) of the ArSH (**2**) changed significantly upon addition of excess β-cyclodextrin at ambient temperature. The H^a and H^c protons of aromatic region in ^{1}H NMR spectra showed significant upfield shift compared to the spectrum of the ArSH (**2**) alone. The H^b proton showed significant downfield shift, however, indicating that a moderate increase in water exposure of H^b proton of aromatic region. The H^a and H^b protons of aromatic region showed remarkable cleavage compared to the spectrum of the ArSH (**2**) alone, indicating that the H^a and H^b protons of aromatic region were locating in different microenvironment in the presence of excess β-cyclodextrin in water. In addition, in ^{1}H NMR spectra the peaks of outside protons of β-cyclodextrin region in the presence of ArSH (**2**) became obviously wide compared to the spectrum of β-cyclodextrin alone (see Supporting Information). This experiment revealed that the interaction of β-cyclodextrin and ArSH (**2**) did exist in the complexation. At the same time, a 2D NMR spectrum also provided a further proof for the inclusion complexation of β-cyclodextrin and ArSH (**2**), in which the interaction of the aryl protons of ArSH (**2**) and inside protons of β-cyclodextrin had been observed (see Supporting Information).

Molecular Simulation of Complexation of β-Cyclodextrin and Derivative with ArSH (2). The Dreiding 2.21 force field used for molecular simulation was able to capture much of the observed experimental trends for the complexation of β-cyclodextrin and derivative with ArSH (**2**). The structure of β-cyclodextrin was kept fixed during the entire simulation. The total potential energy (E_{total}) acted as a criterion of complexation stability of β-cyclodextrin and derivative with ArSH (**2**). There were four accessible orientations (S to A, S to B, N to A, N to B)[28] in the simulation of ArSH (**2**) (size 5.68−5.91 Å) accessed

(28) The prepositive "S" implied the sulfur headgroup of ArSH (**2**) and "N" implied the nitrogen headgroup of ArSH (**2**); the latter "A" implied the primary side of β-cyclodextrin and "B" implied the secondary side of β-cyclodextrin.

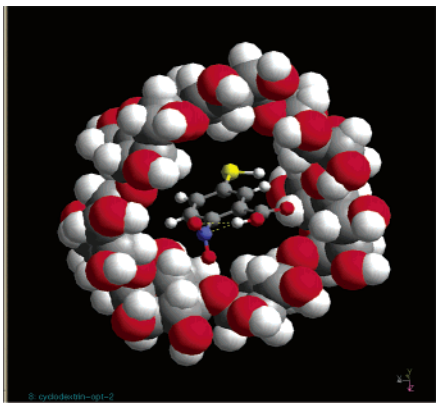


Figure 7. Lowest E_{total} structural model of the complexation of β-cyclodextrin corresponding to the accessible orientation of sulfur headgroup of ArSH (**2**) toward the secondary side of β-cyclodextrin.

the cavity of β-cyclodextrin, and each of the orientations resulted in a stable structure, and accordingly produced an E_{total} (see Supporting Information). We found that ArSH (**2**) can enter into the cavity of β-cyclodextrin easily and the orientations of "S to B" and "S to A" were much easier than those of "N to B" and "N to A", as evidenced by the lower E_{total}. It was expected that the orientation of "S to B" was easiest among them and the complexation structure was shown in Figure 7. When we optimized the structure of the intermediate CDTeSAr (**5**), the aryl group in CDTeSAr (**5**) could not enter into the cavity of cyclodextrin but located upon its secondary side. This result was proved experimentally by ^{1}H NMR spectrum of CDTeSAr (**5**). In the ^{1}H NMR spectrum the chemical shifts of the aryl and cyclodextryl protons of CDTeSAr (**5**) had no any change compared to the spectrum of ArSH (**2**) or β-cyclodextrin alone, indicating no self-inclusion of CDTeSAr (**5**) (see Supporting Information). It was essential that the intermediate CDTeSAr (**5**) could bind another ArSH molecule to facilitate the catalytic cycle of the enzymatic reaction, otherwise it would be self-inhibited. To insight into the effects of geometric preference on the catalysis, the tellurium atom was introduced to the primary side of β-cyclodextrin. 6, 6′-Ditellurobis(6-deoxy-β-cyclodextrin) (6-TeCD, unpublished) also displayed thiol per-

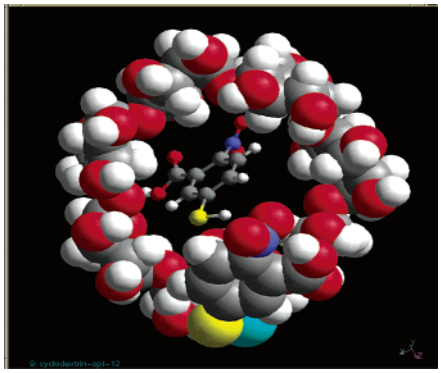


Figure 8. Lowest E_{total} structural model of the complexation of CDTeSAr (**5**) corresponding to the accessible orientation of sulfur headgroup of ArSH (**2**) toward the primary side of β-cyclodextrin.

oxidase activity, but was obviously lower than 2-TeCD (**3**).[29] The difference of catalytic efficiency might be made by geometric preference. Inoue and co-worker recently reported a similar system and showed that the type of substituent introduced to cyclodextrin drastically affects the molecular recognition ability.[30] We did a similar molecular simulation using CDTeSAr (**5**) as a β-cyclodextrin alternative. The Te−S−Ar moiety of CDTeSAr (**5**) was flexible, and another ArSH (**2**) molecule was easy to locate in the cavity of cyclodextrin. However, we found that the preferential orientation preferred "S to A" to "S to B", as evidenced by the lower E_{total}, and "S to A" was easiest orientation among them (Figure 8). Consequently, the cavity of β-cyclodextrin oriented the thiolate ion of ArSH (**2**) toward active site for nucleophilic attack, and this procedure was necessary for obtaining high catalytic activity in 2-TeCD catalysis. From molecular simulation we found that ArSH (**2**) could easily enter into the cavity of cyclodextrin to form complex with a favorable orientation for catalysis.

Discussion

2-TeCD had been shown as a GPx mimic and its thiol peroxidase activity was assessed in two assay systems: classical coupled reductase assay and ArSH assay. When GSH (**1**) was used as a thiol substrate in a classical coupled reductase assay system, the thiol peroxidase activity of 2-TeCD was only 24 times than that of PhSeSePh. However, in ArSH (**2**) assay system, as shown in Table 1, it was apparent that 2-TeCD reduces H_2O_2, *t*-BuOOH and CuOOH effectively and the thiol peroxidase activity was almost 10^5 times than that of PhSeSePh. This rate enhancement was remarkable, and reflected the recognition action for thiol substratre in 2-TeCD catalysis. 2-TeCD seemed to be a preferential scaffold for the compound ArSH (**2**) (the aryl group in ArSH) rather than the hydrophilic compound GSH (**1**). It was generally known that cyclodextrins have been extensively exploited in the past as enzyme models and molecular receptors because of their capacity to accommodate various guest molecules in their hydrophobic cavities through host−guest chemistry, and partly because of large numbers of hydroxyl groups in all directions around the cavity.[12a,b] Inoue and co-workers 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 on how the size and/or shape of a guest molecule fit into the host cavity.[31] The complexation of ArSH (**2**) with β-cyclodextrin was investigated through UV spectral titrations, fluorescence spectrum, ^{1}H NMR and molecular simulation, and these results indicated that ArSH (**2**) fits well to the size of the cavity of β-cyclodextrin. Consequently, in our miniature enzyme model, 2-TeCD have cavities that (a) provide maximum hydrophobic interaction with a substrate to form complexes, (b) fit the aromatic ring of the bound substrate, and (c) orient the thiolate ion of the bound substrate toward active site for nucleophilic attack. All these results indicated that the thiol substrate ArSH (**2**) takes some advantage of the binding site of 2-TeCD and improves the catalytic efficiency of 2-TeCD, but there was no reason to believe that this activity was optimal. A semisynthetic enzyme selonosubtilisin retained some of the substrate specificity of its natural template and favored ArSH (**2**) as a preferential thiol substrate, however, for thiol substrate GSH (**1**), the enzymatic efficiency was even lower.[15a] Similarly, 2-TeCD also seemed to have a preference for ArSH (**2**), although possibly for different reasons. Cytosolic GPx exhibits a strong specificity for its thiol substrate GSH (**1**), with small structural changes in the thiol leading to large reductions in catalytic efficiency.[5,32] Cytosolic GPx, selenosubtilisin, and 2-TeCD all exhibited dramatically different thiol substrate specificity and catalytic efficiency. It was clear that 2-TeCD which has strong hydrophobic interaction with ArSH (**2**) as thiol substrate produced large rate accelerations.

A comparison of kinetic parameters (Table 2) obtained from kinetic analyses of 2-TeCD using a variety of structurally distinct ROOH, such as H_2O_2, *t*-BuOOH, and CuOOH, as an oxidative reagent indicated the following conclusions. First, because the maximal k_{cat} value for the enzymatic reaction (k_{max}) was altered, the hydroperoxide substrate must be involved in a step which is at least partially rate-determining. Second, the variety of k_{max}/K_{ROOH} values and saturation kinetics together with different K_{ROOH} values suggested that 2-TeCD has substrate specificity for hydroperoxides. Third, the high k_{max}/K_{ArSH} values and saturation kinetics together with the low K_{ArSH} values indicated that ArSH (**2**) is a preferential thiol substrate of 2-TeCD. Finally, the variational orders of the k_{max}/K_{ROOH} values and k_{max}/K_{ArSH} values revealed that the 2-TeCD can recognize and bind these substrates and the competitive binding affinity of these substrates for 2-TeCD did exist in the enzymatic reaction. In the natural GPx the selenolate locates in a shallow depression on the protein's surface and may essentially react with any approaching hydroperoxide: the enzyme has no real substrate specificity for ROOH, provided that steric hindrance does not prevent their reaction.[33] However, the thiol peroxidase activity of 2-TeCD depended upon the nature of ROOH. In the presence of 2-TeCD, CuOOH was at least 10 times faster than hydrogen peroxide.

(29) The GPx-like activity of 6, 6′-ditellurobis(6-deoxy-β-cyclodextrin) (6-TeCD) catalyzed the reduction of hydrogen peroxide by ArSH or GSH was found to be 4083 and 1.98 respectively (calculated based upon GPx-like activity of PhSeSePh equal to 1) in two assay systems under identical experimental conditions.

(30) Liu, Y.; You, C. C.; Wada, T.; Inoue, Y. *J. Org. Chem.* **1999**, *64*, 3630.

(31) (a) Inoue, Y.; Hakushi, T.; Liu, Y.; Tong, L.; Shen, B.; Jin, D. *J. Am. Chem. Soc.* **1993**, *115*, 475. (b) Inoue, Y.; Liu, Y.; Tong, L.; Shen, B. *J. Am. Chem. Soc.* **1993**, *115*, 10637.

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(33) Flohé, L. In *Glutathione: Chemical, Biochemical, and Medical Aspects*; Dolphin, D., Poulson, R., Avamovic, O., Eds.; *Wiley: New York*, 1989, p 644.

Our kinetic data also suggested that the attack of ArS^- on CDTeSAr may be rate-determining to some degree and hence the CDTeSAr would accumulate, while the concentration of $CDTe^-$ would be low, under steady-state conditions. Such a low concentration of the tellurolate may be responsible for the slow rate of consumption of hydroperoxide, which reacts with this enzymatic intermediate. The natural GPx was believed to have evolved to near optimal efficiency for the decomposition of ROOH. All of the GPx family contained an active triad consisting of selenocysteine, glutamine, and tryptophan residues,[34] and the cytosolic GPx contained a GSH binding site consisting of one lysine and four arginine residues.[3d] Our small molecular enzyme model 2-TeCD lacked these features of binding site and was relatively simpler than the natural GPx. However, in the GPx mimic 2-TeCD the hydrophobic cavities could accommodate accurately ArSH (**2**) to enhance the catalytic efficiency. 2-TeCD displayed a higher catalytic efficiency than that of the semisynthetic enzyme selenosubtilisin which had evolved to bind specific substrate. It was very interesting that a small molecular enzyme model exhibits such a remarkable thiol peroxidase activity. This case successfully corroborated our strategy of imitating GPx in small molecular enzyme model.

The turnover reaction of 2-TeCD-catalyzed reduction of ROOH by ArSH (**2**) may proceed via the mechanism shown in Scheme 4, in analogy with the natural GPx, and exerted its thiol peroxidase activity via tellurol, tellurenic acid, and tellurosulfide in ArSH assay system.

In conclusion, we had shown that 2-TeCD catalyzes the reduction of ROOH by an aryl thiol ArSH (**2**) with remarkable catalytic efficiency. Studies of the kinetics of the 2-TeCD-catalyzed reduction of ROOH by ArSH (**2**) suggested that binding substrate was essential for the thiol peroxidase activity of GPx mimics. The high catalytic efficiency and selectivity, together with water-soluble and thermal stability gave 2-TeCD a real advantage compared to other GPx mimics. 2-TeCD represented an excellent alternative for the study of enzymatic specificity and potential pharmaceutical application.

Exceprimental Section

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. 2-TeCD[13] was prepared as described previously and characterized in detail. *p*-Toluenesulfonyl chloride and iodoacetate acid were also purchased from Tianjin Chemical Plant. *Tert*-butyl hydroperoxide (*t*-BuOOH) and reduced glutathione (GSH) were purchased from Merck. 1, 4-Dithio-DL-threitol (DTT) was obtained from Bebco. Cumene hydroperoxide (CuOOH) and 1-adamantaneethanol were purchased from Fluka. Diphenyl ditelluride (PhTeTePh) was obtained from Aldrich. Sodium hydroborate, diphenyl diselenide (PhSeSePh), 2-phenyl-1, 2-benzoisoselenazol-3(*2H*)-one (ebselen), 5, 5'-dithiolbis(2-nitrobenzoic acid), β-nicotinamide adenine dinucleatide phosphate reduced form (NADPH), and glutathione reductase were purchased from Sigma. Sephadex G-15 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). Fluorescence spectral measurements were performed on a Shimadzu RF-5301PC Spectrofluorophotometer. The spectrometric measurements were carried out with a Shimadzu 3100 UV−vis−near-IR Recording Spectrophotometer or Lambda 800 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 3-Carboxy-4-Nitrobenzenethiol (ArSH, 2). 3-carboxy-4-nitrobenzenethiol was prepared by reduction of the corresponding disulfide 5, 5'-dithiolbis(2-nitrobenzoic acid) following the procedure of Silver[35] and was characterized by ^{1}H NMR: (500 MHz, D_2O) δ 7.91 (d, 1 H, J = 8.5 Hz), 7.44−7.42 (m, 2 H, J = 8.5 Hz); UV/vis (PBS, pH 7.0): λ_{max} (ϵ) = 410 nm (13600 mol^{-1} dm^3 cm^{-1}).

Synthesis of Compound 4. Method 1. 2-TeCD (100 mg, 0.04 mmol) and iodoacetate acid (149 mg, 0.8 mmol) were dissolved in 30 mL of phosphate buffer (pH 4.0) under nitrogen, and large excess amount of 3-carboxy-4-nitrobenzenethiol was added dropwise. After the addition was complete, the mixture was allowed to stir at room-temperature overnight. The mixture was purified by centrifugation. The resulting solution was freeze-dried and the lyophilized powder was washed with acetone three times. The residue was purified on a column of Sephadex G-15 with distilled water as the eluent. The resulting solution was again freeze-dried and a pure sample was obtained in 27% yield as a colorless solid. ^{1}H NMR (500 MHz, D_2O): δ 3.23−4.08 (m, 44 H), 4.94−5.27 (m, 7 H); ^{13}C NMR (500 MHz, D_2O): δ 39.6, 60.4, 72.4, 72.6, 73.1, 81.4, 101.7, 161.0; IR (cm^{-1}, KBr): $\bar{\nu}$ = 3340 (OH), 2960, 2928, 2855 (CH, CH_2), 1680 (COOH), 1620, 1110, 1080, 1030 (−O−); MALDI−MS: calcd. 1303.6 found 1304.0; Anal. Calcd. for $C_{44}H_{72}O_{36}Te\cdot 7H_2O$: C, 36.91; H, 6.01. Found: C, 36.55; H, 5.65.

Method 2. Under a nitrogen atmosphere, 6.1 mg (0.16 mmol) of $NaBH_4$ was added to 15 mL (0.04 mmol) of the stock solution of 2-TeCD and the colorless mixture stirred at room temperature for 15 min. Iodoacetate acid (25 mg, 0.4 mmol) was then added dropwise via syringe. After the addition was complete, the colorless mixture was stirred at room temperature for 1 h. Acetone was added and the compound was allowed to precipitate. The residue was purified by a similar way above and a pure sample was obtained in 92% yield as a colorless solid. Its characteristics agreed well with the results above.

Synthesis of CDTeSAr (5). 2-TeCD (100 mg, 0.04 mmol) was dissolved in 10 mL of distilled water, and 2 equiv (16 mg, 0.08 mmol) of 3-carboxy-4-nitrobenzenethiol was added dropwise. After the addition was complete, the mixture was allowed to stir at room temperature under air for 2 h. The resulting mixture was purified by centrifugation and Sephadex G-15

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column chromatography (Φ5 × A80 cm; $\lambda = 254$ nm) with distilled water as the eluent. The resulting solution was freeze-dried and the lyophilized powder was washed with ethyl ether three times. After residue was dried in vacuo, a pure sample was obtained in 39% yield as a light yellow solid. ^{1}H NMR (500 MHz, D_2O): δ 3.22−4.18 (m, 42 H), 4.88−5.26 (m, 7 H), 7.15−8.05 (m, 3H); IR (cm^{-1}, KBr): $\bar{\nu} = 3340$ (OH), 2928 (CH, CH_2), 1688, 1562, 1525 (Ar), 1625, 1154, 1080, 1030 (−O−); UV/vis (PBS, pH 5.5): λ_{max} (ϵ) = 336 nm (9000 mol^{-1} dm^3 cm^{-1}); MALDI-MS: calcd. 1442.6 found 1443.1; Anal. Calcd. for $C_{49}H_{73}O_{38}NSTe\cdot 6H_2O$: C, 37.86; H, 5.47; N, 0.90; S, 2.06. Found: C, 38.01; H, 5.72; N, 1.04; S, 2.21.

Synthesis of Cyclodextrin-Based Telluronic Acid. 2-TeCD (100 mg, 0.04 mmol) was dissolved in 15 mL of distilled water, and a large excess amount (30%, 150μL) of H_2O_2 was added. After the addition was complete, the mixture was allowed to stir at room-temperature overnight. The resulting mixture was purified by Sephadex G-15 column chromatography with distilled water as the eluent. The resulting solution was freeze-dried and a pure sample was obtained in 80% yield as a colorless solid. ^{1}H NMR (500 MHz, D_2O): δ 3.29−4.28 (m, 42 H), 4.76−5.19 (m, 7 H); ^{13}C NMR (500 MHz, D_2O): δ 60.5, 72.3, 72.5, 73.6, 81.5, 102.4; IR (cm^{-1}, KBr): $\bar{\nu} = 3342$ (OH), 2926 (CH, CH_2), 1621, 1155, 1080, 1031 (−O−); MALDI−MS: calcd. 1295.6 found 1296.3; Anal. Calcd. for $C_{42}H_{70}O_{37}Te\cdot 6H_2O$: C, 35.96; H, 5.89. Found: C, 36.18; H, 6.03.

Coupled Reductase Assay. The GPx-like activities of these compounds were measured using the Wilson's method[16] with minor modification. The assay mixture contained 50 mM phosphate buffer, 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 was recorded for a few minutes to calculate the rate of NADPH consumption. Methanol (10%, v/v) was used to increase the solubility of PhSeSePh and PhTeTePh in the enzymatic reactions.

In the coupled reductase assay system, the consumption of NADPH in the absence of catalyst was shown as follows: ROOH [NADPH consumption (μM/min) ± standard deviation], H_2O_2 [1.28 ± 0.05], *t*-BuOOH [0.61 ± 0.02], CuOOH [0.75 ± 0.03].

In the enzymatic reactions, the following NADPH consumptions corrected for the background reactions were obtained: catalyst [concentration of catalyst (μM), ROOH, NADPH consumption (μM/min) ± standard deviation], Ebselen [5, H_2O_2, 7.28 ± 0.43], PhSeSePh [10, H_2O_2, 30.33 ± 1.69], PhTeTePh [10, H_2O_2, 27.16 ± 1.13], β-cyclodextrin [100, H_2O_2, 0.16 ± 0.02], 2-TeCD [1, H_2O_2, 72.40 ± 3.13; *t*-BuOOH, 98.66 ± 4.26; CuOOH, 135.33 ± 3.82].

Kinetic Analysis. The reactions of the enzyme-catalyzed reduction and the nonenzymatic reduction of hydroperoxides by ArSH (**2**) were studied by following the disappearance of the thiolate absorption at 410 nm, at pH 5.5 (50 mM phosphate buffer, 1 mM EDTA) and 25 °C. To investigate the dependency of rate on substrate concentration, the reaction rates were determined at several concentration of one substrate while keeping the concentration of the other constant. All kinetic experiments were performed in a solution containing phosphate buffer (50 mM, pH 5.5), ethylendiaminetetraacetate (EDTA, 1 mM), and appropriate concentrations of ArSH (**2**), hydroperoxides, and 2-TeCD. The reaction rates were determined on Shimadzu 3100 UV/vis-near-IR Recording Spectrophotometer. The reaction was initiated by addition of ROOH. The enzymatic rates were corrected for the background reactions between ROOH and ArSH (**2**). The initial concentration of ArSH (**2**) was measured from the 410-nm absorbance ($\epsilon = 12\,600$ M^{-1} cm^{-1}, pH 5.5). Each initial rate was measured at least 5 times and calculated from the first 5∼10% of the reaction. Lineweaver−Burk plots were obtained by using the Origin 7.0 (professional version) program. For each set of experiments a straight line was drawn with the best-fit method.

Molecular Simulation. We had used the molecular modeling program CERIUS^2 4.6 (Accelrys Inc.; San Diego, CA)[36] to carry out our molecular simulation. The Dreiding 2.21 force field from CERIUS^2 software package was used in the entire simulation which had been found to be reliable for many organic system.[37] All of the simulations were started from energy-minimized structures obtained through the Smart Minimizer method and Convergence Level was set to high. The spline function was chosed for switching function in nonbond interaction. The partial charges were assigned with Charge Equilibration method before each Minimization.

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Supporting Information Available: The details of kinetics of 2-TeCD, UV spectral titrations, fluorescence spectrum, ^{1}H and 2D NMR measurements, and molecular simulation. This material is available free of charge via the Internet at http://pubs.acs.org.

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