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Gastroprotective effect of a type I arabinogalactan from soybean meal

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ABSTRACT

A highly purified type I arabinogalactan (AG) from soybean meal was prepared. It contained galactose, arabinose, galacturonic acid and rhamnose in a 52:41:4:3 molar ratio and had M_w 124,000 g/mol. Methylation analysis and ^{13}C NMR spectroscopy indicated that AG is constituted by a (1→4)-linked β-Gal*p* main chain with substituents of α-Ara*f* at O-3, which are in turn substituted at O-5, O-3,5, and O-2,5. It is probably linked to O-4 of some rhamnosyl units of a type I rhamnogalacturonan, formed by repeating (1→4)-α-D-Gal*p*A-(1→2)-α-L-Rha*p* groups. AG significantly inhibited ethanol-induced gastric lesions in rats at an ED_{50} of 35 mg/kg, indicating that it has a gastroprotective effect.

1. Introduction

Soybean meal, a byproduct of industrial oil extraction, is rich in proteins and polysaccharides. Some of their structures have been partly elucidated during the sixties (Aspinall, Begbie, Hamilton, & Whyte, 1967; Morita, 1965). The most intensively studied is an arabinogalactan, the major component of the hot-water-extraction (Morita, 1965). It was shown to contain a main chain of (1→4)-linked β-D-galactopyranose residues in which some are substituted at O-3 by side chains containing (1→5)-linked L-arabinofuranose residues (Aspinall et al., 1967). The soybean arabionogalactan is defined as type I, whereas the type II consists of a (1→3)-linked β-D-Gal*p* main chain, substituted at O-6 by (1→6)-linked β-D-Gal*p* side chains (Carpita & Gibeaut, 1993).

Plant polysaccharides have been reported to have antiviral, antitumor, immunostimulating, anti-inflammatory, anticomplementary, anticoagulant, hypoglycemic and anti-ulcer activities (Srivastava & Kulshveshtha, 1989; Nergard et al., 2005; Yamada, 1994). The last has been attributed to pectic polysaccharides from *Panax ginseng* (Sun, Matsumoto, & Yamada, 1992) and *Bupleurum falcatum* (Yamada, 1994), which have a high galacturonic acid content, acidic heteroxylans from *Maytenus ilicifolia* and *Phyllanthus niruri* (Cipriani et al., 2008), and type II arabinogalactans from *Cochlospermum tinctorium* (Nergard et al., 2005) and *M. ilicifolia* (Cipriani et al., 2006).

Although arabinogalactans have been related to an anti-ulcer protective effect, this has not been described for those of type I. Since soybean meal is widely consumed as a human food supplement, and is a great source of this polysaccharide, we now report the isolation and characterization of the type I arabinogalactan of soybean (AG) and evaluate its protective anti-ulcer activity.

2. Materials and methods

2.1. Plant material

Soybean meal (defatted soybean flour) was obtained from Vitao® (Curitiba/PR, Brazil).

2.2. General analytical methods

All extracts were evaporated at <40 °C under reduced pressure. Centrifugation was at 10,000 rpm for 15 min, at 25 °C. Uronic acid contents were determined using the *m*-hydroxybiphenyl method (Filisetti-Cozzi & Carpita, 1991). AG (10 mg) was carboxy-reduced by the carbodiimide method (Taylor & Conrad, 1972), using $NaBH_4$ as the reducing agent, giving a product (AG-CR) with the $-CO_2H$ groups of its uronic acid residues reduced to $-CH_2OH$.

2.3. Extraction and purification of arabinogalactan from soybean meal

Soybean meal (100 g) was extracted with water (1 l) under reflux for 2 h (× 3); extract was evaporated to a small volume, and added to EtOH (× 3 vol.). The resulting precipitate (18.2 g) was dissolved in water (200 ml), and the solution was treated with 10% aqueous TCA (200 ml) to precipitate protein. The supernatant was neutralized with aq. NaOH, dialyzed and freeze–dried. The residue (2 g) was dissolved in water, and was submitted to freeze-thawing until no more precipitate appeared, and the soluble component (1.2 g) was then dialyzed against distilled water in a

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closed system with a 100 kDa cut-off membrane. The water in the system (1 l) was renewed every 12 h (× 3). The retained fraction on dialysis contained purified polysaccharide (AG, 550 mg).

2.4. HPSEC analysis

Homogeneity and average molar mass (M_w) of AG were determined by high-performance size-exclusion chromatography (HPSEC) coupled to refractive index and multi-angle laser light scattering detectors. Four ultrahydrogel (Waters) columns were used in series, with exclusion sizes of 7×10^6, 4×10^5, 8×10^4, and 5×10^3 Da. The eluent was 0.1 M aq. $NaNO_2$ containing 200 ppm aq. NaN_3 at 0.6 ml/min. The sample, previously filtered through a membrane (0.22 μm), was injected (250 μl loop) at a concentration of 1 mg/ml. The specific refractive index increment (d*n*/d*c*) was determined and the results were processed with software provided by the manufacturer (Wyatt Technologies).

2.5. Monosaccharide analysis

AG (2 mg) was hydrolyzed with 2 M TFA (1 ml) at 100 °C for 8 h; the solution was then evaporated, and the residue dissolved in water (1 ml). The resulting monosaccharide mixture was examined by silica-gel 60 thin-layer chromatography (TLC; Merck), the plates being developed by ethyl acetate:*n*-propanol:acetic acid:water (4:2:2:1, v/v) and stained with orcinol-sulfuric acid (Sassaki, Souza, Cipriani, & Iacomini, 2008). The hydrolyzate was also treated with $NaBH_4$ (2 mg) and, after 18 h, HOAc was added, the solution evaporated to dryness and the resulting boric acid removed as trimethyl borate by co-evaporation with MeOH. Acetylation was carried out with Ac_2O-pyridine (1:1, v/v; 2 ml) at room temperature for 12 h, and the resulting alditol acetates extracted with $CHCl_3$ (Wolfrom & Thompson, 1963a,b). These were analyzed by GC–MS (Varian Saturn 2000R – 3800 gas chromatograph coupled to a Varian Ion-Trap 2000R mass spectrometer), using a DB-225 column (30 m × 0.25 mm) programmed from 50 to 220 °C at 40 °C/min, with helium as carrier gas. Components were identified by their typical retention times and electron ionization spectra.

2.6. Methylation analysis

AG and AG-CR (5 mg) were each methylated according to the method of Ciucanu and Kerek (1984), using powdered NaOH in DMSO-MeI. The per-*O*-methylated derivative was hydrolyzed with 50% v/v H_2SO_4 (0.5 ml) at 0 °C for 1 h, which was then diluted to 5.5% v/v and maintained at 100 °C for 17 h. The resulting mixture of *O*-methyl aldoses was neutralized with $BaCO_3$, filtered, reduced with $NaBD_4$ and acetylated as described above to give a mixture of partially *O*-methylated alditol acetates. These were analyzed by GC–MS, using the same conditions described for alditol acetates, except the final temperature that was 215 °C. They were identified by their typical retention times and electron ionization spectra (Sassaki, Gorin, Souza, Czelusniak, & Iacomini, 2005).

2.7. Partial acid hydrolysis

AG (150 mg) was partially hydrolyzed with 0.2 M TFA (20 ml) at 100 °C for 2 h. The mixture was evaporated to dryness and redissolved in water (10 ml), and the resistant main chain of AG (AG-MC, 45 mg yield) was precipitated with excess acetone (30 ml), centrifuged, and freeze–dried.

2.8. NMR spectroscopy

^{13}C NMR spectra were obtained using a 400 MHz Bruker model DRX Avance spectrometer with a 5 mm inverse probe, at 30 °C in D_2O. Chemical shifts (δ) are expressed in ppm relative to acetone, at δ 30.2.

2.9. Animals

Female Wistar rats (180–200 g) were maintained under standard laboratory conditions (12 h light/dark cycle, at 22 ± 2 °C). Standard pellet food and water were available *ad libitum*. The animals were deprived of food 15–18 h prior to the experiment. The experimental protocol was performed according to the "Principles of Laboratory Animal Care" (NIH Publication 85–23, revised 1985).

2.10. Induction of acute gastric lesions in rats

Fasted rats ($n = 6$) were orally fed with vehicle (water, 0.1 ml/100 g body weight), omeprazole (40 mg/kg), or AG (10, 30, 100 mg/kg), 1 h before administration of 80% EtOH (0.5 ml/200 g, p.o.). They were killed by cervical dislocation 1 h after treatment (Robert, Nezamis, Lancaster, & Hauchar, 1979). The gastric lesion area (mm^2) was determined as length × width of lesion.

2.11. Statistical analysis

Results were expressed as means ± standard error of the mean (SEM) and statistical significance was determined using one-way analysis of variance (ANOVA) followed by Bonferroni's test. Data were considered different at a significance level of $p < 0.05$. The effective dose 50 (ED_{50}) was calculated by fitting the data into the equation: $Vi/Vo = 1/(1 + [I]/IC_{50})$, using the KhaleidaGraph 3.0 for a Windows program (Synergy Software, PA, USA). *Vi* = total activity, *Vo* = remaining activity.

3. Results and discussion

3.1. Structural characterization of the arabinogalactan

Soybean meal (100 g) was extracted with water under reflux, and the extract was treated with excess EtOH to obtain a crude precipitate of polysaccharides and proteins (18.2 g). It was deproteinated and submitted to the freezing–thawing process. The resulting supernatant was then submitted to dialysis with a 100 kDa cut-off membrane to give AG (550 mg). HPSEC showed it to be homogeneous, with M_w 124,000 g/mol (d*n*/d*c* = 0.157). It contained galactose, arabinose, uronic acid and rhamnose in a 52:41:4:3 molar ratio. TLC analysis of the AG hydrolyzate indicated that galacturonic acid was present.

The ^{13}C NMR spectrum of AG (Fig. 1A) showed typical signals of C-1 of α-Ara*f* units at δ 107.7, 107.2, and 106.5, and of C-1 of β-Gal*p* units at δ 104.4 (Cipriani, Mellinger, Gorin, & Iacomini, 2004). Although AG contained 4% uronic acids, no carboxy signal appeared due to the spectral conditions. The presence of CH_3-6 of Rha*p* units was shown by a small signal at δ 16.8 (Gorin & Mazurek, 1975). Partial acid hydrolysis of AG was carried out to form its resistant main chain (AG-MC). This contained almost 100% of galactose and its ^{13}C NMR spectrum (Fig. 1B) contained six signals at δ 104.3 (C-1), 77.6 (C-4), 74.5 (C-5), 73.3 (C-3), 71.8 (C-2), and 60.7 (C-6) (Fransen et al., 2000; Hinz, Verhoef, Schols, Vincken, & Voragen, 2005), typical of a (1→4)-linked β-Gal*p* main chain.

Methylation analysis (Table 1) showed that AG contained non-reducing end-units of Ara*f* and Gal*p*, due to the formation of alditol acetates of 2,3,5-Me_3-Ara (13%) and 2,3,4,6-Me_4-Gal (3%), respectively. The arabinosyl units were substituted at O-5, O-3,5, and O-2,5, in accord with formation of alditol acetates of 2,3-Me_2-Ara (15%), 2-Me-Ara (6%), and 3-Me-Ara (6%), respectively. The galactopyranosyl units are 4-*O*- and 3,4-di-*O*-substituted, since alditol

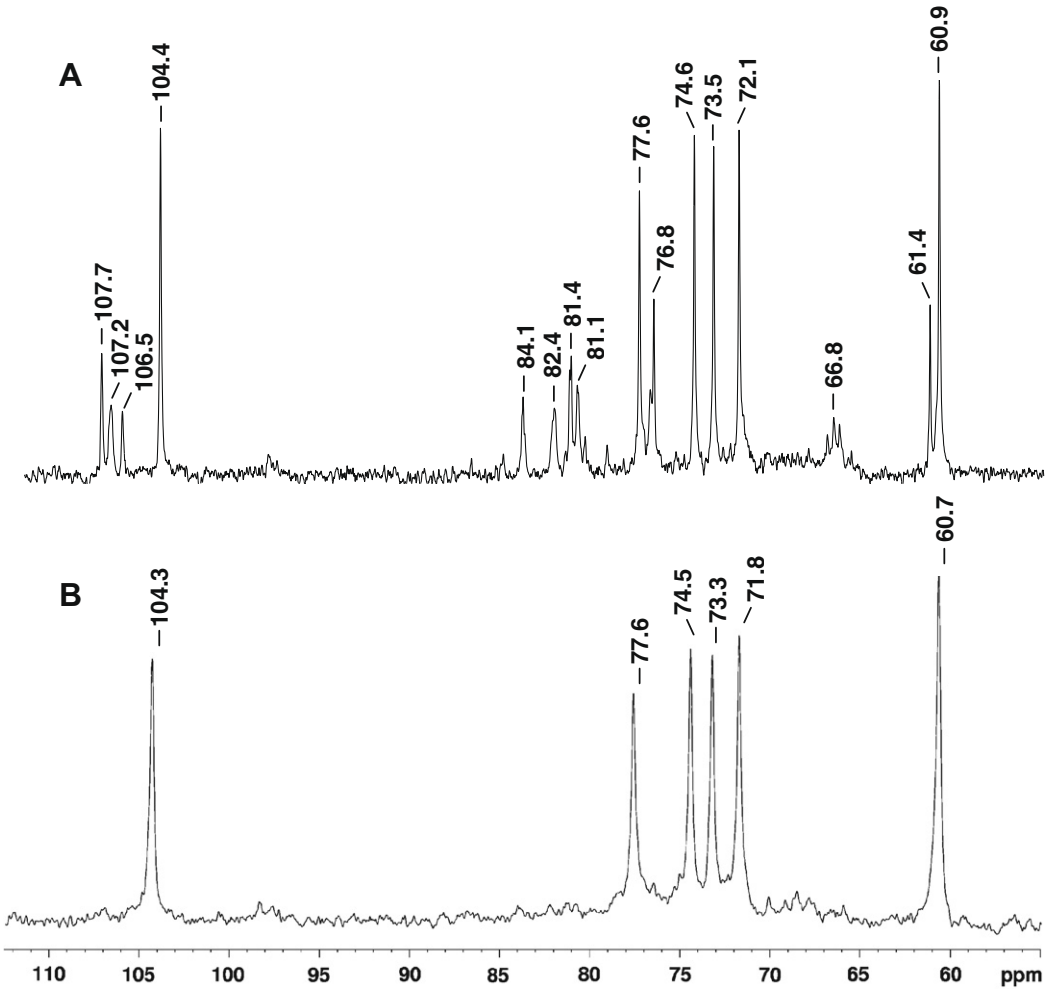

Fig. 1. ^{13}C NMR spectra of AG (A) and AG-MC (B). Solvent D_2O at 30 °C; numerical values are in δ ppm.

Table 1
Profile of partially *O*-methylated alditol acetates obtained by methylation analysis of AG[a].

O-Me-alditol acetate	linkages	mol%
2,3,5-Me_3-Ara	Ara*f*-(1→	13
3,4-Me_2-Rha	→2)-Rha*p*-(1→	1
2,3-Me_2-Ara	→5)-Ara*f*-(1→	15
2,3,4,6-Me_4-Gal	Gal*p*-(1→	3
3-Me-Rha	→2,4)-Rha*p*-(1→	2
2-Me-Ara	→3,5)-Ara*f*-(1→	6
3-Me-Ara	→2,5)-Ara*f*-(1→	6
2,3,6-Me_3-Gal	→4)-Gal*p*-(1→	45
2,6-Me_2-Gal	→3,4)-Gal*p*-(1→	5

[a] The uronic acid content was 4%.

acetate of 2,3,6-Me_3-Gal (45%) and 2,6-Me_2-Gal (5%) were formed. The rhamnosyl units were 2-*O*- and 2,4-di-*O*-substituted, as demonstrated by the presence of alditol acetates of 3,4-Me_2-Rha (1%) and 3-Me-Rha (2%), respectively. Methylation analysis on carboxy-reduced AG (AG-CR) showed an increase in the 2,3,6-Me_3-Gal derivative, indicating that 4-*O*-substituted galacturonic acid residues were present. These, plus 2-*O*- and 2,4-di-*O*-substituted Rha*p* residues, strongly suggest a type I rhamnogalacturonan structure. These are formed by the repeating (1→4)-α-D-Gal*p*A-(1→2)-α-L-Rha*p* group, often having O-4 of the rhamnosyl units substituted by an arabinogalactan sequence (Carpita & Gibeaut, 1993).

AG is therefore an arabinogalactan containing a (1→4)-linked β-Gal*p* main chain, with substituents of arabinosyl units at O-3, as described by Aspinall et al. (1967). These are in turn substituted at O-5, O-3,5, and O-2,5. This arabinogalactan is probably linked to a type I rhamnogalacturonan through O-4 of some of the rhamnosyl units. A proposed structure of AG is shown in Fig. 2.

3.2. Anti-ulcer activity

Studies have shown that type II arabinogalactans are polysaccharides having anti-ulcer protective effect (Cipriani et al., 2006; Nergard et al., 2005), but this property has not yet been described for type I arabinogalactans. In order to determine whether AG has anti-ulcer activity, oral treatments with female Wistar rats of 10, 30, and 100 mg/kg AG were tested. The gastric lesions induced by EtOH were reduced in a dose-dependent manner by 33, 48, and 71%, respectively, with ED_{50} of 35 mg/kg. Omeprazole (40 mg/kg), the positive control for the test, showed a 47% reduction of the lesions (Fig. 3).

This result suggests a potential activity of AG to act as a direct cytoprotective agent. Possible mechanisms suggested for anti-ulcer effects of polysaccharides are in their ability to, (1) bind to the mucosa surface and function as a protective coating, (2) diminish secretory activities of acid and pepsin, and (3) protect the mucosa by increasing mucus synthesis and/or scavenging radicals (Matsumoto, Moriguchi, & Yamada, 1993; Nergard et al., 2005; Yamada, 1994).

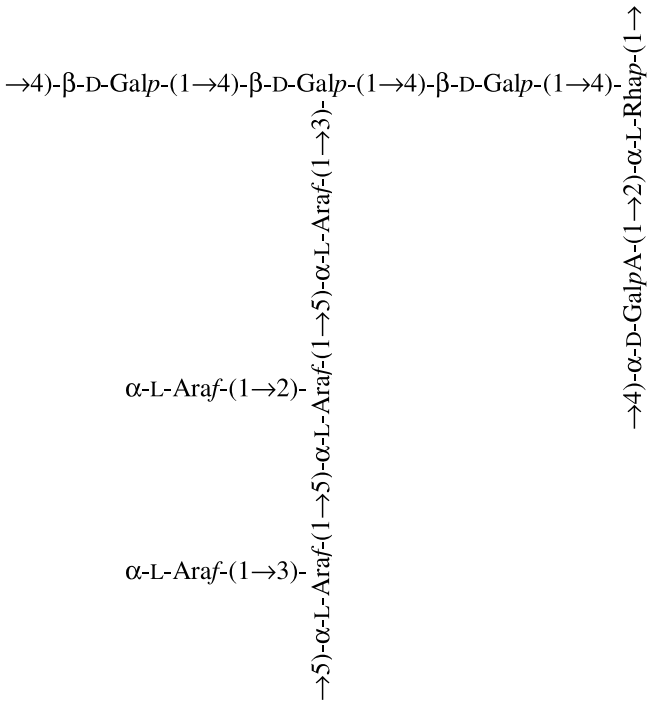

Fig. 2. Depiction of AG structure.

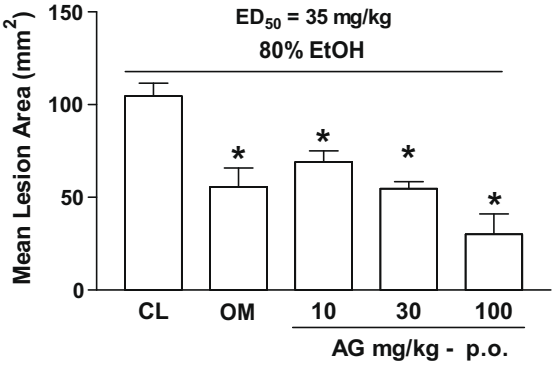

Fig. 3. Protective effect of AG (10, 30, and 100 mg/kg, p.o.) against ethanol-induced gastric lesions (CL: control, water 0.1 ml/100 g, p.o.; OM: omeprazole 40 mg/kg, p.o.). The results are expressed as means ± SEM ($n = 6$), with $^{*}p < 0.05$ when compared to control group.

4. Conclusion

We show a detailed chemical structure of the highly purified type I arabinogalactan from soybean and that it is able to inhibit ethanol-induced gastric lesions, indicating that it could be an effective gastroprotective agent. Our data suggest that, as well as the nutritional value of soybean meal, attributed to its high protein content, it could also have pharmacological value, as a nutraceutical food.

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