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Characterisation and properties of *Acacia senegal* (L.) Willd. var, *senegal* with enhanced properties (Acacia (sen) SUPER GUM™): Part 3 Immunological characterisation of Acacia (sen) SUPER GUM™

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Abstract

Four *Acacia senegal* samples, one control (M_w 6.2×10^5 g/mol) and three enhanced samples with different molecular weights ranging from 1.2×10^6–2.5×10^6 g/mol were fractionated using hydrophobic interaction chromatography (HIC) into two fractions, hydrophilic (fraction 1, yield ~80%) and hydrophobic (fraction 2, yield ~2%). The elution profile and weight average molecular weight of fraction 1 were similar to the starting materials but contained slightly more arabinogalactan protein (AGP) component. On the other hand, the AGP peak was almost completely removed from Fraction 2. The M_w for fraction 2 was ~1.1×10^5 g/mol and contained <0.5% (of the total injected mass) of aggregated materials with $M_w > 4.9 \times 10^7$ g/mol. These fractions plus the whole gum were also analysed by ELISA (enzyme linked immunosorbent assay). The results showed that the interaction with an *A. senegal* specific antibody (SY CC7) is the same for the whole gum sample and its fractions, indicating a common, widely distributed epitope. One sample with the highest molecular weight (2.5×10^6 g/mol) showed a slightly different interaction, displaying a lower sensitivity, attributed to the formation of a more compact hydrophobic form of AGP. This is in accord also with the observations on the same sample using spectroscopic methods which was attributed to dehydration of the COOH uronic acid group.

Examination of the commercially available Acacia(sen) SUPER GUM™ (EM2—M_w ~1.8×10^6 g/mol) with three different antibodies (SY CC7, UC-SEN-PS-01 and UC-SEY-PS-01) showed the response to be identical to that of control *A. senegal* gum. These results demonstrate how immunological techniques, in this instance ELISAs, can be utilised to indicate differences between gum samples and to determine the limit of maturation of Acacia(sen) SUPER GUM™.

Keywords: Gum Arabic; *Acacia senegal*; SUPER GUM™; Hydrophobic fractionation; GPC-MALLS; ELISA

1. Introduction

The definition, composition and uses of Acacia gums have been reviewed in Part 1 of this series (Al-Assaf, Phillips, & Sasaki, 2006). Gums and hydrocolloids generally, have been subjected to immunological characterisation , allowing gums with identical chemical composition to be distinguished with the use of antibodies (Ireland et al., 2004; Pickles, Ireland, Al-Assaf, & Williams, 2004). For example, exudate gums from *Acacia seyal* and *A. nilotica* have the same sugar and amino acid composition,

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but have quite different arabinogalactan (AG) and arabinogalactan-protein (AGP) epitopes (Baldwin, Quah, & Menzies, 1999). *A. nilotica* does not contain an epitope recognised by the monoclonal antibody (MAb), MAC 207, whereas *A. seyal* contains high levels of this epitope. Conversely, *A. nilotica* contains epitopes which can be recognised by MAbs JIM 4, JIM 14, JIM 15 and JIM 16 but *A. seyal* contains none of them (Baldwin et al., 1999).

A panel of MAbs raised against gum arabic were able to distinguish between gums from *A. senegal*, *A. seyal* and *Combretum erythrophyllum* (Dewey, Thurston, & Cronk, 1997; Ireland et al., 2004; Thurston et al., 1998). Three MAbs with differing specificities were selected: SY CC7 (*A. senegal*), SY HH3 (*A. seyal*) and SY J1A1 (*Combretum*). These antibodies were able to determine adulteration of *A. senegal* samples with either *A. seyal* gum (0.6 w/w%) or *C. erythrophyllum* gum (0.2 w/w%) (Ireland et al., 2004). The assays used were reproducible (inter-assay CV < 15%) and were applied successfully to confectionary (Ireland et al., 2004). The assays are of practical value since Combretum gums are not food approved and there has been concern that since they are laevo-rotatory, they could be used as adulterants to replace *A. senegal.* Although *A. seyal* has been approved for use in food, the gum has different emulsification properties to *A. senegal* gum. The assays therefore have potential for use in evaluating gum quality.

Chemical characterisation has shown that the maturation process to improve the content of AGP protein in commercial gum arabic does not change the chemical character of the matured samples (designated Acacia (sen) SUPER GUM^{TM}) and that no new chemical groups have been introduced. The matured samples contained exactly the same specific optical rotation, sugar moieties and amino acids in the same proportions as control gum, which has not been subjected to the accelerated maturation process (Aoki, Katayama, Al-Assaf, & Phillips, 2006). We have shown that the controlling feature of the maturation process is the agglomeration of the proteinaceous components, already present in the complex material of *A. senegal*, which leads to an increased amount of AGP by aggregating the protein and carbohydrate. The weight average molecular weight (M_w) of the matured samples increased from 0.6 to 2.5 million; with the AGP M_w value increasing from 2.5 to 11.6 million. The amount of AGP increased from 10.6% to 18.6% whereas the M_w of the AG component changes very little—from 0.40 to 0.45 million.

Here the same matured samples have been subjected to immunological evaluation. The samples were also fractionated into hydrophobic and hydrophilic fractions, so separating the main protein components from the bulk carbohydrate components. The fractions were subjected to the same immunological analysis, in order to establish whether any new epitopes had been introduced by the maturation process. The evidence from previous studies have shown that ELISAs are extremely sensitive to structural modifications between closely related chemical gum structures and so are an important step in characterising any changes that might be induced by the maturation process.

2. Materials and methods

2.1. Materials

The gum arabic samples used were FR-2876 (control *A. senegal*), the matured samples FR-2877, FR-2878 and FR-2879 and the product Acacia (sen) SUPER GUM^{TM} EM2 were used as described previously (Aoki et al., 2006).

Methanol (99.5 > %), NaOH (98 > %), and NaCl (99.9 > %) were obtained from Fisher Scientific UK. Trifluoroacetic acid (TFA, 98 > %) was purchased from Sigma. DIAION HP-20 was provided from Mitsubishi Chemical Corporation. Distilled water (Bibby Merit W4000) was used for all experiments.

2.2. Fractionation by hydrophobic interaction chromatography

Previous hydrophobic fractionation studies of gum arabic have mainly used column packed with Phenyl-Sepharose CL-4B, phenyl group covalently coupled to 4% cross linked agarose matrix (Fauconnier et al., 2000; Randall, Phillips, & Williams, 1989). The fractionation was achieved by successive elution with 4.2 M NaCl, 2 M NaCl and water to yield the AG, AGP and glycoprotein (GP) fractions, respectively.

In this study, the gums were separated by HIC fractionation on a DIAION HP-20 column (200 ml, d30 mm × h280 mm). DIAION HP-20 has been widely used in the variety of applications such as adsorption of proteins and decolourisation of natural products. It is a highly porous styrenic adsorbent resin with relatively large size pores (52 nm) and particle size distribution of > 250 μm. Based on our experiences, HP-20 resin was shown to hold around 20 g or more of hydrophobic material per 1000 ml resin (4 g/200 ml). Eluents were applied at varying space velocities (SV, SV = 1 is equivalent to 200 ml/h). Column conditioning was undertaken with 4 w/v% NaOH/70 v/v% methanol (SV = 2; 400 ml), followed by distilled water (SV = 3; 1 L). Then, 1 v/v% TFA aqueous solution was applied (SV = 2; 400 ml), followed by distilled water (SV = 4; 2 L).

Test materials (50 g) were dissolved with 450 ml of distilled water. The solution was applied to the column (SV = 2) and the eluate collected immediately. The column was then washed with distilled water (SV = 3–5, 2 L). Only 100 ml of this wash was added to the collected fraction and made the total collected volume ~600 ml. This constituted the first fraction (hydrophilic). Finally, 70 v/v% methanol (SV = 1, 400 ml) was applied to the column and the eluate collected, which constituted the second fraction (hydrophobic).