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PURIFICATION AND CHEMICAL COMPOSITIONS OF A PROTEOGLYCAN ISOLATED FROM *Aconitum coreanum*

Haibin Tong*, Kai Feng, Xiaoqi Zhang, Dan Tian, Yang Liu, Xiaodan Chu, and Xin Sun*

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Proteoglycans represent a special class of glycoproteins that are heavily glycosylated. In nature proteoglycans have a wide variety of biological functions, such as maintenance of protein conformation and stability, immune and surface or intracellular recognition, inflammation, cell adhesion, pathogenicity, metastasis, and other cellular processes [1]. These functions may be dependent on either the protein part or the carbohydrate part, or on both.

Aconitum coreanum (Levl.) Rapaics (Guanbaifu in Chinese) is one of the most documented centuries-old Chinese herbs in ancient Chinese medicinal literature. To date, *A. coreanum* has been used to treat various kinds of disorders such as cardialgia, facial distortion, epilepsia, migraine headache, vertigo, tetanus, infantile convulsion, and rheumatic arthralgia [2, 3]. Some active components from *A. coreanum*, such as alkaloids and flavonol glycosides, have been widely investigated [4]. However, no specific studies on proteoglycan isolated from *A. coreanum* have been carried out. In this study a more refined water-soluble proteoglycan was isolated and purified, and some of its properties and chemical compositions were determined.

In this study, an AKTA Explorer 100 purification system, equipped with a P-900 series pump, Monitor UV-900, Monitor pH/C-900, Fraction collector Frac-950, Auto-sampler A-900, and various kinds of columns, was employed to purify *A. coreanum* proteoglycan. It can simplify the experiment and improve the purity of proteoglycan. At the same time, the operations of data collection were driven by UNICORN software, which guarantees quick, simple communications between systems and users and meets the stringent control and data handling procedures of modern laboratories [5].

The yield of the crude water-soluble polysaccharide extracted with hot water from *A. coreanum* was 4.62%. The main fraction (ACP-I) was purified with DEAE-cellulose anion-exchange and Sepharose CL-6B gel filtration chromatography to yield 45% of the crude polysaccharide.

ACP-I appeared as a white powder, $[\alpha]_D^{20}$ +148° (*c* 0.2, H_2O). The ACP-I was identified as consisting mainly of polysaccharide and protein by the phenol-sulfuric acid method and the Bradford method. As shown in Table 1, the percentages of polysaccharide and protein were 84.3% and 12.4%, respectively. ACP-I contained 20.3% uronic acid evaluated by the *m*-hydroxydiphenyl colorimetric method.

The average molecular weight of ACP-I was calculated as 24 kDa by HPGPC according to the calibration curve with standard dextrans and glucose. The HPGPC profile also demonstrated that ACP-I had a single and symmetrically sharp peak, revealing that ACP-I was a homogeneous proteoglycan. GC analysis showed ACP-I was composed of five kinds of monosaccharides, namely fucose, arabinose, xylose, glucose, and galacturonic acid with molar ratios of 0.8:0.8:1:3.9:1.6. The results indicated Gal was the predominant monosaccharide.

In the UV spectrum of the ACP-I, the intense peak at 209 nm was the characteristic band of double bonds or triple bonds. However, the peak at 278.9 nm should be attributed to protein. The IR spectrum of ACP-I revealed a typical major broad stretching peak around 3440 cm^{-1} for the hydroxyl group, and a weak band at 2930 cm^{-1} showing the C–H stretching vibration. The strong absorption peak at around 1630 cm^{-1} and a weak one near 1430 cm^{-1} were indicative of the presence of carboxyl groups and carbonyl groups that indicated the characteristic IR absorption of uronic acids. The band at 849 cm^{-1} was ascribed to α-pyranoses in the polysaccharide. These observations further confirmed that the ACP-I was composed of polysaccharide, protein, and uronic acids.

Life Science Research Center, Beihua University, JiLin, 132013, P. R. China, fax: + 86 432 4608350, e-mail: tonghaibin@gmail.com; sxin1122@yahoo.com.cn. Published in Khimiya Prirodnykh Soedinenii, No. 2, pp. 274–275, March–April, 2010. Original article submitted October 21, 2008.

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TABLE 1. Molecular Weight and Composition of ACP-I

Sample	*M*w (Da)	Total sugar, %	Protein, %	Uronic acid, %	Molar ratios of monosaccharides (mol%)				
					Fuc	Ara	Xyl	Glu	GalUA
ACP-I	24.000	84.3	12.4	20.3	0.8	0.8	1	3.9	1.6

Spectroscopic Methods. Ultraviolet-visible spectra of ACP-I were recorded with a Varian Cary-100 ultraviolet-visible spectrophotometer. Fourier transform infrared spectra of ACP-I were recorded on a Nicolet 5700 FT-IR spectrometer in the range 400–4000 cm^{-1} using KBr disks.

Plant Material and Reagents. *A. coreanum* were purchased from a medicine market in Jilin City and identified according to the identification standard of the Pharmacopeia of the People′s Republic of China. Sepharose CL-6B was purchased from Amersham Pharmacia Co. (Sweden). T-series dextrans and trifluoroacetic acid (TFA) were purchased from Fluka. DEAE-cellulose, standard sugars, and bovine serum albumin (BSA) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All other chemical reagents used were of analytical grade.

Isolation and Purification of ACP-I. The dried *A. coreanum* were defatted with 85% alcohol for 24 h. After filtration, the residues were extracted with distilled water. The aqueous extracts were filtered and concentrated. The supernatant was treated with alcohol for precipitation. The precipitate was deproteinated by the Sevag method [6]. Finally the supernatant was lyophilized to give crude *A. coreanum* polysaccharides (ACP).

The ACP was dissolved in distilled water, applied to a DEAE–cellulose column, and eluted successively with distilled water and a gradient of 0→1 mol/L NaCl. Fractions were collected and monitored using the phenol–sulfuric acid method at 490 nm absorbance for polysaccharides, and protein absorption at 280 nm was measured for each fraction. The eluate fraction was further fractionated on a Sepharose CL-6B column and eluted with 0.15 mol/L NaCl to yield one main fraction (ACP-I).

Analysis of Monosaccharide Composition, Carbohydrate, and Protein Contents. Gas chromatography (GC) was used for identification and quantification of the monosaccharides. ACP-I was hydrolyzed with 2 M TFA at 120°C for 2 h. The hydrolyzed product was converted into alditol acetates as described in [7] and analyzed by GC. GC was performed on a Varian 3400 instrument equipped with a DM-2330 capillary column and flame-ionization detector (FID). The total carbohydrate contents in ACP-I were determined by the phenol–sulfuric acid colorimetric method [8]. Total uronic acid contents were measured by the *m*-hydroxydiphenyl method [9]. In addition, proteins in the proteoglycans were quantified according to the Bradford method [10].

Homogeneity and Molecular Weight. The homogeneity and molecular weight of ACP-I was evaluated and determined by HPGPC as described in [11].

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