A novel dicyclodextrinyl ditelluride compound with antioxidant activity

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Abstract Reactive oxygen species (ROS) primarily arise from products of normal metabolic activities and are thought to be the etiology of many diseases. A novel dicyclodextrinyl ditelluride (2-TeCD) compound was designed to be a functional mimic of the glutathione peroxidase that normally removes ROS. 2-TeCD exhibited highly catalytic efficiency and good water solubility. Antioxidant activity was studied by using ferrous sulfate/ascorbate-induced mitochondria damage model system. 2-TeCD protected the mitochondria against oxidative damage in a dose-dependent manner and exhibited also great antioxidant ability in comparison with 2-phenyl-1,2-benziososelenazol-3(2H)-one. The mimic may result in better clinical therapies for the treatment of ROS-mediated diseases. © 2001 Published by Elsevier Science B.V. on behalf of the Federation of European Biochemical Societies.

Key words: Antioxidant activity; Artificial enzyme; Cyclodextrin; Glutathione peroxidase; Tellurium

1. Introduction

Reactive oxygen species (ROS), such as superoxide anion, H_2O_2, organic peroxide, and hydroxyl radical are products of normal metabolic activities and are produced in response to various stimuli. Under normal conditions, there is a balance between the production of ROS and their destruction. In certain diseases, the production of ROS is enhanced and then ROS damage biomacromolecules [1], resulting in ROS-mediated cell injury. Examples of such oxidative stress-related diseases include reperfusion injury, inflammatory process, age-related diseases, neuronal apoptosis, cancer, and cataract [2–4]. To prevent undesired ROS-induced damage, organisms are equipped with several lines of antioxidant defense. These act either as non-enzymatic action (vitamine E, ascorbate, glutathione (GSH), and uric acid) or as enzymatic action (superoxide dismutase, catalase, and glutathione peroxidase (GPX)).

Because of the limitations associated with enzyme therapies (solution instability, short half-lives, costs of production, and proteolytic digestion), considerable efforts have been made to find compounds that could mimic the properties of the GPX [5,6]. 2-Phenyl-1,2-benziososelenazol-3(2H)-one (Ebselen) was the first compound found to have this capacity [7] and is currently undergoing phase III clinical trial in Japan as an antioxidant [5], but it has some drawbacks, such as low GPX activity and water insolubility.

General conclusion from work on models is that an efficient enzyme mimic involves the substrate binding and the subsequent intracomplex catalysis [8,9]. Cyclodextrins (CDs) have been extensively exploited in the past as a variety of enzyme models and molecular receptors because of their abilities to bind various hydrophobic compounds into their hydrophobic cavities via host–guest chemistry [10,11]. Based on CD as an enzyme model, we here reported a novel GPX mimic (dicyclodextrinyl ditelluride (2-TeCD)) that catalyzes the decomposition of hydroperoxides with remarkably high GPX activity exceeding other small mimics of GPX. The biological effect of 2-TeCD was evaluated by the protection of mitochondria against oxidative damage and it was found to be a better antioxidant than Ebselen. 2-TeCD exhibited high GPX activity and good water solubility, therefore has obvious advantages for pharmacological application.

2. Materials and methods

2.1. Apparatus and materials

The characterization of structure of the mimic was performed with a Varian Unity-400 NMR spectrometer, a Bruker IFS-FT66V Infrared spectrometer and a Perkin-Elmer 240 DS Elemental Analyzer. The content and valence of tellurium in the mimic were determined using an ESCALAB MKII X-ray photoelectron spectrometer. The spectrometric measurements were carried out using a Shimadzu 3100 UV-Vis-near-IR Spectrophotometer interfaced with a personal computer.

β-CD was purchased from Tianjin Chemical Plant, recrystallized twice from water. 2-SeCD was prepared as described previously [12]. *p*-Toluene sulfochloride and *tert*-butylhydroperoxide (*t*-BuOOH) were also obtained from Tianjin Chemical Plant. Tellurium, sodium borohydride, GSH, β-nicotinamide adenine dinucleotide phosphate (NADPH), cumene hydroperoxide (CuOOH), Ebselen, and GSH reductase (type III) were purchased from Sigma. Thiobarbituric acid (TBA) and ferrous sulfate were obtained from Shanghai Second Reagent Plant. Ascorbic acid was purchased from Fluka. Sephadex G-25 was purchased from Amersham Pharmacia Biotech, Uppsala, Sweden.

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Abbreviations: ROS, reactive oxygen species; GPX, glutathione peroxidase; Ebselen, 2-phenyl-1,2-benziososelenazol-3(2H)-one; 2-TeCD, dicyclodextrinyl ditelluride; *t*-BuOOH, *tert*-butylhydroperoxide; CuOOH, cumene hydroperoxide; GSH, glutathione; β-CD, β-cyclodextrin; NADPH, β-nicotinamide adenine dinucleotide phosphate; TBARS, thiobarbituric acid reactive substances

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All other chemicals were of the analytic purity commercially available and were used without further purification.

2.2. Synthesis of 2, 2′-tellurium bridged β-CD (2-TeCD)

The 2-OTs-2-deoxy-β-CD (2-OTs-β-CD) was prepared as described previously [12]. Finely ground elemental tellurium (1.27 g) and sodium borohydride (0.9 g) were heated in ethanol (20 ml) at reflux under nitrogen for 1 h. After cooling to ambient temperature, acetic acid free of oxygen (1.2 ml) was added to the solution. 2-OTs-β-CD (2 g) dissolved in 50 mM potassium phosphate buffer, pH 7.0 (20 ml), was bubbled using pure nitrogen for 30 min and added to the above solution. Under the protection of nitrogen, the mixture was kept for 48 h at 60°C, and then was oxidized in air, finally purified by centrifugation and Sephadex G-25 column chromatography. The product solution was freeze-dried and the lyophilized powder was yellow product with 62% yield. The structure of 2-TeCD was analyzed by means of elemental analysis, IR, ^{1}H NMR, ^{13}C NMR. The data were shown as follows: Anal. ($C_{84}H_{138}O_{68}Te_2{\cdot}6H_2O$) C, H. Calcd: C, 38.32; H, 5.58. Found: C, 37.87; H, 5.65. IR (KBr): 3367 (–OH), 2928 (CH, CH_2), 1630, 1154, 1083, 1027 (–O–), 947, 753, 705, 583. ^{1}H NMR (400 MHz, DO) δ: 4.99 (1-H), 4.0–3.65 (3-, 5-, 6-H), 3.65–3.28 (2-, 4-H). ^{13}C NMR (400 MHz DO) δ: 100.8 (1-C), 98.5 (1′-C), 80.1 (4-c), 75.9 (2′-C), 72.3 (2-c), 70.9 (3-, 5-, 5′-C), 68.3 (3′-C), 59.5 (6-, 6′-C). The content and valence of tellurium in the mimic were determined by X-ray photoelectron spectroscopy. The energy of the exciting X-ray was 1253.6 eV (Mg, Kα). C_{1s} = 285.0 eV was served as standard. The scans were performed 10 times.

2.3. Assay of enzymatic property of 2-TeCD

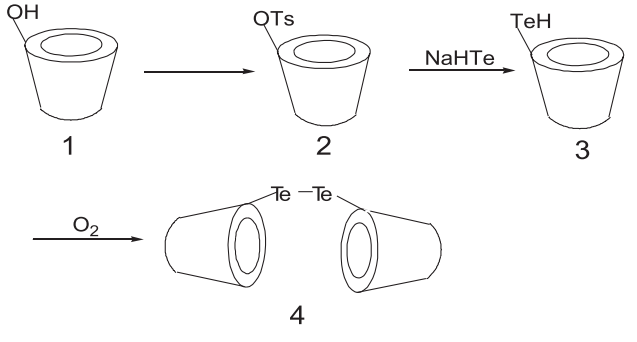
GPX activities were examined by following the oxidation of NADPH in the presence of GSH reductase, which catalyzes the reduction of oxidized GSH formed in the catalytic cycle. Both samples and control cuvettes contained 50 mM potassium phosphate buffer, pH 7.0, 1 mM EDTA, 1 mM sodium azide, 1 mM GSH, 1 U of GSH reductase in a total volume of 0.5 ml. An aliquot of 2-TeCD was added to the sample cuvette only. The reaction mixture was started by the addition of 0.5 mM hydroperoxide in both cuvettes. The reaction was followed by the decrease of NADPH absorption at 340 nm. The activity was corrected for control experiment. One unit of enzyme activity is defined as amount of mimic that utilizes 1 μmol of NADPH per minute.

2.4. Preparation of mitochondria

Bovine heart mitochondria were isolated from fresh bovine heart according to ref. [13] and suspended in 0.25 M sucrose, 10 mM EDTA and 25 mM HEPES–NaOH buffer, pH 7.4, and maintained at 0°C. The concentration of the mitochondria protein was determined by Coomassie brilliant blue [14] using bovine serum albumin as the standard.

2.5. Ferrous sulfate/ascorbate-induced mitochondria damage

The incubation mixture consisted of 0.125 M KCl, 1 mM $MgCl_2$, 5 mM glutamate, mitochondria (0.5 mg protein/ml), 1 μM GSH, and appropriate enzyme mimic in 10 mM potassium phosphate buffer, pH 7.4, 37°C. TBA reactive substances (TBARS) and swelling of mitochondria were determined at some intervals after addition of 0.5 mM ascorbate and 12.5 μM ferrous sulfate. Damage experiments have been done without enzyme mimic; control experiments have been performed without enzyme mimic, ascorbate and ferrous sulfate.



Scheme 1.

Table 1
Comparison between GPX activities of the 2-TeCD-catalyzed reduction of hydroperoxides by GSH and other species

Enzyme mimic	Hydroperoxide	Activity (U/μmol of mimic)
Ebselen	H_2O_2	0.99
	t-BuOOH	0.35 (0.03)
	CuOOH	1.28 (0.12)
PhSeSePh	H_2O_2	1.95
2-SeCD	H_2O_2	7.4
2-TeCD[a]	H_2O_2	46.7 (1.2)
	t-BuOOH	32.3 (0.8)
	CuOOH	87.3 (0.7)

[a]Reactions were carried out in 50 mM potassium phosphate buffer, pH 7.0, at 37°C, 1 mM GSH, 0.5 mM hydroperoxide. One unit of enzyme activity is defined as amount of mimic that utilizes of 1 μmol of NADPH per minute. All values are means of at least five times, and standard deviations are shown in parentheses.

2.6. Biological analysis of 2-TeCD against mitochondria damage

TBARS content in ferrous sulfate/ascorbate-treated mitochondria was analyzed by TBA assay [15].

Swelling of mitochondria was assayed as described by Hunter at al. [16]. The swelling of mitochondria was measured as the decrease in turbidity of the reaction mixture at 520 nm. The decrease of the absorbance indicates an increase in the mitochondria swelling and a decrease in the mitochondria integrity.

3. Results

3.1. Synthesis and characterization of 2-TeCD

The route of the synthesis of 2-TeCD is shown in Scheme 1. 2-TeCD was characterized by elemental analysis, IR, ^{1}H NMR, ^{13}C NMR. The content and valence of tellurium in 2-TeCD were measured by X-ray photoelectron Spectroscopy. The $Te3d_{5/2}$ electronic binding energy of 2-TeCD is 574.2 eV, which approaches the binding energy of diphenyl ditelluride of 573.9 eV, indicating that the tellurium in 2-TeCD was presented as the form of −1 valence (ditellurium bridge, –Te–Te–). The experiment also gave the C/Te, which is 42.3:1 (calculated 42:1), indicating that the mimic contains 2 mol of tellurium per mole mimic.

3.2. Enzymatic property of 2-TeCD

The GPX activities of 2-TeCD and other species are listed in Table 1. As shown in Table 1, the GPX activity of 2-TeCD for the reduction of H_2O_2 by GSH was determined to be 46.7 U/μmol of 2-TeCD, indicating that 2-TeCD displays more efficient catalysis than Ebselen [17], diphenyl diselenide (PhSeSePh) [17], and 2-SeCD [12]. The 2-TeCD also catalyzes the reduction of a variety of structurally distinct hydroperoxides, from the hydrophilic H_2O_2 to the bulky aromatic CuOOH. The GPX activities of the 2-TeCD-catalyzed reduction of *t*-BuOOH and CuOOH by GSH were determined to be 32.3 and 87.3 U/mol of 2-TeCD, respectively. These results show that 2-TeCD has different substrate specificity for hydroperoxides, and the preferred substrate is CuOOH. The GPX activities of the Ebselen-catalyzed reduction of *t*-BuOOH and CuOOH were determined to be 0.35 and 1.28 U/μmol, respectively. As discussed above, the catalytic efficiency of 2-TeCD for the reduction of organic hydroperoxides was also higher than that of Ebselen. To gauge further the catalytic efficiency of 2-TeCD, we compared 2-TeCD with model compound Eb-

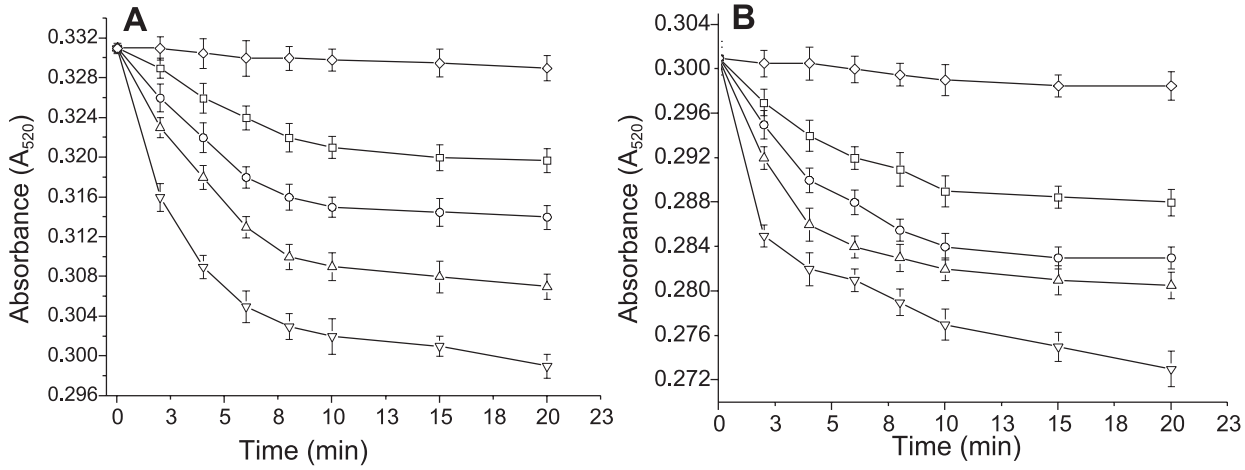


Fig. 1. A: Effect of concentration of 2-TeCD on the swelling of mitochondria. (◇) Control; (□) damage+16 μM of 2-TeCD; (○) damage+8 μM of 2-TeCD; (△) damage+2 μM of 2-TeCD; (▽) damage. B: Effect of the different GPX mimics on the swelling of mitochondria. (◇) Control; (□) damage+8 μM of 2-TeCD; (○) damage+8 μM of 2-SeCD; (△) damage+8 μM of Ebselen; (▽) damage. The damage condition is explained in Section 2.

selen, a well-studied GPX mimic. At 37.0°C and pH 7.0, the initial rate of the reduction of H_2O_2 (0.5 mM) by GSH (1 mM) in the presence of 1.2 μM of 2-TeCD is 3.2×10^{-5} M/min, but under the same conditions, when 1.2 μM of Ebselen was used as the catalyst, the initial rate is only 6.9×10^{-7} M/min. These data indicate that 2-TeCD is at least 46-fold more efficient than Ebselen.

3.3. 2-TeCD protection of mitochondria against oxidative damage

Fig. 1A shows that the mitochondria swell greatly by ferrous sulfate/ascorbate-induced mitochondria damage and the swelling of mitochondria was decreased by the addition of 2-TeCD. The absorbance at 520 nm for the control group was basically constant, whereas the absorbance for the damage group was decreased considerably with time, indicating the mitochondria swelling was considerably increased. But, the swelling for the protection group, which contained a certain concentration of 2-TeCD, was apparently inhibited, and the swelling of mitochondria was decreased with an increase of 2-TeCD concentration. The ability of GPX mimics, 2-TeCD, 2-SeCD and Ebselen, to inhibit the swelling of mitochondria was different, as evidenced by Fig. 1B, whereas the 2-TeCD was the best protective among them. This is in agreement with H_2O_2 removal activity of these GPX mimics.

Fig. 2A shows the extent of protection afforded by 2-TeCD. The TBARS amount accumulated during damage of mitochondria was considerably reduced in the presence of 2-TeCD, and the decrease of TBARS amount was increased with the increase of the concentration of 2-TeCD. When the 2-TeCD concentration was 16 μM, in 50 min the TBARS content was only 35.8% of the damage group, indicating that 64.2% of TBARS production was inhibited. In order to gauge the ability of the three GPX mimics, 2-TeCD, 2-SeCD and Ebselen, to inhibit TBARS accumulation, their antioxidant activities were undertaken under the identical condition. As evidenced by Fig. 2B, the ability of 2-TeCD to decrease the TBARS accumulation was greater than that of 2-SeCD and Ebselen.

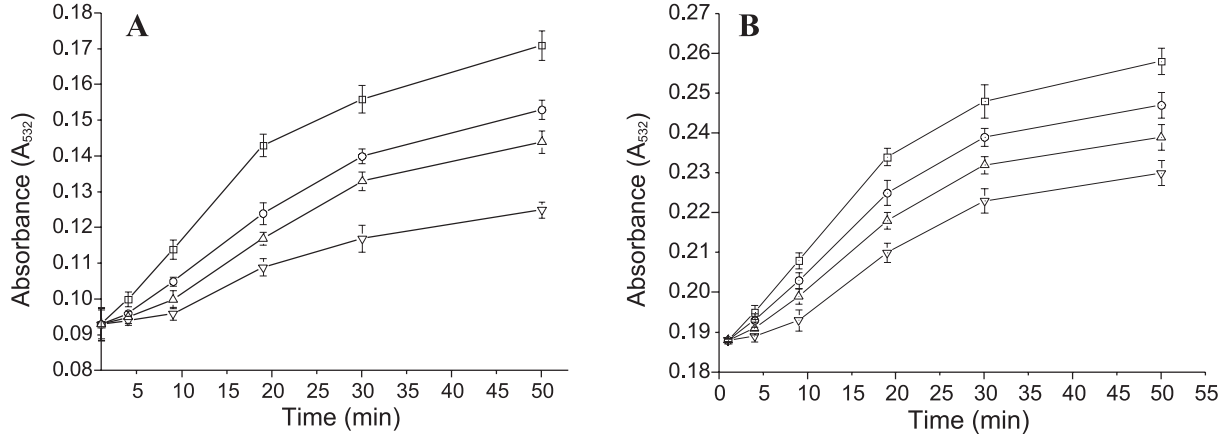


Fig. 2. A: Dependence of extent of TBARS accumulation on concentration of 2-TeCD. (□) Damage; (○) damage+2 μM of 2-TeCD; (△) damage+8 μM of 2-TeCD; (▽) damage+16 μM of 2-TeCD. B: Effect of different GPX mimics on the TBARS accumulated during damage of mitochondria. (□) Damage; (○) damage+8 μM of Ebselen; (△) damage+8 μM of 2-SeCD; (▽) damage+8 μM of 2-TeCD. The damage condition is explained in Section 2. The absorbance values represent TBARS equivalents and are means of three determinations.

4. Discussion

Exposing mitochondria in vitro to redox active xenobiotics can mimic the oxidative damage of mitochondria in vivo. The reaction for ferrous sulfate/ascorbate-induced mitochondria damage can be proposed:

$$\text{Ascorbic acid} + O_2 \rightarrow \text{dehydroascorbic acid} + H_2O_2 \quad (1)$$

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + {}^{\bullet}OH \quad (2)$$

$$\text{Ascorbic acid} + 2Fe^{3+} \rightarrow \text{dehydroascorbic acid} + 2Fe^{2+} + 2H^+ \quad (3)$$

where H_2O_2 was produced by oxidation of ascorbic acid to dehydroascorbic acid, and then hydroxyl radical was produced via Fenton reaction. The biological molecules in mitochondria were easily attacked by hydroxyl radical, then changes of mitochondria in composition, morphology, structure, integrity, and function took place. GPX mimics can scavenge hydroperoxides and block the production of hydroxyl radical, and therefore protect mitochondria against oxidative damage.

In ferrous sulfate/ascorbate-induced mitochondria damage model system, swelling extent and TBARS content were choose as standard, which was used to determine the injury and protection extent of mitochondria. 2-TeCD reduced the swelling of mitochondria during its damage and decreased the maximal level of TBARS accumulation and also the slope of rapid phase of TBARS accumulation. The swelling of mitochondria and TBARS accumulation decreased by 2-TeCD is a dose-dependent manner. The reason that 2-TeCD inhibited TBARS accumulation and decreased the swelling of mitochondria can be explained by 2-TeCD acting as a GPX mimic, which effectively scavenged hydroperoxides and protected mitochondria against oxidative damage.

In conclusion, we have prepared a novel class of GPX mimic. 2-TeCD is an excellent GPX mimic, as evidenced by enzymatic properties. These investigations on the mitochondria damage system induced by ferrous sulfate/ascorbate reveal that the mimic is a better antioxidant than other GPX mimics. We anticipate that the mimic may have potential for the treatment of diseases ranging from acute and chronic inflammation to cataract, cancer, cardiovascular and age-related diseases.

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