JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

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Characterization of Selenium-Enriched Mycelia of *Catathelasma ventricosum* and Their Antihyperglycemic and Antioxidant Properties

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ABSTRACT: This is the first report concerning the selenium enrichment of *Catathelasma ventricosum* mycelia. The selenium-containing proteins present in selenium-enriched mycelia (Se-MC) were identified using size-exclusion chromatography−inductively coupled plasma−mass spectrometry (SEC-ICP-MS). The selenium-containing amino acids liberated by hydrolysis of these proteins were identified using anion exchange-ICP-MS. Se-MC was found to contain selenoproteins with molecular weights ranging from 1.7 to 60.5 kDa. The main selenium-containing amino acids within them were selenomethionine and selenocysteine. Furthermore, Se-MC possessed excellent antihyperglycemic and antioxidant properties. Se-MC normalized biochemical parameters like insulin level, blood glucose level, body weight, and antioxidant enzyme activity in streptozocin-induced diabetic mice. It also inhibited the α-amylase and α-glucosidase activities present in in vitro gastric and intestinal models. In conclusion, Se-MC has the potential to serve as a dietary supplement of selenium, an antioxidant, or an ingredient for the formulation of nutraceuticals.

KEYWORDS: *mushroom, antihyperglycemic activity, antioxidant activity, chemical composition, selenium speciation*

INTRODUCTION

Diabetes mellitus, a chronic metabolic disease caused by insulin deficiency or dysfunction, is characterized by high blood glucose levels.[1] Hyperglycemia has been shown to increase oxidative stress through the overproduction of reactive oxygen species.[2] These oxygen radicals cause lipid oxidation and play a role in the development of diabetic complications. Oxidative stress is thought to link diverse mechanisms for the pathogenesis of diabetic complications.[3] Although several hypoglycemic drugs are available to treat diabetes mellitus, many of them have serious side effects.[4] Relatively low cost management of hyperglycemia with minimal clinical side effects remains a challenge for the medical system.[5] Phytochemicals from relatively low cost bioresources have relatively few side effects. Exploiting this resource offers a new approach for the treatment of various diseases, including diabetes.[6]

Catathelasma ventricosum, an edible fungus, is a delicious source of valuable nutrition. Its fruiting body provides an excellent source of nutrients because of its high protein, polysaccharide, and mineral content, as well as its low content of toxic metals, fat, and energy. A previous report has demonstrated that the fruiting body of wild *C. ventricosum* has antihyperglycemic and antioxidative effects.[7] Selenium (Se), a trace element important for human health, is a component of several selenoproteins that perform essential biological functions, including glutathione peroxidase (GSH-Px) and selenoprotein P. The Se-dependent enzyme GSH-Px reduces oxidized glutathione and minimizes lipid peroxidation by catalyzing the reduction of both lipid peroxides and hydrogen peroxide. Selenoprotein P, an extracellular antioxidant present in the vascular endothelium, lowers the level of peroxinitrile ($ONOO^-$), a reactive nitrogen species.[8] Se deficiency has been associated with several diseases, including hepatocyte damage, cardiovascular disease, and cancers.[9−11] Se supplementation using microorganisms may be an excellent solution to the problem of Se deficiency because organic Se compounds are generally believed to be better and safer dietary supplements than inorganic Se compounds. A variety of Se-enriched food supplements have been commercialized, including garlic, yeast, and lactic acid bacteria.[12]

Submerged basidiomycete culture is currently the preferred technique for producing stable, safe mycelia and mushroom biometabolites. Importantly, submerged culture is less time-consuming than mushroom cultivation and leads to the production of products with mushroom metabolite contents that are more consistent than those of products consisting of fruiting bodies.[1] Furthermore, based on our preliminary experiment, the capacity of *C. ventricosum* for selenium enrichment is greater than that of some famous mushrooms, like *Lentinula edodes, Pleurotus ostreatus, Ganoderma lucidum, Coriolus versicolor, Tremella fuciformis,* etc. (data not shown). In order to assess the suitability of Se-enriched mycelia from *C. ventricosum* (Se-MC) as a candidate for diabetes treatment, the

Received: October 17, 2014
Revised: December 22, 2014
Accepted: December 22, 2014
Published: December 22, 2014

DOI: 10.1021/jf5050316
J. Agric. Food Chem. 2015, 63, 562−568

chemical composition and Se speciation in Se-MC was determined. The antihyperglycemic and antioxidant properties of Se-MC were also investigated.

MATERIALS AND METHODS

Chemicals. Sodium selenite and sodium selenate were obtained from Alfa Aesar (Ward Hill, MA, USA). Working solutions for the determination of total selenium were prepared daily by appropriate dilution of a 1 mg/mL Se (IV) standard solution (Merck, Darmstadt, Germany). Glibenclamide, streptozocin (STZ), 4-nitrophenyl-α-D-glucopyranoside (PNPG), selenocysteine (SeCys), selenomethionine (SeMet), and amino acid standards were purchased from Sigma-Aldrich Co. LLC (St. Louis, MO, USA). Reagent kits for the determination of catalase (CAT), maleic dialdehyde (MDA), superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and insulin were obtained from the Jianchen Bioengineering Institute (Nanjing, Jiangsu, China). The SXT Blood Glucose Monitoring System and Test Strip were from Sinocare Co., Ltd. (Changsha, Hunan, China). All other materials were of the highest grade commercially available and obtained from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China).

Se-Enriched Fermentation *C. ventricosum* Mycelia. The seed of *C. ventricosum* was purchased from Mianyang Edible Fungi Research Institute (Mianyang, Sichuan, China) and maintained on synthetic potato dextrose agar (PDA).

C. ventricosum was initially grown on PDA at 22 °C for 4 days. Aliquots (2.5 mL) of this 4-day-old liquid culture were then transferred to two hundred 250 mL flasks. Each flask contained 47.5 mL of Se-enriched fermentation culture medium (medium components were based on our previous reports[13]) and incubated at 22 °C on a shake platform at 100 rpm for 15 days. The harvest mycelia are referred to as Se-MC. *C. ventricosum* was also incubated in an otherwise identical fermentation culture medium without Na_2SeO_3 as a control. This control material is referred to as MC.

In Vitro Simulation of Gastrointestinal Digestion. Gastrointestinal digestion was simulated using a method based upon a published procedure that mimicks the physiological events taking place in the upper gastrointestinal tract (stomach and small intestine).[14] Three-gram samples of powdered *C. ventricosum* mycelia (Se-MC and MC were freeze-dried and ground to a fine powder (40 mesh)) were mixed with 18 mL of saline (140 mM NaCl, 5 mM KCl and 150 μM butylated hydroxytoluene (BHT)), acidified to pH 2 with 0.1 M HCl, and then mixed with 500 μL of pepsin solution. After incubation at 37 °C for 1 h in a shaking water bath, 0.1 M $NaHCO_3$ was added to increase the pH to 6.9. At this point, 2.5 mL of pancreatin-bile solution was added. After incubation at 37 °C for 2 h in a shaking water bath, the simulated gastrointestinal digestion (GI) extract was centrifuged, and then the supernatant was dried under nitrogen gas. The dried powder was used for the in vitro assay. The samples of Se-MC and MC were treated in triplicate.

Chemical Composition. *Proximate Analysis.* The crude protein, crude fat, moisture, ash, and total phenolic contents of Se-MC and MC were determined in triplicate using the Association of Official Analytical Chemists (AOAC) procedures (1995).[15] The determination of carbohydrate content (g) and energy content (KJ) were performed based on the methods of Liu et al. (2012).[7]

Amino Acid Analysis. Extracts were prepared according to the method of Ribeiro et al. (2008).[16] Amino acid analysis was performed using our previously published methods,[7] with slight modifications. Briefly, the amino acid composition was determined using a reversed-phase high-performance liquid chromatography (HPLC, Agilent 1100) system equipped with a 250 × 4.6 mm, 5 μm ODS HYPERSIL column (Agilent) (Santa Clara, CA, USA). The analysis was conducted using gradient elution at 40 °C and a flow rate of 1 mL/min. The mobile phase gradient was formed from buffer A (8 g of sodium acetate dissolved in 1000 mL of deionized water plus 225 μL of triethylamine and 5 mL of tetrahydrofuran; pH adjusted to 7.2 ± 0.05 with acetic acid) and buffer B (8 g of sodium acetate dissolved in 400 mL of deionized water plus 800 mL of acetonitrile and 800 mL of methanol; pH adjusted to 7.2 ± 0.05 with acetic acid). A/B ratios were 92:8, 40:60, 0:100, 0:100, 0:100, and 92:8, at run times of 0, 27.5, 31.5, 32, 34, and 35.5 min, respectively. Samples were derivatized with *o*-phthaldialdehyde (OPA, Sigma) (St. Louis, MO, USA) prior to HPLC; the detection wavelength was set to 338 nm, or 262 nm for proline. Stock solutions of individual amino acids, except for tryptophan, were prepared in 0.1 M HCl. Tryptophan solutions were prepared in water. The concentrations of the individual amino acids were approximately 1 mM. The samples of Se-MC and MC were analyzed in triplicate.

The Content of Se and Its Speciation. *Determination of Total Se.* Dried samples (approximately 0.1 g) were digested with a mixture containing 0.5 mL of H_2O_2 (30%) and 2 mL of HNO_3 (65%). After 24 h, the solutions were digested using a microwave system. A mineralization program was performed based on the method of Wrobel et al. (2004).[17] After digestion, the final solutions were diluted to a total volume of 25 mL with distilled water. The standard solutions and reagent blanks were digested using the same procedure. The samples of Se-MC and MC were treated in triplicate.

The total selenium concentration was determined using inductively coupled plasma mass spectrometetry (ICP-MS, Agilent 7500ce) (Tokyo, Japan). Isotopes ^{77}Se, ^{78}Se, ^{80}Se, and ^{82}Se were monitored. The instrument settings were as follows: forward power, 1590 W; carrier gas flow rate, 1.2 L/min; plasma gas flow rate, 15 L/min; and dwell time, 0.3 s per isotope. All samples were analyzed in triplicate.[18]

Investigation of Selenoproteins in Mycelia. Mycelia samples (1 g) were added to 100 mL of buffer containing 20 mM Tris-HCl (pH 7.5) and 150 mM NaCl. The mixtures were extracted with magnetic stirring at 37 °C for 24 h. After centrifugation (8000 rpm for 10 min), 1 mL of supernatant was transferred to 5 mL of acetone. This mixture was extracted with magnetic stirring at 4 °C for 24 h, and then placed at 5 °C for 24 h. After centrifugation (8000 rpm for 10 min), the supernatant was discarded and the protein precipitate was dried under nitrogen gas. The samples of Se-MC and MC were treated in triplicate.

The dry precipitate was resolubilized with 1 mL of mobile phase buffer for analysis by size exclusion chromatography (SEC)-ICP-MS. Chromatographic separations were performed using an Agilent 1100 liquid chromatograph (Palo Alto, CA, USA) equipped with a Superdex peptide 10-300 GL (300 mm × 10 mm × 15 μm) column. The mobile phase was composed of 20 mM Tris-HCl and 150 mM NaCl (pH 7.5), the injection volume was 50 μL, and the flow rate was 0.5 mL/min.[18] The SEC column was calibrated using a standard mixture of albumin (66 kDa), myoglobin (16 kDa), cytochrome *c* (12.5 kDa), methylcobalamin (1.34 kDa), and $(Gly)_6$ (0.36 kDa). All samples were analyzed in triplicate.

Se Speciation in the Mycelia. Enzymatic hydrolysis was performed to identify the species that possess covalent bonds between Se and proteins or peptides.[19] In this method, the precipitated selenoproteins were resolubilized with 1 mL of a solution containing 20 mM Tris-HCl (pH 7.5), 150 mM NaCl, and 0.03 g of trypsin. After incubation at 50 °C for 24 h in a shaking water bath, 0.03 g of proteinase K was added to the mixture described above, and the resulting solution was kept in a shaking water bath at 50 °C for an additional 24 h. After centrifugation (8000 rpm for 10 min) and filtration (0.45 μm polyvinylidene fluoride filter), the supernatant was passed through 5 kDa molecular weight cutoff filters (Ultrafree-0.5, Millipore) to remove high molecular weight proteins from the extracts before the analysis of the Se-containing species. The samples were treated in triplicate.

Se species were analyzed by anion exchange-HPLC-ICP-MS and identified by comparing their retention times with those of available standards (sodium selenite, sodium selenite, SeCys, and SeMet).[20] Chromatographic separations were performed using an Agilent 1100 liquid chromatograph (Palo Alto, CA, USA) equipped with a Hamilton PRP-X100 (250 mm × 4.1 mm × 10 μm) column. The mobile phase consisted of 5 mM ammonium citrate in 2% methanol (pH 5.2), the flow rate was 1 mL/min, and the injection volume was 50 μL. All samples were analyzed in triplicate.[18]

Antihyperglycemic Assays in Vitro. *α-Glucosidase Inhibitory Activity.* Inhibition of α-glucosidase activity was assayed using the

DOI: 10.1021/jf5050316
J. Agric. Food Chem. 2015, 63, 562−568

Table 1. Moisture (g/100 g of Fresh Weight), Macronutrients (g/100 g of Dry Weight), and Total Energy (kJ/100 g of Dry Weight) of *C. ventricosum*

	components						
C. ventricosum	moisture	ash	crude protein	crude fat	total carbohydrates	total energy	total phenols
selenium-enriched mycelia	93.03 ± 3.67[a]	10.64 ± 1.48	31.22 ± 2.59	2.17 ± 0.34	55.97 ± 4.94	1562.52 ± 87.42	0.44 ± 0.04
mycelia	93.55 ± 2.07	9.29 ± 1.17	21.42 ± 5.03	6.19 ± 0.29	63.10 ± 7.39	1665.87 ± 102.53	0.83 ± 0.08

[a]Each value is expressed as the mean ± SD (n = 3).

method of Palanisamy et al. (2011), with minor modifications.[3] Various dilutions of the GI extracts described above were mixed with α-glucosidase (0.4 IU) dissolved in 2 mL of phosphate buffer (0.1 M, pH 6.8) containing 50 μL of glutathione (1 mg/mL) and 50 μL of PNPG (0.1 M). These reaction mixtures were incubated at 37 °C for 15 min. The reactions were stopped with 10 mL of 0.1 M sodium carbonate, and the extent of reaction was determined by measuring the absorbance at 400 nm using a UV−visible spectrophotometer. Acarbose was used as the positive control, while the absence of sample was used as a negative control. The percentage inhibition was calculated as % inhibition = {1 − [(absorbance of sample)/(absorbance of negative control)]} × 100. The α-glucosidase inhibitory activity was expressed as an EC_{50} value (amount of sample causing 50% inhibition). All samples were analyzed in triplicate.

α-Amylase Inhibitory Activity. Porcine pancreatic α-amylase (1 IU) was dissolved in 0.5 mL of buffer (20 mM phosphate, pH 6.9) containing various dilutions of the GI extracts described above. These mixtures were incubated at 37 °C for 15 min. Each reaction mixture was subsequently added to 0.5 mL of soluble starch solution (1.5%) and incubated at 37 °C for 5 min. Each reaction was stopped at this point using 1.0 mL of 3,5-dinitrosalicylic acid reagent. The samples were then incubated in a boiling water bath for 10 min, cooled to room temperature, and diluted with distilled water. The absorbance was measured at 520 nm. Acarbose was used as the positive control, and reactions lacking sample were used as a negative control. The percentage inhibition was calculated as % inhibition = {1 − [(absorbance of sample)/(absorbance of negative control)]} × 100. The α-amylase inhibitory activity was expressed as an EC_{50} value (amount of sample causing 50% inhibition). All samples were analyzed in triplicate.

Animals. The male ICR mice (body weight 18 ± 2 g) used in this study were purchased from SLAC Laboratory Animal Company (Shanghai, China). The mice were acclimatized for at least 1 week before the experiment was started. Before and during the experiment, the mice were housed in a segregated air-conditioned room at 25 °C with a 12 h light and dark cycle. Mice were provided a basal diet (Shanghai Laboratory Animal Company) and free access to drinking water. This experiment was approved by the Institutional Animal Ethics Committee of Jiangnan University (Wuxi, China). All experimental animals were overseen and approved by the Animal Care and Use Committee of our Institute before and during experiments.

Establishment of a Diabetic Mouse Model. Diabetes was induced in mice by the intraperitoneal (ip) injection of a freshly prepared solution of STZ at a dose of 150 mg/kg body weight.[21] Mice displaying polydipsia and polyuria after 72 h were chosen for measurement of their fasting glucose levels using blood glucose test strips. Hyperglycemic mice (blood glucose level >16.8 mmol/L) were used as the diabetic mice for further studies.

Experimental Design. Based on a preliminary experiment (data not shown), the STZ-induced diabetic mice were randomly divided into five groups (6 mice per group). Group I: normal mice treated with saline solution as control. Group II: STZ-induced diabetic mice treated with saline solution as diabetic control. Group III: STZ-induced diabetic mice receiving oral glibenclamide (20 mg/kg/d) as a positive control. Group IV: STZ-induced diabetic mice receiving oral MC at a dose of 500 mg/kg/d. Group V: STZ-induced diabetic mice receiving oral Se-MC at a dose of 500 mg/kg/d. After 30 days of treatment, the animals were fasted overnight and sacrificed by cervical dislocation. Their blood glucose levels and body weight were measured. Blood collected from their eyes was immediately centrifuged for 5 min to obtain serum (stored at −70 °C). Kidney and liver tissues were excised from the animals, washed with saline solution, and stored at −70 °C.

Biochemical Analysis. Blood glucose levels were determined with blood glucose test strips using blood samples taken from the tail veins of overnight fasted mice. The serum insulin concentration was determined using commercially available kits according to the manufacturer's instructions. Liver and kidney homogenates were immediately assayed for SOD, CAT, GSH-Px, and MDA activities using the procedures described in the instructions contained in their corresponding assay kits.

Statistical Analyses. All of the assays described above were carried out in triplicate. The results are expressed as the mean ± SD. Data were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's test. Statistical analyses were performed using SPSS software, version 16.0. Differences leading to values of $p < 0.05$ were considered statistically significant.

RESULTS

Chemical Composition. The proximate compositions of the investigated mushrooms are shown in Table 1. The order of the contents (g/100 g dry weight) was total carbohydrate > crude protein > ash > crude fat > total phenols. These data demonstrate that Se-MC contained slightly more protein and ash than the control MC, and slightly less total carbohydrate. The crude fat and total phenol contents of Se-MC were about half or less of those of the MC control. The total energy content of the Se-MC was slightly lower than that of the MC control.

The percentage composition of 17 amino acids was investigated in the mycelia of *C. ventricosum*; the results are presented in Table 2. Se-MC and MC were found to contain 13 and 12 amino acids, respectively. Se-MC contained seven of the essential amino acids, lacking only isoleucine. The ratios of the essential amino acids to nonessential amino acids were 0.87 and 0.80 in Se-MC and MC respectively.

The Content of Se and Its Speciation. The total Se contents of Se-MC and the MC control were determined by digesting samples and quantifying the Se by ICP-MS. The total Se content of Se-MC was 867.11 μg/g of dry mycelia. The mycelial yield was about 12.64 g of dry mycelia per L of culture. The conversion of organic Se was approximate 11%. No Se was detected in the MC control.

The SEC-ICP-MS chromatograms of the Se-MC extracts are presented in Figure 1a. The molecular weights (MW) of the three main Se-containing fractions were about 60.5, 11.2, and 1.7 kDa. Based on the description of Kapolna et al. (2007),[18] the fractions with high molecular weights (HMW; 60.5 and 11.2 kDa) represent Se-containing proteins, and those with the low molecular weight (LMW) of 1.7 kDa correspond to Se-containing peptides, while the molecular weights of selenoamino acids and inorganic selenium are less than 1.7 kDa.

A chromatogram of the Se species standards, obtained using anion exchange-HPLC-ICP-MS, is displayed in Figure 1b. The

DOI: 10.1021/jf5050316
J. Agric. Food Chem. 2015, 63, 562−568

Table 2. Free Amino Acid Composition (Wt %) of *C. ventricosum*

amino acids[a]	*C. ventricosum*	
	Se-enriched mycelia (Se-CM)	mycelia (CM)
Val[b]	13.59 ± 0.63[c]	12.14 ± 0.53
Thr[b]	1.45 ± 0.49	7.92 ± 0.82
Met[b]	16.37 ± 0.51	4.06 ± 0.31
Leu[b]	1.07 ± 0.05	ND[d]
Ile[b]	ND	ND
Trp[b]	4.19 ± 0.63	8.63 ± 0.77
Phe[b]	2.73 ± 0.34	5.19 ± 0.49
Lys[b]	7.11 ± 0.39	6.48 ± 0.67
Gly	1.06 ± 0.08	1.53 ± 0.08
His	10.52 ± 0.96	12.15 ± 0.75
Tyr	ND	ND
Asp	ND	ND
Ser	8.74 ± 1.02	17.53 ± 1.24
Cys	9.74 ± 0.18	1.46 ± 0.16
Arg	ND	ND
Ala	18.62 ± 2.74	12.18 ± 0.21
Glu	4.81 ± 0.43	10.73 ± 1.11

[a]Valine (Val); threonine (Thr); methionine (Met); leucine (Leu); isoleucine (Ile); tryptophan (Trp); phenylalanine (Phe); lysine (Lys); glycine (Gly); histidine (His); tyrosine (Tyr); aspartic acid (Asp); serine (Ser); cysteine (Cys); arginine (Arg); alanine (Ala); glutamic acid (Glu). [b]Essential amino acids. [c]Each value is expressed as mean ± SD (n = 3). [d]ND: not detected.

species were identified by comparing their retention times to those of available standards. The selenium species profile of the Se-MC extracts is shown in Figure 1c. Selenomethionine (SeMet) was the predominant selenium species in the Se-MC extracts, comprising approximately 68% of the total selenium content. Other selenium species identified in Se-MC samples included selenocysteine (SeCys, 6%), selenite (Se−IV, 2%), and selenate (Se−VI, 5%).

Antihyperglycemic Activity in Vitro. The antihyperglycemic properties of Se-MC and MC were evaluated using two assays that measured the inhibition of α-glucosidase and α-amylase activities, respectively. Based on the results, the highest α-glucosidase inhibitory activity (EC_{50} = 8.3 μg/mL) was found in Se-MC, and the highest α-amylase inhibitory activity (EC_{50} = 19.4 μg/mL) was found in MC, while the α-glucosidase and α-amylase inhibitory activities of acarbose (positive control) were 15.9 and 26.7 μg/mL, respectively.

Effects of Se-MC and MC on Blood Glucose, Body Weight, and Insulin in STZ-Induced Diabetic Mice. Mice in the diabetic control group (group II) exhibited a severe decrease in insulin level and body weight and a significant increase in blood glucose level, compared with mice in the normal group (group I; $p < 0.05$) (Table 3). After administration of Se-MC, MC, or glibenclamide (positive control) for 30 days, the levels of blood glucose in groups III, IV, and V were much lower than that in group II. On the other hand, the insulin levels and body weights of the mice in groups III, IV, and V showed a significant increase compared with those of the mice in group II.

Effects of Se-MC and MC on MDA and Antioxidant Enzymes in Kidney and Liver of STZ-Induced Diabetic Mice. The MDA levels in the kidney and liver of untreated STZ-induced diabetic mice were significantly increased ($p < 0.05$), whereas the activities of antioxidant enzymes in the kidney and liver of untreated STZ-induced diabetic mice were significantly decreased ($p < 0.05$) compared with those of the mice in group I (Table 4). After administration of Se-MC or MC for 30 days, the MDA levels present in the mice in groups IV and V were significantly decreased ($p < 0.05$), while SOD, GSH-Px, and CAT activity were significantly increased ($p < 0.05$) in the kidney and liver, compared with those of the mice in groups II and III.

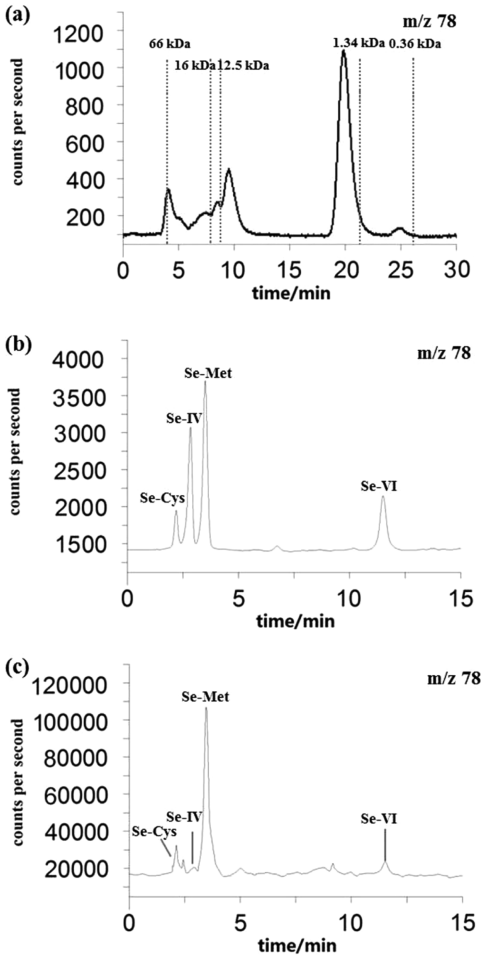

Figure 1. HPLC-ICP-MS chromatograms of the mycelia of Se-enriched *C. ventricosum*. (a) Protein fractions of Se-MC were analyzed by SEC-ICP-MS. (b) Chromatogram of Se species standard obtained by anion-exchange ICP-MS. (c) Chromatogram of Se species profile in Se-MC obtained by anion-exchange ICP-MS.

DISCUSSION

The moisture content of mushrooms ranges from 86−94 g/100g fresh weight. The moisture contents of Se-MC and MC were 93.03 and 93.55 g/100 g fresh weight, respectively, which is higher than that of the fruiting body of *C. ventricosum*.[7] It seems that liquid culture conditions can increase the moisture content of *C. ventricosum*. Kalač (2009) indicated that low dry matter and lipid contents result in mushrooms with low energy values.[22]

The concentration order of the components in the proximate analyses of Se-MC and MC is consistent with that revealed in our previous report.[7] This indicates that the physical state of the culture medium (liquid state or solid state) may have a limited effect on the chemical composition of *C. ventricosum*.

DOI: 10.1021/jf5050316
J. Agric. Food Chem. 2015, 63, 562−568

Table 3. Effects of Selenium-Enriched Mycelia and Mycelia on Body Weight, Blood Glucose, and Insulin in Diabetic Mice[a]

	group I (control)	group II (diabetic mice)	group III (positive control)	group IV (diabetic mice + MC)	group V (diabetic mice + Se-MC)
insulin level (μIU/mL)	28.7 ± 1.7[c]	8.3 ± 0.8 a	19.1 ± 2.0 b	11.2 ± 0.7 a	18.3 ± 1.2 b
blood glucose (mmol/L)	5.8 ± 0.6 a	26.3 ± 3.1 d	11.5 ± 1.1 b	17.7 ± 1.4 c	13.1 ± 0.9 b
body weight (g)	35.3 ± 1.5 d	25.8 ± 3.2 a	32.5 ± 2.4 c	28.7 ± 1.4 b	28.2 ± 1.7 b

[a]Each value is expressed as the mean ± SD (n = 6). Means with different letters within a row are significantly different (p < 0.05).

Table 4. Effect of Selenium-Enriched Mycelia and Mycelia on Antioxidants in Liver and Kidney of Diabetic Mice[a]

	group I (control)	group II (diabetic mice)	group III (positive control)	group IV (diabetic mice + CM)	group V (diabetic mice + Se-CM)
			Liver		
GSH-Px (U/mg protein)	877.1 ± 74.1 d	413.5 ± 32.3 a	442.0 ± 29.7 a	593.6 ± 30.1 b	797.4 ± 59.6 c
SOD (U/mg protein)	478.1 ± 39.5 c	277.3 ± 14.6 a	234.9 ± 10.7 a	299.7 ± 19.4 b	424.5 ± 21.7 c
CAT (U/mg protein)	194.4 ± 20.1 c	41.7 ± 2.6 a	49.9 ± 2.8 a	92.7 ± 10.7 b	167.4 ± 17.3 c
MDA (nmol/mg protein)	4.2 ± 0.8 a	9.0 ± 0.7 c	8.5 ± 0.8 c	6.2 ± 1.1 b	4.7 ± 0.7 a
			Kidney		
GSH-Px (U/mg protein)	564.2 ± 34.9 c	277.4 ± 17.7 a	263.7 ± 20.4 a	400.3 ± 35.2 b	584.5 ± 67.6 c
SOD (U/mg protein)	277.3 ± 14.9 c	104.4 ± 8.5 a	115.8 ± 11.0 a	197.6 ± 20.4 b	283.7 ± 17.7 c
CAT (U/mg protein)	83.8 ± 7.5 c	37.5 ± 3.7 a	42.1 ± 3.5 a	66.3 ± 4.7 b	89.1 ± 9.5 c
MDA (nmol/mg protein)	3.1 ± 0.5 a	7.3 ± 0.4 d	7.3 ± 0.6 d	4.9 ± 0.2 c	3.8 ± 0.7 b

[a]Each value is expressed as the mean ± SD (n = 6). Means with different letters within a row are significantly different (p < 0.05); glutathione peroxidase (GSH-Px); superoxide dismutase (SOD); catalase (CAT); maleic dialdehyde (MDA).

The concentration of protein in Se-MC (31.22% of dry matter) is very high compared with those of most plants and mushrooms. The results are consistent with the conclusions of Belitz and Grosch (1999):[23] Se concentrations are positively correlated with protein content in food. Moreover, Pappa et al. (2006)[24] indicated that the Se concentration is negatively correlated with food fat content. The concentration of crude fat in Se-MC (2.17% of dry matter) is very low compared with those of most plants and mushrooms.[22] In a word, *C. ventricosum* is an ideal candidate for Se enrichment.

The content of crude protein in Se-MC, on the other hand, was 30% higher than that in MC. It seems that the presence of Se in the culture medium can affect protein metabolism in *C. ventricosum*. One possibility is that Se can replace sulfur in amino acids like selenomethionine (SeMet) and selenocysteine (SeCys), due to their physicochemical similarity, which may stimulate the synthesis of protein (especially Met and Cys). The results of the amino acid analysis also support this assumption. The concentration of methionine (Met) and cysteine (Cys) in Se-MC was much higher than that in MC. Moreover, Dumont et al. (2006)[25] indicated that most plants lack the ability to accumulate large amounts of Se (they rarely exceed 100 μg/g dry weight). However, some mushrooms have been recognized as Se accumulators. These mushrooms can take up a large amount of Se (>1000 μg/g dry weight) without exhibiting any negative effects because they increase their intracellular concentrations of SeCys and SeMet, which are normally incorporated into proteins.[8,25]

Although selenium is beneficial to normal biological function when supplied in appropriate quantities, excessive selenium intake can cause toxicosis.[26] The essential and toxic levels of Se are similar. The tolerable upper Se intake level for adults is set at 400 μg/day, while the recommended dietary allowance for Se is 55 μg/day for both men and women.[27] Therefore, determination of the total Se content of Se-MC is of great significance. The total Se content of Se-MC (867.11 μg/g of dry mycelia) is higher than that of some other Se-enriched plants and mushrooms, like *Allium schoenoprasum* (1.07 μg/g), *Lentinula edodes* (770.7 μg/g), and *Cordyceps militaris* (42 μg/g).[18,19,28]

The bioavailability of Se depends strongly on the chemical form of the Se that is ingested. Organic Se compounds are generally more bioavailable than inorganic Se compounds.[8] Ferri et al. (2007)[29] indicated there is a lack of information about the chemical forms of selenium in foods. This lack is mainly the result of the difficulties inherent in their identification. The application of different hyphenated techniques (HPLC-MS, HPLC-ICP-MS, etc.) has begun to change this situation. In this study, we employed HPLC-ICP-MS to analyze the Se species. Previous reports have identified selenate, selenite, SeMet, and SeCys as the primary selenocompounds in mushrooms.[19,28] These reports agree well with the results of this study. In addition, the ratio of organic Se to inorganic Se in Se-MC is 74:7, which is higher than the ratio seen in some Se-enriched foods.[20,28]

Two in vitro assays were carried out to evaluate the antihyperglycemic activity of a simulated gastrointestinal digestion extract of Se-MC and MC: an α-glucosidase inhibition assay and an α-amylase inhibition assay. α-Glucosidase, located in the brush-border surface membrane of intestinal cells, is a key enzyme of carbohydrate digestion. α-Amylase catalyzes the hydrolysis of the α-1,4-glucosidic linkages in glycogen, starch, and other oligosaccharides. Inhibition of the α-glucosidase and α-amylase activities in the human digestive tract can effectively control diabetes by diminishing the absorption of glucose derived from the degradation of starch by these enzymes.[30] There are two main concepts to explain the interaction mechanism between α-glucosidase/α-amylase and their inhibitor: (1) The inhibitor inhibits α-glucosidase/α-amylase activity by hydrolyzing them.[31] (2) α-Glucosidase/α-amylase and their inhibitor combine to form a soluble complex, which inactivates the enzymes.[32] Yasuda (2003)[33] indicated

DOI: 10.1021/jf5050316
J. Agric. Food Chem. 2015, 63, 562−568

that α-glucosidase/α-amylase inhibitor is not a competitive inhibitor that competes with α-glucosidase/α-amylase for starch, disaccharides, or various oligosaccharides. However, the inhibitor will combine with α-glucosidase/α-amylase directly and change their conformations, inactivating them. Acarbose, an inhibitor of a-glucosidase and α-amylase used in the treatment of diabetes mellitus, was chosen as the positive control.[3] The inhibitory activities of Se-MC and MC were significantly greater than those of acarbose ($p < 0.05$), which indicates that Se-MC and MC possess excellent antihyperglycemic activity. Inhibition of intestinal α-glucosidase and α-amylase activities is an important antihyperglycemic mechanism of Se-MC and MC. However, the antihyperglycemic activities of Se-MC and MC are very similar. It seems that adding Se into the culture medium does not have has much of an influence on the α-glucosidase and α-amylase inhibitory activities of *C. ventricosum* mycelium.

Animal models in which diabetes mellitus is induced by the administration of STZ or other chemicals have been reported.[21] The advantages of STZ are its greater stability and relative lack of extrapancreatic toxicity. In the present study, the diabetic mouse model was established by intraperitoneal injection of STZ, and all of the STZ-induced diabetic mice displayed polydipsia and polyuria.

Stapleton (2000)[34] reported that selenium has been found to have insulin-mimetic activities in vitro and in vivo. Based on the results presented here, Se-MC possesses better antihyperglycemic properties than MC. The levels of blood glucose and insulin in the mice in groups III and V were very close ($p > 0.05$), although they did not recover completely (compared with the normal group, $p < 0.05$). However, the body weight of the mice in groups IV and V was significantly lower than that of the mice in the positive control (group III) and normal mice (group I). It seems that both Se-MC and MC lack a strong ability to increase body weight in STZ-induced diabetic mice. Chen and Ianuzzo (1982)[35] suggested that the decrease in body weight observed in diabetic mice might be the result of protein wasting caused by the inability to use carbohydrates as an energy source. Zhao et al. (2011)[36] reported that administration of *Opuntia dillenii* polysaccharides significantly increased the body weight of STZ-induced diabetic mice.

Diabetes mellitus is associated with the generation of reactive oxygen species that cause oxidative damage to the liver and kidney.[37,38] Major antioxidant enzymes, including GSH-Px and SOD, are the first line of defense against the reactive oxygen species generated during oxidative stress in vivo.[39,40] In the present study, a significant increase in MDA levels and a significant decrease in GSH-Px, SOD, and CAT activity were found in kidney and liver of mice with STZ-induced diabetes (group II; compared with mice in group I), which suggests that severe oxidative stress occurred in liver and kidney tissues. However, after treatment with Se-MC (mice in group IV) or MC (mice in group V), MDA levels were significantly decreased ($p < 0.05$) and GSH-Px, SOD, and CAT activity were significantly increased ($p < 0.05$) in liver and kidney, compared with those of mice in group II. In particular, except MDA levels in kidney, activities of GSH-Px, SOD, CAT, and MDA in group V almost returned to their normal level, which indicated that Se-MC possesses a strong effect on increasing the synthesis of these antioxidant enzymes. MDA, the end product of lipid peroxidation, is used as an index of cellular damage and cytotoxicity. The MDA generated by high levels of free radicals can damage proteins and inactivate membrane-bound enzymes.[41] Chen et al. (2013)[39] indicated that decreasing MDA levels in diabetic rats may be associated with a drug's potential organ protective effect through improved glycometabolism and lipid metabolism. Therefore, it can be inferred that Se-MC probably exhibits its antihyperglycemic property by increasing these antioxidant enzymes in diabetic mice. These enzymes protect tissues from peroxidative damage and maintain their function, improving the sensitivity and response of target cells to insulin. Simultaneously, after the administration of glibenclamide, there was no difference among these biochemical parameters in group III compared with group II, which reveals that glibenclamide does not have an obvious ability to stimulate the synthesis of these antioxidant enzymes. This observation is consistent with those of a previous study, which found that glibenclamide is not capable of modulating antioxidant enzymes and diminishing STZ-induced damage to the liver, which is the main organ to synthesize antioxidant enzymes.[13]

In conclusion, the selenoproteins in Se-MC possess a wide range of molecular weights and SeMet and SeCys were identified as the main selenium compounds bound to these proteins. Moreover, Se-MC was found to be an effective antihyperglycemic and antioxidant. These results demonstrate that the mycelia of Se-enriched *C. ventricosum* can be used as a source of nutrients because of their high protein and carbohydrate content and their low fat and energy content. Mushrooms can also be uesd as a natural source of Se supplements for both animals and humans, especially in Se-deficient areas. Future studies may show that the Se-enriched mycelium of *C. ventricosum* is a source of new antidiabetic drugs, chemopreventive preparations, or food supplements.

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Funding

This work was supported by Scientific Research Foundation of Sichuan Agricultural University (No. 06021400).

Notes

The authors declare no competing financial interest.

ABBREVIATIONS USED

CAT, catalase; GSH-Px, glutathione peroxidase; HPLC, high performance liquid chromatography; MDA, maleic dialdehyde; Se-MC, selenium-enriched mycelia; SOD, superoxide dismutase; SEC-ICP-MS, size-exclusion chromatography−inductively coupled plasma−mass spectrometry

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