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The analysis of Acacia gums using electrophoresis

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Abstract

Acacia gum is a ubiquitous water-soluble material that has been analyzed using a variety of techniques. The absence of a high-resolution technique for the separation of the components of the gum has been an impediment to its characterization. Two complementary electrophoretic methods for the separation and detection of the polysaccharides, glycoproteins, and proteins that constitute Acacia gum and other gums have been developed. The first uses sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) with 2-[*N*-Morpholino] ethanesulfonic acid (MES) buffer and silver staining for the analysis of the proteins in Acacia gum. Over 10 proteinaceous bands were observed in the M.W. range of 2×10^3–2×10^5. The second uses agarose electrophoresis with Tris-Borate (TB) buffer and dansyl hydrazine staining for the analysis of the polysaccharides and glycoproteins in Acacia gum. One to two carbohydrate-containing bands were observed in the high M.W. ($<5\times10^6$) region. The advantages of electrophoresis over gel filtration chromatography for gum analysis are discussed.

Keywords: Acacia gum; Gum arabic; *Acacia senegal*; *Acacia seyal*; Gum tragacanth;*Astralagus gummifer*; Polyacrilamide gel electrophoresis (PAGE); Agarose gel electrophoresis

1. Introduction

Acacia gum, the gum exudate from Acacia trees, is a water-soluble gum that is widely used because of its unique emulsification, film forming, and encapsulation properties. It is extensively used in foods (confectionery, baked goods, beverages and dry packaged products), pharmaceuticals (as a carrier in capsules and in high soluble fiber supplements), cosmetic products (creams and lotions) and lithographic inks. It is also used in sprayed glazes, high-tech ceramics, paper coatings, textile sizing and finishes, metal corrosion inhibitors, office glues, emulsion prints, artists' materials (water colors and inks), pesticides and in flocculating agents used in ore refining.

Acacia gum is used in the food and pharmaceutical industries. It is known to be a mixture of complex carbohydrates with molecular weights between 2.5×10^5 and 1×10^6, which contains a small proportion of proteinaceous material (2%) and has been classified as an arabinogalactan–protein complex (Islam, Phillips, Sljivo, Snowden, & Williams, 1997). The gum has a highly variable composition and the physical and chemical properties can vary considerably depending on the source of the nodules (Islam et al., 1997).

The main obstacle to the identification of the components of Acacia gum is the absence of an efficient separation technique. Several liquid chromatographic techniques, such as hydrophobic interaction chromatography (HIC) (Ray, Bird, Iacobucci, & Clark, 1995; Siddig, Osman, Al-Assaf, Phillips, & Williams, 2005) and weak anion-exchange chromatography (Osman, Menzies, Martin, Williams, & Phillips, 1995), have been used with partial success. The most widely used technique has been size exclusion/gel filtration chromatography (GFC); however, even GFC does not provide a reasonable separation (Islam et al., 1997; Flindt, Al-Assaf, Phillips, & Williams, 2005) of the components of the gum.

The first attempt to separate the components of Acacia gum using electrophoresis was made by Lewis and Smith (1957). They reported a 10 h-long separation of the gum of *Acacia senegal* (and *Acacia seyal*) using glass-fiber paper in conjunction with a 2 M NaOH buffer. However, no mention was made of the possibility of hydrolysis of the gum by the strong base. In 1982, Pechanek, Blaicher, Pfannhauser, & Woidich, (1982)

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encountered problems with the PAGE separation of Acacia gum. Instead, they used agarose and cellulose acetate membranes with a boric acid buffer and fuchsin, toluidine blue and methylene blue stains (all carbohydrate stains) and obtained only one band. Most recently in 1993, Osman, Menzies, Williams, Phillips, and Baldwin (1993) performed a SDS-PAGE (Tris-Glycine buffer system with a uniform gel) separation of three HIC fractions of *A. senegal* gum and, after silver staining (a protein stain), obtained a total of four broad bands. One at 7×10^4 Da, a second in the 3×10^4–4.5×10^4 Da region, a third at 2.9×10^4 Da and a fourth at 1×10^4 Da.

We have developed two complementary procedures for the analysis of gums using electrophoresis based on our initial results (Motlagh, Ravines, Ma, & Jaksch, 2000). The first procedure uses SDS-PAGE with a MES buffer system, a gradient gel, and a silver stain for the separation and detection of the low molecular weight (2×10^3–2×10^5 Da) protein portion of the gum. Up to 30 samples can be run and stained in parallel in a little over 2 h. The stain can detect less than 1 ng of protein. The second procedure uses agarose gel electrophoresis with a SDS-TB buffer system, a uniform gel and a dansyl hydrazine stain (Furlan, Perret, & Beck, 1979) for the high molecular weight (up to 5×10^6 Da) glycoprotein and carbohydrate portions of the gum. Up to 24 samples can be run and stained in parallel in just over 2 days. The stain can detect down to approximately 10 μg of carbohydrate. The technique was successfully used to analyze gum samples from *A. senegal* (var. *senegal* and var. *karensis*), *A. seyal* (var. *seyal* and var. *fistula*), *Acacia polyacantha*, *Acacia gerrardii*, *Acacia laeta*, *Acacia nilotica*, *Acacia fischeri*, and *Astralagus gummifer* (gum tragacanth).

2. Experimental—materials and methods

2.1. Apparatus

The Xcell II Mini-Cell for PAGE and all PAGE precast gels were purchased from Novex (San Diego, CA), except for the 2–15% Tris-Glycine gel that was purchased from Owl Separation Systems (Portsmouth, NH). The Wide Mini-Sub Cell GT System for agarose electrophoresis was purchased from Bio-Rad (Hercules, CA). The UV Transilluminator (365 nm) was purchased from Glyko (Novato, CA). 1% agarose precast gels with Tris-Borate-EDTA (TBE) buffer were purchased from BioWhittaker Molecular Applications (Rockland, ME). Digital imaging was performed using a Nikon D1 camera. For fluorescence imaging, a yellow filter was used to cut down on the UV background and the resulting fluorescence of the lens.

2.2. Reagents

Sample and running buffers and molecular weight standards for PAGE were purchased from Novex, now Invitrogen. The Novex Mark 12 Wide Range Standard used contains 12 polypeptides in the range of 2.5–200 kDa: Myosin (200 kDa), β galactosidase (116.5 kDa), Phosphorylase b (97.4 kDa), Bovine serum albumin (66.3 kDa), Glutamic dehydrogenase (55.4 kDa), Lactate dehydrogenase (36.5 kDa), Carbonic anhydrase (31 kDa), Trypsin inhibitor (21.5 kDa), Lysozyme (14.4 kDa), Aprotinin (6 kDa), Insulin B chain (3.5 kDa) and Insulin A chain (2.5 kDa). Two silver stain kits were used; Sammons' silver stain was purchased from Pierce (Rockford, IL) and Merril's silver stain from Bio-Rad. Sample buffer for agarose electrophoresis was prepared by adding 2% SDS to the TBE sample buffer (diluted to 1X) purchased from Bio-Rad. Running buffer for agarose electrophoresis was 90 mM Tris base, 90 mM Boric acid, and 0.1% SDS. All other reagents and stains were either electrophoresis or ACS reagent grade and were purchased from Sigma (St Louis, MO), Aldrich (Milwaukee, WI) or Fluka (Buchs, Switzerland).

2.3. Biological materials

Table 1 provides the origins, sources and other details of the Acacia gums used. The gum samples of *A. senegal* var. *karensis*, *A. seyal* var. *seyal*, and *A. seyal* var. *fistula* were gifts from Dr G.O. Phillips (North East Wales Institute, Wrexham, UK). The gum samples of *A. nilotica* and *A. fischeri* were provided by Dr Sarah Taylor (Natural Resources Institute, Chatham, UK). One commercially purchased sample was used, Gum arabic (spray dried) from Fluka. All other Acacia gum samples were obtained from Gandil Agricultural Company (Khartoum, Sudan). The *A. gummifer* sample was purchased in the Egyptian (Spice) Market, Istanbul, Turkey.

2.4. Procedure

Unless otherwise noted, PAGE experiments were performed in non-reducing SDS-MES buffer using a 4–12% gradient gel at 200 V constant voltage and stained with Merril's silver stain while agarose gel electrophoresis experiments were performed in non-reducing SDS-TBE buffer using a 1% uniform gel at 100 V constant voltage and stained with dansyl hydrazine stain. Runs were stopped when the bromophenol blue dye front reached the end of the gel. Gels were loaded with 10 μl of a 1% Acacia gum solution.

3. Results and discussion

The effects of the buffer system, the presence of denaturants in the buffer, and the gel density on the separation efficiency of Acacia gum were investigated. The staining efficiency of a few stains was compared, a SDS-PAGE separation and detection of the carbohydrates in Acacia gum was attempted.

3.1. PAGE of proteins in gums

The influence of the buffer system on the separation of Acacia gum was investigated by using eight different PAGE buffer systems. The TBE, the Tris-Acetate (TA), the MES, the 3-[Morpholino]propanesulfonic acid (MOPS), and the SDS-Tris-Glycine buffer systems did provide separations of the protein