Carbohydrate Research 352 (2012) 166–176

Contents lists available at SciVerse ScienceDirect

Carbohydrate Research

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

A new arabinomannan from the cell wall of the chlorococcal algae *Chlorella vulgaris*

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ARTICLE INFO

Article history:
Received 24 November 2011
Received in revised form 9 February 2012
Accepted 10 February 2012
Available online 21 February 2012

Keywords:
Chlorococcal algae
Chlorella vulgaris
Cell wall polysaccharide
Arabinomannan
Polysaccharide structure
Electrospray ionisation mass spectrometry/collision induced dissociation (ESI–MS/CID)

ABSTRACT

A new arabinomannan has been isolated from the cell wall of the green algae *Chlorella vulgaris* by extraction with 0.1 M NaOH, dialysis and SEC fractionation. M_w was about 8000 u. Terminal, 2- and 5-O-linked arabinofuranosyl residues, and 2,6-O-linked mannopyranosyl residues were detected as the main constituents beside some minor mannose components by methylation analysis. Electrospray ionisation mass spectrometry (ESI–MS) with collision induced dissociation (CID) up to MS^3 experiments of oligosaccharides obtained by partial methanolysis or partial acid hydrolysis indicated the presence of Man→Man- and Ara→Ara- as well as Ara→Man- and also Man→Ara-sequences. MS^2 experiments gave evidence of a (1→6)-linked mannan, probably also including some (1→2)-linked mannosyl and 5-linked arabinofuranosyl residues. Mannooligosaccharides up to DP5 with mainly (1→6)-, but at higher DP also (1→2)-linkages were obtained by acid hydrolysis, and arabino oligomers up to DP4 could be detected after mild methanolysis. In accordance with results from methylation analysis and ESI–MS/CID *t*-β-Ara*f*, 2-α-Ara*f*, 5-α-Ara*f* and 2,6-α-Man*p* were identified from homo- and heteronuclear 1D- and 2D NMR experiments in a molar ratio of ~2:2:1:2. A highly branched structure is suggested with a 6- and 2-O-linked mannan main chain, comprising 5-Ara*f* residues. Ara*f*-β-[(1→2)-Ara*f*-α-(1→]$_n$ side chains with an average chain length of 2 are linked to the mannan main chain. The configuration of Ara is D. The new polysaccharide shows structural similarities with the (lipo)arabinomannans of *Mycobacterium tuberculosis*.

1. Introduction

In the last decades, microalgae, for example, chlorococcal algae, have drawn more attention as sources of biologically active compounds. Morimoto et al.[1] reported on anti-tumour-promoting mono- and digalactosyl diacylglycerols in *Chlorella*. Chlorophyll, carotenoids and unsaturated fatty acids, and vitamins are further valuable constituents. Therefore, microalgae are considered as biomass, as food supplement, as additive for food and animal feeding or for cosmetic preparations.[2] Beside their nutritional applications, *Chlorella* species are also discussed for waste water treatment due to their heavy metal complexing properties, which are related to the cell wall composition.[3] A water-soluble antitumour glycoprotein has been purified from the culture media of *Chlorella vulgaris* and found to contain a β-(1→6)-linked galactan backbone.[4] In order to find chemotaxonomic indicators to differentiate the morphologically very similar species of the genus *Chlorella*, cell wall composition has been considered besides other properties.[5,6] Rigid cell walls with incorporated matrix carbohydrates have been isolated and submitted to a cascade of hydrolysis procedures. From the results two groups of chlorococcal algae species are distinguished: one with glucan-mannan in the rigid wall, and a second with glucosamine as the main constituent, to which *Chlorella vulgaris* belongs.[7,8] Comparison of matrix sugar composition was useful for further classification. The glucosamine-type species were dominated by rhamnose and galactose,[9] while for those of the glucose–mannose-rigid wall group mannose was the preferred one.[6] The matrix carbohydrates of four from five strains of *Chlorella vulgaris* contained 42–50% rhamnose, 4–9% arabinose, 5–17% xylose, 2–10% mannose, 22–30% galactose and 0–4% glucose. The uronic acid content, which correlates with ruthenium red stainability, also varied for different species and was in the range of 8–11% of dry mass for the *Chlorella vulgaris* strains. Kapaun et al.[7] reported rough data for the composition of a cell wall preparation of *Chlorella* species ranging from 23% to 29% for neutral sugars, 15–20% for uronic acids, 7–17% for glucosamine and 6–10% for protein.[10] An acidic polysaccharide, mainly consisting of 4-O-linked α-D-glucuronic acid, terminal, 3-O-, 2-O- and 2,3-di-O- linked (branched) α-L-rhamnosyl residues, has been isolated by extraction of the defatted cell wall with 4% KOH (0.7 M) by Ogawa et al.[11] Xylose, mannose, glucose, and galactose were further constituents.[12] Some uncommon sugars as 2-*O*-methyl- and 3-*O*-methyl-L-rhamnose were also identified in an acidic polysaccharide

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0008-6215/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carres.2012.02.007

fraction,[13] while a further O-methylated sugar, 6-O-glycosylated 3-*O*-methyl-D-galactose, was isolated from a neutral β-D-galactan having (1→3)-linkages in the backbone and (1→6)-linkages in the side chain.[14] Brunner and Loos[15] identified 4-*O*-methyl xylose as a constituent of the cell wall of *Chlorella vulgaris*. Thus, the carbohydrate profile of *Chlorella* cell walls is very complex, and it must be kept in mind that composition of isolates and detection of constituents depend on the extraction and fractionation conditions, and type of hydrolysis applied to the isolated polysaccharides as well.

We here report on a new arabinomannan, which has been isolated from the commercial spray-dried *Chlorella vulgaris* by extraction with 0.1 M NaOH.

2. Results and discussion

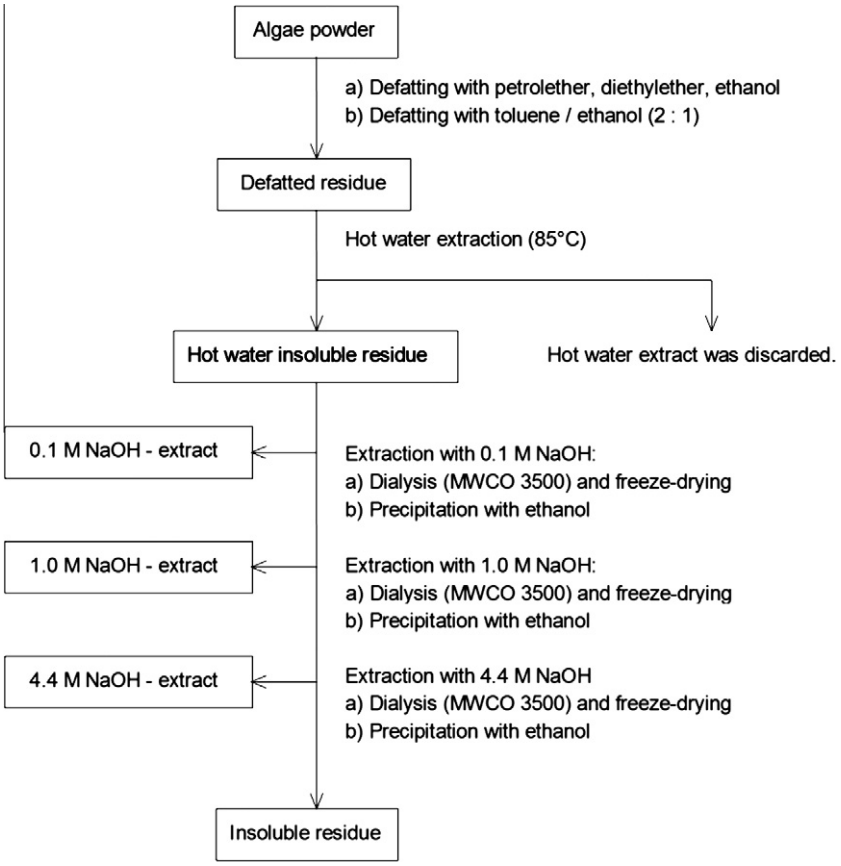
2.1. Isolation of polysaccharides

Polysaccharides were isolated from the spray-dried algae of *Chlorella vulgaris* according to procedures reported in the literature[16,17] (see Scheme 1). The spray-dried algae was first defatted and then extracted with hot water to remove water soluble material. The residue was subsequently extracted with aqueous base of increasing concentration at rt. The extracts obtained with 0.1 M, 1.0 M and 4.4 M NaOH were dialysed and freeze-dried. In a second approach, polysaccharides were precipitated with ethanol from the neutralised extracts. Yields for the salt-free dialysed extracts were 3.9%, 5.7% and 3.4% (w/w), respectively, yields for the precipitated extracts were 7.5%, 18.5%, 1.0% (w/w). For comparison, Takeda[6] obtained 3–6% referred to dry mass of algae by his cell-wall preparation protocol, including cell disruption and α-amylase digestion to remove starch. Extracts from precipitation, especially the 1.0 M NaOH extract, contained a huge amount of low molecular weight and the apparent yield was therefore much higher compared to the dialysed sample.

2.1.1. Sugar composition

Neutral sugars were determined by the alditol acetate method.[18] Rhamnose, fucose, arabinose, xylose, mannose, glucose and galactose were identified. A 6-deoxy-3-*O*-methyl-hexose and traces of a presumably 6-deoxy-2-*O*-methyl-hexose were assigned as 3- and 2-*O*-methyl-L-rhamnose, according to the results of Ogawa et al.[13] 3-*O*-Methyl-pentose is assumed to refer to xylose, due to the finding of 4-*O*-methyl-D-xylose by Brunner and Loos.[15] The *O*-methyl-hexoses most probably are D-galactose derivatives, because at least 3-*O*-methyl-D-galactose has been reported as a constituent of 1,6-β-D-galactan by Ogawa et al.[14] Differences in composition between dialysed and precipitated fractions were small, although some minor *O*-methyl sugars were missing in the precipitate. Figure 1 shows the relative molar composition of the various extracts. Mannose and arabinose were the main constituents of the 0.1 M NaOH extract, while fucose, xylose and the *O*-methyl sugars occurred at only low amounts. The aminosugars 2-amino-2-deoxy-D-glucose and -galactose could be detected from the TFA hydrolysates, but were recovered to a much higher extent by hydrolysis with 6 M HCl.

The qualitative results for the 1.0 M NaOH and 4.4 M NaOH on one side, and the 0.1 M NaOH extract on the other side were very similar, but the quantitative composition differed significantly. The 3- and 4-*O*-methyl-hexoses present at 0.1 M NaOH were no longer detected for the more alkaline extracts, while the contribution of 3-*O*-methyl-pentose and the 3-*O*-methyl rhamnose together increased up to 8.6 mol % for the more alkaline extracts. Mannose is the main constituent in all extracts, while arabinose strongly



Scheme 1. Isolation of polysaccharides from spray-dried *Chlorella vulgaris*—methods (a) and (b) are applied alternatives.