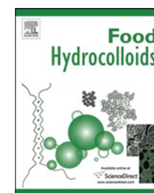
Food Hydrocolloids 31 (2013) 42–48

Contents lists available at SciVerse ScienceDirect

Food Hydrocolloids

journal homepage: www.elsevier.com/locate/foodhyd



A further amendment to the classical core structure of gum arabic (*Acacia senegal*)

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ARTICLE INFO

Article history:
Received 19 July 2012
Accepted 24 September 2012

Keywords:
Gum arabic (*Acacia senegal*)
Structural characterization
Methylation analysis
2D NMR

ABSTRACT

Using the more recently available techniques such as methylation–GC–MS, 1D (^{1}H, ^{13}C) and 2D (COSY, TOCSY, HMQC and HMBC) NMR spectral analysis, we have revisited the classical structure of gum arabic (*Acacia senegal*). Methylation and GC–MS analysis confirmed that gum arabic (*A. senegal*) is a highly branched polysaccharide with the backbone composed of 1,3-linked galactopyransyl (Gal*p*) residues substituted at O-2, O-6 or O-4 positions. The terminal sugar residues are 59.5% of the total sugars. The residues of →2,3,6-β-D-Gal*p*1→, →3,4-Gal*p*1→, →3,4,6-Gal*p*1→ and substitutions at O-2 and O-4 position were not identified in previous studies.

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1. Introduction

Gum arabic is a tree exudate, mainly from African Sahelian countries, and is an important additive and ingredient used in the food and pharmaceutical industries (Phillips & Williams, 2009, chap. 11). Some 93% of the structure is associated with the core carbohydrate. It was the classical work of the late Douglas Anderson in the UK and Alistair Stephens in South Africa that led to the structure that we are currently using for this core arabinogalactan carbohydrate component (Anderson & Stoddart, 1966; Churms, Merrifield, & Stephen, 1983). Subsequently, we have seen, of course, that gum arabic (*Acacia senegal*) is a polydisperse molecule which includes morphological structures in the form of a compact arabinogalactan protein (AGP) (Mahendran, Williams, Phillips, Al-Assaf, & Baldwin, 2008) and a disk arabinogalactan (AG) (Mahendran et al., 2008). The AGP consists of a polypeptide chain possibly containing ~250 amino acids with short arabinose side chains and much larger blocks of carbohydrate of molecular mass ~4.0 × 10^4 Da attached. The carbohydrate is highly branched. The molecule adopts a very compact conformation with Rg of ~36 nm (Fig. 1) (Mahendran et al., 2008). Sanchez (2010) has also proposed a structure for the AGP which is consistent with this model. A thin oblate ellipsoid structure has been proposed for the AG component (Fig. 2). The branched structure of this disc-like structure is mainly composed of 1,3-linked β-D-galactopyranosyl units with 1,6-linked β-D-galactopyranosyl side chains to which there are linked many α-arabinosyl, uronic acid and rhamnose residues (Anderson & Stoddart, 1966; Churms et al., 1983; Sanchez et al., 2008). The classical Anderson–Stephen structure has stood the test of time (Fig. 3) (Street & Anderson, 1983; Wang, Burchard, Cui, Huang, & Phillips, 2008). Here we revisit the structure of these carbohydrate blocks of *A. senegal* using more recently available techniques. The methods used were methylation analysis and 2D NMR spectroscopy including homonuclear ^{1}H/^{1}H correlation spectroscopy (COSY, TOCSY), and heteronuclear ^{13}C/^{1}H multiple quantum coherence experiments (HMQC, HMBC).

2. Materials and methods

2.1. Materials

The *A. senegal* var. *senegal* samples used in this investigation were from the Gum Arabic Company, Sudan and had been well authenticated (Al-Assaf, Phillips, & Williams, 2005a, 2005b).

2.2. Methylation and GC–MS for gum arabic

Uronic acids were reduced to neutral polysaccharide before methylation following the previous procedure (Taylor & Conrad, 1972; York, Darvill, McNeil, Stevenson, & Albersheim, 1986), with minor modifications. Duplicated samples of gum arabic (5 mg)

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0268-005X/$ – see front matter © 2012 Published by Elsevier Ltd.
http://dx.doi.org/10.1016/j.foodhyd.2012.09.014

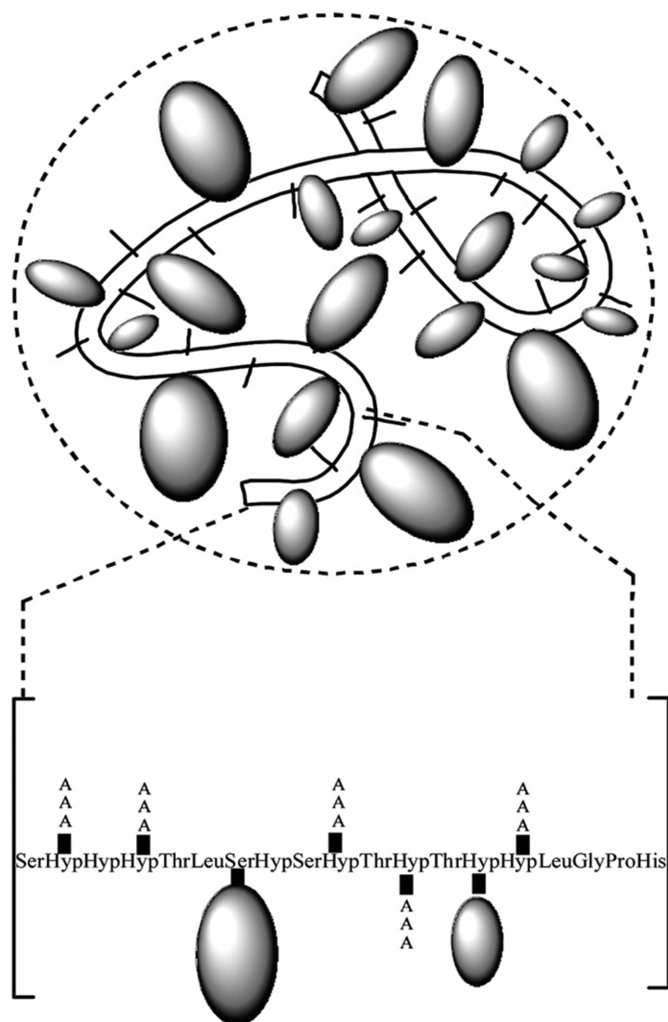

Fig. 1. Schematic illustration of the structure of the gum arabic arabinogalactan protein complex (Mahendran et al., 2008).

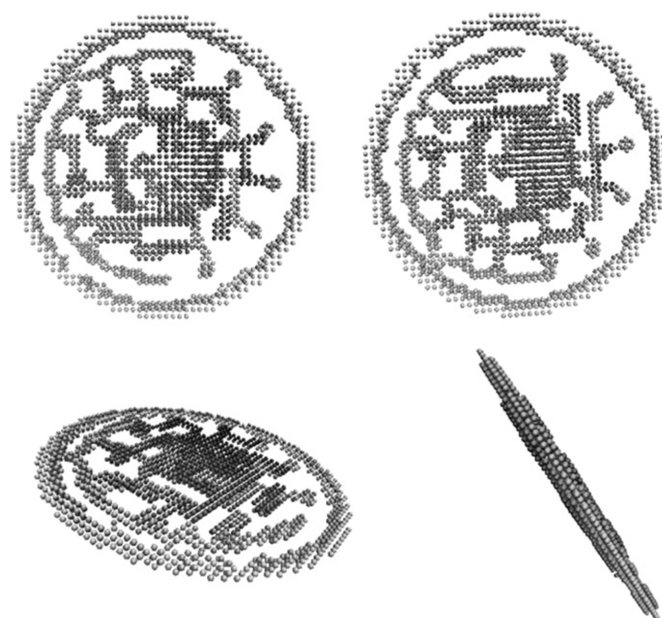

Fig. 2. Different views of the thin oblate ellipsoid AG structural component of gum arabic (Sanchez et al., 2008).

were dissolved in deuterium oxide D_2O (2 mL). To the solution, 50 mg of 1-cyclohexyl-3-(2-morpholinoethyl)-carbodiimide methyl-*p*-toluenesulfonate was added while using 0.1 mol/L HCl in D_2O to keep the pH at 4.75. The solution was left for 1 h while stirring, and followed by adding 5 mL of sodium borodeuteride (160 mg/mL) drop wise over a period of 0.5 h, and the reaction mixture pH was maintained at 7.0, using 2.0 mol/L HCl in D_2O during the reduction reaction. The reaction continued with constant stirring for 0.5 h at pH 7.0 after the addition of sodium borodeuteride. The solution pH was then brought back to pH 4.0. The reduced polysaccharide was separated from salts by dialysis against distilled water overnight at 25 °C (3500 Da molecular weight cut off), then lyophilized. The polysaccharide was re-dissolved in 0.5 mL distilled water and 0.5 mL 10% acetic acid in methanol was added. The mixture was dried under a stream of nitrogen to remove boric acid. Another 0.5 mL of 10% acetic acid in methanol was added to the residue and evaporated under nitrogen stream. This process was repeated 3–4 times to ensure that most of the boric acid was removed. Finally, a few drops of methanol were added and the solution evaporated (two times) to remove any boric acid remaining.

Methylation analysis of gum arabic was conducted according to the method of Ciucanu and Kerek (1984) with slight modification. The dried samples (about 2–3 mg) were dissolved in anhydrous DMSO (0.5 mL), sonicated at 50 °C for 6.5 h, then heated at 85 °C for 2 h with constant stirring. The sample in DMSO solution was stirred at room temperature overnight to obtain a clear solution. Dry sodium hydroxide (20 mg) was added, and the mixture was stirred for 3 h at room temperature before introducing methyl iodide. The mixture was stirred for another 2.5 h after adding 0.3 mL methyl iodide. The methylated sample was then extracted with 1.5 mL methylene chloride. The methylene chloride extract was passed through a sodium sulphate column (0.5 × 15 cm) to remove water, and evaporated to dry by a stream of nitrogen. The dried methylated sample was hydrolysed in 0.5 mL of 4.0 M trifluoroacetic acid (TFA) in a sealed test tube at 100 °C for 6 h and the TFA was removed by evaporation under a stream of nitrogen and the residue was dissolved in 0.3 mL distilled water. The hydrolysate was reduced, using 5 mg sodium borodeuteride and borate was removed by repeated additions and evaporations first of 19:1 methanol–acetic acid then methanol alone. After that, the residue was acetylated with acetic anhydride (0.5 mL) for heating at 100 °C for 2 h. The resultant partially methylated alditol acetates (PMAA) were passed through a sodium sulphate column again to remove water. Aliquots

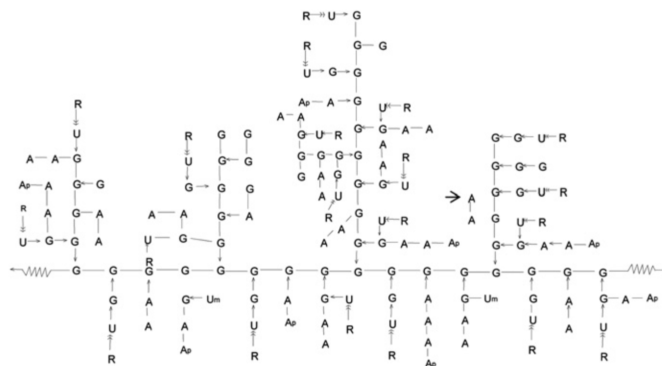

Fig. 3. Illustration of a possible structural fragment of *A. senegal* gum (Street & Anderson, 1983). R – rhamnose; Um – 4-O-methylglucuronic acid; U – glucuronic acid; Ap – arabinopyranose; A – arabinose; G – galactose.

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