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Some properties of an acidic protein-bound polysaccharide from the fruit of pumpkin

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Abstract

To contribute toward our understanding of therapy mechanism of pumpkin protein-bound polysaccharides, homogeneity and weight-average molecular weight of an acidic polysaccharide from the fruit of pumpkin (APBPP) was determined by HPLC. The amino acid and monosaccharide composition of APBPP were tested. In the acid hydrolysates of APBPP, analysis by HPLC showed the presence of mannose and arabinose in molar ratios of 1:2. Eighteen amino acids were identified to be components of the polymer. Alanine was the main amino acid (0.13%), followed by glutamic acid (0.113%) and serine (0.088%). But the relationship between the contents of amino acids and hypoglycemic activity of APBPP is not clear.

Keywords: Protein-bound polysaccharide; Pumpkin; HPLC; Monosaccharide; Amino acid; Homogeneousness; Weight-average molecular weight

1. Introduction

The fruit of pumpkin (*Cucurbita moschata*) has been widely accepted as a dietary constituent among peasants in China; it is green when unripe and turns yellow on ripening. Pumpkin has received considerable attention in recent years because of the nutritional and health protective value of the proteins and oil (Barbara & Michael, 2004; Murkovic, Piironen, Lampi, Kraushofer, & Gerhard, 2004) from the seeds as well as the polysaccharides from the fruits. Fluted pumpkin seed flours were used as protein supplements in a variety of local foods (Giami & Bekebain, 1992; Sunday et al., 1999). In vitro protein digestibility of bread improved when pumpkin seed proteins were added (El-Soukkary, 2001). Preliminary investigations were shown that a pumpkin-rich dietary could reduce blood glucose and polysaccharides from pumpkin had hypoglycemic activity (Cai, Li, Yan, & Li, 2003; Chen, Wang, Jie, Huang, & Zhang, 1994; Li, Tian, & Cai, 2003; Xiong & Cao, 2001; Zhang & Yao, 2002). We also reported that protein-bound polysaccharides from the fruit of pumpkin could obviously increase the levels of serum insulin, reduce the blood glucose levels and improve tolerance of glucose and hence could be developed as new antidiabetic agent (Li, Fu, Rui, Hu, & Cai, 2005).

Several protein-bound polysaccharides have been shown to function as a nonspecific immunostimulating biological response modifier in cancer and herpetic patients (Matsunaga et al., 1992; Seong-Kug, Young-So, Chong-Kil, & Seong-Sun, 1999, 2000; Eo, Kim, Lee, & Han, 2000; Hattori, Komatsu, Shichijo, & Itoh, 2004; Tsukagoshi et al., 1984). An understanding of the properties of the protein-bound polysaccharide of pumpkin should contribute to our understanding of therapy mechanisms. Constituent monosaccharide residues of complex polysaccharide were often determined after chemical hydrolysis of the native polymers. Because it can hydrolyze glycosidic bonds and is volatile, which minimises its interference with subsequent procedures such as acetylation of the monosaccharide components, trifluoroacetic acid has become the preferred acid for most carbohydrate analysis (Benhura & Chidewe,

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2002). Hydrolysis of polysaccharide with trifluoroacetic acid and extensive destruction of the resulting monosaccharide components would not occur and hence the accuracy will increase.

The purpose of the present work was to determine some properties of APBPP, such as weight-average molecular weight, amino acid and monosaccharide composition.

2. Materials and methods

2.1. Materials

The fruits of pumpkin were harvested from the region of Qiqihar (North-east of China). The samples were thoroughly washed with tap water, air-dried and finely powdered.

2.2. Activity-guided isolation and purification of the acidic protein-bound polysaccharide from pumpkin fruit (APBPP)

The acidic protein-bound polysaccharide was extracted and purified as previously described (Li et al., 2005). Briefly, the fruits of pumpkin were extracted with water at 45 °C for 16 h, following treatment with SEVAG reagent. Then the mixture was concentrated and precipitated with three volumes of ice cold EtOH. It was centrifuged after standing overnight at 4 °C and the obtained precipitate was lyophilized and denoted PPEW. Then the PPEW, a brownish powder with high molecular weight component of water soluble substances, was separated by using the DEAE–Sepharose fast flow column chromatography and the acidic protein-bound polysaccharide from pumpkin fruit (APBPP) was obtained when linear gradients of sodium phosphate buffers were applied.

2.3. Determination of the molecular weight

One milligram APBPP was dissolved in 0.5 ml deionised water, applied to an HPLC system of Agilent 1100 (America) equipped a gel-filtration chromatographic column of Shodex Sugar KS-805(Japan), maintained at a temperature of 60 °C, eluted with the deionised water at a flow rate of 1.0 ml/min and detected by a SEDEX 75 evaporation light scatter detector. In the estimation of the molecular weight of the APBPP by gel permeation HPLC, dextran standards (MW 2,000,000, 500,000, 70,000, 40,000 and 10,000, Sigma) and glucose(MW 180, Sigma) were used under the conditions described above.

2.4. Hydrolysis of polysaccharide using 2 M trifluoroacetic acid (Benhura & Chidewe, 2002)

The polysaccharide (10 mg) was suspended in 1 ml of 2 M trifluoroacetic acid (TFA) in a screw-cap vial. The vial was tightly sealed, heated for up to 3 h at 120°C allowed to cool and the contents centrifuged for 5 min at 2000*g*. The TFA in the supernatant was allowed to evaporate off in the fume hood and the remaining mixture was freeze-dried, after which the dried material was dissolved in 50 ml of distilled water. Samples of the hydrolysed mixtures were also analysed by HPLC using a Agilent HPLC system, a refractive index detector and a Sugar-pak-1 column. Deionised water was used as the solvent at a flow rate of 0.7 ml/min and 25 μl of samples or standards were injected.

2.5. Amino acid analysis

APBPP was hydrolyzed in vacuoat 110 °C in 6 M HCl for 24 h. The amino acids, except for tryptophan, were determined with an Hitachi 835-50 amino acid analyzer. For the analysis of tryptophan, APBPP was hydrolyzed in 4.2 N barium hydroxide, including 3% thiodiethylene glycol, in a sealed tube at 110 °C for 12 h and then the tryptophan was tested as described previously(Matsumura et al., 2003).

3. Results and discussion

3.1. General

In an attempt to find hypoglycemic substances, an acidic protein-bound polysaccharide from pumpkin fruit (APBPP) was isolated and purified previously (Li et al., 2005). APBPP was identified as consisting mainly of polysaccharide and protein by the anthrone test and the Lowry–Folin test. Further study of APBPP should contribute to an understanding of therapy mechanisms.

3.2. Homogeneity and weight-average molecular weight of APBPP

APBPP was eluted as a single symmetrical peak, as determined by HPLC, which indicated that the polysaccharide was homogeneous. The weight-average molecular weight was calculated to be 9.19×10^6 Da, according to the calibration curve with standard dextrans and glucose (Fig. 1a and b).

3.3. Monosaccharide and amino acid composition of APBPP

HPLC analyses of the samples that had been treated with trifluoroacetic acid for 2 h showed that APBPP was composed of the monosaccharides, mannose and arabinose in molar ratios of 1:2.

The content of total amino acid is 0.742 g/100 g in the acidic protein-bound polysaccharide from pumpkin fruit (Table 1), 18 amino acids was identified to be components of the polymer. Alanine was the main amino acid (0.13%), followed by glutamic acid (0.113%) and serine (0.088%). During our investigation of the hypoglycemic substances in pumpkin, we found that proteins of the fresh pumpkin seeds do not reduce the blood glucose levels of