The Separation and Purification of Se-enriched Mycelium Polysaccharides of *Catathelasma Ventricosum* and Their Anti-hyperglycemic and Antioxidant Activity

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Abstract. Selenium (Se) is an essential nutrient for humans as it plays an important role in anti-hyperglycemic and antioxidant activities. The objective of this work was to evaluate anti-hyperglycemic and antioxidant activities of Se-enriched mycelium polysaccharides. The Se-enriched mycelium polysaccharides were separated from aqueous extract, and then purified by DEAE-52 (obtained four components, the bioactivities of SPC-2 was highest among them) and Sephadex G-100 column, a component of polysaccharide (SPC-2s) was found to play a main role in anti-hyperglycemic and antioxidant activities (these bioactivities of SPC-2s were higher than positive control (CVP-1s) and contain organic selenium with 41.77 μg/g.

Introduction

Diabetes is a serious metabolic disease; about 3% of the world population is suffering from the disease. It will produce a lot of reactive oxygen species, which cause oxidative damage in the liver, kidney, pancreas tissues and other organs [1].

Selenium is a necessary to the health of the human body trace element, and also an important part of Se dependent enzyme such as: glutathione peroxidase and other antioxidant activity of selenium protease. On the other hand, if human body excessive intake of selenium may also cause the body damage. It is generally think that Se toxicity, mainly determined by the state of Se and organic selenium toxicity is far lower than inorganic selenium [2].

Catathelasma ventricosum is the mushroom that widely consumed in southwest of our country, and also has many biological activities. Many studies have proved its fruiting body and mycelium have anti-hyperglycemic, antioxidant activity [3, 4]. Especially its Se-enriched mycelium polysaccharides, the purpose of this paper are to purification of the main bioactive polysaccharide configuration in it, and further explore the Se-enriched mycelium polysaccharides mechanism for the treatment of diabetes.

Materials and Methods

Materials and Reagents

C. ventricosum were purchased from Mianyang edible fungi institute. All chemical reagents were analytical grade.

The Preparation of Se-enriched Polysaccharide Mycelium of *C. ventricosum*

Liquid fermentation medium: the stock culture prepared by modified PDA medium; Se-enriched fermentation liquid medium: C. *ventricosum* seeds were inoculated in PDA liquid medium, in 22^{o}C under the condition of 4 d growth [4].

The Content Determination of Se-enriched Crude Polysaccharide Mycelium of *C. ventricosum*(SPCs)

The extract determination of reducing sugar in SPCs: the proper dilution of extract was introduced, and the DNS method was used for the determination of reducing sugar; the extract determination of total sugar in SPCs: the phenol- sulfuric acid method was used for the determination of total sugar; the determination of crude polysaccharide in SPCs: the calculating formula for the coarse polysaccharide content extract as (1):

$$\text{Crude polysaccharide}=\text{Total sugar}-\text{Reducing sugar}. \quad (1)$$

The Determination of Se Content

We used 3, 3-2 amino benzidine (DAB) colorimetric method to determine the content of Se [5].

The total content of Se in SPCs: Se content calculated in DAB colorimetric method; the organic selenium in SPCs: put 0.5 g SPCs in the dialysis bag, after 24 h adequate dialysis (removal of inorganic selenium), take the dialysis bag material. Se content was determined according toDAB colorimetric method.Considering the rich Se and organic selenium conversion rate, then determine the optimal culture medium Se concentrations, the calculating formula as (2) and (3):

$$\text{Selenium accumulation } (\mu g/g)=\frac{\text{Total Se content}}{\text{Mycelial dry weight}}. \quad (2)$$

$$\text{Organic selenium transformation rate } (\%)=\frac{\text{Content of organic selenium}}{\text{Total content of selenium}}\times 100. \quad (3)$$

The Separation and Purification of Se-enriched Mycelium Polysaccharides

DEAE-52 column (3.5 × 20 cm) and Sephadex G-100 column (2.6 × 80 cm) were used for purification of Se-enriched mycelium polysaccharides. Finally, collected the components with the highest anti-hyperglycemic and antioxidant activity of the peak, concentration after freeze-drying [3].

The Activity of in Vitro Anti-hyperglycemic Determination

α-GlucosidaseInhibitory Activity. Theα-glucosidase enzyme inhibition activity determination methods based on Palanisamyand modified slightly [6].The inhibition rate was calculated according to the formula given in (4):

$$\text{Inhibition rate } (\%)=\frac{A_{400}\text{ of negative control}-A_{400}\text{ of sample}}{A_{400}\text{ of negative control}}\times 100. \quad (4)$$

α-Amylase Inhibitory Activity. The α-amylase inhibitory activity determination methods based on Palanisamy and modified slightly [6].The inhibition rate was calculated according to the formula given in (5):

$$\text{Inhibition rate } (\%)=\frac{A_{520}\text{ of negative control}-A_{520}\text{ of sample}}{A_{520}\text{ of negative control}}\times 100. \quad (5)$$

Advanced Glycosylation End-products (AGEs) Inhibitory. TheAGEs inhibitory activity determination methods based on Palanisamy and modified slightly [6].The inhibition rate was calculated according to the formula giv212en in (6):

$$\text{The inhibition rate of AGEs (\%)} = \frac{A - B}{A}. \quad (6)$$

The Determination of Antioxidant Activity in Vitro

DPPH free radical-scavenging activity. DPPHradical scavenging activity determination methods based on Blois and modified slightly [7].

$$\text{Free radical scavenging rate (\%)} = \frac{A_{51} \text{ of negative control} - A_{51} \text{ of sample}}{A_{51} \text{ of negative control}}. \quad (7)$$

Reducing Power. The determination methods of reducing power based on Chenand modified slightly [7].

Metal Chelating Activity. The determination methods of metal chelating activity based on Dinis and modified slightly [7].

$$\text{Metal chelating rate (\%)} = \frac{A_{562} \text{ of negative control} - A_{562} \text{ of sample}}{A_{562} \text{ of negative control}} \times 100. \quad (8)$$

Results and Discussions

The Content of the Mushroom and its Molecular Weight Distribution

SPCs showed 99.76% sugar content, and Bradford test were negative. And infrared scanning results showed that in 883 cm^{-1} has absorbed, prove that SPCs as the β-configuration polysaccharide. Simultaneously by high performance liquid gel permeation chromatography (HPGPC) for SPCs' molecular weight distribution were determined, and the results show that there are four main peak was cleared out, corresponding to the molecular weight of about 3.6×10^7, 8.3×10^6, 1.4×10^5 and 4.8×10^3 Da in turn. The data to our late purification, gel column type choice has a guiding significance.

It's showed that the mycelium of *C. ventricosum* have very strong transforming ability of organic selenium, at the same time Shang reported that Se combined with polysaccharide in the main existence form:C_2–Se(–O)(=O), C–Se–H and C–Se$(=O)_2$ [8]. According to the results of the infrared scanning, Se-enriched pure polysaccharide mycelium of *C. ventricosum*onSephadexG-100 column chromatography obtained a single symmetrical peak (SPC-2s), mostly is for β-glucan. Roupas pointed out that the health benefits of edible mushroom and its extract such as antibacterial, antitumor and enhance immunity, etc., mainly because they contain polysaccharide in the structure of β-glucan [9].

Despite various molecular weights, monosaccharide composition and configuration of the reported, but the structure of polysaccharides and their bioactivities of correlation still are unclear.

The Purification of *C. ventricosum* Polysaccharides.

Ion Exchange Chromatography Separation of Active Polysaccharide. Use anion exchange column DEAE-52 to early separation SPCs. As shown in Fig. 1A, four components of polysaccharide was separated in total, the SPC-1, the SPC-2, SPC-3 and SPC-4, respectively on the total sample amount of 32.6%, 14.3%, 21.3%, 14.3%, and a total of 77.7% of the polysaccharide was recycling.

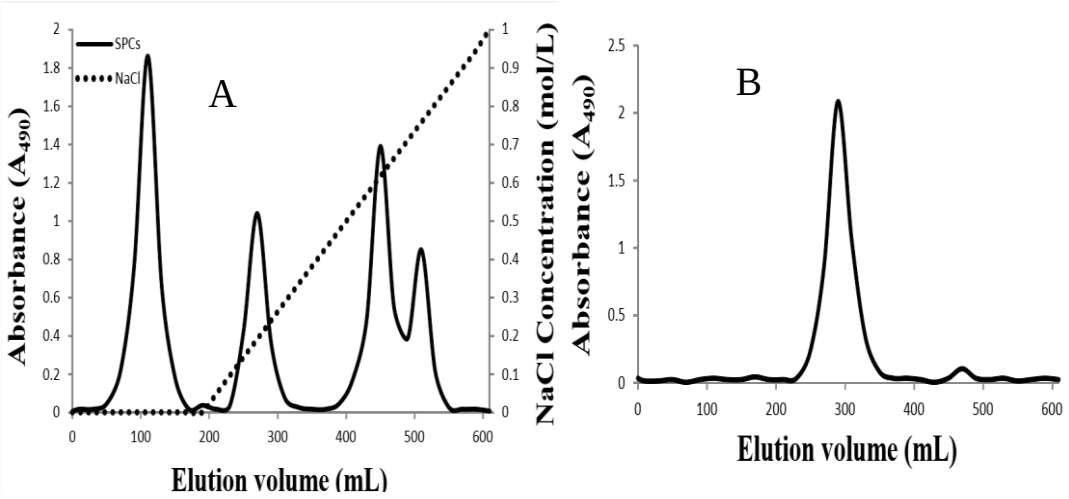

Figure 1.Fractionation of Se-polysaccharides on DEAE-52 column (A) and Sephadex G-100 column(B)

The Comparison of Anti-hyperglycemic and Antioxidant Activity in Different Selenium Polysaccharides through Ion Exchange Chromatography Separation. The Fig. 2A shows the inhibition rate of each early separation SCPs component on DEAE-52 column. By the graph, you can see that the SPC-2 inhibitory activity of which is the strongest, in which 11 days after reaction peak inhibition rate of 91%, while the other components in the reaction of the 11 days of which inhibitory activity is very limited. The four components of the C. *ventricosum* polysaccharides(CVPs) was early separated by weak anion exchange column DEAE-52, CVP-1, CVP-2, CVP-3 and CVP-4, and the highest inhibition rate CVP-4 was also only 59%.

The results also show that although the SPC-4 metal chelating activity is higher than the SPC-2 ($P > 0.05$), but there is no significant difference. And the two anti-hyperglycemic activity indicators of SPC-3 are very close to SPC-2 ($P > 0.05$), but its antioxidant activity index were significantly weaker than the SPC-2.Besides, the other blood sugar and antioxidant indicators of SPC-2 were all the most prominent ($P < 0.05$). Therefore, we think the SPC-2 in SPCs plays a main role in anti-hyperglycemic and antioxidant activity.

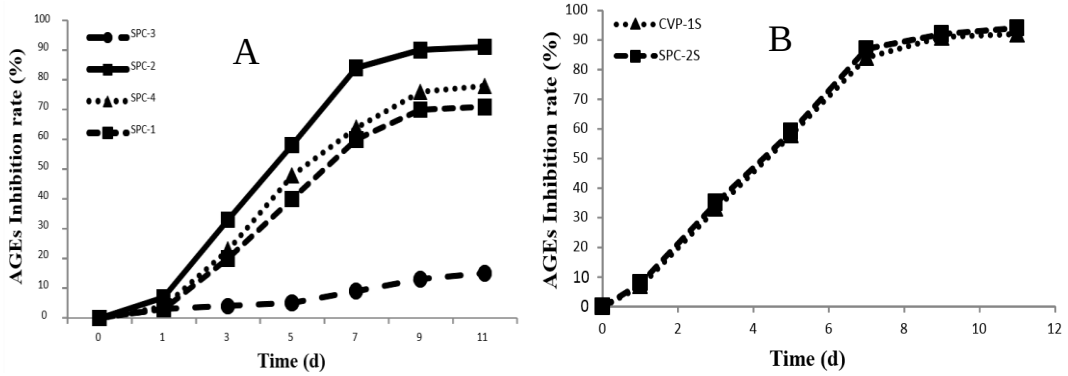

Figure 2.AGEs inhibition activity offractionation of Se-polysaccharides on DEAE-52 column (A) and Sephadex G-100 column (B)

Table 1. Anti-hyperglycemic and antioxidantactivity of fractionation of Se-polysaccharides on DEAE-52 column

Bioactivity	Components			
	SPC-1	SPC-2	SPC-3	SPC-4
α-GI	3.74±0.11b[A]	2.37±0.13a	2.58±0.15a	6.35±0.77c
α-AI	3.54±0.36b	2.14±0.15a	2.45±0.98a	5.73±0.85c
DPPH	4.71±0.28c	2.58±0.27a	11.35±1.09d	3.96±0.88b
RP	12.46±0.95c	1.14±0.03a	12.86±1.09c	5.04±0.75b
FI	7.46±0.79c	4.39±0.45a	6.23±1.01b	4.26±0.42a

[A] Each numerical are expressed as the average of three tests ± standard deviation.

Gel Column Separation of Se-enriched Polysaccharide. In order to further explore the structure-activity relationship, we choose SPC-2 for further purification. As shown in Fig. 1B, the SPC-2s (each purification on Sephadex G-100 column that about 15% of the loss) after DABcolorimetric method to determine the Se content of SPC - 2, the results showed 1g SPC-2 contains organic selenium 41.77 μg.

As shown in Fig. 2B, which reflects a single component of inhibition rate of the SPC-2s, which is purified in SPC-2 on Sephadex G-100 column? By the graph, we can see that the SPC-2s and CVP-1s which the inhibitory activity about the same, and the SPC-2s in 11 days after reaction to a peak of 94%, slightly higher than that of CVP-1s 92%.

According to Table2, the anti-diabetic indicators of SPC-2s that to be measured is all superior to CVP-1s. The results show that the anti-diabetic activity of SPC-2s is better than that of CVP-1s to a certain extent.

Table 2.Antihyperglycemic and antioxidantactivity of CVP-1S and SPC-2S

Purepolysaccharides	Bioactivity				
	α-GI	α-AI	DPPH	RP	FI
SPC-2S	2.21±0.13A	2.38±0.15	2.76±0.19	1.39±0.09	5.24±0.34
CVP-1S	2.56±0.18	2.86±0.21	3.01±0.17	1.77±0.11	5.73±0.44

[A] Each numerical are expressed as the average of three tests ± standard deviation.

Conclusions

The mycelium of *catathelasmaventricosum* has very strong transforming ability of organic selenium, and is of benefit to antibacterial, antitumor and enhances immunity, etc. because of its β configuration. 1) We analysis the inhibition rate of four components of SPCs, which early separated on DEAE-52 column, found that the SPC-2 which inhibitory activity is the strongest, in 11 days after reaction to a peak of 91%, generally higher than the crude polysaccharide mycelium of *catathelasmaventricosum*. 2) We believed that the SPC-2 plays a main role in SPCs toanti-hyperglycemic and antioxidant. 3) Meanwhile, we measured the SPC-2 containing organic selenium 41.77 μg per 1 g. 4) Then we purification of SPC-2 to get SPC-2s, analysis and found its inhibition rate to a peak of 94% in 11 days, slightly higher than that of CVP-1s 92%. As a result, we considered SPC-2s is superior to the CVP-1 s on anti-diabetic activity to a certain extent.

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