Journal of Molecular Catalysis B: Enzymatic 55 (2008) 99–103

Contents lists available at ScienceDirect

Journal of Molecular Catalysis B: Enzymatic

journal homepage: www.elsevier.com/locate/molcatb

Letter

Studies on telluric hyaluronic acid (TeHA): A novel antioxidant☆

ARTICLE INFO

Keywords:
GPX mimic
Hyaluronic acid
Tellurium
Mitochondria
Glutathione peroxidase

ABSTRACT

A novel telluric hyaluronic acid (TeHA) compound was synthesized to imitate the antioxidant enzyme glutathione peroxidase (GPX). The GPX activity of the mimic was found to be 164 U/μmol, which is 165 times as active as Ebselen [PZ51], a well-known GPX mimic. Antioxidant activity was studied by using a ferrous sulfate/ascorbate (Fe^{2+}/Vc)-induced mitochondria damage model system. TeHA markedly decreased the swelling of mitochondria, inhibited the lipid peroxidation, maintained the activity of cytochrome *c* oxidase, and exhibited excellent antioxidant ability.

1. Introduction

Reactive oxygen species (ROS), such as superoxide anion, H_2O_2, organic peroxide, and hydroxyl radical, are constantly being generated in aerobic organisms during normal respiration. In addition, environmental factors (such as ionizing radiation) and pathological compounds (such as β-amyloid in Alzheimer's disease) can generate ROS. Although ROS at physiological concentration may be required for normal cell function, excessive amount of ROS can damage cellular components, such as lipids, protein, and DNA [1,2]. Glutathione peroxidase (GPX) is one of the most crucial antioxidant enzymes in a variety of organisms. It distributes extensively in cells, blood, and tissues, and can remove various ROS produced by free radical reactions. However, GPX, a therapy enzyme, has some limitations: limited sources, solution instability, short half-life, and proteolytic digestion. Therefore, more attention has been paid to artificial imitation of GPX [3,4]. Some mimics have been synthesized, like Ebselen, 6-selenium bridgedβ-cyclodextrin (6-SeCD), dicyclodextrinyl ditelluride (2-TeCD) [5–7], etc. But most of them have low GPX activity. Hyaluronic acid (HA), a high molecular mass mucopolysacchride, consists of *N*-acetyl-D-glucosamine (GLcNAc) and D-glucuronic acid. HA is the main component of extracellular and intercellular matrix of mammal tissues [8,9]. We incorporated the catalytic group —the tellurium bound (-TeH) into HA and synthesized a novel telluric hyraluronic acid compound TeHA, which exhibited good water solubility and high GPX activity. We constructed a ferrous sulfate/ascorbate (Fe^{2+}/Vc)-induced mitochondria damage model system and investigated the capacity of TeHA in protecting mitochondria from oxidative damage. We found that HA is a strong antioxidant. This is the first report about imitating GPX by using HA.

2. Experimental

2.1. Materials

Medical standard hyaluronic acid was obtained from Huayuan Bioengineering Corporation (Shandong, China). P-Toluene sulfonychloride (p-TsCL) and tellurium were purchased from Tianjin Chemical Plant (Tianjin, China). Sodium borohydride, reduced glutathione (GSH), NADPH, glutathione reductase (Type III), cytochrome *c*, and ascorbate were products of Sigma. Hydroxyl peroxide (H_2O_2) and potassium phosphate were obtained from Beijing Chemical Factory (Beijing, China), and Sephadex G-25 was purchased from Bio-Rad. Shanghai Biochemistry Reagent Corporation (Shanghai, China) supplied us with thiobarbituric acid (TBA). Bovine serum albumin was from Shanghai Biochemistry Institute (Shanghai, China). All other chemicals were of the highest purity commercially available and were used without further purification.

2.2. Apparatus

JJ-2 high-speed tissue blender is made in Changzhou Guohua Electrical Appliance Corporation (Changzhou, China), J-25 low temperature high speed centrifuger is Beckman Company's product. TGL-16 bench-top centrifuge is from Shanghai Anting Science Instrument Plant (Shanghai, China). 752N ultraviolet and visual light spectrophotometer and 760CRT double beam ultraviolet and visual light spectrophotometer are both from Shanghai Exactitude Science Instrument Corporation (Shanghai, China). H2S-H constant-temperatured water bath shaker is made in Harbin Donglian Electron Technique Exploiture Corporation (Harbin, China).

2.3. Methods

2.3.1. Synthesis of mimic TeHA

2.3.1.1. Preparation of HA Toluene sulfonyl ester. A 2.0 g of HA was dissolved in 80 ml of 0.15 M NaOH (0.15 mol/l). While maintaining the mixture pH > 12.5 with an addition of 1.0 M NaOH solution,

☆ This research is one of scientific research items of Ministry of Education of Heilongjiang (No. 11521307), and it was sponsored by Ministry of Education of Heilongjiang.

1381-1177/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.molcatb.2008.02.003

10 ml of acetonitrile solution containing 4.0 g of p-TsCl was dropped slowly into the mixture at ambient temperature. After being stirred for an hour, the mixture was adjusted to pH 7.0 with 1 M HCl. Then a 100 ml of methanol was added into the mixture. The mixture was condensed to 80 ml by methanol vaporization under decompressing condition after filtration with 3 mm Whatman filter. Then it was stored in a 4 °C refrigerator. Eight days later, the mixture was filtered to remove insoluble substances. The mixture was centrifuged at 900 × *g* (20 min), and the HA Toluene sulfonyl ester in the supernatant was purified by filtration chromatography with a Sephadex G-25 column. The first peak of the elution containing HA Toluene sulfonyl ester was collected and freeze-dried by lyophilization.

2.3.1.2. Preparation of NaHTe. NaHTe was prepared according to reference [10]. Finely ground elemental tellurium (1.3 g) and $NaBH_4$ (0.9 g) were heated in ethanol (30 ml) at reflux under nitrogen for 1.5 h. After cooling to ambient temperature, acetic acid (3 ml) was added to the solution. After heading the above solution to 100 °C, dark precipitate was found in the solution and the topper achromatous transparent solution is ethanol solution including NaHTe.

2.3.1.3. Synthesis of TeHA. A 100 mg of HA Toluene sulfonyl ester was fully dissolved in 10 ml of H_2O, then 5 ml of NaHTe was added under the protection of pure nitrogen. Next, the mixture was incubated at 60 °C for 48 h. After being oxidized in air, the reaction mixture was centrifuged at 900 × *g* (10 min). The supernatant was loaded onto Sephadex G-25 column for filtration chromatography. The first elution peak containing TeHA was collected and lyophilized into dried powder.

2.3.2. The GPX activity of TeHA

The TeHA can catalyze the reduction of hydroperoxides such as ROOH.

$$2GSH + ROOH \xrightarrow{TeHA} GSSG + ROH + H_2O$$

The catalytic activities were measured according to Wilson's method [13]. As shown in the following reactions, the presence of GSSG reductase and NADPH allows the progress of the TeHA-catalyzed reduction reaction to be spectrophotometrically monitored at an observance of 340 nm.

$$2GSH + H_2O_2 \xrightleftharpoons{TeHA} 2H_2O + GSSG \xrightarrow[\text{Reductase}]{\text{Glutathione}} \begin{matrix} NADPH \\ NADP^+ \end{matrix} \quad 2GSH$$

The reactions were carried out at 37 °C in 0.5 ml of solution containing 50 mM potassium phosphate buffer, pH 7.0, 1 mM EDTA, 1 mM sodium azide, 1 mM GSH, 0.25 mM NADPH, 1 U of GSH reductase, and an aliquot of TeHA. The mixtures were preincubated for 10 min. The reactions were then initiated by addition of 0.5 mM H_2O_2, and the GPX activities of TeHA were detected by measuring the decrease of NADPH at a 340 nm observance. Appropriate controls were run without TeHA. One unit of enzyme activity is defined as the amount of mimic that utilizes 1 μmol of NADPH per minute. The activity is expressed in U/μmol of enzyme mimic.

2.3.3. Establishment of ferrous sulfate/ascorbate (Fe^{2+}/Vc)-induced mitochondrial damage model

Bovine heart mitochondria were prepared and stored according to the procedures described in Xue's report [12]. Mitochondria protein concentration was determined by the method of Folin [14] with bovine blood serum albumin as standard protein. A ferrous sulfate/ascorbate (Fe^{2+}/Vc)-induced mitochondrial damage model was constructed according to the published protocols [15]. The incubation mixture consisted of 0.125 M KLC, 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. MDA content and swelling of the mitochondria were determined at intervals after addition of 0.5 mmol/l ascorbate and 12.5 μmol/l ferrous sulfate. Damage experiments were carried out without the enzyme mimic as the damage group; experiments without enzyme mimic, ascorbate, and ferrous sulfate were known as the control group.

2.3.4. Assays for mitochondria swelling

When the membrane of mitochondria is being damaged, mitochondrial swelling occurs. The swelling of mitochondria was assayed according to the method presented in reference [16]. Changes in light scattering are correlated with the degree of mitochondrial swelling. The swelling of mitochondria was measured as the decrease in turbidity of the reaction mixture at 520 nm. A decrease of the absorbance indicates an increase in the mitochondrial swelling and a decrease in the mitochondrial integrity.

2.3.5. Measurement of lipid peroxidation

The level of lipid peroxidation was determined by measuring the formation of malondialdehyde (MDA), the final product of lipid peroxidation. MDA content in ferrous sulfate/ascorbate-treated mitochondria was analyzed by a thiobarbituric acid assay. In this assay, TBA reacts with MDA and/or other carbonyl by-products of free radical-mediated lipid peroxidation to give colored conjugates in acid environment under heating condition. The colored conjugates absorb the most light at 532 nm; the absorbance was directly proportional to the contents of MDA and/or other carbonyl by-products of free radical-mediated lipid peroxidation. The experiment process was as followed: 1. A sample of 350 μl reaction mixture was taken at different time points. 2. The sample was mixed with 350 μl of 700 g/l trichloroacetic acid (TCA) and 350 μl of 5 g/l TBA. 3. The mixtures were heated for 40 min at 80 °C. After cooling and centrifugation, the supernatant was measured at 532 nm.

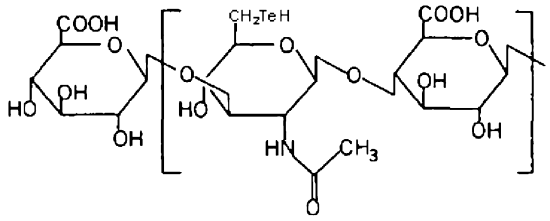
2.3.6. Detection of cytochrome c oxidase (CCO) activity

Samples of 900 μl incubation mixture was taken at different time points and centrifuged (10,000 × *g*, 2 min, 4 °C). The precipitate was washed with 10 mM potassium phosphate buffer, pH 7.4 containing 125 mM KCl, 1 mM $MgCl_2$, and 5 mM glutamate. Then the precipitate was suspended in a small amount of 100 mM potassium phosphate buffer, pH 7.0, and samples of 100 μl was taken for CCO activity assay [17]. The CCO activity was measured at 550 nm in a 1 ml reaction volume, in which the cytochrome *c* concentration was 15 μM. The absorbance decreases as the oxidation of cytochrome *c* processes in the sample. A 5 μl of 10 mM $K_3Fe(CN)_6$ was added into the reaction to oxidize the cytochrome *c* thoroughly when the reaction was nearly complete. The absorbance intensity at this time was recorded as A_∞. A plot of in $(A_t - A_\infty)$ versus time was made (A_t = absorbance at time t). The absolute values of the line slope and K_{app}, the apparent rate constants of cytochrome *c* oxidation, were used for indicating CCO activity.

3. Results and discussion

3.1. Structure and characterization of TeHA

TeHA was characterized by elemental analysis, IR, ^1H NMR, ^{13}C NMR (see Scheme 1) [11,12].



Scheme 1.

Table 1
The GPX Activity of TeHA and other species

Species	Substrate	Activity (U/μmol)
Ebselen	H_2O_2	0.99
2-SeCD	H_2O_2	7.4
6-diSeCD	H_2O_2	13.5
2-TeCD	H_2O_2	46.7
TeHA	H_2O_2	163.56

Reactions were carried out in 50 mM potassium phosphate buffer, pH 7.0, 1 mM GSH, 0.5 mM H_2O_2. One unit of enzyme activity is defined as amount of mimic which utilizes 1 μmol of NADPH per minute. The activity is expressed in U/μmol of enzyme mimic.

3.2. The GPX activity of TeHA

The GPX activity of TeHA and other species was determined (as described above in Section 2.3.2) by using H_2O_2 as substrate. The results are summarized in Table 1.

The GPX activities of Ebselen, 2-SeCD, 6-diSeCD, and 2-TeCD are 0.99, 7.4, 13.5, and 46.7 U/μmol, respectively. Surprisingly, the GPX activity of TeHA in reduction of H_2O_2 with GSH is 163.56 U/μmol, which has a remarkably higher catalytic efficiency than those of Ebselen, 2-SeCD, 6-diSeCD, and 2-TeCD.

3.3. The effect of telluric hyaluronic on the swelling of damage mitochondria

The model of ferrous sulfate/ascorbate (Fe^{2+}/Ve)-induced mitochondrial swelling was established for evaluating the antioxidant function of TeHA. Mitochondrial swelling can be correlated with changes in light scattering. The results are shown in Fig. 1A. A decrease in the absorbance at 520 nm reflects an increase in mitochondrial swelling and a decrease in mitochondrial integrity. The absorbance at 520 nm for the control group (without TeHA as well as ferrous sulfate/ascorbate) remained basically constant, whereas the absorbance for the damage group (with ferrous sulfate/ascorbate) decreased considerably with time, indicating that the Fe^{2+}/Vc-induced damage resulted in extensive mitochondrial swelling. In other words, in the absence of TeHA, mitochondria were severely damaged by ferrous sulfate/ascorbate, and such damage increased as the incubation time increased. The reason for mitochondria swelling is that H_2O_2 produced by Fe^{2+}/Vc was converted into •OH which initiates lipid peroxidation and destroys the structure of membrane. However, in the presence of TeHA, the swelling of mitochondria decreased, showing the protection effect of TeHA on mitochondria against the damage from ferrous sulfate/ascorbate. Such protection effects increased significantly as the concentration of TeHA increased. That is, the reactive oxygen radicals produced by ferrous sulfate/ascorbate were eliminated by TeHA. The GPX mimics TeHA, 2-TeCD, and Ebselen displayed different levels of ability to inhibit the swelling of mitochondria. As evidenced by Fig. 1B, TeHA was the best ROS scavenger among those studied. This is in agreement with the H_2O_2 removal activities of these GPX mimics.

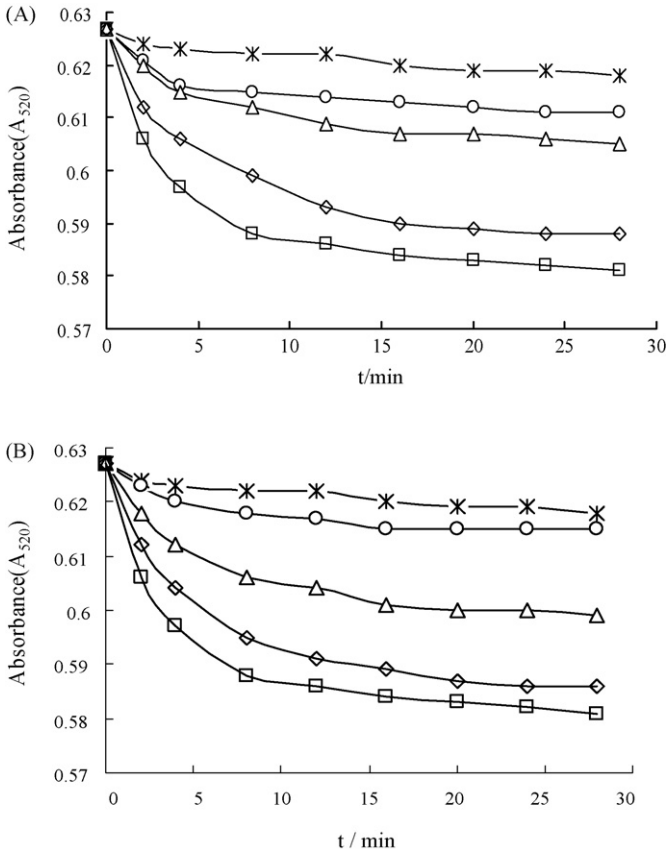


Fig. 1. (A) Effect of concentration of TeHA on the swelling of mitochondria. (□) Damage; (◊) damage +0.25 μmol/l TeHA; (△) damage +0.5 μmol/l TeHA; (○) damage +1 μmol/l TeHA; (*) control. (B) Effect of the different GPX mimics on the swelling of mitochondria. (□) Damage; (◊) damage +2 μmol/l Ebselen; (△) damage + 2 μmol/L2-TeCD; (○) damage + 2 μmol/l TeHA; (*) control. For damage conditions, see Section 2.3.4. The ordinate axes have been interrupted in both (A) and (B).

3.4. The effect of telluric hyaluronic on lipid peroxidation

The antioxidant effects of TeHA were also evaluated in protecting mitochondria against lipid peroxidation. The polyunsaturated fatty acid in mitochondrial membrane is readily attacked by ROS, producing MDA. MDA therefore was used to measure the extent of lipid peroxidation. Fig. 2A shows that TeHA effectively protects membrane lipids from Fe^{2+}/Vc-induced oxidative damage. The inhibition of lipid peroxidation by TeHA was strongly dependent on the concentration of TeHA. The amount of MDA accumulation increased with time and decreased with the increase of TeHA concentration. That is, lipid peroxidation in mitochondria was considerably reduced in the presence of TeHA. To gauge the capacity of the three GPX mimics, TeHA, 2-TeCD, and Ebselen to inhibit MDA accumulation, their antioxidant activities were determined under identical conditions. As evidenced by Fig. 2B, the capacity of TeHA to decrease the MDA accumulation was greater than that of 2-TeCD and Ebselen.

3.5. The protective effects of telluric hyaluronic on CCO

CCO is not only one of the key redox enzymes of electron transport chain, but is also a marker enzyme of mitochondria. The integrity of mitochondrial membrane is very important for the activity of this enzyme. Mitochondria exposed to Fe^{2+}/Vc-induced oxidative stress leads to peroxidization. The integrity of mitochondria is destroyed, resulting in a decrease in CCO activity. Fig. 3 shows that the CCO activity was decreased by ferrous sulfate/ascorbate

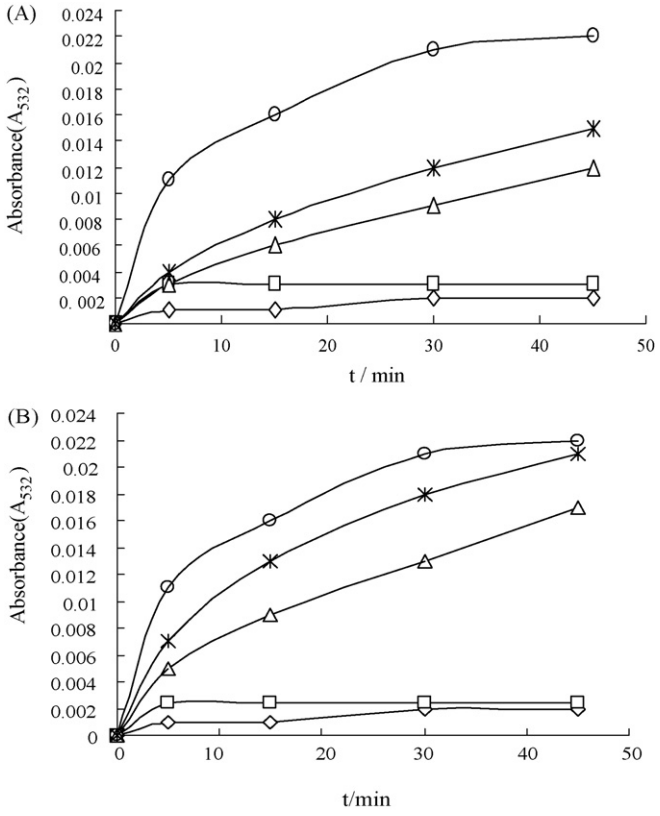


Fig. 2. (A) Dependence of extent of MDA accumulation on concentration of TeHA. (◊) Control; (□) damage + 1 μmol/l TeHA; (△) damage + 0.5 μmol/l TeHA; (*) damage + 0.25 μmol/l TeHA; (○) damage. (B) Effect of different GPX mimics on the MDA accumulated during damage of mitochondria. (◊) Control; (□) damage + 2 μmol/l TeHA; (△) damage + 2 μmol/l 2-TeCD; (*) damage + 2 μmol/l Ebselen; (○) damage. For damage conditions, see Section 2.3.5. The optical density (absorbance) values represent MDA equivalents and each is the mean of three determinations. The ordinate axes have been interrupted in both (A) and (B).

(Fe^{2+}Vc)-mediated mitochondria damage. After 45 min, the CCO activity of the damaged group was 59.7% of that of the control group, whereas 91% of CCO activity was retained in the presence of 2 μmol TeHA. After 45 min under identical conditions, the CCO activities retained in the presence of 2 μmol of 2-TeCD or Ebselen were 74.6% and 65.6% of the control group activity, respectively.

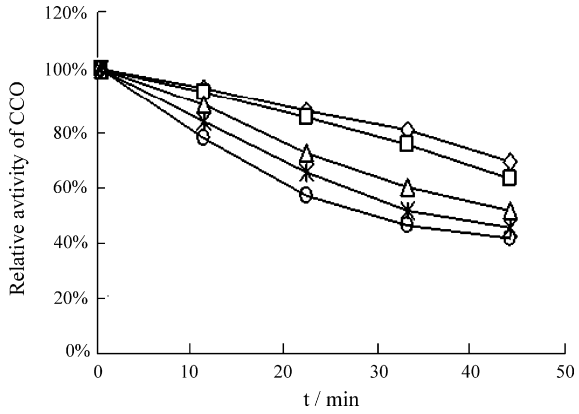


Fig. 3. Effect of different GPX mimics on CCO activity in damaged mitochondria. (◊) Control; (□) damage + 2 μmol/l TeHA; (△) damage + 2 μmol/l 2-TeCD; (*) damage + 2 μmol/l Ebselen; (○) damage. Activity of CCO in the control group is defined as 100%. For damage conditions, see Section 2.3.6. Values are mean values from three sets of experiments. The ordinate axis has been interrupted.

This results indicates that TeHA was the most effective GPX mimic among those tested.

The involvement of ROS in a wide variety of diseases and the ageing process is now widely accepted [18,19]. Mitochondria are one of the major sources of ROS production in cells and are particularly susceptible to oxidative stress [18,20]. It has been revealed that ROS of mitochondria regulate the physiological state of the cell and influence cell death [21]. Therefore, we chose the mitochondrion as the model for our oxidative damage experiments. Exposing mitochondria in vitro to redox active xenobiotics can mimic the oxidative damage of mitochondria in vivo.

In the ferrous sulfate/ascorbate-induced mitochondrion damage model system, the extent of swelling, MDA content, and CCO activity of the mitochondria were chosen as standards to evaluate the antioxidant effects of TeHA. Swelling and shrinking of mitochondria is a normal physiological phenomenon during respiration. However, abnormal swelling will disrupt the mitochondrial membrane and result in cell death. Mitochondrial swelling therefore characterizes its integrity. Polyunsaturated fatty acids, which are found predominantly in mitochondrial membranes, are especially vulnerable to ROS attack because of the high concentration of allylic hydrogen in their structure. The resulting lipid hydroperoxides can affect membrane fluidity and the function of membrane proteins. The end products of lipid peroxidation are reactive aldehydes such as 4-hydroxyl nonenal and malondialdehyde, many of which are highly toxic to cells. Malondialdehyde therefore was used to measure the extent of lipid peroxidation. TeHA reduced the swelling of mitochondria resulting from its oxidative damage and decreased the maximal level of MDA accumulation and the slope of rapid phase of MDA accumulation. The swelling of mitochondria and MDA accumulation decreased by TeHA is a dose-dependent manner. Increasing concentration of TeHA prevented MDA accumulation and mitochondrial swelling and preserved CCO activity. The reason that TeHA inhibited MDA accumulation preserved CCO activity and decreased the swelling of mitochondria can be explained by TeHA acting as a GPX mimic, which effectively scavenged hydroperoxides and protected mitochondria against oxidative damage. In order to further evaluate the GPX-like activity and the capacity of antioxidation of TeHA, we compared TeHA to other mimics. The GPX activities are shown in Table 1. The activity of TeHA for the reduction of H_2O_2 by GSH was measured to be 163.56 U/μM, a catalytic efficiency remarkably higher than those of Ebselen, 2-SeCD, 6-diSeCD, and 2-TeCD. The investigations of mitochondrial damage induced by ferroussulfate/ascorbate (Fe^{2+}Vc) indicate that TeHA is a more excellent antioxidant than other GPX mimics. A GPX mimic (2-TeCD) which is superior to Ebselen was reported in reference [10]. According to their report, in the experiment of assaying mitochondrial swelling, when both TeCD and Ebselen concentration was 8 μmol/l in 20 min of incubation, the absorbance was 55.2 and 89.6% of that of damage group, respectively, However, in our experiment, under the same conditions, when TeHA concentration was 1 μmol/l with a 20 min of incubation, the absorbance only was 36.2% of that of damage group; In the lipid peroxidation experiment reported by Xue et al. [10] when both 2-TeCD and Ebselen concentration was 8 μmol/l, in 30 min, the absorbance was 56.7 and 83.3%, of that of damage group, respectively, whereas in our experiment, under identical conditions, when TeHA concentration was 1 μmol/l, the absorbance only was 14.3% of that of damage group. This further indicated that the protection effect provided by TeHA is much greater than that by 2-TeCD and Ebselen. Our data presented here strongly indicates that TeHA, as a novel PGX mimic, is superior to other reported GPX mimics.

4. Conclusions

In summary, TeHA is an excellent GPX mimic with the advantages of good water solubility and high GPX activity. The investigation of mitochondrial damage model induced by ferrous sulfate/ascorbate suggested that TeHA has stronger antioxidant activity than other GPX mimics. TeHA, as a novel GPX mimic, may have a widely application prospect of enzyme therapy.

Acknowledgements

This research was supported by scientific research item of Ministry of Education of Heilongjiang (No. 11521307). We are grateful to Dr. Shanyu Li for the creative critiques and editing help in preparing this manuscript.

References

[1] D. Salvemini, Z.Q. Wang, J.L. Zweier, A. Samouilov, H. Macarthur, T.P. Misko, M.G. Currie, S. Cuzzocrea, J.A. Sikorski, D.P. Riley, Science 286 (1999) 304–306.
[2] S. Meloy, J. Ravenscroft, S. Malik, M.S. Gill, D.W. Walker, P.E. Clayton, D.C. Wallace, B. Malfroy, S.R. Doctrow, G.J. Lithgow, Science 289 (2000) 1567–1569.
[3] G. Mugesh, H.B. Singh, Chem. Soc. Rev. 29 (2000) 347–358.
[4] G. Mugesh, W.-W.D. Mont, Chem. Eur. J. 7 (2001) 1365–1370.
[5] H. Sies, Methods Enzymol. 234 (1994) 476–482.
[6] Jun-Qiu Liu, Shu-Juan Gao, Gui-Min Luo, Gang-Lin Yan, Jia-Cong Shen, Biochem. Biophys. Res. Commun. 247 (1998) 397–400.
[7] Xiaojun Ren, Yan Xue, Junqiu Liu, Kun Zhang, Jian Zheng, Guimin Luo, Canhui Guo, Ying Mu, Jiacong Shen, Chem. Bio. Chem. 3 (2002) 356–363.
[8] A.M. Gressner, R. Haarmann, J. Hepatol. 7 (3) (1988) 310–318.
[9] B. Weissman, K. Meyer, J. Am. Chem. Soc. 76 (1954) 1753–1754.
[10] Y. Xue, X.J. Ren, K. Zhang, Y. Mu, J.Q. Liu, P. Cao, C.H. Guo, G.M. Luo, J.C. Shen, Chin. J. Biochem. Mol. Biol. 18 (4) (2002) 506–510.
[11] Boxun Zhang, Zhibo Chen, Yang An, Qinglin Peng, Jia Chen, Jianguo Zhang, Lanying Liu, Chin. J. Catal. 27 (10) (2006) 875–879.
[12] Zhibo Chen, Boxun Zhang, Zhongxiu Huang, Qinglin Peng, Jia Chen, Yu Wang, Jianguo Zhang, Guangzhi Jiang, Wenshu Li, Lanying Liu, Chin. Chem. Lett. 17 (7) (2006) 969–972.
[13] S.R. Wilson, P.A. Zucker, R.C. Huang, A. Spector, J. Am. Chem. Soc. 111 (1989) 5936–5939.
[14] O.H. Lowry, N.J. Rosebrough, A.L. Farr, et al., J. Biol. Chem. 193 (1) (1951) 265–275.
[15] Xiaojun Ren, Yan Xue, Kun Zhang, Junqiu Liu, Guimin Luo, Jian Zheng, Ying Mu, Jiacong Shen, FEBS Lett. 507 (2001) 377–380.
[16] Edmund Hunter Jr., A. Scott, J. Weinstein, A. Schneider, J. Biol. Chem. 239 (1964) 622–630.
[17] T. Yonetani, G.S. Ray, J. Biol. Chem. 240 (1965) 4503–4508.
[18] M.K. Shigenaga, T.M. Hagen, B.N. Ames, Proc. Natl. Acad. Sci. U.S.A. 91 (1994) 10771–10778.
[19] R.A. Floyed, Proc. Soc. Exp. Biol. 222 (1999) 236–245.
[20] G. Lenaz, Biochim. Biophys. Acta 1366 (1998) 53–67.
[21] J.P. Thomas, M. Maiorino, F. Ursini, A.W. Girotti, J. Biol. Chem. 265 (1990) 454–461.

Zhibo Chen*
College of Life Science and Engineering, Qiqihar University, Qiqihar 161000, PR China

Jianwei Lv
College of Life Science and Engineering, Qiqihar University, Qiqihar 161000, PR China

Fengqing Chen
Department of Biology, Baicheng Teacher College, Baicheng 137000, PR China

Lidong Lin
College of Life Science and Engineering, Qiqihar University, Qiqihar 161000, PR China

* Corresponding author. Tel.: +86 452 8266631.
E-mail address: zhibochen1962@yahoo.com.cn (Z. Chen)

30 May 2007
Available online 4 March 2008