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Arabino-Galactan Proteins from *Pistacia lentiscus* var. *chia*: isolation, characterization and biological function

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Summary. Arabino-Galactan Proteins (AGPs) were isolated from Chios mastic gum (CMG) by using a buffer containing 0.1 M NaCl, 20 mM Tris–HCl, pH 7.5. Protein analytical methods, combined with specific procedures for carbohydrate characterization, indicated the presence of highly glycosylated protein backbone. In particular, staining by Yariv reagent of the electrophoretically separated molecules revealed the existence of arabinose and galactose and such a modification is characteristic for AGPs.

After experiments involving extensive dialysis of the isolated extracts against water and atomic absorption, there was evidence of the existence of zinc ions that are probably covalently bound to the AGPs. By using anion-exchange chromatography, capillary electrophoresis, colorimetric methods and GC-MS, it was found that the extracts were separated into three major populations (A, B, and C), which were consistent with their respective negative charge content namely, uronic acid. The characterization of neutral sugars that was investigated with GC-MS showed the existence of arabinose and galactose in different amounts for each group.

Experiments concerning the inhibition of growth of *Helicobacter pylori* in the presence of AGPs, as is shown for other CMG constituents, showed that the extracts of at least 1.4 g CMG affected the viability of the bacterium. There is no evidence as to whether the AGPs provoke abnormal morphologies of *H. pylori*, as is reported for the total CMG, or for O-glycans that possess terminal α1, 4-linked N-acetylglucosamine and are expressed in the human gastric mucosa; this has to be further investigated.

Keywords: Arabino-Galactan Proteins (AGPs) – Chios mastic gum (CMG) – Biological function

Introduction

Mastic is a white, semitransparent, natural resin that is obtained as trunk exudates from mastic trees. Its scientific name is *Pistacia lentiscus*, and it belongs to the Anacardiaceae family. The plant *Pistacia lentiscus* var. *chia*. grows particularly and almost exclusively in the south region of Chios Island, Greece, and produces a resin, known as Chios mastic gum (CMG). CMG, like other exudate gums, is produced from the trunk and the thickest branches of the tree when it is injured in order to seal the wound, preventing the infection and dehydration of the plant. The chemical composition of the mastic oil and the essential oil of the resin have been reported (Papageorgiou et al., 1991; Magiatis et al., 1999). Its biological activity can be attributed to a variety of compounds. It contains triterpenes of the oleanane, euphane and lupine type (Andrikopoulos et al., 2003; Assimopoulou and Papageorgiou, 2005), alpha-tocopherol (Kivcak and Akay, 2005) and polyphenols (Romani et al., 2002); the latter have been associated with a hypotensive effect of mastic (Sanz et al., 1992).

CMG possesses anti-bacterial properties (Iauk et al., 1996; Magiatis et al., 1999; Koutsoudaki et al., 2005) and its in vivo-demonstrated antiplaque action in the oral cavity has been attributed to its inhibitory action against overall bacterial growth (Takashi et al., 2003), and especially against *S. mutants* (Aksoy et al., 2006). As far as the effectiveness of *Pistacia lentiscus* against *Helicobacter pylori* and peptic ulcers is concerned, data remain controversial. Clinical studies have initially indicated that CMG is effective against gastric and duodenal ulcers (Al-Habbal et al., 1984; Al-Said et al., 1986); mastic has also been proven to be bactericidal against *H. pylori* in vitro (Huwez et al., 1998; Marone et al., 2001). However, other studies show that a total mastic extract without polymer led to an approximately 30-fold reduction in *H. pylori* colonization of infected mice (Paraschos et al., 2007), while the results of Loughlin et al. (2003) showed that *H. pylori* in infected

mice was not eradicated after CMG receipt. In addition, the inability of CMG to eradicate *H. pylori* from infected humans (Bebb et al., 2003) contradicted the findings reported by Al-Habbal et al. (1984) and Al-Said et al. (1986).

All the above activities concerning the therapeutic properties of CMG were explored by using either the total resin or the constituents that were extracted with buffers containing organic solvents. However, it is well known that exudate gums also contain substances that are water soluble and some of them are rich in arabinogalactan proteins (AGPs) (Showalter, 2001). AGPs are a family of extensively glycosylated hydroxyproline-rich glycoproteins, analogous to animal proteoglycans that are thought to have important roles in various aspects of plant growth and development. At the organ level, AGPs are found in leaves, stems, roots, floral parts and seeds (Fincher, et al., 1983; Nothnagel, 1997). Knowledge of the protein moieties of AGPs has mostly come from purifying AGPs, deglycosylating them and analyzing their respective core proteins by amino acid analysis and, to a more limited extent, by sequence analysis (Du et al., 1994; Gao et al., 1999). As their name implies, AGPs are rich in arabinose and galactose and in some cases glucuronic acid, along with other less abundant sugars. AGPs are thought to function in various aspects of plant growth and development namely, vegetative, reproductive and cellular growth and development (Cheung et al., 1995), programmed cell death and social control (Shindler et al., 1995) and molecular interactions and signaling (Schultz et al., 1998).

Recently it has been reported that AGPs from *Echinacea purpurea* stimulate phagocytosis and release of TNF by macrophages (Wagner et al., 1988; Classen et al., 2000), and a pilot study suggested that this AG might be effective in vivo in reducing chemotherapy-induced leucopenia (Melchart et al., 2002), as another AGP from *Echinacea purpurea* was found to possess complementary-stimulating activities in vitro (Alban et al., 2002), while interactions with leucocytes or the complementary system have been demonstrated by Diallo et al. (2001) and Thude et al. (2006), respectively.

Within this work we present the existence of AGPs in CMG extracts which are linked to zinc, as evidenced by atomic absorption. Their isolation as well as their partial characterization have shown that they consist of 3.1% protein, 78.5% neutral sugars (arabinose and galactose) and 18.4% uronic acids. In addition, it is evidenced by MIC experiments that they exhibit antibacterial activity in vitro against *H. pylori.*

Materials and methods

Preparation of water-soluble gum extracts and verification of their protein content

Dry Chios mastic gum (resin, CMG) was pulverized to a fine powder and 5 g were mixed with 0.1 M NaCl, 20 mM Tris–HCl, pH 7.5, and after overnight stirring at 4 °C were centrifuged to separate soluble (supernatant) material from insoluble (pellet) material. The soluble material was filtered by using 0.45 μm HA filters and dialyzed extensively against water. The zinc content of the extracts was estimated by atomic absorption on a Perkin-Elmer atomic absorption spectrometer (Model 403) at 214.5 nm and by using a Zn EDL lamp. The extracts were analysed on SDS-PAGE 12% and visualized with Coomassie blue. The presence of the protein was verified after incubation of the extracts with proteinase K. In particular, the extract from 2 g CMG was solubilized in 50 μl 100 mM Tris–HCl, pH 7.5, 0.5% SDS. Freshly prepared proteinase K stock solution (10 mg/ml in water) was added to a final concentration of 20 μg/ml and the mixture was incubated at 37 °C for 4.5 h. The sample was then analyzed in 12% SDS-PAGE.

Polyclonal antibodies production

The isolated extracts from CMG that were prepared as described above were used for polyclonal antibodies production. A 3-month-old rabbit was injected subcutaneously with the extracts from 1 g mastic as described by Harlow and Lane (1998). The resulted rabbit serum was collected after the second and third injection and was stored in 1ml aliquots at −20°C. The isolated CMG extracts were separated onto SDS-PAGE 12%, transferred onto PVDF membranes and visualized by using the produced antiserum in a dilution of 1:1000.

Glycosylation investigation of the CMG extracts

In order to investigate the modification of the proteins within the CMG extracts, a procedure was followed that concerns the glycoprotein oxidation, as described by Rothfus and Smith (1963). The protein extract (from 1 g CMG) was solubilized in 250 μl of 0.1 M Na-acetate buffer, pH 4.5 and was pre-cooled on ice. A fresh stock of 0.5 M $NaIO_4$ pre-cooled on ice and stored in the dark was prepared and was added to the sample to a final concentration of 10 mM periodate. The mixture was then incubated in the dark for 1 h at room temperature, the reaction was stopped by adding 1/10 volume of 0.5 M ethylene glycol and the excess of the glycol reagent was removed by dialysis against PBS.

The biotinylation of oxidized glycoproteins with the commercially available biocytin hydrazide (Pierce Chemical) was performed according to Spector et al. (1998). A freshly prepared stock solution of biocytin hydrazide (1 mg/ml in DMSO) was added to the dialyzed sample at a ratio of 1:10. This was followed by incubation of the samples at room temperature for 1 h, and the resulting biotinylated glycoproteins were analyzed on 12% SDS-PAGE and transferred on a nitrocellulose membrane, and the biotin was detected with the streptavidin-horseradish peroxidase protein.

Characterization of the CMG polysaccharide content

Extraction and purification of polysaccharides

CMG was extracted for 24 h at 4 °C under continuous stirring with 20 mM Tris/HCl, pH 7.5 which contained 0.1 M NaCl. After the end of extraction, the extract was filtered and centrifuged in order to remove the solid particles. Salts and other molecules of low molecular weight were removed from the extract with dialysis against water and gel filtration chromatography on PD-10 columns (Sephadex G-25, Pharmacia). Separation and isolation of carbohydrates was performed using an FPLC (Pharmacia) system with anion-exchange chromatography on Mono Q (Pharmacia)

column with a flow-rate of 1.0 mL/min. Elution was performed with gradient from 0.0 to 0.3 M NaCl for 25 min and from 0.3 to 1.0 M NaCl for 20 min in 20 mM Tris/HCl buffer, pH 7.5.

Colorimetric methods

The characterization of the crude extract and the isolated fraction was performed with colorimetric methods. Total neutral sugars and uronic acid were determined with the anthrone method, using galactose as standard (Scott and Melvin, 1953; Laurentin and Edwards, 2003), and the borate-carbazole method of Bitter and Muir, using the glucuronic acid lactone as standard (Bitter and Muir, 1962), respectively, after correcting for the interference of each of these substances with the other reaction. Total protein was determined with the Bradford method (Bradford, 1976), using bovine serum albumin as standard, and sialic acid with the orcinal reagent, using N-acetyl-neuraminic acid as standard (Schauer, 1978).

Other analytical methods

Purity and homogeneity of polysaccharide fractions was obtained using capillary electrophoresis. Capillary electrophoresis was performed in an HP3DCE instrument from Agilent Technologies in uncoated silica-fused capillaries (50 µm i.d. × 54.6 cm effective length). Separation was performed in a 25 mM borate buffer, pH 10.0, containing 25 mM SDS at 20 kV (normal polarity), and detection was at 200 nm. SDS-PAGE was performed as described by Laemmli using a 10% polyacrylamide gel in a vertical slab gel electrophoresis apparatus (Bio-Rad Laboratories). Staining of the gel was achieved with a combination of polysaccharide oxidation and Alcian-Blue staining (Moller and Poulsen, 1995). Determination of neutral and amino sugars was performed with GC-MS as TMS derivatives by the UCSD GRTC Glycotechnology Core Resource. In brief, methanolic HCl was used to hydrolyze glycosidic bonds and concomitantly form the methyl glycoside derivative (methanolysis). Free hydroxyl groups are trimethylsilylated and the resulting volatile derivatives are fractionated by gas chromatography on a DB-1 column using a temperature gradient and detected by electron ionization mass spectrometry (Kakehi and Honda, 1989).

Verification of the arabino-galactan presence in CMG extracts

Samples of mastic extract (from 1 g of mastic) were dissolved in Laemmli buffer and analysed on a 12% SDS-PAGE. The gel was then immersed for 15 min in β-glucosyl Yariv reagent. The concentration of Yariv reagent was 0.15 mg/ml in 1% NaCl (Baldwin et al., 1993).

Bactericidal activity of the water extracts against Helicobacter pylori

H. pylori strains were grown on agar plates containing 10% horse serum in a microaerophilic atmosphere (generated by Campy-Gen, Oxoid, Basingstoke, U.K.) at 37 °C for 48 h. Bacteria were harvested in phosphate-buffered saline (pH 7.4), diluted to a concentration of 2×10^9cells/ml and 10 µl were plated on 6 cm plates plus 10 µl BHI (Brain heart infusion – growth medium) containing agar. The mastic gum water soluble extracts of 1, 2 and 3 g were dissolved in 50 µl PBS and added to the cultures.

Results and discussion

Preparation and partial characterization of the proteinaceous nature of the water soluble CMG extracts

The pulverized resin (5 g each time), as described under the experimental session, was mixed with a buffer containing 0.1 M NaCl, 20 mM Tris–HCl, pH 7.5 and after overnight stirring at 4 °C was centrifuged to separate the soluble (supernatant) from the insoluble (pellet) material. Here it has to be pointed out that the above-used buffer was found to be more successful than others (data not shown) and therefore its use was adopted. After the centrifugation step, filtering as well as extensive dialysis against water were very important steps in order to remove salts and everything else that was co-purified without being covalent bound to the extracted substances. The extracts were analyzed with SDS-PAGE 12% and visualized with Coomassie blue (Fig. 1a). It is clearly shown that several bands with different molecular masses, ranging from 14 to 150 kDa, were present and that some of them were highly diffused. This electrophoretical behaviour was thought to be attributed to post translational modifications and in this particular case to glycosylation. The presence of glycosylated molecules within the isolated extracts as well as their partial characterization are described in detail below in this section.

These extracts were used for the production of polyclonal antibodies. Figure 1b shows a Western blot analysis of CMG extracts which were separated by SDS-PAGE 12% and transferred onto PVDF membranes. Their visualization was performed by using the produced antiserum in a dilution of 1:1000. The protein character of the analyzed substances was verified with proteinase K. Thus, the extracts obtained from 2 g CMG were incubated with

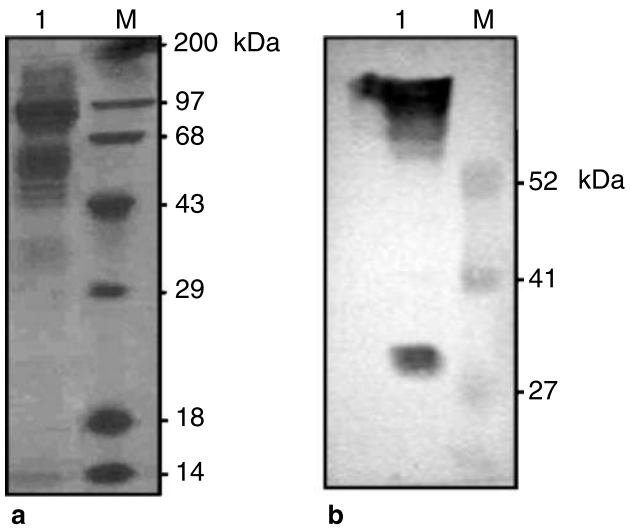

Fig. 1. Analysis of mastic gum extracts onto 12% SDS-PAGE. The extraction was performed with a buffer of 20 mM Tris–HCl pH 7.5, 100 mM NaCl, followed by extensive dialysis against water and drying in a Speedvac. **a** The existence of several bands indicates the presence of proteinacious molecules in the sample. **b** Western blot analysis of mastic gum extracts. Detection was performed with rabbit anti-mastic serum

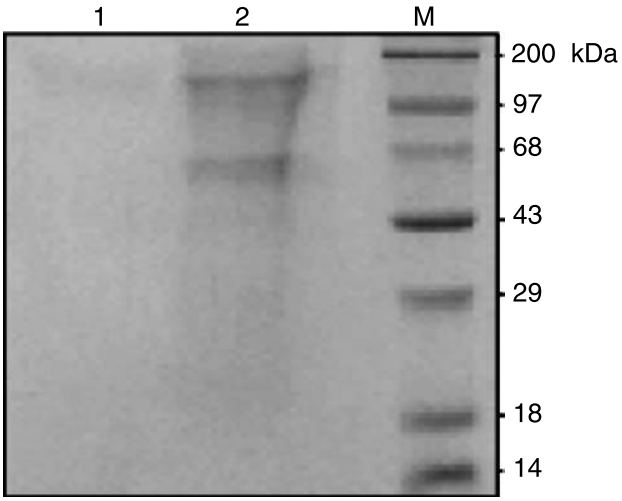

Fig. 2. Treatment of the extracts with Proteinase K. Mastic extracts were subjected to proteolysis with proteinase K at 37 °C for 4.5 h, analyzed onto 12% SDS-PAGE and visualized by staining with coommassie blue. *1* shows the sample after incubation with Proteinase K, *2*, the sample without protease treatment and *M* indicates the molecular weight markers

freshly prepared enzyme at 37 °C for 4.5 h and analyzed onto SDS-PAGE 12%. Figure 2 shows that when the extracts are incubated with proteinase K (lane 1) the bands disappeared, in contrast to the extracts that were not subjected to incubation with the enzyme (lane 2).

Another aspect that has also to be discussed here is the zinc assessment. The zinc content of CMG was reported several years ago. However, no evidence was reported until now for the co-existence of zinc within the different extracted substances. Figure 3 presents the zinc amount which was estimated with atomic absorption. CMG was subjected to extraction by using different buffers or solvents, as described in the legend of Fig. 3. Thus, it is clearly shown that the CMG extracts obtained by using the buffer containing 20 mM Tris–HCl, pH 7.5, 0.1 M NaCl exhibit the highest Zn concentration (Fig. 3, bar 2). It should also be noticed that the above assessments took place after extensive dialysis of the extracts against water by using the Spectrapor 3 dialysis membranes that allow molecules with MW lower than 3000 to penetrate them and therefore to be removed. The detection of Zn even under these conditions is indicated in Fig. 3. Its existence could probably be attributed to its specific or non-specific strong or covalent interactions with some parts of the isolated substances that inhibit its removal during dialysis.

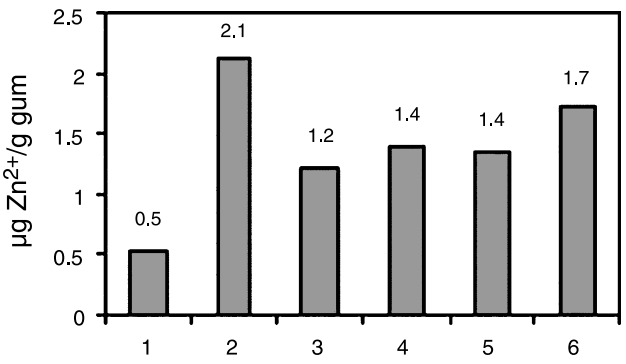

Fig. 3. Zinc assessments. The barograms show the zinc content after treatment of the extracts with different buffers/solvents and estimation with atomic absorption. *1* CMG extract with H_2O; *2* CMG extract with 0.1 M NaCl, 20 mM Tris–HCl pH 7.5; *3* CMG extract with HCl; *4* CMG extract with 0.5N HNO_3; *5* CMG extract with 0.1 M citrate-phosphate buffer; *6* CMG extracts with 6 M guanidine hydrochloride

Investigation of the carbohydrate moiety of the isolated substances

It has been mentioned above that some protein bands, which were visualized with Coomassie blue, were extremely diffused and this behaviour can be attributed to modifications such as glycosylation. In a first attempt, in order to characterize the modification, a $NaIO_4$-biocytin hydrazide coupled reaction was performed, as described under the experimental session. This methodology allows carbohydrate oxidation to occur by the addition of the $NaIO_4$ reagent. The formed aldehydes react with the hydrazide group of the biocytin-hydrazide reagent, which results in the labeling of carbohydrate molecules with biotin.

After labeling, the produced sample was analyzed by SDS-PAGE 12% and transferred onto PVDF membranes, and the detection was performed by using the streptavidin-horseradish peroxidase. In Fig. 4a, lane 1 clearly shows the presence of the carboxydrates. Positive and negative controls were used namely, fetuin (lane 2), a protein that is highly glycosylated, and albumin (lane 3), which is known not to carry such a modification, respectively. Figure 4b shows the deglycosylation of CMG extracts after treatment with $NaBH_4$ according to Gemmil and Trimble (1999). Visualization of the bands was performed after Western blot analysis by using the polyclonal antibodies that were raised against CMG extracts.

Further characterization of the highly glycosylated CMG extracts

In order to characterize the carbohydrates of CMG, chromatographic methods were carried out, as described under the experimental session. Neutral carbohydrate, uronic acid, sialic acid and protein analysis were tested for, according to Scott and Melvin (1953), Bitter and Muir (1962), and Bradford (1976) and Schauer (1978), respectively,

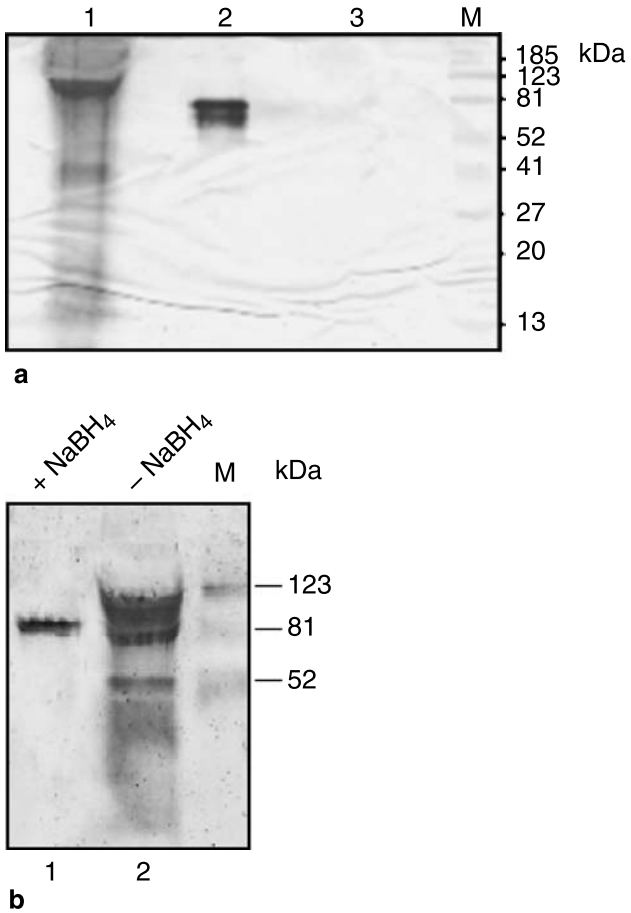

Fig. 4. Western blot analysis of the isolated proteins from GMG after biotinylation and treatment with $NaBH_4$. After treatment of the extracts with $NaIO_4$ for oxidizing the cis-1,2-diol groups of the carbohydrates and labeling with the biotin, the samples were analyzed onto SDS-PAGE 12%, transferred onto PVDF membranes and detected with the streptavidin-horseradish peroxidase. Positive reaction indicates the existence of carbohydrates in the sample. Deglycosylation with $NaBH_4$ was performed according to Gemmil and Trimble (1999). **a** *1* Glycosylation of the CMG extracts; *2* glycosylation of fetuin, which was used as positive control; *3* absence of glycosylation of albumin, which was used as negative control. *M* Molecular weight markers. **b** *1* CMG extracts after treatment with $NaBH_4$; *2* extracts without treatment

using galactose, glucurolactone, N-acetyl-D-neuraminic acid and bovine serum albumin as standards. The results of these experiments (Table 1) show that the major part of

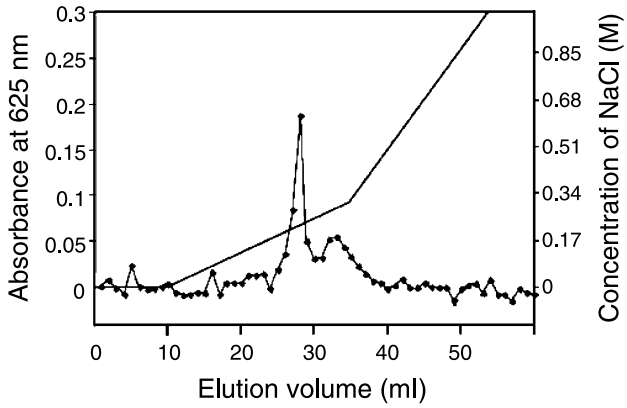

Fig. 5. Separation of the CMG extracts with ion-exchange chromatography. On this chromatogram there are 3 peaks dividing the fractions into three groups (Group A–C). Group A consists of the fractions eluted with 0.1–0.14 M NaCl and represents 7% of the eluted carbohydrates; group B consists of the fractions eluted with 0.18–0.23 M NaCl and represents 58% of the eluted carbohydrates; and group C consists of the fractions eluted with 0.26–0.37 M NaCl and represents 35% of the eluted carbohydrates

the extracts consists of neutral sugars (78.5%), uronic acids (18.4%) and proteins (3.1%), while there is a complete lack of sialic acids. The obtained CMG extracts were separated by using an ion-exchange MonoQ column, as shown in Fig. 5. On this chromatogram there are 3 peaks dividing the fractions in three groups namely, Group A, Group B and Group C. Group A consists of the fractions eluted with 0.1–0.14 M NaCl and represents 7% of the eluted carbohydrates; Group B consists of the fractions eluted with 0.18–0.23 M NaCl and represents 58% of the eluted carbohydrates; and Group C consists of the fractions eluted with 0.26–0.37 M NaCl and represents 35% of the eluted carbohydrates. As expected, the elution pattern of the three groups is consistent with their respective negative charge content namely, uronic acid. Chromatometric analysis of the three fraction groups is shown in Table 2. Thus Group A consists of 73.1% neutral sugars and 1.2% uronic acids, Group B consists of 50.5% neutral sugars and 2.54% uronic acids, while Group C consists of 49.3% neutral sugars and 3.3% uronic acids.

Table 1. Chromatometric analysis of CMG extract

	Concentration (μg/g of mastic)	Percentage
Proteins	21 ± 5	3.1
Carbohydrates	668	96.9
Neutral	541 ± 6	78.5
Uronic acids	127 ± 4	18.4
Sialic acids	–	–

Table 2. Carbohydrate analysis of fraction Groups A, B and C. All analyses were performed with chromatometric methods

	Group A (%)	Group B (%)	Group C (%)
Neutral sugars	73.1	50.5	49.3
Uronic acids	1.2	2.54	3.3
Proteins	Not detected	Not detected	Not detected

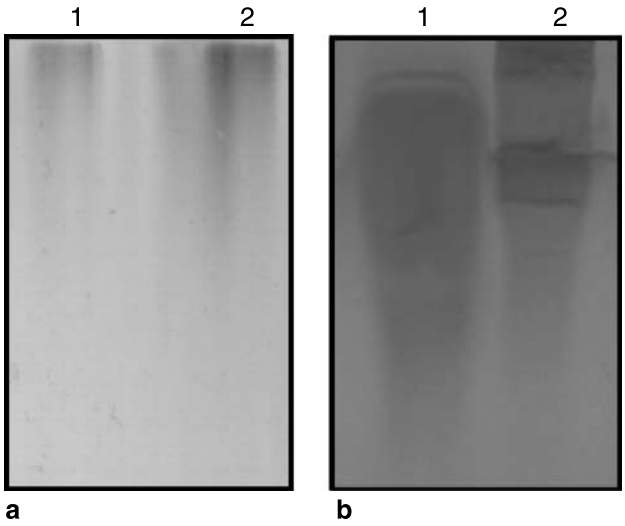

Fig. 6. Characterization of the carbohydrates with different staining procedures. **a** *1* Fraction group B, *2* fraction Group C. Both were visualized with Alcian blue. **b** Staining of the total CMG extracts with the Yariv reagent. *1* Arabic gum arabinogalactans (positive control), *2* CMG extracts

Proteins were not detected, probably due to their low amount and maybe due to the low sensitivity of the Bradford method. The existence of high molecular weight molecules in Groups B and C was additionally confirmed by Alcian blue staining after their separation onto SDS-PAGE 10% (Fig. 6a).

In order to elucidate their carbohydrate content, the macromolecules of groups B and C were subjected to GC-MS analysis. As shown in Table 3, the carbohydrates that were detected are arabinose and galactose. This is an indication that the glycosylated proteins of CMG belong to the family of AGPs, which is consistent with other proteinaceous macromolecules isolated from other gums (Verbeken et al., 2003). Experiments concerning the detection of CMG extracts by using the Yariv reagent, which is specific for AGPs detection, after their separation onto SDS-PAGE (Fig. 6b), have led to the same suggestions.

The homogeneity of Groups B and C was examined with capillary electrophoresis. Both B and C each form a single peak, migrating in 9.3 and 9.2 min, respectively

Table 3. Determination of neutral and amino sugars of fraction Groups B and C with GC-MS analysis

Neutral sugars	Group B (%)	Group C (%)
Arabinose	51.1	8.5
Galactose	48.9	91.5

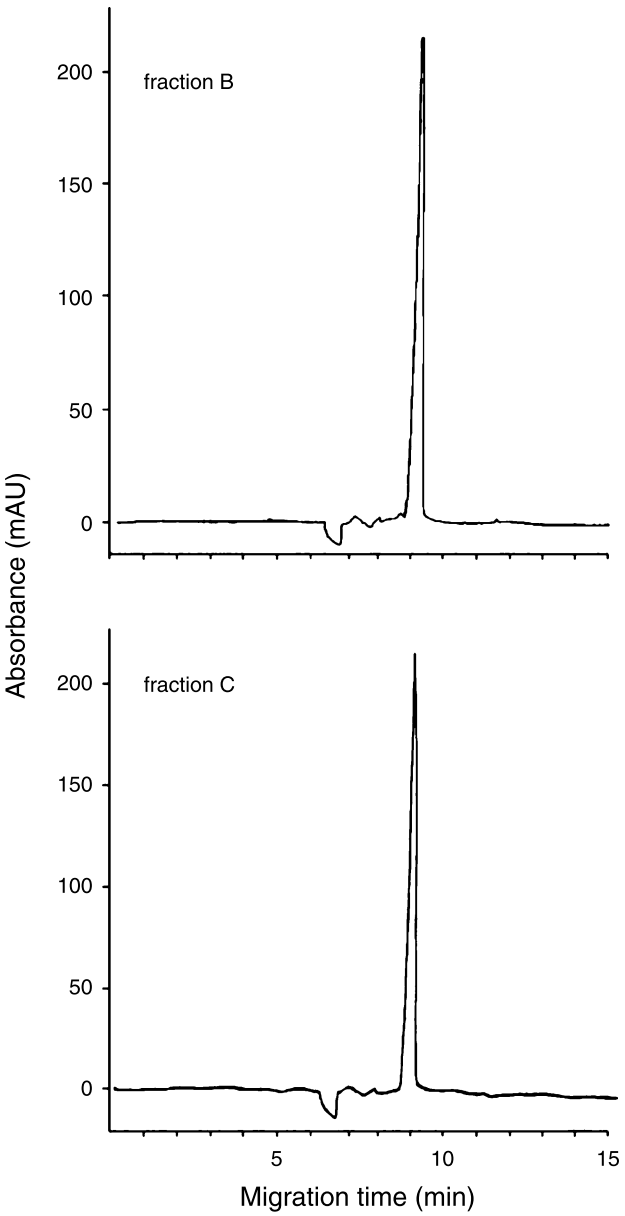

Fig. 7. Performance of purity and homogeneity of polysaccharide fractions by capillary electrophoresis. Capillary electrophoresis was performed in an HP3DCE instrument from Agilent Technologies in uncoated silica-fused capillaries (50 µm i.d. × 54.6 cm effective length). Separation was performed in a 25 mM borate buffer, pH 10.0, containing 25 mM SDS at 20 kV (normal polarity) and detection was at 200 nm. The sample from Group B migrates in 9.3 min and that from Group C migrates in 9.2 min, forming one peak each, indicating that the population in each sample is homogeneous

(Fig. 7), which verifies the homogeneity of the AGP populations.

Antibacterial activity

The bacteriostatic activity of AGPs against *H. pylori* was tested as described in the experimental session. According

Table 4. Growth of *H. pylori* in the presence of various CMG extracts

CMG extract	*H. pylori* growth
From 1 g CMG	+
From 1.4 g CMG	−
From 2 g CMG	−
From 3 g CMG	−
From 4 g CMG	−

to Table 4, the AGPs obtained from at least 1.4 g CMG inhibited the growth of the cells. However, such an effect of other AGPs has never been reported.

On the contrary, the antimicrobial activity of mastic against several pathogens was confirmed by several researchers, as mentioned in detail in the introduction. Although some of them reported that the inhibition efficiency of mastic against *H. pylori* in vitro and in vivo was effective in reducing *H. pylori* colonization and that the major triterpenic acids in the acid extract may be responsible for this activity (Paraschos et al., 2007), some others (Loughlin et al., 2003) showed that *H. pylori* in infected mice was not eradicated after CMG receipt. In addition, the inability of CMG to eradicate *H. pylori* from infected humans (Bebb et al., 2003) contradicted the findings reported by Al-Habbal et al. (1984) and Al-Said et al. (1986).

H. pylori colonizes the gastric mucosa by association solely with surface mucous cell-type mucin (Hidaka et al., 2001) and two carbohydrate structures, Lewis b and sialyl dimeric Lewis x in surface mucous cells that are serving as specific ligands for *H. pylori* adhesions BabA and SabA, respectively (Mahdavi et al., 2002). The pathogenic bacterium rarely colonizes the deeper portions of gastric mucosa, where the gland mucous cells producing mucins have terminal α1,4-linked N-acetyl-glucosamine residues attached to core 2-branched O-glycans (Nakayama et al., 1999).

Interestingly, Kawakubo et al. (2004) reported that these glycan chains that are produced by human gastric gland mucous cells raise the possibility of having protective properties against *H. pylori* infection. They found that this natural protection could be attributed to several abnormal morphologies of *H. pylori*, such as elongation, segmental narrowing and folding. The above-mentioned abnormal morphologies were observed by scanning electron micrographs of *H. pylori* after its incubation with the O-glycans that possess terminal α1, 4-linked N-acetylglucosamine.

In addition, Marone et al. (2001) evidenced in vitro the CMG total killing activity against *H. pylori,* attributing this effect to several morphological abnormalities, blebbing and cellular fragmentation that were caused by CMG.

By taking into account the observation reported by Marone et al. (2001) concerning the observed abnormal morphologies of *H. pylori* after its treatment with mastic, as well as the similar observations reported by Kawakubo et al. (2004), we suggest that the AGPs from CMG could provoke such alterations in *H. pylori* and therefore inhibit its growth in vitro. However, we cannot postulate that AGPs from CMG function in a similar way in vivo by altering the *H. pylori* morphology, as the mucin O-glycans protects the gastric mucous cells from infection. Their in vitro activity against *H. pylori* has to be further investigated in vivo.

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