

# Effect of selenium on increasing free radical scavenging activities of polysaccharide extracts from a Se-enriched mushroom species of the genus *Ganoderma*

Lei Zhao · Guanghua Zhao · Ming Du ·  
Zhengdong Zhao · Lixia Xiao · Xiaosong Hu

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**Abstract** Chemiluminescent analysis indicates that the polysaccharide extracts from Se-enriched *G. lucidum* (Se-GLPs) could protect DNA from hydroxyl radical oxidative damage in dose dependent manner. Moreover, Se-GLPs exhibited higher activities of scavenging superoxide and hydroxyl radicals than its analog from common *G. lucidum* extract as suggested by EPR measurement, indicating that Se plays an important role in increasing the antioxidant activities of the polysaccharide extracts. This was confirmed by spin-trapping experiments showing that at the same polysaccharide concentration, the activities of all the Se-GLPs samples in scavenging hydroxyl radical increased with the increase of Se content. Additionally,

all Se-GLPs samples showed stronger activities of attenuating the production of superoxide radical than that of hydroxyl radical.

**Keywords** Selenium · Antioxidant activity · Polysaccharide extract · Se-enriched *Ganoderma lucidum* · Chemiluminescence analysis · EPR

## Introduction

Reactive oxygen species produced by sunlight, ultraviolet, ionizing radiation, chemical reactions and metabolic processes have a wide variety of pathological effects, such as causing DNA damage, carcinogenesis and cellular degeneration related to aging [1, 2]. Superoxide and hydroxyl radicals are inevitably formed in almost all aerobic organisms. In cellular oxidation reactions, superoxide radical is normally formed first, and its effects can be magnified by production of other kinds of cell-damaging free radicals and oxidizing agents. However, the damaging action of the hydroxyl radical seems to be the strongest among free radicals due to its highest reactivity [3, 4]. Although almost all organisms have evolved a variety of complex defense and repair systems that have evolved to protect them against oxidative damage, only these systems are insufficient to entirely prevent the damage [5]. Fortunately, antioxidant supplements containing antioxidants can help human body prevent or reduce the oxidative damage caused by these toxic radicals.

Selenium is an essential element for human body and can only be obtained from food or other source of supplementation. A high incidence of cancer and other

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L. Zhao  
China National Institute of Standardization, Beijing 100088,  
China  
e-mail: sunnylei@126.com

G. Zhao · X. Hu (✉)  
College of Food Science and Nutritional Engineering, China  
Agricultural University, Beijing 100083, China  
e-mail: huxiaos@263.net

M. Du  
College of Food Science and Engineering, Harbin Institute  
of Technology, Harbin 150090, China

Z. Zhao  
Northwest Sci-Tech University of Agriculture and Forestry,  
Yangling 712100, China

L. Xiao  
College of Food Science, Yangzhou University, Yangzhou  
225200, China

disease is attributed to a lower selenium level in the body [6]. The biological role of selenium has received considerable attention since early times. Its biological functions are at least in part associated with its antioxidant activity [7–9]. As known, many biological molecules such as glutathione peroxidase, thioredoxin reductases, as well as numerous small organic molecules show promising antioxidant activity.

However, to date, the antioxidant activities of polysaccharides containing Se, especially effects of Se on antioxidant activity of biological molecules, have received little attention.

On the other hand, *G. lucidum* (GL) has been used as a traditional Chinese medicine since ancient times. It has been proved to have extensive physiological effects on chronic hepatopathy, hypertension, bronchitis, arthritis, neurasthenia, neoplasin, tumorigenic diseases and so on [10–12]. Polysaccharide is considered as one of the most important bioactive components of GL, which also exhibits antitumor, immuno-modulating, liver protective, hypoglycemic and platelet aggregation-inhibiting activities [13]. Se-enriched *G. lucidum* (Se-GL) has been successfully cultivated in our group [14]. Previous studies showed that *G. lucidum* could absorb 20–30% of inorganic selenium in substrate and transformed most of them into organic Se. Moreover, 11.2–18.0% of the organic Se was found in the polysaccharide fraction [15]. We have found that selenium can pronouncedly increase the antioxidative activity of the protein extracts from the Se-GL [16]. These raises questions of whether selenium in polysaccharide extracts from Se-GL can have the same function with expectation to find a component with higher bioactivity.

Based on these thinking, the antioxidant activity of polysaccharide extracts from Se-GL and common GL was determined, respectively, by chemiluminescence method [17–19] and electron paramagnetic resonance (EPR) in conjunction with spin trapping techniques. The polysaccharide extracts from Se-GL showed stronger antioxidant activity than those from common GL, implying that selenium in the polysaccharide extracts plays a positive role in enhancing antioxidant activity of polysaccharide extracts.

## Materials and methods

### Materials

All solvents/chemicals used were of analytical grade. Hypoxanthine (HPX) and xanthine oxidase (XOD)

were obtained from Sigma Chemical Co. (Beijing, People's Republic of China). 5,5-dimethyl-1-pyrroline-*N*-oxide (DMPO) was obtained from Labtec (Tokyo, Japan), diethylenetriamine-*N, N, N', N', N''*-pentaacetic acid (DTPA), FeSO<sub>4</sub>, and H<sub>2</sub>O<sub>2</sub> were purchased from Beijing Chemical Co. (Beijing, People's Republic of China). The cultivation method of Se-enriched *G. lucidum* (Se-GL) was basically the same as that of *G. lucidum* (GL) [20]. The only difference was that a series of 100, 200, and 250 µg of Se in the form of sodium selenite per gram of substrate was added to the substrate for obtaining Se-enriched *G. lucidum* samples 1 (Se-GLPs 1), 2 (Se-GLPs 2), and 3 (Se-GLPs 3), respectively [14].

### Preparation of polysaccharides from GL and Se-GL

Freeze-dried and powdered GL or Se-GL (10 g) was stirred into 100 mL of 1.0 M NaOH at 4 °C. After 4 h, the supernatant was obtained by filtration. The protein in the supernatant was removed using the Sevag method [21]. Ethanol was added to the supernatant until 75% (v/v) of ethanol was obtained. The resultant precipitate was collected by centrifugation, and dissolved in 5.0 mL of distilled water. This solution was then filtrated and dialyzed against distilled water at 4 °C three times. After 24 h, the solution was lyophilized and stored at –20 °C for use [22].

### Determination of selenium and polysaccharide contents

The selenium concentration of tested samples was determined by atomic fluorescence spectrophotometry (AFS-1201, Haiguang Analytical Co., China) as reported recently [16]. The Se content was given by the Se concentration detected by AFS over the exact weight of lyophilized samples.

The polysaccharide concentration was determined by the phenol-sulfate method [23]. The polysaccharide content was obtained by the polysaccharide concentration over the exact weight of lyophilized polysaccharide extracts.

### Evaluation of antioxidant capacity of GLPs and Se-GLPs by chemiluminescence

The CuSO<sub>4</sub>-Phen-Vc-DNA solution was prepared with acetate buffer (pH = 5.5) as described previously [19]. The final concentrations of Cu<sup>2+</sup>, Phen, Vc, and DNA were 50 µM, 350 µM, 350 µM, and 1.0 µg/mL, respectively. Exactly 0.8 mL of the above solution was added

to a 1 mL cuvette of the Ultra Weak Chemiluminescence Analyzer (BPCL-G-K, Inst. of Biophysics, Academia Sinica, Beijing, People's Republic of China). Then each 0.2 mL solution of polysaccharides, concentrations indicated above was injected into the cuvette. The same volume of distilled water in place of the polysaccharide solution was used as control. After 1 min, 20  $\mu\text{L}$   $\text{H}_2\text{O}_2$  (3%) was added to the cuvette while the kinetic curve of chemiluminescence was measured at  $22.0 \pm 0.1$  °C [19]. The depression rate of chemiluminescence (CL) due to both DNA damage and Phen oxidation was calculated using the following formula: depression rate (%) =  $(C - C_1)/C \times 100\%$ , where  $C$  is the value of photon count of CL for control and  $C_1$  is the value of photon count of CL for test samples.

#### Evaluation of activity of scavenging hydroxyl radical and superoxide radical of GLPs and Se-GLPs by the EPR method

EPR was used to monitor  $\text{HO}^\bullet$  and  $\text{O}_2^{\bullet-}$  formation by using DMPO as a spin trapping reagent. EPR spectra were recorded on an EPR spectrometer (model JES-TE200, JEOL Co., Tokyo, Japan). Samples (1-mm-i.d.) of quartz capillaries were placed in a  $\text{TE}_{102}$  cavity for measurement at room temperature. Typical spectrometer parameters for monitoring  $\text{HO}^\bullet$  were: central field, 336.5 mT; microwave power, 10.0 mW; modulation frequency, 9.44 GHz; scanning width, 10 mT; modulation amplitude, 0.2 mT; time constant, 0.1 s; and scanning period, 120 s. All spectra were recorded exactly 2.0 min after addition of the last reagent. The concentrations of reactants are indicated in the figure captions. The scavenging effects of GLPs and Se-GLPs on hydroxyl radical were calculated by  $E$  (the scavenging effect) =  $[(h_o - h_x)/h_o] \times 100\%$ , where  $h_o$  and  $h_x$  are the amplitude of EPR signal of control containing DTPA + DMPO + Fe (II) +  $\text{H}_2\text{O}_2$  and samples containing DTPA + DMPO + Fe (II) + different polysaccharide extracts +  $\text{H}_2\text{O}_2$ , respectively [24].

Superoxide anion radicals were generated from an HPX-XOD reaction system in 10 mM phosphate buffer solution (pH 7.4) [24, 25]. Briefly, 5  $\mu\text{L}$  of 1.0 M DMPO, 5  $\mu\text{L}$  of 6 mM HPX, 5  $\mu\text{L}$  of 4 mM DTPA and 5  $\mu\text{L}$  of tested sample were put into a test tube. The amount of  $\text{DMPO-O}_2^{\bullet-}$  formed was determined exactly 80 s after the addition of 5  $\mu\text{L}$  of 50 mU/mL XOD using the EPR spectrometer. The EPR spectrometer conditions were central field, 336.3 mT; microwave power, 10.0 mW; modulation frequency, 9.44 GHz; scanning width, 5.0 mT; modulation amplitude, 0.079 mT; time constant, 0.1 s; and scanning period, 120 s. The scav-

enging activity was denoted as the percentage inhibition of the peak intensity of the control in which 5  $\mu\text{L}$  of phosphate buffer solution was used instead of sample solution [26]. The scavenging effects of GLPs and Se-GLPs were calculated the same way for superoxide radical as for hydroxyl radical.

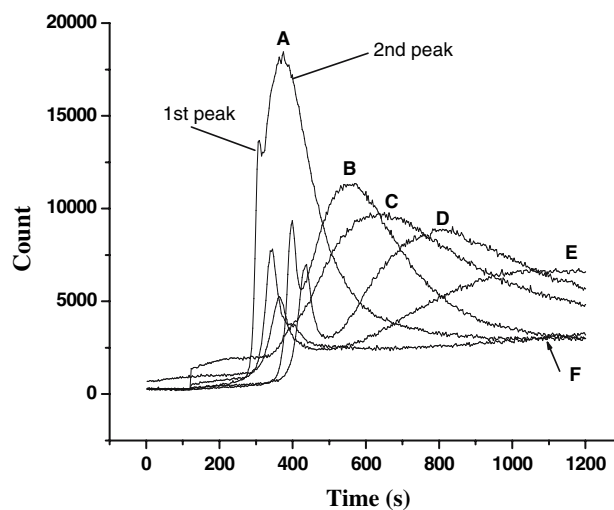
#### Statistics analysis

The data were analyzed using the statistical analysis system (SAS 9.0) package software for the analysis of variance and Duncan's test. All experiments were carried out in triplicate. The significance was established at  $p \leq 0.05$ .

## Results and discussion

### Protective effects of Se-GLPs on DNA and Phen against oxidative damage by free radicals

The protective effects of Se-GLPs on DNA and Phen from oxidative damage by free radicals were assayed with a  $\text{CuSO}_4$ -Phen-Vc- $\text{H}_2\text{O}_2$ -DNA chemiluminescent system [19]. The kinetic traces of inhibition of Se-GLPs to chemiluminescence induced by both DNA damage and Phen oxidation are shown in Fig. 1 and have the same general profile as were reported for inhibition of selenium-enriched protein extracts and green tea po-

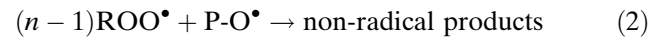
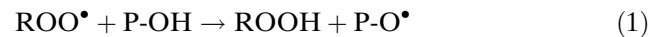


**Fig. 1** Inhibition of Se-GLPs to chemiluminescence due to both DNA damage (peak 2) and Phen oxidation (peak 1) in  $\text{CuSO}_4$ -Phen-Vc- $\text{H}_2\text{O}_2$ -DNA-Se-GLPs system. Curve A distilled water alone. Curve B 0.0514 mg/mL Se-GLPs. Curve C 0.103 mg/mL Se-GLPs. Curve D 0.206 mg/mL Se-GLPs. Curve E 0.410 mg/mL Se-GLPs. Curve F 1.03 mg/mL Se-GLPs. Conditions: 50  $\mu\text{M}$   $\text{Cu}^{2+}$ , 350  $\mu\text{M}$  Phen, 350  $\mu\text{M}$  Vc, 14.7 mM  $\text{H}_2\text{O}_2$ , and 1.0  $\mu\text{g}/\text{mL}$  DNA, 22 °C

lyphenols to chemiluminescence, respectively [16, 19]. There are two peaks on the spectra; the first peak represents the chemiluminescence due to Phen possibly oxidized by  $O_2^{\bullet-}$  and the second peak corresponds to the chemiluminescence of DNA damage likely induced by  $HO^{\bullet}$  [17, 19]. Compared with control (curve A), the time of appearance of the second peak was delayed, accompanied by a decrease of its intensity upon the addition of Se-GLPs to the chemiluminescent system. The increase of the Se-GLPs concentration, from 0.0514 mg/mL (curve B), 0.103 mg/mL (curve C), and 0.206 mg/mL (curve D), to 0.410 mg/mL (curve E) caused an increase in the inhibiting effect of Se-GLPs on the chemiluminescence due to DNA damage (peak 2) and Phen oxidation (peak 1). Moreover, the chemiluminescence due to DNA damage (peak 2) can hardly be detected within experiment time when the concentration of Se-GLPs was up to 1.030 mg/mL (curve F), indicating that 1.030 mg/mL of Se-GLPs could totally quench the producing of hydroxyl radicals which lead to the oxidative damage of DNA. Additionally, it can also be observed that the inhibiting effect of Se-GLPs (expressed in depression rate and delay time) on the chemiluminescence due to DNA damage is more pronounced than that on the chemiluminescence due to Phen oxidation ( $p < 0.05$ ) (Table 1, Fig. 1). On the whole, Se-GLPs exhibited DNA protective effects from oxidative damage in dose dependent manner, though the delay time of Phen did not show the dose-dependency significantly. This is similar to the result we have recently reported on the protein extracts from Se-enriched *G. lucidum* [16].

The contribution of all the Se-GLPs samples to prevent DNA from oxidative damage by hydroxyl radical possibly comes from two parts, namely, polysaccharides itself and selenium moiety in the polysaccharides. Polysaccharides might act as hydrogen donors by providing hydrogen atom from HO group in polysaccharide chains to free radical such as peroxy

radicals ( $ROO^{\bullet}$ ), forming a new polysaccharide radical. The producing polysaccharide radical can react with the peroxy radicals, producing non-radical as following [19, 27, 28].



where P-OH is the polysaccharide,  $n$  is chemically calculating factor of antioxidant.

However, another possibility cannot be excluded that hydroxyl group (-OH) in polysaccharide ring could form coordinate with free metal ions such as copper and iron ions, which are necessary for free radical production [27, 28]. Resulting complexes are inactive for the production of free radicals.

The contribution of the selenium moiety in the Se-GLPs samples to antioxidant activity probably also stems from, in part, the ability to complex the free metal ion. It was proposed that selenium possibly exists in the polysaccharides containing Se in the form of selenyl group (-SeH) or seleno-acid ester [29]. The new groups may be able to complex  $Cu^+$  or  $Cu^{2+}$ , thereby inhibiting the generation of hydroxyl radicals ( $HO^{\bullet}$ ) and superoxide radicals ( $O_2^{\bullet-}$ ) in  $CuSO_4$ -Phen-Vc-DNA system. However, the real mechanism of Se-GLPs' protecting effect of DNA from oxidative damage is beyond the scope of the present study and need further testifying.

The role of selenium in Se-GLPs in scavenging hydroxyl radical

Electron paramagnetic resonance (EPR) is a powerful tool for detection of all kinds of radical. In order to characterize the role of selenium in Se-GLPs, EPR in conjunction with spin trap technique was used to determine the ability of GLPs and Se-GLPs with different Se content to attenuate hydroxyl radical

**Table 1** Depression rate and delay time of CL due to DNA damage by Se-GLPs

Concentration of Se-GLPs (mg/mL)	Depression rate of DNA (%)	Delay time of DNA (s)	Depression rate of Phen (%)	Delay time of Phen (s)
0.0514	38.5 ± 1.0a	195 ± 5a	31.6 ± 0.8a*	90 ± 2a**
0.103	47.1 ± 1.3b	288 ± 9b	42.8 ± 1.4b*	36 ± 1b**
0.206	51.2 ± 1.5c	462 ± 14c	48.9 ± 1.4c*	129 ± 4c**
0.410	63.4 ± 1.5d	681 ± 19d	61.5 ± 1.3d*	41 ± 1d**
1.03	100	N/A	72.6 ± 1.9e*	96 ± 3e**

All values are mean ± SD ( $n = 3$ ). Values followed by different letters in same column are significantly different ( $p < 0.05$ )

\* $p < 0.05$  compared to depression rate of DNA at the same concentration of Se-GLPs

\*\* $p < 0.01$  compared to delay time of DNA at the same concentration of Se-GLPs

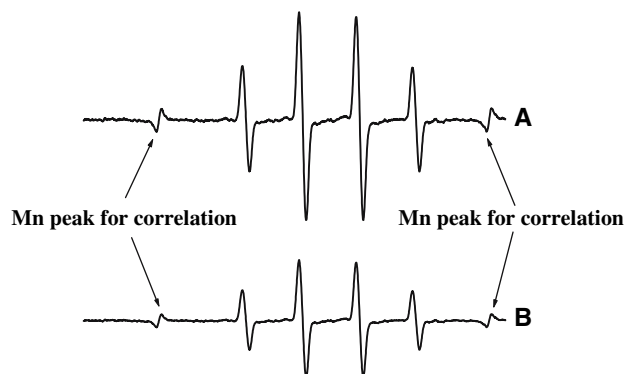
production from the Fenton reaction. The results are shown in Tables 2 and 3; Figs. 2 and 3. Spectrum A of Fig. 2 is that of the DMPO-OH adduct in a control experiment adding reagents in the sequence DTPA + DMPO + Fe (II) + H<sub>2</sub>O<sub>2</sub>. The one-electron oxidation of Fe (II) by H<sub>2</sub>O<sub>2</sub> is an efficient generator of hydroxyl radical [24] and an intense spectrum from trapped HO• was observed (spectrum A). With Se-GLPs 3, a weakest EPR signal was obtained (spectrum B).

It is evident that the polysaccharide contents of GLPs (86.5%), Se-GLPs 1 (85.9%), Se-GLPs2 (86.3%), and Se-GLPs 3 (87.1%) are nearly same (*p* > 0.05), the activity of scavenging HO• increases from 34.7%, 44.1%, 47.7% to 52.5%, respectively (Fig. 3) as the content of Se increase in all the samples, and following the order GLPs (1.45 µg selenium/g polysaccharide extract) < Se-GLP 1 (20.53 µg selenium/g polysaccharide extract) < Se-GLPs 2 (49.62 µg selenium/g polysaccharide extract) < Se-GLPs 3 (60.50 µg selenium/g polysaccharide extract). Because the polysaccharide concentration was kept constant, the different activity of GLPs and Se-GLPs stems from the different Se-content contained in the Se-GLPs, and not from the polysaccharide itself, a finding indicating that Se plays an important role in enhancing the antioxidant capacity of the polysaccharide extracts. The higher activity of the Se-enriched polysaccharide extracts in scavenging HO• comes from the higher reactivity of the selenium moiety including possibly formed selenyl group (-SeH) [29] in the Se-GLPs as compared to hydroxyl group (-OH) in the regular analogues. A similar result was obtained with protein extracts from the same Se-enriched *G. lucidum* (Se-GL) in this section of experiment [16]. This implies that the antioxidation mechanism of protein extracts from Se-GL might be similar to that of the polysaccharide extracts from the same source, namely, Se-contained moiety in both kinds of extracts plays an important role in attenuating the hydroxyl radical.

**Table 3** The activity of scavenging hydroxyl radical of Se-GLP 3

Sample	EPR amplitude for DMPO-OH <sup>a</sup>	Scavenging effect of •OH (%)
Control	1142 ± 30	N/A
Se-GLP 3	542 ± 8	52.5 ± 0.5

<sup>a</sup> EPR amplitude of the second DMPO-OH adduct peak in EPR spectrum



**Fig. 2** EPR spectra of the DMPO-OH. The EPR spectrum was recorded 2.0 min after the addition of H<sub>2</sub>O<sub>2</sub>. Curve A control (DTPA + DMPO + Fe (II) + H<sub>2</sub>O<sub>2</sub>). Curve B DTPA + DMPO + Fe (II) + 1.03 mg/mL Se-GLPs 3+ H<sub>2</sub>O<sub>2</sub>. Conditions: 0.8 mM H<sub>2</sub>O<sub>2</sub>, 20 µM Fe<sup>2+</sup>, 0.8 mM DTPA, and 100 mM DMPO in 10 mM phosphate buffer (pH 7.4)

Comparison of the antioxidant activity of sodium selenite, GLPs, GLPs plus sodium selenite, and Se-GLPs

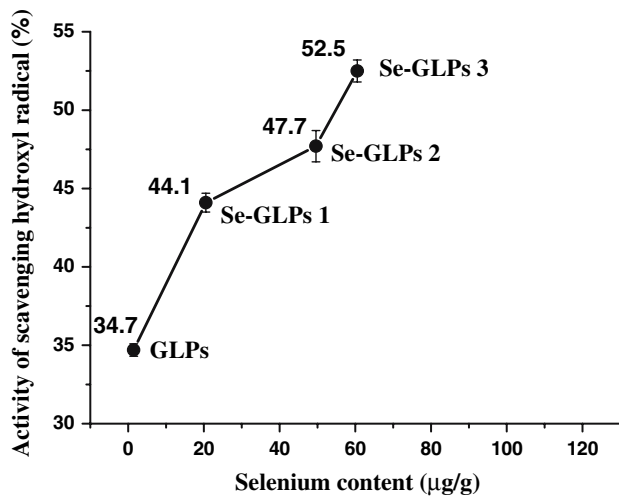
In order to determine whether there is a synergetic effect between selenium and Se-GLPs, the activities of scavenging free radicals (O<sub>2</sub><sup>•-</sup> and HO•) of selenite, GLPs, GLPs plus selenite and Se-GLPs 3 were compared by spin trapping experiments and the results are shown in Table 4. All samples reduced the amount of both O<sub>2</sub><sup>•-</sup> and HO• trapped by DMPO, a result consistent with the above observation that Se-GLPs had the ability to prevent DNA damage and Phen oxida-

**Table 2** The role of selenium in Se-GLPs in scavenging hydroxyl radical

Sample	Se-content (µg/g polysaccharide extract)	Polysaccharide content (% , w/w)	Scavenging effect of HO• (%)
GLPs <sup>a</sup>	1.45 ± 0.02a	86.5 ± 0.5a	34.7 ± 0.3a
Se-GLPs 1	20.53 ± 0.35b	85.9 ± 0.6a	44.1 ± 0.9b
Se-GLPs 2	49.62 ± 0.06c	86.3 ± 0.8a	47.7 ± 0.6c
Se-GLPs 3	60.50 ± 3.08d	87.1 ± 0.6a	52.5 ± 0.5d

All values are mean ± SD (*n* = 3). Values followed by different letters in same column are significantly different (*p* < 0.05)

<sup>a</sup> The concentration of polysaccharide of all samples is 1.03 mg/mL



**Fig. 3** Scavenging effects of GLPs, Se-GLPs 1, Se-GLPs 2, and Se-GLPs 3 on hydroxyl radical generated from the Fenton reaction. Values are mean  $\pm$  SD of three independent experiments. Conditions: 0.8 mM  $\text{H}_2\text{O}_2$ , 20  $\mu\text{M}$   $\text{Fe}^{2+}$ , 0.8 mM DTPA, 100 mM DMPO in 10 mM phosphate buffer (pH 7.4), and polysaccharide of 1.03 mg/mL for all samples

tion from hydroxyl and superoxide radicals, respectively (Fig. 1, Table 1). When the Se concentration of  $\text{Na}_2\text{SeO}_3$ , GLPs +  $\text{Na}_2\text{SeO}_3$ , and Se-GLPs 3 stayed constant, Se-GLPs 3 showed the strongest activities of attenuating  $\text{O}_2^{\bullet-}$  and  $\text{HO}^\bullet$  production by 82.0% and 73.3%, respectively, agreeing with the above observation that Se could increase the antioxidant activity of Se-GLPs. However, the sum of the scavenging effect of  $\text{Na}_2\text{SeO}_3$  (31.0%) on  $\text{HO}^\bullet$  and that of GLPs (51.0%) on  $\text{HO}^\bullet$  was approximate 10% bigger than that of Se-GLPs 3 on  $\text{HO}^\bullet$  (73.3%); similarly, the sum of the scavenging effect of  $\text{Na}_2\text{SeO}_3$  (13.4%) on  $\text{O}_2^{\bullet-}$  and that of GLPs (71.2%) on  $\text{O}_2^{\bullet-}$  was also a little higher than that of Se-GLPs 3 on  $\text{O}_2^{\bullet-}$  (82.0%) (Table 4), suggesting that there may be no synergetic effect between Se in the Se-GLPs and the polysaccharide itself on scav-

enging free radicals. In contrast, an obvious synergetic effect between the protein extracts from Se-enriched *G. lucidum* and their Se components has been observed [16]. Furthermore, the radical scavenging activities of Se-GLPs is significantly higher than that of GLPs +  $\text{Na}_2\text{SeO}_3$  ( $p < 0.05$ ) (Table 4), a result implying that the chemical form of selenium seems also be crucial for polysaccharide's antioxidative activities, which is similar to what we have found in the protein extracts from Se-enriched *G. lucidum* [16]. In addition, it is of interest to note that except for  $\text{Na}_2\text{SeO}_3$ , other three samples with polysaccharide exhibited stronger activities of attenuating the production of superoxide radical than hydroxyl radical. This might be due to the inhibition of polysaccharides on activity of the XOD enzyme, which catalyzes the formation of superoxide radical [30], besides their activity against superoxide radical. Illustrating the mechanism of the free radical scavenging activities of Se-enriched polysaccharide extracts and their analogs are under investigation.

## Conclusions

The polysaccharide extracts from Se-enriched *G. lucidum* (Se-GLPs) exhibited DNA protective effects from hydroxyl radical oxidative damage in dose dependent manner. Moreover, at the same polysaccharide concentration, the polysaccharide extracts from Se-enriched *G. lucidum* showed much stronger properties against hydroxyl and superoxide radicals than those from unenriched *G. lucidum* as suggested by spin-trapping experiment. These results demonstrate that selenium in polysaccharide extracts plays an important role in enhancing their antioxidant activities. The idea was further confirmed by the observation that with increasing the Se content in the polysaccharide extracts, the antioxidant activities of all the Se-GLPs

**Table 4** Activities of scavenging hydroxyl radical and superoxide radical of sodium selenite, GLPs, GLPs plus  $\text{Na}_2\text{SeO}_3$ , and Se-GLPs<sup>a</sup>

Sample	EPR amplitude for DMPO-OH <sup>b</sup>	EPR amplitude for DMPO-O <sub>2</sub> <sup>•-</sup>	Scavenging effect of HO <sup>•</sup> (%)	Scavenging effect of O <sub>2</sub> <sup>•-</sup> (%)
Control	1163 $\pm$ 19	1925 $\pm$ 17	N/A	N/A
$\text{Na}_2\text{SeO}_3$ (0.144 $\mu\text{g}/\text{mL}$ Se)	802 $\pm$ 5	1668 $\pm$ 21	31.0 $\pm$ 0.4 <sup>a</sup>	13.4 $\pm$ 1.0 <sup>a</sup>
GLPs (2.06 mg/mL)	570 $\pm$ 25	554 $\pm$ 20	51.0 $\pm$ 2.1 <sup>b</sup>	71.2 $\pm$ 1.0 <sup>b</sup>
GLPs (2.06 mg/mL) + $\text{Na}_2\text{SeO}_3$ (0.144 $\mu\text{g}/\text{mL}$ Se)	342 $\pm$ 12	422 $\pm$ 2	70.6 $\pm$ 1.0 <sup>c</sup>	78.1 $\pm$ 0.1 <sup>c</sup>
Se-GLPs 3 (0.144 $\mu\text{g}/\text{mL}$ of Se; 2.06 mg/mL of polysaccharide)	310 $\pm$ 5	346 $\pm$ 8	73.3 $\pm$ 0.4 <sup>d</sup>	82.0 $\pm$ 0.4 <sup>d</sup>

<sup>a</sup> All values are mean  $\pm$  SD ( $n = 3$ ). Values followed by different letters in same column are significantly different ( $p < 0.05$ )

<sup>b</sup> EPR amplitude of the second DMPO-OH adduct peak in EPR spectrum

<sup>c</sup> EPR amplitude of the first DMPO-O<sub>2</sub><sup>•-</sup> adduct peak in EPR spectrum

samples increased. However, unlike their protein analogues [16], there is no synergetic effect observed between Se moiety and polysaccharide itself in antioxidant activity.

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