International Journal of Biological Macromolecules 67 (2014) 446–451

Contents lists available at ScienceDirect

International Journal of Biological Macromolecules

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

Interaction of arabinogalactan with mucins

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ARTICLE INFO

Article history:
Received 12 January 2014
Received in revised form 30 March 2014
Accepted 1 April 2014
Available online 12 April 2014

Keywords:
Arabinogalactan
Mucin
Mucoadesivity

ABSTRACT

Arabinogalactan is a naturally-occurring, densely branched, polysaccharide mainly made-up of galactose and arabinose with variable amounts of uronic acids, which received attention for several industrial and biomedical applications. The ability of Western Larch arabinogalactan to interact with mucins was assessed by both classical gel filtration chromatography and frontal chromatography on Sephacryl S300 resin. The shift of arabinogalactan elution volume in classical gel filtration chromatography induced by both bovine submaxillary mucin and porcine gastric mucin resulted useful for revealing the occurrence of an interaction between arabinogalactan and mucins. A frontal gel chromatography, in which arabinogalactan is used as eluent, enabled a dissociation constant of 5×10^{-6} M to be measured for the arabinogalactan-bovine submaxillary mucin complex, with approximately 50 equivalents of arabinogalactan bound per mucin mole.

The mucoadhesivity of arabinogalactan may be a relevant feature for its biomedical and industrial applications.

1. Introduction

Natural polysaccharides have been widely used for many industrial and biomedical applications [1]. The mucoadhesivity of polysaccharides is generally considered advantageous for several of these applications. This is the case, for instance, in the development of ophthalmic preparations in which the mucoadhesivity of polysaccharides allows them to adhere to ocular surfaces [2,3].

The aim of this work was to assess the mucoadhesive properties of western larch (*Larex occidentalis*) arabinogalactan by evaluating its ability to interact with mucins.

Arabinogalactan is a long-chain, densely branched polysaccharide abundantly present in plants as part of cell walls [4]. A significant presence of arabinogalactan has been detected in many herbs with assessed immuno-stimulant properties [5,6]. In addition, arabinogalactan has been approved by the U.S. Food and Drug Administration as a source of dietary fiber [7]. The structure of the arabinogalactan backbone allows the identification of two types of the polysaccharide; the type I has β-1,4-galactan backbones, while the type II has β-1,3-galactan backbones [4]. The type II is often present also as the carbohydrate moiety of a class of highly glycosylated hydroxyproline-rich glycoproteins, known as arabinogalactan proteins, which are involved in several cell functions [8].

The woody tissues of western larch are especially rich in arabinogalactan type II (AG) and represent the main source of the commercially available arabinogalactan. It has been reported to be mainly composed of galactose and arabinose units in a molar ratio of approximately 6:1 [9]. The presence of glucuronic acid residues has also been detected and it has been demonstrated that the presence of uronic acids affect the behavior of AG on size exclusion chromatography [10].

One third of the AG molecule is composed of (1→3)-β-D-galactopyranose units, which constitute the main chain, while the rest consists of side groups (whose size varies from monosaccharides to oligosaccharides) which are (1→6)-linked to each galactose unit [11,12]. AG exists naturally as an ordered assembly of molecules in which the (1→3)-β-D-galactan main chain is organized in triple helices [13,14]. Side groups do not interfere with the stability of the chain triple helices, but generate a wide flexible branching surface with many exposed hydroxyl groups, which are able to interact via hydrogen bonds with both neighboring helices and several polysaccaridic targets. These features have been proposed to be responsible for the hydrocolloid nature of AG [13,15].

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http://dx.doi.org/10.1016/j.ijbiomac.2014.04.001
0141-8130/© 2014 Published by Elsevier B.V.

The values reported of AG molecular weight (MW) vary considerably, ranging from 3 to 100 kDa. Evidence has been presented of the existence of two AG components characterized by different MW [9,16,17]. At the same time other authors have described a preparation of AG which was homogeneous in terms of MW [18,19].

Mucins are extracellular proteins with a high MW ranging from 5×10^2 to 2×10^4 kDa and are among the largest known glycoproteins (for review see Ref. [20]). They are usually divided in two classes: membrane associated and secreted. The protein core of mucins of both classes displays a molecular mass ranging from 200 to 600 kDa, representing only approximately 20% of the mass of the mature glycoprotein. A common characteristic of all mucins is the high level of glycosylation, which occurs through glycans O-linked to serine and threonine residues. The hydrophilicity of mucins is associated with the heavy glycosylation and this helps to hold fluids onto epithelial surfaces and to clean the surfaces and/or to block surface microbial binding [21]. In the present study, through different gel filtration chromatographic approaches, it was possible to evidence and quantify an interaction between AG and mucins.

2. Materials and methods

2.1. Materials

Arabinogalactan (AG), pharmaceutical grade, was supplied by Opocrin S.p.A. Modena, Italy. The product, a white dry powder, was 96.3% in AG with a galactose to galactose plus arabinose molar fraction of 0.86 and with a protein content less than 0.004%. Mucin from bovine submaxillary glands (BSM), mucin from porcine stomach (PSM), ferritin, bovine serum albumin (BSA), anthrone and fluorescein isothiocyanate were purchased from Sigma–Aldrich, Italy. Alpha-crystallin was isolated from bovine lens as previously described [22]. Sephacryl S300 HR, was purchased from GE Healthcare Europe GmBH, Germany. Peak areas and overlapping peak regions were quantitated by Image Measurement software KLONK, Ringsted, Denmark.

2.2. Determination of carbohydrates by anthrone method

Carbohydrate content was evaluated spectrophotometrically upon reaction with the anthrone reagent [23]. Aliquots of 0.25 mL of samples were mixed with 1.25 mL of the anthrone reagent (2.5 mM anthrone, 70% H_2SO_4 and 130 mM thiourea) and kept in boiling water for 15 min. The solution was then allowed to cool and the absorbance at 620 nm was measured in a Beckman DU-7 spectrophotometer. The absorbance measured for a sample in the absence of carbohydrates was subtracted as a blank. The amount of sugars in the sample was evaluated referring to a calibration curve obtained using different known concentrations of AG ranging from 0 to 0.40 mg mL^{-1}. An extinction coefficient of 13.43 ± 0.13 (standard error, S.E.) mL mg^{-1} cm^{-1} was obtained.

2.3. Preparation of mucins

Commercial mucins were subjected to gel filtration on Sephacryl S-300 HR at 4 °C prior to testing their ability to interact with AG. Aliquots of 2 mL of 1 mg mL^{-1} of protein solutions in 10 mM phosphate buffer pH 7 (Standard Buffer, SB) were applied to a Sephacryl S-300 column (1.6 cm × 83 cm) and the elution was performed with the same buffer at a flow rate of 20 mL h^{-1}, collecting fractions of 2 mL. BSM eluted as a high MW single symmetrical peak evaluated both by absorbance at 280 nm and by anthrone assay, with a ratio A_{620}/A_{280} of 0.24 (data not shown). When PSM was subjected to gel filtration chromatography as above, its elution profile revealed two well-resolved peaks (data not shown). The higher MW peak was detectable, as BSM, by absorbance at 280 nm and by anthrone assay, while the lower MW peak was exclusively revealed by the anthrone assay, with essentially no absorbance at 280 nm. The carbohydrate content of the high molecular weight peak of PSM was higher with respect to BSM with an A_{620}/A_{280} ratio of 0.74. While commercial preparations of BSM were used for further study, the glycosylated high MW fractions of PSM were pooled, concentrated on Amicon YM30 membrane to a final concentration of 1 mg mL^{-1}, and used for further study.

2.4. Arabinogalactan fractionation

AG was subjected to gel filtration chromatography at 4 °C on a Sephacryl S300 column (1.6 cm × 83 cm). Two mL samples of 1 mg mL^{-1} AG solution were loaded onto the column at a flow rate of 20 mL h^{-1}, and 2 mL fractions were collected. The AG elution was monitored using the anthrone assay method. When using SB as an equilibrating and eluting medium, only one peak with an elution volume of approximately 113 mL was observed (see below, Section 3.1). When using deionized water as equilibrating and eluting medium, two well-resolved components, AG_A and AG_B, were revealed, with elution volumes of approximately 116 mL and 80 mL, respectively (Fig. 1S). Both AG_A and AG_B, when individually re-chromatographed in deionized water in the above conditions, eluted as symmetrical peaks with no changes in their respective elution volumes. When AG_A and AG_B were individually re-chromatographed as above in SB, both displayed the same elution volume of approximately 115 mL (data not shown), similarly to what observed for the unfractionated AG (see below, Section 3.1).

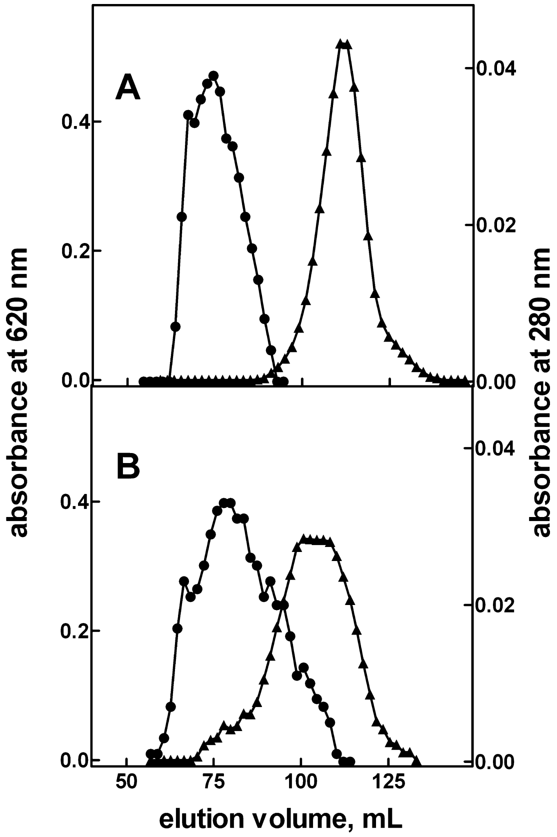

Fig. 1. Gel filtration chromatography of BSM and arabinogalactan. BSM (1 mg mL^{-1}) and AG (2 mg mL^{-1}) were chromatographed on Sephacryl S300 either alone (Panel A) or in a mixture (Panel B). The mucin elution profile was monitored by measuring the absorbance at 280 nm (circles); the AG elution profile was monitored by measuring the absorbance at 620 nm after reaction with the anthrone reagent (triangles). When analyzing the mixture, the contribution of mucin to the absorbance at 620 nm was subtracted in each collected fraction.