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Structural analysis and anti-tumor activity comparison of polysaccharides from *Astragalus*

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ABSTRACT

Two polysaccharides: APS-I and APS-II, were obtained after a successive purification consisting of (1) fractional precipitation using ethyl alcohol; (2) filtration through DEAE-Sephadex A-25 and Sephadex G-100 eluted by water. The molecular weights of APS-I and APS-II were 4.77×10^6 and 8.68×10^3 Da, respectively, and they were mainly composed of glucose, and a small amount of arabinose and xylose, with their molar ratios of 0.54:1:18.14 and 0.23:1:29.39, respectively for APS-I and APS-II. FTIR, methylation, periodate oxidation, Smith degradation, ^{1}H NMR and ^{13}C NMR were used in their structure analysis. The results indicated that structurally, APS-I and APS-II were similar, with their main chains mainly composed of major α-(1 → 3) glucose and a few 1 → 4, 1 → 6 glucoses, while the side chain contained arabinoses and xyloses. The tumor inhibition ratios of APS-I and APS-II were 55.47% and 47.72%, respectively.

1. Introduction

Radix *Astragalus* is the dry root of *Astragalus membranaceus* (Fisch.) Bge. var. *mongholicus* (Bge.) Hsiao or *A. membranaceus* (Fisch.) Bge. It belongs to Leguminous plant which has been used as a health-promoting herb in China for more than 2000 years. Many pharmacological functions of different components from *Astragalus* have been recognized in "Chinese Pharmacopoeia". Particularly for its polysaccharide fractions which show a variety of biological activities, such as anti-tumor, immune modulation and hypoglycemic effects (Lin & Chiang, 2008; Liu, Wu, Mao, Wu, & Ouyang, 2009; Shao et al., 2004; State Pharmacopoeia Committee, 2010).

Polysaccharides from the functional fractions of *Astragalus* have received much attention in the research community. Previously, one kind of polysaccharides (APS) has been isolated from *Astragalus*, which is an α-(1 → 4)-D-glucan, containing one single α-D-glucose at the C-6 position of every nine residue, with a molecular weight of approximately 3.6×10^4 Da (Li, Chen, Wang, & Tian, 2009). The animal experiments indicated that APS could have a glomerulonephritis therapeutic potential (Li & Zhang, 2009). Others reported that APS was an α-(1 → 4)-D-glucan with α-(1 → 6)-linked branches attached to the O-6 of branch points and biological tests showed that APS had significant immune modulating activity, thus can be used to treat the gastric cancer (Li et al., 2009).

Recently, we isolated several kinds of polysaccharides from *A. mongholicus* and studied their effects on cancer cells. Interestingly, two novel polysaccharides, named APS-I and APS-II, have a backbone composed of (1,3)-β-D-glucopyranosyl (Glcp) residues. It has been shown that the bioactivities of polysaccharides are most closely related to their chemical composition, configuration and molecular weight, as well as their physical properties. In this paper, we report the extraction and purification of two novel polysaccharides from *A. mongholicus* based on the DEAE-A25 and Sephadex G-100 column chromatography, and their structure were identified by a series of chemical and instrumental analyses. In addition, the correlations between the structural characteristics and anti-tumor activities *in vitro* were also assayed.

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Table 1
The GC result of standard monosaccharides.

Name	Rha	Ara	Xyl	Man	Glu	Gal	Internal standard
Retention time (min)	6.682	7.307	7.902	17.359	18.682	20.066	28.898

2. Materials and methods

2.1. Materials

The dry roots of *A. mongholicus* were commercially obtained from Datong, Shanxi, China, and were crushed to coarse powder.

D-Glucose, D-galactose, D-xylose, L-arabinose, D-mannose, T-series dextran were purchased from Solarbio Corp. (Beijing, China). DEAE-Sephadex A-25 and Sephadex G-100 were purchased from Sigma (St, Louis, MO, USA). All other chemicals were of analytical reagent grade, and were used as received.

2.2. Extraction and purification of polysaccharides

The pre-degreased powdered roots (50 g) of *A. mongholicus* were extracted three times by using CaO solution (pH 9–10, 300 mL) for 90 min at 100 °C (Li, 2000), the filtrate was combined and concentrated to 200 mL using a rotary evaporator at 60 °C. The protein was removed by following the Sevag method (Alam & Gupta, 1986). The mixture was precipitated by using 3 volumes of ethanol overnight after removal of the Sevag reagent. The crude polysaccharide (8.95 g) was obtained by centrifugation at 2000 r/min for 20 min and washed using ethanol and acetone alternately 5 times.

The polysaccharide was re-dissolved and fractioned by using 30% and 70% ethanol solutions, successively. Subsequently, the fractions were separately by applying a DEAE-Sephadex A-25 column (30 cm × 3 cm) that was eluted with distilled water. The yielded fractions were concentrated and combined according to the phenol–sulfuric acid method. The solutions were concentrated in a rotary evaporator and applied to Sephadex G 100 (30 cm × 3 cm) and equilibrated with distilled water. Each fraction had only one main peak, and it was collected and freeze-dried.

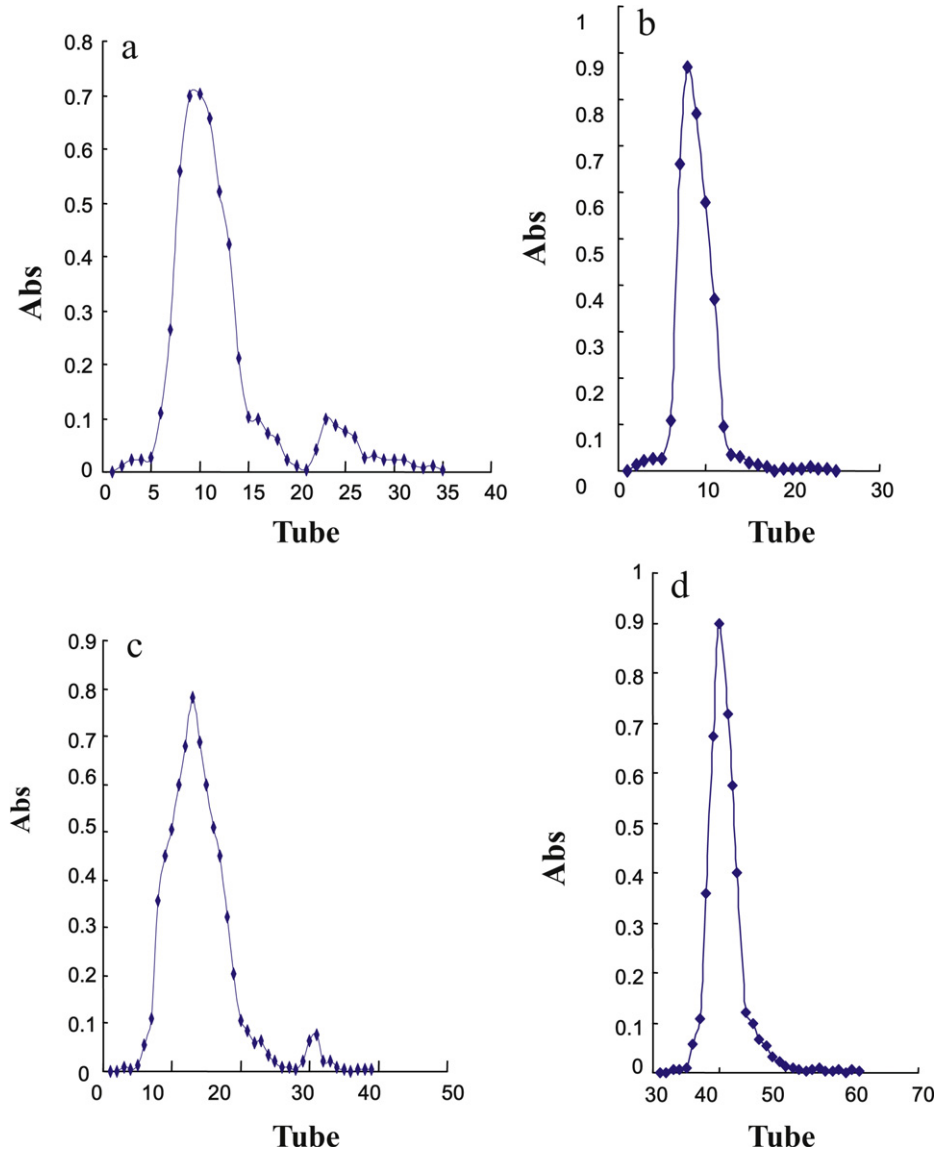

Fig. 1. Elution profiles of APS-I on DEAE-Sephadex A-25 (a), APS-I on Sephadex G-100 (b), APS-II on DEAE-Sephadex A-25 (c), and APS-II on Sephadex G-100 (d).