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Chemical composition and antitumor activity of different polysaccharides from the roots of *Actinidia eriantha*

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ABSTRACT

Four polysaccharides (AEPA, AEPB, AEPC and AEPD) isolated and purified from the roots of *Actinidia eriantha* using gel filtration were subjected to composition analysis and valuated for the antitumor and immunomodulatory activity. The predominant neutral monosaccharides in four fractions were identified as galactose, arabinose and fucose, while the composition and ratio of the monosaccharides were different from one another. All four polysaccharides could significantly not only inhibit the growth of transplantable S180 sarcoma in mice, but promote splenocytes proliferation, natural killer cells activity, interleukin-2 production from splenocytes and serum tumor antigen-specific antibody levels in tumor-bearing mice, indicating that their antitumor activity might be achieved by improving immune response. Among four polysaccharides, AEPC and AEPD showed the higher antitumor and immunomodulatory activity. Taken together, the chemical composition of these polysaccharides could affect their antitumor and immunomodulatory activity, and AEPC and AEPD could be explored as antitumor agent with immunomodulatory activity.

1. Introduction

Malignancies are one of the most serious diseases that damage human health in the modern world and the second largest deadly disease just below heart disease. Currently chemotherapy is still the standard treatment method. However, most of the anticancer drugs currently used in chemotherapy are cytotoxic to normal cells, leading to multiple organ toxicity that includes hemopoetic suppression and immunotoxicity (Ehrke, 2003). There exists close relationship between the occurrence, growth and decline of tumor and immune states. The low immune function of an organism may not only result in the generation and development of a tumor, but also be one of the most important factors that prevent the tumor patients' recovery. The discovery and identification of new antitumor drugs, which can potentiate the immune function, has become an important goal of research in immunopharmacology and oncotherapy (Mitchell, 2003). A wide variety of compounds are capable of potentiating immune responses. Classical adjuvants of bacterial origin, such as Bacillus Calmette Guerin (BCG), have been shown to exert therapeutic effects in the treatment of cancer. However, the effect is limited due to a number of undesirable side effects in host, like liver dysfunction, induction of hepatic granuloma, and enhancement of tumor growth when large doses of BCG are administered. Although the immunomodulating property of IL-2, IL-4, and IL-7 promoted their use in the treatment of cancer patients, their diverse side effects, such as cardiovascular toxicity, pulmonary toxicity, hematological toxicity, etc., made limitations in their use (Ognibene et al., 1988; Rosenberg et al., 1994). Immunomodulators, which can be used for long period without or less side effects, are appreciable in the cancer therapy.

Most polysaccharides derived from higher plants are relatively nontoxic and do not cause significant side effects, which is a major problem associated with immunomodulatory bacterial polysaccharides and synthetic compounds. Thus, plant polysaccharides are ideal candidates for therapeutics with immunomodulatory and antitumor effects and low toxicity (Schepetkin & Quinn, 2006). Recently, lentinan, schizophyllan and krestin have been accepted as immunoceuticals in several oriental countries (Wasser, 2002).

The genus *Actinidia* (Actinidiaceae) consists of over 58 species and widely distributed in the Asian continent. Most species are native to temperate regions of south-western China. *Actinidia* fruits are utritious fruits distinguishable from other fruits by the attractive green color of their flesh (Boldingh, Smith, & Klages, 2000; Nishiyama, Fukuda, & Oota, 2005). Some *Actinidia* species, such as *A. macrosperma*, are the important traditional medicine (Zhao et al., 2006). They were widely used as health foods and medical products (Yang, Bai, & Qiu, 2007). *A. eriantha* Benth is a liana plant

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that commonly grows in temperate climate zones. Its roots have been used for gastric carcinoma, nasopharyngeal carcinoma, breast carcinoma, and hepatitis in traditional Chinese medicine (Jiangsu New Medical College, 1977). Pharmacological experiments also indicated that the water extracts of this drug possessed the antitumor and immunopotentiating activities (Lin, Yu, Zhu, Wu, & Yu, 1987). However, the antitumor constituents of the roots of *A. eriantha* have not yet been reported. To elucidate the principles for the antitumor action and to acquire high-performance polysaccharide products, the crude polysaccharide from the roots of *A. eriantha* (CAEP) was isolated and purified using gel filtration to afford four polysaccharides, namely AEPA, AEPB, AEPC and AEPD, and the chemical composition and antitumor activity of these polysaccharides as well as their immunomodulatory potential on the immune response in tumor-bearing mice were investigated.

2. Materials and methods

2.1. Materials and reagents

The roots of *A. eriantha* were collected in Wuyi county, Zhejiang province, China in August 2007. A voucher specimen (No. 20070806) has been deposited at the Laboratory of Nature Drug, College of Animal Sciences, Zhejiang University, China. DEAE-Sephadex A-50 and Sephacryl S-400 was from Amersham Biosciences Co., Piscataway, NJ, USA. Arabinose, fucose, galactose, glucose, rhamnose, rhamnose, xylose, glucuronic acid, trifluoroacetic acid (TFA), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), concanavalin A (Con A), lipopolysaccharide (LPS) and rabbit anti-mouse IgG peroxidase conjugate were purchased from Sigma Chemical Co., Saint Louis, MO, USA; RPMI 1640 medium, fetal calf serum (FCS) and dimethyl sulfoxide (DMSO) were from Gibco, Grand Island, NY, USA; goat anti-mouse IgG1, IgG2a and IgG2b peroxidase conjugate were from Southern Biotech. Assoc., Birmingham, AL, USA. Cyclophosphamide (CTX) was provided by Jiangsu Hengrui Company, China. Inositol was purchased from Huamei Biochemistry Co., Shanghai, China. All other chemicals and solvents used were of analytical grade.

2.2. Isolation and purification of polysaccharides

The plant material (1 kg) was extracted with boiling water three times under reflux. The aqueous extract was filtered through Whatman filter paper. The filtrate was concentrated in a rotary evaporator under reduced pressure, and then centrifuged at 3000 rpm for 15 min. The supernatant was precipitated with three volumes of 95% ethanol, and stored overnight at 4 °C. The precipitate was collected by vacuum filtration in büshi funnel, and then washed sequentially with ethanol, acetone and petroleum ether to defat. The resulting precipitate was dissolved in distilled water and dialyzed against distilled water (cut-off M_w 7000 Da). The retentate portion was concentrated under a reduced pressure and lyophilized to afford crude *A. eriantha* polysaccharide (CAEP). CAEP was dissolved in 0.1 M NaCl solution and filtered through 0.45-μm Millipore filter, and then the solution was subjected to DEAE-Sephadex A-50 column chromatography and eluted with NaCl gradients (0.1–2.0 M). The elution fraction (5 ml) were collected and monitored for carbohydrate content based on phenol–sulfuric acid method at 492 nm absorbance. Finally, the eluted fractions were concentrated, dialyzed and lyophilized. The products were further chromatographed on a Sephacryl S-400 gel filtration column with water and lyophilized to afford four white polysaccharides, namely AEPA, AEPB, AEPC and AEPD. These polysaccharides were endotoxin free with Limulus amebocyte lysate (LAL) test.

2.3. Measurement of carbohydrate and protein contents

Total carbohydrate contents in purified samples were determined by phenol–sulfuric acid colorimetric method using glucose as the standard (Dubois, Gilles, Hamilton, Rebers, & Smith 1956). Uronic acid contents were measured by the carbazole–sulfuric acid method using glucuronic acid as standard (Bitter & Muir, 1962). In addition, proteins in the polysaccharides were quantified according to the Lowry method using bovine serum albumin (BSA) as the standard (Lowry, Rosebrough, Farr, & Randall, 1951), combined with the method of UV absorption on a TU-1800PC spectrophotometer (Beijing Purkinje General Instrument Co., Ltd., China).

2.4. Molecular weight determination

The average molecular weight were determined by the HPGPC, which was performed on a Waters HPLC system (Allances 2695, Waters, USA) equipped with a Waters Ultrahydrogel 250 column (7.8 × 300 mm) and a Waters 2410 differential refractometer. The mobile phase was 0.1 mol/L $NaNO_3$, and the flow rate was 0.9 mL/min. The sample (2 mg) was dissolved in the mobile phase (0.2 ml) and centrifuged. A 20 μl sample was injected in each run. The molecular weight was estimated by reference to the calibration curve made from a Dextran T-series standard of known molecular weight (6100, 16,500, 26,290, 40,000, 84,000, 158,000) (Sun, Tang, Gu, & Li, 2005).

2.5. Analysis of monosaccharide composition

Neutral monosaccharide composition was analyzed according to the following procedure: the polysaccharide samples (5 mg) were hydrolyzed with 5 ml of 2 M TFA at 110 °C for 5 h, followed by evaporation and addition of MeOH to the residue. The hydrolyzate was reduced with $NaBH_4$ for 3 h at room temperature. The excess $NaBH_4$ was decomposed with HOAc and removed by repeated evaporation to dryness with the addition of 10% (v/v) HOAc in MeOH (Mawhinney, Feather, Barbero, & Martinez, 1980). Alditol acetates of the reduced sugars and authentic standards (rhamnose, fucose, arabinose, xylose, mannose, glucose and galactose with inositol as the internal standard) were prepared with AC_2O at 100 °C for 1 h and subjected to gas chromatography (GC) on an Agilent 4890D system (Agilent Technologies, Palo Alto, CA, USA) equipped with a flame-ionization detector (FID) and a DB-35 capillary column (30.0 m × 0.32 nm × 0.25 μm). The injector temperature was kept at 250 °C (split injection 40:1). The operation was performed at a column temperature program from 110 °C to 190 °C at 5 °C/min, holding for 5 min at 190 °C, then increasing to 250 °C at 10 °C/min and finally holding for 5 min at 250 °C. The molar percentage of the component monosaccharides was calculated as follows. The correction factor is shown in the equation: $f_{i/s} = (W_i/W_s)/(A_i/A_s)$, where A_s and A_i are the values of peak areas for inositol and standard monosaccharide, respectively. W_s and W_i are the values of weights for inositol and standard monosaccharide, respectively. The molar ratio value is shown in the equation: $R_{i/s} = f_{i/s} \times (A_i/A_s)/M$, where A_i/A_s is the ratio value of peak area for the component monosaccharide of tested samples and inositol. M is the molecular weight of the monosaccharides and $f_{i/s}$ is the correction factor.

2.6. Animals and cell lines

ICR mice (Grade II, 5 weeks old) weighing 18–22 g were purchased from Zhejiang Experimental Animal Center (Certificate No. 22-2001001, Hangzhou, China) and acclimatized for 1 week before use. Half of them were male and the others were female. Ro-