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Antioxidant properties and PC12 cell protective effects of APS-1, a polysaccharide from *Aloe vera* var. *chinensis*

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Abstract

Through a combination of anion-exchange and repeated gel chromatographies, APS-1 was isolated from fresh leaves of *Aloe vera* L. var. *chinensis* (Haw.) Berger (an edible and medicinal plant widely cultivated and consumed in China) as a principal polysaccharide composed of mannose and glucose (ca. 18 : 5) with its molecular weight around 2.1×10^5. In a dose-dependent manner, APS-1 was demonstrated to be free radical scavenging in superoxide and hydroxyl radical assays, inhibitory to the copper-mediated oxidation of human low density lipoprotein (LDL), and protective against hydrogen peroxide (H_2O_2)-induced lesion to rat PC12 cell (pheochromocytoma cell line). The result suggested that APS-1 could be of considerable preventive and therapeutic significance to some free radical associated health problems such as coronary heart ailments, Parkinson's and Alzheimer's diseases. Furthermore, the finding shed as well fresh light helpful for a better understanding of the health-benefiting potential of the edible plant consumed by the Chinese people for a couple of centuries.

Keywords: *Aloe vera*; APS-1; Polysaccharide; Antioxidant; Free radicals; PC12 cells

Introduction

An increasing pile of evidences highlights that the oxidative stress-induced cell damage triggers both the physiological process of aging (Harman, 1993) and many pathological progression leading eventually to some serious health problems such as Parkinson's and Alzheimer's diseases (Benzi and Moretti, 1995; Finkel and Holbrook, 2000). For a normal glucose metabolism in the brain, neuronal cells have to consume a large quantity of oxygen (Kawai et al., 1989) for maintaining energy-intensive biochemical processes (Nohl, 1987). Frequently as concomitant by-products, oxygen-derived free radicals are inevitably generated during the normal and/or aberrant consumption of molecular oxygen. And these free radicals are ascertained to be able to attack lipid membranes, proteins and deoxynucleic acids, and exert some detrimental effects, including lipid peroxidation of cell membranes, alteration of lipid–protein interactions, enzyme inactivation (Kim et al., 1985), and DNA breakage (Imlay and Linn, 1988). Cells protect themselves from oxidative damage through several defense mechanisms such as the enzymatic conversion of reactive oxygen species (ROS, e.g., $O_2^{\cdot}$ and HO·) into none/less toxic substances (Cotgreave et al., 1988), and through detoxification based on the reaction with antioxidants (Aruoma, 1996). Practically, application of antioxidants might be an acceptable effective therapeutic strategy to cure disorders initiated by ROS.

Referred to as a miracle plant, *Aloe vera* possesses confirmed curative or healing actions (Davis et al., 1989). A total of 360 *Aloe* species (commonly accepted as Aloe vera) are growing in the dry regions of North American, Europe and Asia. These plants were demonstrated to contain a yellow exudate (composed mainly of anthraquinone derivatives) that has been used for centuries as a purging agent; and a clear mucilaginous gel (consisting principally of polysaccharides) that has been utilized since ancient times to treat burns and other wounds where it is thought to be able to speed up the healing rate and to reduce the infection risk (Grindlay and Reynolds, 1986). Furthermore, some specially prepared *A. vera* extracts possess other biological activities such as anti-inflammation, anti-cancer, anti-diabetes and macrophage activation (Reynolds and Dweck, 1999).

A. vera L. var. *chinensis* (Haw.) Berger, a special species in China, has been widely cultivated in many areas such as

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Hainan, Jiangsu and Yunnan provinces. It has been consumed both as a vegetable and as a traditional Chinese medicine in single and compounding prescriptions for treating fever, constipation and ringworm. However, few reports dealing with the characterization of bioactive constituents in *A. vera* var. *chinensis* are available in addition to the preliminary attention to its antitumor action (Wang et al., 1989). As a follow-up to our previous investigation of fungal polysaccharides (Sun et al., 2004, 2005), we have found that the glycan extract of *A. vera* var. *chinensis* is significantly antioxidative. The subsequent ion-exchange and gel chromatographies of the antioxidant extract afforded a main polysaccharide named APS-1. We wish hereby to present the fractionation, compositional analysis, antioxidant action and PC12 cell protecting effect of the glycan.

Materials and methods

Reagents and materials

Phenazin methosulfate (PMS), β-nicotinamide adenine dinucleotide (NADH), butylated hydroxytoluene (BHT), 2-deoxy-D-ribose, reduced glutathione and human low density lipoprotein (LDL) were purchased from Sigma-Aldrich Chemical Co., USA. Nitroblue tetrazolium (NBT) and bovine serum albumin (BSA) were purchased from Shanghai Biochemical Factory, Shanghai, China. Lactate dehydrogenase (LDH) Detection Kit was purchased from Nanjing Jiancheng Bioengineering Institute, Nanjing, China. Other chemicals used in the study were of analytic grade.

Fresh *A. vera* var. *chinensis* was harvested from the suburb of Suzhou, Jiangsu Province, China. A voucher specimen, identified by Assoc. Prof. L. X. Zhang, was deposited under the registration number NJU-758 in the herbarium of Nanjing University, Nanjing, China.

Fractionation of APS-1 from A. vera var. chinensis extract

The leaves (300 g) of the title plant, after ground on an electrical grinder, were extracted thrice with 2500 ml of distilled water at 70–80 °C for 2 h. The filtrate of the obtained extract was condensed in vacuo to a syrup (ca. 500 ml), to which cold 95% ethanol (approximately 1500 ml) was added. The crude polysaccharide part was precipitated from the alcoholic liquor during its subsequent standing at 4 °C overnight. The precipitate that formed was collected by centrifugation at 12,000 $\times g$ and repeatedly washed sequentially with possibly less amounts of ethanol, acetone and ether, respectively. The refined crude polysaccharide (2.4 g) was redissolved in distilled water (240 ml) at a concentration of 1% (w/v) followed by filtration. The filtrate was deproteinized by treating it with trichloroacetic acid following the procedure detailed elsewhere (Yang et al., 1999). After centrifugation at 12,000 $\times g$ for 10 min, the supernatant was then precipitated with 3-fold volumes of 95% ethanol to obtain the polysaccharide-enriched fraction (APS). The fraction APS was subsequently subjected to a DEAE-52 anion-exchange column chromatography (8 × 30 cm) eluting with the deionized water at a flow rate of 50 ml/h. Fractions (8 ml, each) were combined according to the total carbohydrate content determined by the phenol-sulphuric acid method (Dubois, 1956). The discerned major peak (APS-A) was dialyzed, concentrated and fractionated further by gel filtration over a Sephacryl S-400 column (3.6 × 100 cm) eluting with the deionized water at a flow rate of 24 ml/h. The afforded purer product was further purified in the same manner to yield finally a homogeneous polysaccharide named APS-1. Purity of APS-1 was reinforced by its chromatographic profile on Sepharose CL-6B column (1.6 × 80 cm).

Determination of the molecular weight and monosaccharide composition of APS-1

To determine the molecular weight (MW) of APS-1 over a Sephacryl S-400 gel column (1.6 × 80 cm), a set of Dextrans (T-2000, T-110, T-70, T-50, T-40 and T-10) was used as MW references. The monosaccharide composition of APS-1 was determined as described earlier (Dong et al., 2003). Briefly, APS-1 (10 mg) was hydrolyzed with 2 M trifluoroacetic acid (TFA, 3 ml) at 110 °C for 3 h in a sealed tube. After hydrolysis completed, the removal of the excess amount of TFA was accomplished by co-evaporation at reduced pressure with methanol added after reaction. The subsequent treatment of the resultant dry hydrolysate with acetic anhydride and pyridine afforded the corresponding alditol acetate which was analyzed by GLC over HP-5 column (Crosslinked 5% PH ME Siloxane, 30 m × 0.25 mm × 0.25 μm) with a temperature gradient programmed from 150 to 190 °C at a speed of 1 °C/min, and from 190 to 250 °C at 10 °C/min.

Scavenging activity of APS-1 against superoxide radicals

The superoxide radical scavenging activity of APS-1 was evaluated according to the method detailed by Sun et al. (2004). Briefly, superoxide radicals were generated in 3.0 ml of 16 mM Tris–HCl buffer (pH 8.0), containing 78 mM β-nicotinamide adenine dinucleotide (NADH), 50 μM nitroblue tetrazolium (NBT), 10 μM phenazin methosulfate (PMS), and APS-1 at given concentrations of 12.5, 25.0, 50.0, 100.0 and 200.0 μg/ml. The coloration reaction of superoxide radicals with NBT was determined at 560 nm on a Hitachi U-3000 spectrophometer with deionized water as the blank control. The scavenging activity of superoxide radicals (%) was calculated according to the equation: $(A_{560(\text{blank})} - A_{560(\text{sample})}) \div A_{560(\text{blank})} \times 100\%$.

Deoxyribose assay for site-specific and non-site-specific hydroxyl radical scavenging activity

Non-site-specific hydroxyl radical scavenging activity of APS-1 was measured using the deoxyribose assay (Halliwell et al., 1987). Every 0.5 ml sample containing allocated amounts (50.0, 100.0, 200.0, 300.0 and 400.0 μg) of APS-1 were added to 1.0 ml solution composed of 20 mM potassium phosphate buffer (pH 7.4), 2.8 mM 2-deoxy-ribose, 104 μM EDTA, 100 μM $FeCl_3$, 100 μM ascorbate and 1 mM hydrogen peroxide. After