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Selenium Distribution in a Se-Enriched Mushroom Species of the Genus *Ganoderma*

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Data reported here show that *Ganoderma lucidum* could biotransform inorganic selenite in the substrate into organic forms by intergrating Se into proteins (56−61%) and polysaccharides (11−18%) and other components. Furthermore, water- and alkaline-soluble protein components were mainly responsible for the storage of organic Se, and Se-Met accounts for only a minor (8.2−18.3%) amount of the selenocompounds present in proteins. The molecular mass of most proteins or protein subunits containing Se was no more than 16 kDa. A low concentration of Se (<100 μg/g) in the substrate facilitated the synthesis of total protein and amino acids in *G. lucidum*, but a high concentration of Se (>150 μg/g) played a reverse role. Additionally, Se concentration in the culture had no significant effect on the distribution of the amino acids and proteins.

KEYWORDS: Selenium; distribution; Se-enriched *Ganoderma lucidum*

INTRODUCTION

Four decades ago, Se was recognized as an essential nutrient on the basis of its ability to serve interchangeably with vitamin E in the prevention of vascular or muscular symptoms in experimental animals (*1*). Further research showed that the essentiality of Se was due to the requirement for the 21st amino acid, selenocysteine, which is used for the synthesis of about a dozen selenoenzymes including glutathione peroxidase, iodothyronine deiodinases, and thioredoxin reductasaes (*2*−*4*). There is evidence that Se deficiency may be related to a variety of degenerative diseases including Kashin−Beck, Keshan, and cancer (*5*−*7*). It is therefore suggested that Se supplementation through reinforcement of endogenenous antioxidative systems may be beneficial as an adjuvant therapy for some human pathologies (*8*). Because supplementation of selenium from natural food materials is considered to be safer than directly ingesting inorganic Se, it seems to be very important for human beings to find dietary sources.

According to Combs (*9*), food sources of selenium can be characterized as follows: poor sources are most meat and fish products and soybeans, moderate sources include most plant materials, and good sources include selenium-enriched yeast. Therefore, the most common form of selenium available commercially as a human dietary supplement is Se-enriched yeast (*10*), but the major selenocompound present in this product is selenomethionine (*11*). Because selenomethionine administration tends to accumulate in tissues (*12*), it is desirable to find other effective selenium sources that do not lead to tissue Se accumulation. Although recently some Se-enriched plant resources such as ramps, wheat, broccoli, onion, garlic, and tea have been reported (*13*−*16*), little information is available on the Se enrichment of fungi such as *Ganoderma lucidum* (GL).

G. lucidum is a unique mushroom species and has been used as a tonic and drug for more than 2000 years. It is very famous in China and other countries of Asia for its extensive physiological effects on chronic hepatopathy, hypertension, bronchitis, arthritis, neurasthenia, neoplasin, tumorigenic diseases, and so on (*17*−*22*). Therefore, it is of interest to know whether *G. lucidum* could also be used as an effective carrier for Se enrichment. If this specific mushroom could accumulate inorganic selenium from culture, Se-enriched GL would be explored as a dietary Se supplement source, which possibly possesses even greater health benefits provided by this specific mushroom. The objective of this study was to thoroughly characterize selenium distribution in a Se-enriched mushroom species of the genus *Ganoderma*.

MATERIALS AND METHODS

Materials. All solvents/chemicals used were of analytical grade. Selenomethionine and bovine serum albumin were obtained from Sigma Chemical Co. (Beijing, China).

Cultivation of Se-Enriched *G. lucidum*. The cultivation method of Se-enriched *G. lucidum* (Se-GL) was basically the same as that of *G. lucidum* (*23*). The only difference was that a series of 100, 150, 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 100), 2 (Se 150), 3 (Se 200), and 4 (Se 250), respectively.

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Table 1. Uptake of Selenium by *G. lucidum*[a]

item	Se 100	Se 150	Se 200	Se 250
Se content in substrate (μg/g)	100	150	200	250
total Se content of fruit body of Se-GL (μg/g)	27.91 ± 0.38 a	42.63 ± 0.13 b	44.62 ± 0.45 c	72.44 ± 0.44 d
accumulating rate of Se by Se-GL[b] (%)	27.91 ± 0.38 a	28.42 ± 0.90 a	22.31 ± 0.22 b	28.98 ± 0.18 a
organic Se content of fruit body of Se-GL (μg/g)	23.79 ± 0.22 a	37.48 ± 0.72 b	38.71 ± 0.58 b	59.19 ± 1.09 c
organic Se in total Se (%)	85.23 ± 0.37 a	87.92 ± 1.42 a	86.75 ± 0.42 a	81.71 ± 1.01 b

[a] Values are the means ± standard deviations ($n = 3$). Values followed by different letters in the same row are significantly different ($p < 0.05$). [b] Accumulating rate was calculated by dividing Se content in substrate of Se-GL by total Se content of fruit body.

Analysis of Organic Selenium in Se-GL. One gram of freeze-dried and powdered Se-GL was dialyzed against distilled water for 96 h by changing the water every 12 h until no Se was detected in the dialyzing water. Thus, Se compounds left in the dialyzed sample were considered to be organic selenium (*24*).

Preparation of Nucleic Acid from Se-GL. Ten grams of freeze-dried and powdered Se-GL was stirred into 150 mL of 12% NaCl at 90−95 °C (*25*). After 2 h, the supernatant was obtained by filtration and the protein of the supernatant was removed using the Sevag method (*26*). Then the nucleic acids were precipitated at 4 °C by adjusting the pH value of the supernatant to 2.5. Finally, the precipitate was collected, lyophilized, and stored at −20 °C for use.

Preparation of Polysaccharide from Se-GL. Ten grams of freeze-dried and powdered Se-GL 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 (*26*). Ethanol was added to the supernatant until 75% (v/v) of ethanol was obtained. The resultant precipitate was collected by centrifugation and then dissolved in 5.0 mL of distilled water. This solution was filtered and dialyzed against distilled water at 4 °C three times to remove any other small molecules. After 24 h, the solution was lyophilized and stored at −20 °C for use (*27*).

Preparation of Water-Soluble Protein from Se-GL. Ten grams of freeze-dried and powdered Se-GL was stirred into 100 mL of distilled water. Four hours later, the supernatant was obtained by filtration. After adjustment of its pH to 4.5, the supernatant was made 100% saturated with ammonium sulfate at 4 °C. The resulting precipitate was collected by centrifugation and dissolved in 10 mL of 50 mM Tris-HCl (pH 8.0). This solution was filtered and dialyzed against 50 mM Tris-HCl (pH 8.0) (1.0 L) at 4 °C three times to remove any other small molecules. After 24 h, the solution was lyophilized and stored at −20 °C for use (*28*).

Preparation of Salt-Soluble Protein from Se-GL. After its water-soluble protein was extracted, the residues of Se-GL were stirred into 100 mL of 0.5 M NaCl. Other procedures were the same as those used for the preparation of water-soluble protein except that the supernatant was made 50% instead of 100% saturated with ammonium sulfate at 4 °C (*28*).

Preparation of Alcohol-Soluble Proteins from Se-GL. After water- and salt-soluble proteins in Se-GL were extracted, respectively, the residues were stirred into 100 mL of 75% (v/v) alcohol. After 4 h, the supernatant was obtained by filtration and added to100 mL of distilled water at 4 °C. The resultant precipitate was collected by centrifugation and dissolved in 75% (v/v) alcohol (*28*). This solution was filtered and dialyzed against distilled water (1.0 L) at 4 °C three times to remove any other small molecules. After 24 h, the solution was lyophilized and stored at −20 °C for use.

Preparation of Alkline-Soluble Protein from Se-GL. After the above consecutive extractions by water, 0.5 M NaCl solution, and alcohol, respectively, the final residues of Se-GL were stirred into 100 mL of 0.1 M NaOH. Other procedures were the same as those used for the preparation of water-soluble protein except that the supernatant was made 50% instead of 100% saturated with ammonium sulfate at 4 °C (*28*).

Determination of Selenium Content. The selenium concentration of tested samples was determined by atomic fluorescence spectrophotometry (AFS-1201, Haiguang Analytical Co., China) as reported recently (*29*). The Se content was given by the Se concentration detected by AFS over the exact weight of lyophilized samples.

Sodium Dodecyl Sulfate−Polyacrylamide Gel Electrophoresis (SDS-PAGE). A 1.5 g sample of the freeze-dried and powdered Se-GL was suspended in 6 mL of distilled water. To the solution was added 2 mL of 60 mM Tris-HCl buffer (pH 8.6) containing 25% glycerol, 2% SDS, 14.4 mM β-mercaptoethanol, and 0.1% bromophenol blue. After the solution had been boiled for 3 min, the supernatant was isolated by centrifugation at 6500 rpm for 20 min. SDS-PAGE was used as described previously (*30*). Proteins in the supernatant were concentrated in 5% polyacrylamide gel and electrophoresed in 10% gel at a constant current of 60 mA. In a 200 × 200 × 3 cell for a total of 12 lanes, 7 were added with blanks and others were added with test samples. At end of the electrophoresis, the gel was stained with Coomassie bright blue R250 (*31*) overnight (with 9% acetic acid and 45% methanol) and destained with 9% acetic acid for 2 days. The gel between the first visualized protein band and the last one was cut into nine segments every 2 cm, which corresponded to nine different molecular mass ranges of proteins evaluated by comparison with a molecular mass calibration kit (Pharmacia LKB, Uppsala, Sweden). At the same time, the sliced segment was also used for Se determination. After deducting the parallel blank, the Se content of each segment was analyzed using atomic fluorescence spectrophotometry.

Determination of Protein Content and Amino Acids. Total raw protein content was determined with a model KN-01 Kjeldahl nitrogen analyzer (Mitsubishi Chemical Industries, Inc., Japan) using the national standard method of China (GB-2905, National Standard Bureau). The amino acids and selenomethionine were determined with a Beckman model 121 amino acid analyzer and a Waters model 600E high-performance liquid chromatograph, respectively, after the tested samples were hydrolyzed in airtight ampules with 10 mL of 6 M HCl at 110 °C for 22 h.

Statistics Analysis. The data were analyzed using the Statistical Analysis System (SAS 1998) package software for the analysis of variance, Duncan's test, and Student's test. All experiments were carried out in triplicate. The significance was established at $p \leq 0.05$.

RESULTS AND DISCUSSION

Distribution of Organic Se in Se-Enriched *G. lucidum* (Se-GL). Addition of increasing levels of selenium in the growth mixture generally resulted in proportional increases of the selenium content of the fruit body of Se-GL (**Table 1**). None of these Se levels caused any inhibition of the growth of Se-GL. *G. lucidum* could absorb ∼20−30% of inorganic selenium in substrate and transformed most of it into organic Se, which accounted for 81.71−87.92% of absorbed Se (**Table 1**). As compared to plant sources such as wheat, sunflower, and ramps (*15*, *16*) used for Se enrichment, *G. lucidum* showed a much lower accumulating rate of Se.

Table 2 shows the percentage distribution of organic Se with different concentrations of inorganic selenium in the substrate. There was no significant relationship between the percentage distribution of organic Se and the Se concentration in the culture. From 64.6 to 69.8% of the organic Se was found in the protein fraction, 11.2−18.0% in the polysaccharide fraction, and 0.0672−0.220% in the nucleic acid fraction with 13.8−20.0% remaining in other components, which possibly included lipids and free amino acids. Thus, proteins in *G. lucidum* showed the

Table 2. Distribution of Organic Selenium in Se-Enriched *G. lucidum*[a]

	item	protein-bound Se	polysaccharide-bound Se	nucleic acid-bound Se	other organic Se
Se 100	content (μg/g)	15.9 ± 0.3	3.12 ± 0.05	0.0305 ± 0.0007	4.74 ± 0.16
	percentage in organic Se (%)	66.8 ± 0.6	13.1 ± 0.1	0.128 ± 0.002	20.0 ± 0.5
	percentage in total Se (%)	56.9 ± 0.3	11.2 ± 0.0	0.109 ± 0.001	17.0 ± 0.3
Se 150	content (μg/g)	26.1 ± 0.3	6.14 ± 0.06	0.0825 ± 0.0021	5.16 ± 0.36
	percentage in organic Se (%)	69.6 ± 0.5	16.4 ± 0.2	0.220 ± 0.001	13.8 ± 0.7
	percentage in total Se (%)	61.2 ± 0.5	14.4 ± 0.1	0.193 ± 0.004	12.1 ± 0.8
Se 200	content (μg/g)	25.0 ± 0.4	8.04 ± 0.01	0.0418 ± 0.0017	5.63 ± 0.17
	percentage in organic Se (%)	64.6 ± 0.1	20.8 ± 0.3	0.108 ± 0.003	14.5 ± 0.2
	percentage in total Se (%)	56.0 ± 0.3	18.0 ± 0.2	0.0937 ± 0.003	12.7 ± 0.3
Se 250	content (μg/g)	41.3 ± 0.2	8.23 ± 0.42	0.0398 ± 0.0013	9.62 ± 0.47
	percentage in organic Se (%)	69.8 ± 0.9	13.9 ± 0.5	0.0672 ± 0.001	16.2 ± 0.5
	percentage in total Se (%)	57.0 ± 0.1	11.4 ± 0.2	0.0549 ± 0.001	13.3 ± 0.6

[a] Values are the means ± standard deviations ($n = 3$).

Table 3. Se Distribution in Protein-Bound Se[a]

	item	water-soluble protein	salt-soluble protein	alcohol-soluble protein	alkaline-soluble protein	unextracted protein
Se 100	Se content (μg/g)	7.76 ± 0.09	0.660 ± 0.018	0.930 ± 0.044	4.19 ± 0.11	2.36 ± 0.04
	percentage in protein-bound Se (%)	48.8 ± 0.4	4.15 ± 0.0.5	5.85 ± 0.17	26.4 ± 0.2	14.8 ± 0.0
	percentage in total Se (%)	27.8 ± 0.1	2.36 ± 0.03	3.33 ± 0.11	15.0 ± 0.2	8.46 ± 0.03
Se 150	Se content (μg/g)	15.7 ± 0.2	1.05 ± 0.02	1.52 ± 0.04	4.96 ± 0.07	2.87 ± 0.03
	percentage in protein-bound Se (%)	60.2 ± 0.1	4.02 ± 0.03	5.82 ± 0.09	19.0 ± 0.0	11.0 ± 0.0
	percentage in total Se (%)	36.8 ± 0.4	2.46 ± 0.04	3.57 ± 0.08	11.6 ± 0.1	6.73 ± 0.05
Se 200	Se content (μg/g)	14.9 ± 0.4	1.29 ± 0.08	1.43 ± 0.03	4.88 ± 0.12	2.50 ± 0.23
	percentage in protein-bound Se (%)	59.6 ± 0.6	5.16 ± 0.24	5.72 ± 0.03	19.5 ± 0.2	10.0 ± 0.8
	percentage in total Se (%)	33.4 ± 0.6	2.89 ± 0.15	3.20 ± 0.03	10.9 ± 0.2	5.60 ± 0.46
Se 250	Se content (μg/g)	23.9 ± 0.7	2.39 ± 0.04	2.35 ± 0.01	7.9 ± 0.2	4.76 ± 0.79
	percentage in protein-bound Se (%)	57.9 ± 1.4	5.79 ± 0.07	5.69 ± 0.00	19.1 ± 0.5	11.5 ± 1.9
	percentage in total Se (%)	33.0 ± 0.8	3.30 ± 0.04	3.24 ± 0.01	10.9 ± 0.3	6.57 ± 1.05

[a] Values are the means ± standard deviations ($n = 3$).

strongest ability to incorporate Se, whereas the nucleic acid fraction hardly bound Se. The present results indicated that, similar to other bacteria such as *Flammulina velutipes*, yeast, and *Lactobacillus delbrueckii* subsp. *bulgaricus* (*32*−*34*), *G. lucidum*, a specific mushroom, can also be used to accumulate Se, and Se tends to be incorporated into its protein components. In contrast, Se-bound protein did not make a major contribution to the storage of the Se in the Se-enriched *Agricus bisporus* (*35*).

Se Distribution in Proteins of Se-Enriched *G. lucidum*. The protein extracts of different solubilities were obtained and analyzed for their Se content. The results (**Table 3**) showed that water-soluble protein extracts had the highest Se content (>40% of organic Se), whereas salt-soluble protein extracts had the least (<6% of organic Se). Se content in different protein extracts was in the order of water-soluble protein-bound Se (48.8−60.2%) > alkaline-soluble protein-bound Se (19.0−26.4%) > alcohol-soluble protein-bound Se (5.69−5.85%) > salt-soluble protein-bound Se (4.02−5.79%). Water-soluble protein and alkaline-soluble protein were major proteins to bind Se as the selenium incorporated with them accounted for >75% of total organic Se and ∼45% of total Se of the fruit bodies of Se-GL.

Molecular Mass Distribution of Selenoproteins in Se-Enriched *G. lucidum*. Selenoproteins in Se-enriched *G. lucidum* were separated using SDS-PAGE. The resultant gel with the Se 250 sample was cut according to the molecular mass range of protein. Nine segments containing 13 bands of protein or protein subunits were obtained and, at the same time, analyzed for their Se content. The results (**Figure 1**; **Table 4**) showed that selenium distributed in all proteins or protein subunits and did not affect the protein distribution of all Se-GL samples. The lower the molecular mass of the protein or protein subunit was,

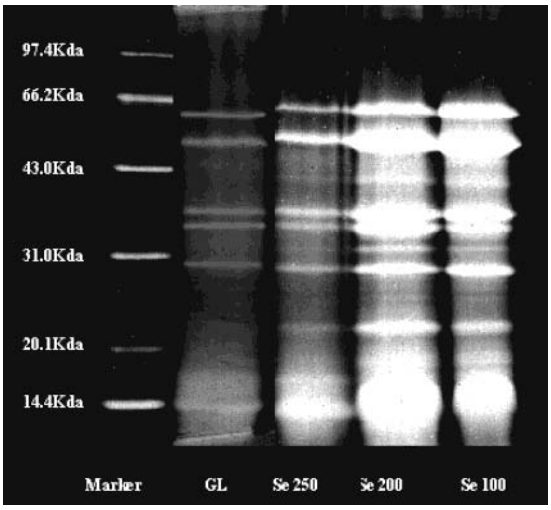

Figure 1. SDS-PAGE analysis of GL and Se-GL samples according to the procedure of Laemmli. The detailed procedures were given under Materials and Methods. The figure shows three sample bands and the standard protein kit of different molecular masses for calibration (from top to bottom: rabbit phosphorylase *b*, 97400; bovine serum albumin, 66200; rabbit actin, 43000; bovine carbonic anhydrase, 31000; trypsin inhibitor, 20100; hen egg white lysozyme, 14400).

Table 4. Molecular Mass Distribution of Selenoproteins or Protein Subunits

segment	molecular mass (kDa)	Se content (μg/g)
1	142.53–104.71	0.0478 ± 0.0007
2	104.71–77.624	0.0340 ± 0.0008
3	77.624–56.885	0.0152 ± 0.0001
4	56.885–41.686	0.0520 ± 0.0009
5	41.686–29.512	0.0641 ± 0.0004
6	29.512–21.877	0.0661 ± 0.0002
7	21.877–16.032	0.0730 ± 0.0001
8	16.032–11.748	0.184
9	11.748–8.709	0.302

the higher the Se content of the protein or protein subunit was. The molecular mass of most proteins or protein subunits containing Se was no more than 16 kDa.

Protein and Amino Acid Contents in *G. lucidum*. **Table 5** gives the amino acid composition and total protein content of the tested samples with different concentrations of inorganic selenium in the culture. The total protein content on the dry weight basis ranged from 7.90 to 10.67%. Significant differences were found between *G. lucidum* (GL) and Se-enriched *G. lucidum* (Se-GL) for total protein and amino acid contents ($p < 0.05$). Selenium in *G. lucidum* seems to have a biphasic effect on the production of its proteins. As compared to common *G. lucidum* hardly containing Se, the Se 100 sample of Se-enriched *G. lucidum* with 27.91 μg/g of Se content (**Table 1**) had pronouncedly higher contents of the protein and all amino acids (**Table 5**). However, as the Se content in Se-enriched *G. lucidum* samples increased from 27.91 (Se 100) to 42.63 (Se 150) to 44.62 (Se 200) to 72.44 μg/g (Se 250) (**Table 1**), the contents of the protein and all amino acids decreased in these samples (**Table 5**). Because selenium in Se-enriched *G. lucidum* came from the culture, the present results suggested that, at low concentrations of Se (<100 μg/g) in the culture, Se may facilitate the synthesis of protein and most amino acids of GL; however, high concentrations of Se (>150 μg/g) in the culture inhibited the synthesis of protein and total amino acids of GL. This finding is inconsistent with previous studies indicating that the increase of Se in soil increased the protein content in both wheat and soybean (*36*, *37*), indicating that *G. lucidum* is more sensitive to selenium in the culture as compared to these plant resources. This may reflect the unique feature of this specific mushroom in absorbing Se from the environment. In addition, the *t* test (**Table 6**) showed that Se had no effect on the distribution of amino acids, but only increased the content of all amino acids except cystine in a certain proportion. The amino acid contents of Se 100, Se 150, Se 200, and Se 250 of Se-GL samples were 1.383, 1.284, 1.189, and 1.195 times of that of *G. lucidum*, respectively.

Percentage of Seleno Amino Acids in Se-Bound Protein. To obtain information on selenocompounds in the protein fraction from three samples of Se-enriched *G. lucidum* with different Se contents, high-performance liquid chromatography (HPLC) was used to measure the content of selenomethionine, and a typical HPLC spectrum is given in **Figure 2**. The results are shown in **Figure 3**. For regular *G. lucidum*, selenomethionine could not be found by HPLC under the present conditions, so the result is not shown in **Figure 2**. In contrast, all samples of Se-enriched *G. lucidum* with different Se contents did contain a certain amount of selenomethionine, a direct result indicating that Se replaced S in methionine to form selenomethionine.

Surprisingly, with the increase of Se content in the samples of Se-enriched *G. lucidum*, the content of selenomethionine in the fruit body of Se-GL decreased, agreeing with the result of amino acid analysis showing that the methionine content of Se-enriched *G. lucidum* decreased as the selenium concentration in the culture increased (**Table 5**). This finding indicated that the high content of Se in *G. lucidum* inhibited the synthesis of selenomethionine. Thus, inorganic Se in the culture seems to have an effect on the synthesis of selenomethionine in Se-enriched *G. lucidum* through a dual mechanism, its stimulating the synthesis of selenomethionine at low concentrations of Se (<100 μg/g) and its retarding the synthesis of selenomethionine at high concentrations of Se (>200 μg/g).

Selenium in selenomethionine accounted for only 8.2−18.3% of the total Se content in protein. The remaining Se was most likely contributed from selenocysteine in the protein fraction on the basis of conclusions of a large body of work indicating that the accumulation of Se is mainly related to S-containing

Table 5. Total Protein and Amino Acid Contents of *G. lucidum* (GL) and Se-Enriched *G. lucidum* (Se-GL)

	content for given samples (g/100 g)				
	GL	Se-GL			
protein or amino acid	CK	Se 100	Se 150	Se 200	Se 250
protein	7.90 ± 0.07	10.67 ± 0.21[a]	9.80 ± 0.11[a]	8.72 ± 0.09[a]	9.73 ± 0.02[a]
total amino acids	4.439	6.005[a]	5.506[a]	5.211[a]	5.237[a]
aspartic acid	0.508	0.67	0.635	0.585	0.597
threonine	0.307	0.379	0.356	0.357	0.368
serine	0.315	0.382	0.356	0.351	0.358
glutamic acid	0.575	0.961	0.927	0.74	0.711
praline	0.256	0.305	0.255	0.261	0.269
glycine	0.282	0.356	0.331	0.31	0.311
alanine	0.268	0.37	0.337	0.329	0.347
cystine	**0.067**	**0.037**	**0.0218**	**0.023**	**0.017**
valine	0.25	0.407	0.326	0.34	0.302
methionine	0.04968	0.08262	0.07505	0.07399	0.06223
isoleucine	0.2292	0.2568	0.2164	0.2495	0.2695
leucine	0.3591	0.3947	0.3555	0.4195	0.4512
tyrosine	0.186	0.238	0.222	0.234	0.214
phenylalanine	0.251	0.336	0.317	0.283	0.294
lysine	0.202	0.29	0.258	0.262	0.265
histine	0.101	0.145	0.134	0.116	0.117
arginine	0.233	0.395	0.383	0.277	0.284

[a] $P < 0.05$ compared to CK.

Table 6. *t* Test[a]

	$\sum x_i y_i$	$\sum x_i^2$	$\sum y_i^2$	a_i	Q	$Q/(n-2)$	Sa	t
CK		1.4529						
Se 100	2.0100		2.8449	1.383	0.06507	0.004338	0.05464	$25.31 > t_{0.01}$
Se 150	1.8657		2.4708	1.284	0.07524	0.005016	0.05876	$21.85 > t_{0.01}$
Se 200	1.7270		2.0664	1.189	0.01300	0.0008667	0.02442	$48.68 > t_{0.01}$
Se 250	1.7361		2.0834	1.195	0.008761	0.0005841	0.02005	$59.60 > t_{0.01}$

[a] y is the amino acid content of Se-GL except cystine, whereas x is the amino acid content of GL except cystine. On the hypothesis that $y = ax$, a is calculated by $\sum x_i y_i / \sum x_i^2$, Q is calculated by $\sum y_i^2 - a\sum x_i y_i$, Sa is calculated by $Q/(n-2)/\sum x_i^2$, and t is calculated by a/Sa.

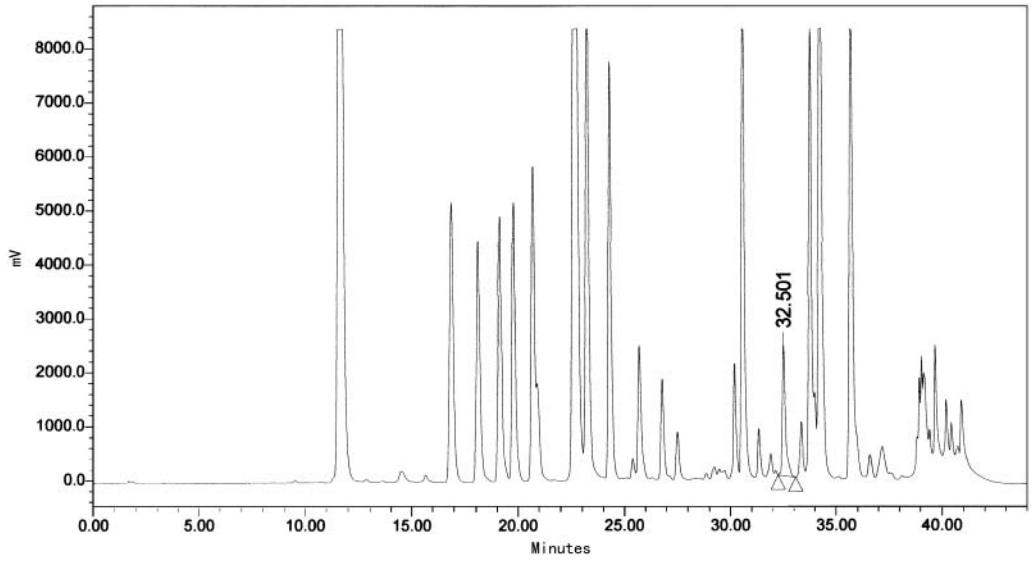

Figure 2. Typical HPLC spectrum for determination of selenomethionine. The retention time of selenomethionine is 32.051 min.

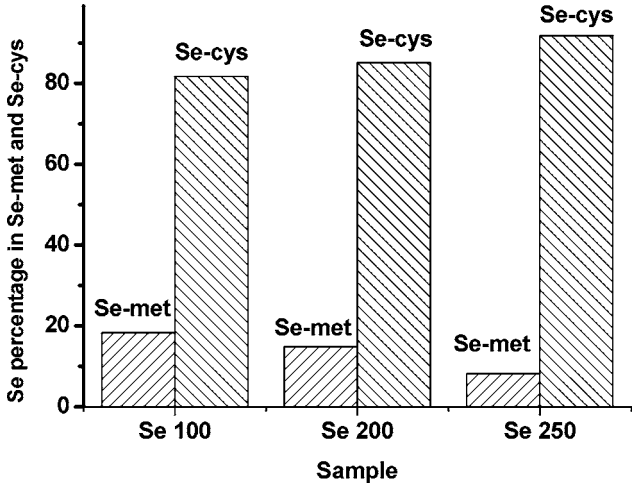

Figure 3. Percentage of Se incorporated with seleno amino acids in protein-bound Se. Percentage of Se incorporated with selenomethionine in protein-bound Se (Se-met%) was calculated using the following formula $\text{Se-met\%} = (C_{\text{Se-met}} \times \text{MW}_{\text{Se}}/\text{MW}_{\text{Se-met}})/C_{\text{protein-Se}} \times 100\%$, where $C_{\text{Se-met}}$ is the content of selenomethionine, MW_{Se} and $\text{MW}_{\text{Se-met}}$ are the molecular weights of Se and selenomethionine, respectively, and $C_{\text{protein-Se}}$ is the content of total Se bound to protein. Percentage of Se incorporated with selenocysteine in protein-bound Se (Se-cys%) was calculated by subtraction of Se-met% from 100%.

amino residues such as cysteine and methionine in proteins (*15*, *38*−*41*). Therefore, the Se content from selenocysteine could be calculated by the total Se content in proteins minus the Se content from selenomethionine, and the result is also shown in **Figure 3**. Because the content of selenomethionine decreased with the increase of Se content in proteins, the content of selenocysteine would increase accordingly. This proposal was consistent with the result of amino acid analysis showing that the cystine content in the protein fraction decreased from 0.067 to 0.017 g/100 g with increasing Se content in the samples of Se-enriched *G. lucidum* (**Table 5**).

We have recently reported that the Se-containing protein extract from Se-enriched *G. lucidum* exhibited much higher activities of scavenging superoxide radical and hydroxyl radical than its analogue, common *G. lucidum* extract (*29*). Because both selenocysteine and selenomethionine are known to show promising biological activities in vitro and in vivo against free radicals including $HO^{\bullet}$, $O_2^{\bullet -}$, and so on (*8*, *42*), they may be responsible, at least partly, for the reported antioxidant activities of the protein extract from this Se-enriched mushroom.

Conclusion. *G. lucidum* could absorb ∼20−30% of inorganic selenium in substrate and transform up to 87.92% of it into organic Se. A large proportion (64.6−69.8%) of organic selenium was incorporated with protein extracts. Water-soluble protein and alkaline-soluble protein were the major proteins to incorporate Se, and selenocysteine might be the major seleno-compound in proteins. The molecular mass of most proteins or protein subunits containing Se was no more than 16 kDa. At low concentration in the substrate, selenium increased the total protein and all amino acid contents; however, a high concentration of Se had an inverse effect. Furthermore, Se had no effect on the distribution of amino acids and proteins of Se-GL no matter what the Se concentration in the culture was.

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