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Influence of the uronic acid composition on the gastroprotective activity of alginates from three different genus of Tunisian brown algae

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ABSTRACT

Alginates from three genus of Tunisian brown algae were isolated and characterized by size exclusion chromatography and Solid-state NMR spectroscopy. Alginate from *Padina pavonica* (APP) had the highest molecular weight (Mw) with 147,000 g/mol while it was 85,000 g/mol for alginate from *Cystoseira compressa* (ACC) and 58,000 g/mol for alginate from *Dictyopteris membranaceae* (ADM). The mannuronate (M) to guluronate (G) ratios were estimated from spectral deconvolution of the ^{13}C CP/MAS spectra and the results has shown that all the extracts are mannuronic acid-rich alginates with M/G ratio increased in the order ADM – ACC – APP. An interesting gastroprotective effect was observed for the extracts; ADM and ACC exhibited the highest inhibition of gastric lesions, at 50 mg/kg, with 83.41% and 75.39% respectively. Otherwise, it has been shown that the gastroprotective effect of alginates depends mainly on their uronic acid composition.

1. Introduction

Gastric ulcer is a disease that affects numerous people around the world. Stress, smoking, alcohol, nutritional deficiencies and frequent use of nonsteroidal anti-inflammatory drugs (NSAIDs) (Khazaei & Salehi, 2006) are the main causes of this disease. Yet, proton pump inhibitors (PPIs) are the most commonly prescribed class of medication to treat heartburn and acid-related disorders. However, like every medication, PPIs causes adverse effects like a potential increased risk of malabsorption of key minerals in the body, namely calcium and magnesium, in addition to an increased risk of infections (Heidelbaugh, 2013). Thus, there is an urgent need to find more safe and effective gastro-protective agents. Several experimental and clinical trials demonstrated that phycocolloids with interesting physical properties have a potent anti-ulcer protective effect on gastric lesions in rats (Asada et al., 1997).

Alginates are a linear copolymers of β-D-mannuronic acid (M) and α-L-guluronic acid (G) linked by 1 → 4 glycosidic bonds. These uronic acid residues, in pyranosic conformation, give three sequences identified after hydrolysis, as the blocks MG consisting of nearly equivalent proportion of both residues, the blocks GG and MM (Fenoradosoa et al., 2010). The biological and physical properties of alginates depend not only upon the uronic acid composition but also upon the relative amount of the three types of block (Stokke, Smidsroed, Bruheim, & Skjaak-Braek, 1991). Therefore, these phycocolloids are in great use in different industries like food, pharmaceutical, cosmetic and textile, notably for their thickening, stabilizing and gelling properties (Lee & Mooney, 2012; McHugh, 1987; Rhim, 2004; Wang et al., 2003). The structure and the quantity of alginates depend on the algae species, the type and age of the matters used for alginate extraction (Haug, Larsen, & Smidsrød, 1974). However, few studies have investigated the influence of M/G ratio on the biological activities (Draget & Taylor, 2011; Iwamoto et al., 2005).

In this work, we present the extraction, purification and analysis of sodium alginates from three brown algae (*Cystoseira compressa*, *Padina pavonica* and *Dictyopteris membranaceae*) collected from the Tunisian coast. The obtained alginates were then characterized by SEC/MALS/VD/DRI and Solid state NMR spectroscopy. At the end, the influence of the M/G ratio of alginates on their anti-ulcerogenic effects was investigated.

2. Materials and methods

2.1. Plant material

Specimens of algae *Cystoseira compressa*, *Padina pavonica* and *Dictyopteris membranaceae* were collected in the coastal waters of

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Monastir and Tabarka (Tunisia) during the year 2007. They were identified by the National Institute of Marine Sciences and Technologies (NIMST) (Salambôo, Tunisia). *C. compressa* belongs to the family of Cystoseiraceae whereas the remaining two are of the family of Dictyotaceae. The collected algae were initially washed to remove the extraneous matters, shade dried and then powdered. The dried seaweeds powder was soaked in Methanol-Dichloromethane (1:1) at room temperature for 48 h then filtered. This process was repeated three times and followed by a sequential extraction with petroleum ether then acetone in a soxhlet apparatus to flush out a substantial portion of lipophilic pigments.

2.2. Extraction and purification of alginates

Alginates were extracted according to the procedure of Rioux, Turgeon, and Beaulieu (2007) with some modification. The seaweeds powders were treated three times with 2% aqueous solution of $CaCl_2$, HCl 0.01 M, pH 2, during 3 h at 70 °C with mechanical stirring. After centrifugation, the pellet is solubilized in 3% aqueous solution of Na_2CO_3. Sodium alginates were finally recuperated after dialysis through tubing of molecular weight cut off 30 kDa and lyophilization.

2.3. Chemical analysis

The micro-titer plate method was used to determinate the uronic acid amount in the extracted sodium alginates (Cesaretti, Luppi, Maccari, & Volpi, 2003). A serial dilution of standard (galacturonic acid) and samples (50 µL, 1 mg/mL) was placed in a 96 well plate. Subsequently, 200 µL of 25 mM sodium tetraborate in H_2SO_4 solution was added. Then, the plate was heated at 100 °C for 10 min in an oven and a volume of 50 µL of 0.125% carbazole in ethanol was carefully added. Finally, the plate was read at 550 nm after a second heating at 100 °C for 10 min.

2.4. ATR-FTIR

Attenuated total reflectance Fourier transform infrared (ATR-FTIR) spectra of the extracted samples were collected at 25 °C using PerkinElmer Spectrum Two™ ATR-FTIR, over the wave number range between 4000 and 400 cm^{-1}.

2.5. Solid state NMR

The solid state ^{13}C CP-MAS NMR spectra were acquired using a BRUKER Avance III 500 MHz spectrometer equipped with an Ultrashiled Plus magnet and Topspin software (version 2:1). The double-resonance probe was tuned respectively to 125.75 MHz for ^{13}C-frequency and to 500.21 MHz for 1H-frequency. Alginate powders were packed into zirconia rotors (5 mm) and sealed with Kel-F caps. Spectra were acquired using ramped-amplitude-cross-polarization (CP), magic-angle spinning (MAS) with total sideband superposition and SPINAL64 decoupling. A contact time of 1 ms, a recycle delays (D1) of 3 s, a MAS frequency of 8.0 kHz, a 1H decoupling field of 100 kHz, and a minimum scan number of approximately 2 K were used in order to attain a high signal-to-noise ratio, was optimized for all spectra.

2.6. ^{13}C CP/MAS spectra deconvolution

A deconvolution procedure was applied to all of the spectra in order to obtain the exact values of chemical shift, line width and intensities of the signals in the spectral region between 50 and 90 ppm (pyranose region). A total number of five peaks were used to reproduce the pyranose region of the experimental spectra, as previously reported by Llanes, Sauriol, Morin, and Perlin (1997) and Salomonsen, Jensen, Larsen, Steuernagel, and Engelsen (2009a, 2009b). The best fitting results were obtained using Gaussian shapes for all the peaks. The spectral fitting procedure was performed using the GAUSS function implemented within the ORIGIN 7.0 software.

The quality of the fit was evaluated through the correlation coefficient (R^2). Therefore, a value of the coefficient close to unity is a sign of good correlation with the experimental spectrum.

The M/G ratios were estimated according to the following equation.

$$(E + F)/(D + G + H)$$

Where E, F, D, G and H are the peak air at about 76, 71, 82, 68, 65 ppm, respectively.

2.7. SEC/MALS/VD/DRI analyses

The macromolecular characteristics (the molar masses distributions, the number- and mass-average molar masses, the hydrodynamic radius and the intrinsic viscosity) of the isolated sodium alginates were determined at 25 °C by coupling on-line a size-exclusion chromatograph (SEC), a multi-angle light scattering system (MALS), a viscometer (VD) and a differential refractive index detector (DRI). The SEC system involves a pump (LC10 Ai Shimadzu, Japan) at a flow rate of 0.5 mL/min and two columns OHPAK SB 804 and 806 HQ. The samples were, firstly, dissolved in $LiNO_3$ (0.1 mol/L) at 2 g/L, filtered on 0.45 µm membrane (regenerated cellulose) and then injected through a 100 µL full loop. The Astra 6.0.1.7 software package was set to collect and extrapolate data according to the known value of the differential refractive index, dn/dc (dn/dc = 0.15 mL g^{-1} (Majdoub, Roudesli, & Deratani, 2001)). The analyses were performed by a data processing Zimm (1948) "order 1" using angles from 34.8° to 142.8°. The gyration radius is too low to be obtained (inferior to 20 nm). Hydrodynamic radii (Rh) are calculated from the intrinsic viscosities using the Einstein-Simha equation with a conformational parameter $\nu = 2.5$ in the case of a spherical conformation (Ali, Rihouey, Larreta-Garde, Le Cerf, & Picton, 2013).

2.8. Animals

All experiments were achieved according to the guidelines recognized by the European Union on Animal Care (CCE Council 86/609). Wistar rats of both sexes weighing between 150 and 200 g were purchased from Pasteur Institute (Tunis, Tunisia). They were divided in groups in cages at 20-25 °C and they were fed a standard pellet diet.

2.9. The effect of sodium alginates on ethanol-induced gastric damage

The gastroprotective propriety of the extracted alginates was studied in HCl/EtOH induced gastric ulcer according to the method described by Okabe, Miyake, and Awane (1986) and in comparison of a commercial alginate. This later was purchased from Degussa Company (Puteaux, Hauts De Seine, France) and have a number- and mass-average molar mass (Mn and Mw) about 195,000 and 350,000 g/mol, respectively (Benykhlef et al., 2012), and a M/G ratio of 0.5.

In brief, rats were distributed into different groups, fasted for 24 h prior and then treated with vehicle (water, 1 mL/kg body weight), ULCAR (200 mg/kg), commercial alginate (CA) (25 and 50 mg/kg) or isolated sodium alginates (25 and 50 mg/kg). An hour later, the animals were treated with gastric ulcer induction solution (1 mL/100 g of 150 mM HCl/EtOH (40:60, v/v)) by gavage and then sacrificed 1 h after the administration. Next, their stomachs were quickly removed and opened along the greater