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Bioactive polysaccharides from *Cordyceps sinensis*: Isolation, structure features and bioactivities

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ABSTRACT

Polysaccharides from *Cordyceps sinensis* have demonstrable bioactivities such as antioxidation, anti-tumor, immunological properties etc. This paper will describe methods used to extract, isolate, purify and characterize polysaccharides which are obtained from *C. sinensis* and review progress in elucidating their structure and related bioactivity.

Contents

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1. Introduction

C. sinensis, a well-known and valued traditional Chinese medicine, is also called "DongChongXiaCao" (winter worm summer grass) in Chinese, and "Tochukaso" in Japanese. *C. sinensis*, a dried fungus grows on the larva of the caterpillar. The parasitic complex of the fungus and the caterpillar is found in the prairie soil of above 2000 m on the Qinghai-Tibetan plateau. In winter, it appears as an old silk worm in the soil, and in summer, hairs grow out of soil, and convert into grass. These need to be collected in the summer, and if not, they will turn into a worm again (Zhu, Halpern, & Jones, 1998a, b). In China, the first known written record of this herbal medicine was written around over 300 years ago in the Ben-Cao-Cong-Xin. This early medical text listed the traditional usage of *Cordyceps* as entering the lung and kidney meridian and being useful as a "lung protectorate", for "kidney improvement" and as a "Yin/Yang double invigorant" (Li, Yang, & Tsim, 2006; Zhu et al., 1998a; Zhu, Halpern, & Jones, 1998b). Modern researches have confirmed that *C. sinensis* possesses wide-ranging beneficial health effects on the circulatory, immune, hematogenic, cardiovascular, respiratory and glandular systems. It can enhance and regulate immune functions, and has anti-aging, anti-tumor, anti-microbial and antioxidant activity. It has also been used for the treatment and protection against renal toxicity, heart diseases, liver disease, and respiratory disease, etc. (Bok, Lermer, Chilton, Klingeman, & Towers, 1999; Ji et al., 2009; Li et al., 2006; Rao, Fang, & Tzeng, 2007; Shi et al., 2009; Yu, Wang, Huang, & Duh, 2006; Zhang, Xie, Li, & Li, 2002). These beneficial effects have been partly attributed to the variety of chemical components, including nucleosides (cordycepin), polysaccharides, alkaloids, amino acids, inorganic elements, etc. However, most of these studies have been concentrated on the nucleosides, alkaloids, mannitol, amino acids and various enzymes components (Huang, Liang, Guo, Zhou, & Cheng, 2003; Li et al., 2004; Li et al., 2006; Yoshikawa et al., 2007; Yuan et al., 2007; Zhang et al., 2002; Zhu et al., 1998a,b). Polysaccharides extracted from many herbs and mushrooms have been shown to have biological activity as do the polysaccharides from *C. sinensis* which possess antioxidation, anti-tumor, and immunological activities. Therefore, the polysaccharides from *C. sinensis* have been extensively studied, but the structure-function relationship has not been unequivocally established (Dong, 2004; Guan & Li, 2008; Guo & Chen, 2006; Zhang, Cui, Cheung, & Wang, 2007; Zhong et al., 2009). Moreover, *C. sinensis* either wild or natural is now an increasingly scarce species due to reckless harvesting and unfavorable weather conditions for its proliferation. Because of the shortage and increasing demand, the price for wild *C. sinensis* has increased sharply, and has nearly doubled in the last 10 years. Mycelial fermentation of *Cordyceps* fungal species is now a feasible and sustainable means of producing the medicinal fungus and its active compounds. The cultivated fungal mycelia of some *C. sinensis* fungal species have been shown to produce similar effects as those of the wild *C. sinensis* species (Hsu, Shiao, Hsieh, & Chang, 2002; Leung, Zhang, & Wu, 2006; Zhu et al., 1998a). This review summarizes the different types of isolation techniques which have been used to investigate the structural features and bioactivities of the polysaccharides from natural and cultured *C. sinensis*.

2. Isolation of polysaccharides from *Cordyceps sinensis*

Polysaccharides are mainly present in the cell wall of *C. sinensis*, and are within the cytoplasm as a structural component. Generally, extraction treatment with different solvents, such as hot water, alkali solution etc., enables isolation and purification of the polysaccharide according to their different solubility in water and organic solvents, or based on their different ionic properties and molecular weight distributions. Other techniques have also been used such as organic solvent precipitation, fractional precipitation, acidic precipitation with acetic acid, adsorption resin, ultra-filtration, dialysis, ion-exchange chromatography, gel filtration, and affinity chromatography method etc. (Izydorczyk, 2005). Miyazaki, Oikawa, and Yamada (1977) firstly isolated and purified a water-soluble polysaccharide by ethanol fractionation and gel filtration chromatography and subsequently more sophisticated methods were developed (Fan, Yin, & Zhou, 2008; Gong et al., 1990; Ji, Tu, & Li, 1993; Wu, Liu, Dou, & Zhao, 2008; Wang et al., 2011; Wang et al., 2011; Sheng, Chen, Li, & Zhang, 2011; Zhang, Liu, Al-Assaf, Phillips, & Phillips, 2012).

To isolate the protein-containing polysaccharide (CT-4N) the crude drug was pulverized, and successively extracted with acetone, hot methanol and hot 70% aqueous ethanol, followed by exhaustive extractions with hot water. The residual material was then suspended in 5% sodium carbonate for 12 h at room temperature. The alkaline suspension was filtered, and the filtrate was neutralized with acetic acid, and dialyzed against distilled water. The solution from inside of the dialysis bag was mixed with 3 volumes of ethanol. The precipitate was dissolved in water, and the solution was deproteinized by the Sevag procedure. The aqueous solution was further purified by column chromatography on DEAE Sephadex A-25. The neutral fraction was dialyzed, and lyophilized, to give the crude polysaccharides in 0.1% yield. These were separated using a column of Sephacryl S-300 to yield the minor protein-containing polysaccharide (CT-4N) as colorless flakes in 0.08% yield. Its molecular weight was estimated by gel filtration to be ~23,000 Da (Kiho, Tabata, Ukai, & Hara, 1986).

Wu et al., combining the use of buffers and column chromatography obtained a series of purified polysaccharides from the mycelium of *C. sinensis*. Dried crushed mycelia were extracted successively with ethanol 95 and 85%, ethanol to defat and decolorize, and then extracted with aqueous 75% ethanol for 12 h. After centrifugation (6700 rpm, 30 min), the residue was dried in air and then treated several times with