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Extraction and characterization of the auricularia auricular polysaccharide

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Abstract. To study a new protein drugs carrier, the Auricularia auricular polysaccharide (AAP) was extracted and purified from Auricularia auricular, and then characterized by the micrOTOF-Q mass spectrometer, UV/Vis spectrophotometer, moisture analyzer and SEM. The results showed that the AAP sample was water- soluble and white flocculence, its molecular weight were 20506.9 Da～63923.7 Da, and the yield, moisture, and total sugar contents of the AAP were 4.5%, 6.2% and 90.12%(w/w), respectively. The results of the SEM revealed that the AAP dried by vacuum were spherical particles with a smooth surface, and the AAP freeze-dried had continuous porous sheet shape with the loose structure.

1. Introduction

To date, over 160 therapeutic protein drugs have been licensed, and increasing protein drugs that will be approved by the regulatory agencies are forecasted to increase significantly in the next few years [1, 2]. The physicochemical and biological properties of protein drugs are unlike those of conventional ones, such as molecular weight, biological half-life, conformational stability, physicochemical stability, solubility, oral bioavailability, dose requirement, and administration [3]. In addition, the disadvantage of the oral protein drugs is that its low bioavailability. The reasons for this are that the poor protein transmembrane transport of gastrointestinal mucosa, easy destruction by the rigor pH environment and the abundant enzyme system of gastrointestinal [4, 5]. Therefore, the oral protein drugs delivery systems have caused the extensive concern.

In order to get over the above hurdles and increasing the gastrointestinal absorption of the oral protein drugs, we have been trying to make some of the nanoparticles (NPs) from natural biodegradable polymers, which have been exploited and measured for protein drugs[6, 7]. However, biodegradable polymer nanoparticles made from polyglycolic acid and polylactide and their copolymers, are usually obtained by using organic solvents, high temperatures, forces and are inactivated by physical and chemical denaturation. Furthermore, after the formulation has been administered, changes in the microenvironment within the nanoparticles due to polymer degradation can dramatically affect the tertiary structure of the protein [8, 9].

A method is to encapsulate protein drugs within Polyelectrolyte polysaccharide nanoparticles, so that the degradation of protein drugs by the gastrointestinal pH and Enzyme System is avoided, they can be transported to the sensitive target for release, enhance their tissue and cell penetrating across the gastrointestinal mucosa , reduced reticuloendothelial system phagocytic [10-12]. Nature polysaccharides possess plenty of promising properties, including excellent biodegradability, high biocompatibility, poor toxicity, safety, abundant availability and low production cost [13]. In

particular, most of the natural polysaccharides have hydrophilic groups such as –OH, –COOH, $–CONH_2$ and $–SO_3H$, they are apt to form non-covalent bonds with the biological tissues, that can prolong residence time in the site of absorption and promote the absorption of the protein drugs [14, 15]. Auricularia auricular polysaccharide (AAP) is found rich in Auricularia auricular, as significant bioactive substances, broad physiological activity, favorable medicinal value, and a research highlight in the field of medicine [16]. However, AAP as a novel nature polysaccharide has been rarely investigated and reported in the application of the drug carriers.

In the present work, the AAP was extracted and purified from Auricularia auricular and characterized. The results of this work should provide valuable information for selecting protein drugs carrier.

2. Materials and methods

2.1. Materials

Bovine serum albumin (BSA) and bovine hemoglobin (BHb) were purchased from Sigma–Aldrich (St. Louis, MO, USA). Coomassie brilliant blue G-250 was obtained from Regent Chemicals Co., Ltd (Tianjin, China). Auricularia auricular was bought from Carrefour supermarket (Shenyang, China), which was cultivated in Liaoning Province, China. Low molecular weight chitosan was purchased from Golden-Shell Pharmaceutical Co., Ltd (Zhejiang, China), and the degree of deacetylation was 90%. All other reagents and chemicals were of analytical grade.

2.2. *Extraction and purification of AAP*

The AAP was extracted and purified by modified water extraction and alcohol precipitation method as follows [17]: The Auricularia auricular was defatted by reflux, and then dried. The resulting powder was weighed and extracted, followed by precipitating and washing with ethanol, and then crude gray polysaccharide was obtained by vacuum-dried. 2% crude polysaccharide solution was prepared by removing protein via the Savage method (chloroform: n-butanol=4:1). With pH adjusted to 8.0 as well as ammonia, color materials were removed by hydrogen peroxide. Afterward, the decolourization of polysaccharide solution was dialyzed against distilled water. The purified AAP was obtained by concentrating or freeze-drying dialysates eventually.

2.3. Characterization of AAP

2.3.1. Polysaccharide content. The AAP contents were measured by a phenol-sulfuric acid method using D-glucose as a standard [18]. The percentage of AAP extraction yield(%) was calculated with the formula as follows:

$$Yield(\%) = \frac{W_2}{W_1} \times 100$$

Where W_2 was the polysaccharides content of extraction, and W_1 represented dried sample weight.

The protein of the polysaccharide was detected by UV-4802 Double Beam UV/Vis Spectrophotometer (Unico, Shanghai, China). The moisture content of the AAP was obtained by a moisture analyzer (Shuangquan, Shanghai, China).

2.3.2. Molecular weight (Mw) The molecular weight of AAP was determined by time-of-flight mass spectrometer (Bruker micrOTOF-Q, Ettlingen, Germany). LC-Q-TOF/MS instrument parameters: electrospray ionization (ESI) detection mode, capillary voltage: 4.5 kV, capillary export voltage: -500V, collision cell RF voltage: 800Vpp, capillary temperature: 180℃, N_2 as the fog and auxiliary gas and flow rate of 4mL/min. Argon as the collision gas for CID experiments, the collision energy was 20eV. The analytical data were collected in full scan mode from 50 m/z to 900 m/z.

2.3.3. SEM. The morphology and structure characteristics of the AAP were observed by SEM (SSX-550, Shimadzu, Japan). The sample was thinly sprinkled onto a metal stub and vacuum coated with a thin layer of gold in an argon atmosphere. The coated samples were examined at an acceleration voltage of 15kV.

2.3.4. DSC. Thermal properties of the samples were surveyed by DSC (DSC-60, Shimadzu, Japan). The samples were scanned at a heating speed of 5℃/min over a temperature range of 20-300℃ with a nitrogen purge of 40ml/min in an aluminum pan and sealed hermetically, using aluminum oxide as the reference. Melting point and crystallization point corresponded to the maximum and minimum of the DSC curves respectively.

3. Results and discussion

3.1. Physicochemical properties of AAP

Purified AAP were obtained by hot-water extraction, ethanol precipitation, removed via Sevag method, dialyzed with distilled water and lyophilized by freeze-drying. The yield, moisture, and total sugar contents in the AAP were 4.5%, 6.2% and 90.12% (w/w), respectively. The AAP sample was water-soluble and white flocculence. AAP was not detected with a significant absorption peak in the wavelength of 280nm by the UV/Vis Spectrophotometer, which indicated that protein impurities have been reduced to the very low amount [19]. This result may be due to the process of vacuum freeze-drying, because of the water precipitated from polysaccharide solution under the condition of the solution freezing, which changed the solution system and made protein molecules denaturation and finally led to its lower solubility [20].

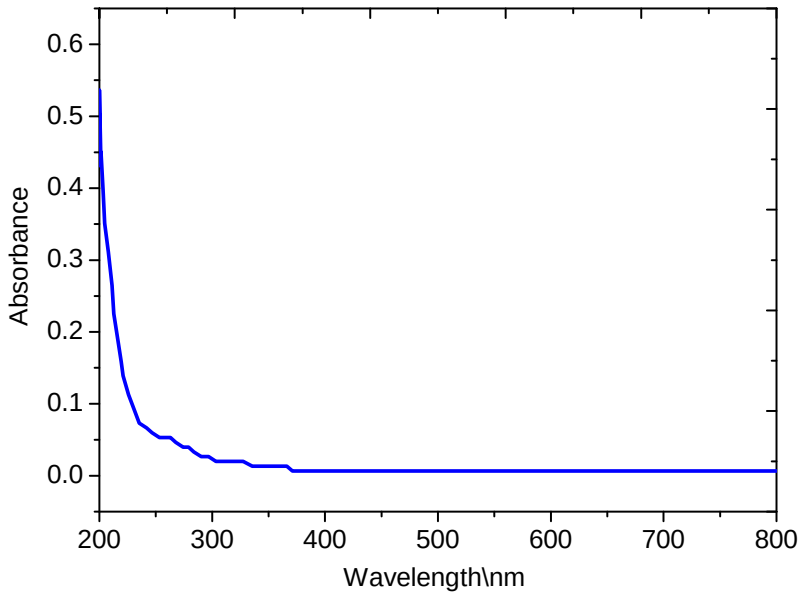

Figure 1. UV Spectrum of AAP in water

3.2. Molecular weight and SEM

The mass spectrometer was applied to determine the molecular weight of AAP. Figure 2, 3 exhibited the mass spectrometer of AAP, and Mw of AAP ranged from 20,506.9Da to 63,923.7Da. The surface topography and structure of a polysaccharide would be affected by different methods of extraction, purification and preparation [21]. Figure 4 revealed the surface morphology of AAP dried by vacuum

and lyophilization. The freeze-dried AAP had continuous porous sheet shape with loose structure, whereas vacuum one had a smooth surface and spherical shape with a tight structure. Comparing the two AAP products, we could speculate that the freeze-dried AAP dissolved much easier and faster than the other one did [22].

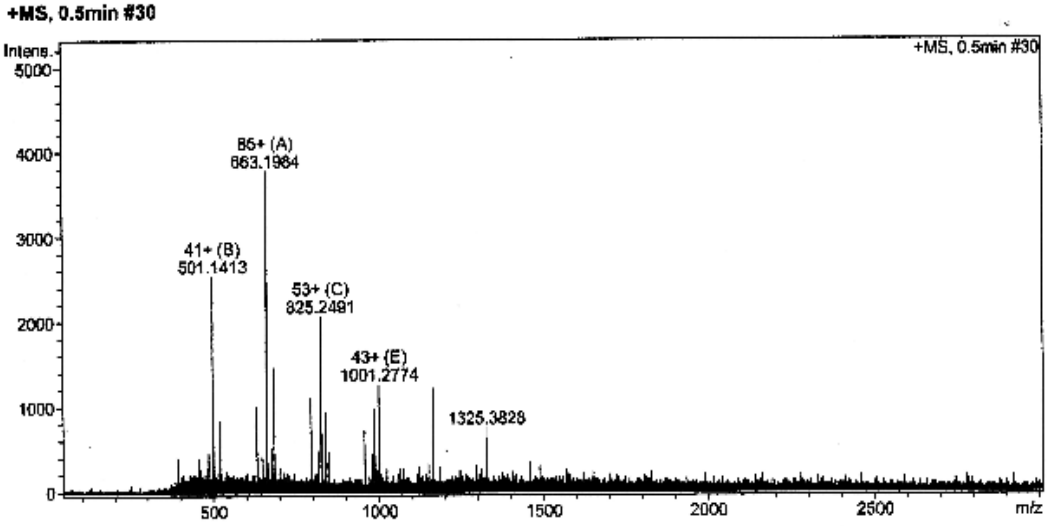

Figure 2. Mass spectrometer of AAP under positive model with ESI source

Component	Molecular Mass	Molecule		Absolute Abundance	Relative Abundance
A	56281.1089	56281.1089	Mr	5927	100.00
B	20506.9015	20506.9015	Mr	3247	54.78
C	43685.0381	43685.0381	Mr	2449	41.33
D	25999.9693	25999.9693	Mr	2315	39.06
E	43015.1381	43015.1381	Mr	2569	43.34
F	63923.7047	63923.7047	Mr	1981	33.42
G	24537.8055	24537.8055	Mr	1236	20.85
H	60160.0246	60160.0246	Mr	1659	27.99
I	23471.2823	23471.2823	Mr	981	16.55
J	25585.7662	25585.7662	Mr	1803	30.42

Figure 3. Mw range of AAP with Flight mass spectrometer

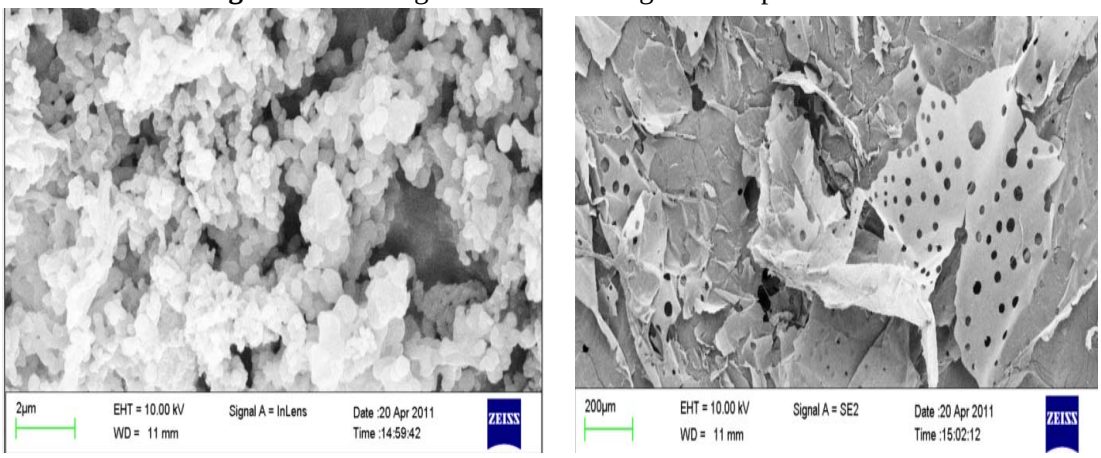

Figure 4. SEM images of AAP dried by vacuum (left) and lyophilization (right).

4. Conclusion

As an innovative idea of the application of AAP serving as the drug carriers, this study demonstrated the polysaccharide extracted and purified from Auricularia auricular. The results revealed that the AAP sample was water-soluble and white flocculence, its molecular weight was 20506.9 Da～63923.7 Da, and the yield, moisture, and total sugar contents of the AAP were 4.5%, 6.2% and 90.12%(w/w), respectively. The AAP dried by vacuum were spherical particles with a smooth surface, and the AAP dried by freeze-drying had continuous porous sheet shape with a loose structure.

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