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Isolation and characterization of poly- and oligosaccharides from the red microalga *Porphyridium* sp.

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ABSTRACT

The current study forms part of an ongoing research effort focusing on the elucidation of the chemical structure of the sulfated extracellular polysaccharide of the red microalga *Porphyridium* sp. (UTEX 637). We report here on the chemical structure of a fraction separated from an acidic crude extract of the polysaccharide, as investigated by methylation analysis, carboxyl reduction–methylation analysis, desulfation–methylation analysis, partial acid hydrolysis, Smith degradation, together with 1D and 2D ^{1}H and ^{13}C NMR spectroscopy. This fraction with a molar mass of 2.39×10^{5} g mol^{-1} is comprised of D- and L-Gal, D-Glc, D-Xyl, D-GlcA, and sulfate groups in a molar ratio of 1.0:1.1:2.1:0.2:0.7. The almost linear backbone of the fraction is composed of (1→2)- or (1→4)-linked D-xylopyranosyl, (1→3)-linked L-galactopyranosyl, (1→3)-linked D-glucopyranosyl and (1→3)-linked D-glucopyranosyluronic acid and comprises a possible acidic building unit:

[(2 or 4)-β-D-Xyl*p*-(1→3)]$_m$-α-D-Glc*p*-(1→3)-α-D-Glc*p*A-(1→3)-L-Gal*p*(1→

Attached to the backbone are sulfate groups and nonreducing terminal D-xylopyranosyl and galactopyranosyl residues, which occur at the O-6 positions of Glc-derived moieties in the main chain.

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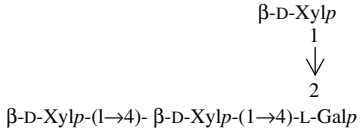
1. Introduction

Porphyridium sp., the most abundant species of red microalga of the division Rhodophyta, has been the subject of intensive study by our group for a number of years.[1,2] The cells of this red microalga are encapsulated within a cell-wall polysaccharide complex. The external part of the complex dissolves continuously into the medium,[3,4] and is thus designated 'soluble polysaccharide'. However, most of the polysaccharide remains bound to the cell-wall and is designated 'bound polysaccharide'.[1,2]

Due to the complexity of the polysaccharide, only limited information is available on its structure. Our group has, however, shown that the soluble polysaccharide, with a molar mass of 2.3×10^{6} g mol^{-1},[5] is composed mainly of a sulfated complex heteropolymer containing a number of neutral sugars (mainly Xyl, Glc and Gal) and GlcA.[2,6,7] Minor amounts of methylated sugars are also present.[6,8] The polysaccharide is anionic (negatively charged) due to the presence of GlcA and half-ester sulfate groups[6,9] and is made up of 67% carbohydrate, ~10% ash, ~9% uronic acid, and ~10% half-ester bound sulfate.[6] Two polysaccharide fractions differing in charge and sugar compositions have been obtained by fractionation.[6] These two fractions accounted for ~89% of the total carbohydrates.[6] A primary disaccharide building block, α-D-glucopyranosyluronic acid-(1→3)-L-galactopyranose has been isolated and characterized from acid hydrolysates of *Porphyridium* sp.[10,11] The monosugar sulfates have also been characterized.[9]

Gloaguen et al.[12] have characterized the chemical structure of an anionic polymer separated from the bound polysaccharide produced by *Porphyridium* sp. Analysis of this polymer showed the presence of three major neutral monosaccharides, Xyl, Glc and Gal and of GlcA. Uronic degradation of this polymer with lithium in ethylenediamine yielded the following two oligosaccharides:



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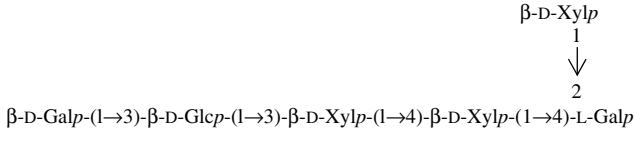

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The studies of our group on the rheology and chemistry[6,7,13–15] of the soluble polysaccharide of *Porphyridium* sp. have revealed that the polysaccharide exhibits special rheological properties, which makes it suitable for a wide range of potential industrial applications.[2,16,17] Biotechnological investigations have also indicated its potential applications in medicine.[18–20]

For advanced biotechnological development, it was necessary to have access to detailed structural information about the polymer complex of *Porphyridium* sp. To supplement the limited information available to date, we isolated a sulfated glucuronoxylan from the soluble polysaccharide of *Porphyridium* sp. and undertook a structural study utilizing classical chemical methods and spectroscopic analysis.

2. Results

The soluble polysaccharide isolated from cultures of *Porphyridium* sp. was subjected to preliminary fractionation by precipitation with cetyltrimethylammonium bromide (CTAB) to produce an acidic crude polysaccharide and a neutral crude polysaccharide. The former (major) fraction comprised about 96% of the total soluble polysaccharide.

Since the high viscosity of the acidic crude polysaccharide complicated further purification and analysis, the crude preparation was first depolymerized by ultrasound to facilitate isolation and purification of smaller fractions. After 40 min of sonication, the viscosity of the acidic crude polysaccharide solution was markedly reduced (by about 98%).

After ultrasonic depolymerization, the crude preparation was separated into four fractions by anion-exchange chromatography on DE-52 by elution with the following media: water (fraction I), 0.5 M NaCl (fraction II), 1.0 M NaCl (fraction III), and hot urea (fraction IV). Fraction III comprised the major fraction of the acidic polysaccharide from *Porphyridium* sp. and accounted for 30.4% of the total soluble polysaccharide. The homogeneity of this fraction was evident from the single symmetrical high-performance size-exclusion chromatography (HPSEC) peak and the absence of proteins, as indicated by negative responses in both the assay by Lowry et al.[21] and the one by Bradford.[22] The molar mass of this fraction was 2.39×10^5 g mol^{-1}, as determined by the gel-permeation chromatography multiple angle laser light scattering (GPC–MALLS) method.

Sugar composition analysis, by thin-layer chromatography (TLC) and gas chromatography (GC) of the alditol acetate derivatives of fraction III, showed this fraction to be composed of Xyl, Glc and Gal in a molar ratio of 2.1:1.0:1.1 (Table 1). Polysaccharide fraction III also contained uronic acid and sulfate groups, as shown by the *m*-hydroxybiphenyl assay[23] and the sodium rhodizonate assay,[24] respectively. IR spectroscopy of fraction III gave absorption bands at 1250 cm^{-1} and 820 cm^{-1}, indicating the presence of sulfate groups and thus confirming the analytical results. The presence of only one absorption band at 820 cm^{-1}, the region characteristic of sulfated primary hydroxyl groups, suggested that the majority of sulfate groups occurred at C-6 of the sugar residues.[25]

It is known that both D- and L-Gal are present in extracellular polysaccharides extracted from other species of red algae.[12,26–31] In our previous studies[10,11], we obtained an α-D-glucopyranosyluronic acid-(1→3)-L-galactopyranose disaccharide from the acid hydrolysate of *Porphyridium* sp., indicating that the absolute configuration of GlcA and Gal was D- and L-, respectively. This disaccharide was shown to be part of a basic building block found in polysaccharides from various red microalgae.[10,11] To reveal whether this disaccharide is related to fraction III, we determined the absolute configuration of the Gal component of fraction III by means of an enzymatic assay with D-Gal oxidase (Fig. 1).[29] D-Gal, determined in this way, accounted for about 50% of the total Gal in the hydrolysate from fraction III (Fig. 1). This finding thus indicated that fraction III contained both D- and L-Gal units, thereby suggesting that other Gal units exist in this polysaccharide with a D-configuration.

The nature of the glycosidic linkage, the number of uronic acid residues, and the position of the sulfate groups in fraction III were elucidated by methylation analysis of the native (designated III), carboxyl-reduced (III-Du) and desulfated (III-Ds) fractions, respectively. For this purpose, fraction III was methylated by the method of Needs and Selvendran,[32] and the fully methylated polysaccharide was then subjected to acid hydrolysis, reduction, and acetylation. The neutral sugar composition of the permethylated fraction III was in good agreement with the GC analytical data for the unmethylated polymer. As shown in Table 2, GC–MS analysis revealed the presence of 2,3,4-tri-*O*- and 3,4- or 2,3-di-*O*-methylxylitol acetates, 2,3,4,6-tetra-*O*- and 2,4,6-tri-*O*-methylgalactitol acetates, and 2,4,6-tri-*O*- and 2,4-di-*O*-methylglucitol acetates. These results indicated that the neutral sugar residues in this polysaccharide existed as (1→2)- or (1→4)-linked xylopyranosyl, (1→3)-linked galactopyranosyl, (1→3)-linked glucopyranosyl,

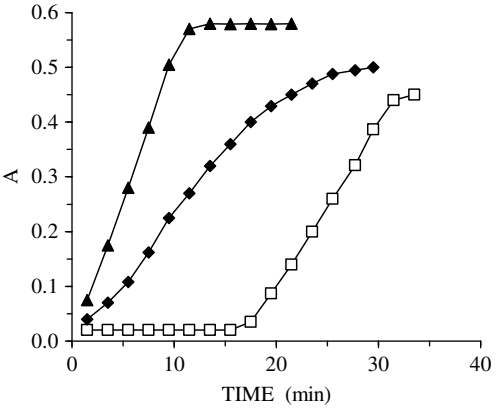

Figure 1. Determination of the absolute configuration of Gal in the polysaccharide III by the D-Gal oxidase assay. Right-most curve (white square symbol): standard L-Gal plus addition of standard D-Gal after 15 min; middle curve (black diamond symbol): standard D-Gal; Left-most curve (black triangle symbol): complete hydrolysate of the polysaccharide III.

Table 1
Molecular weight and sugar composition of fraction III and subfraction III-P

Molar ratio					MW	Samples
SO_2ONa%[a]	Uronic acid[b]	Glu	Gal	Xyl		
0.7	0.2	1.1	1.0	2.1	2.39×10^5	III
0.8	0.5	1.0	1.0	3.0	1.13×10^4	III-P

[a] Determined by the sodium rhodizonate assay.
[b] Determined by the *meta*-hydroxybiphenyl assay.

Table 2
Methylation analysis of the polysaccharide III and its derivatives

Mass fragments (*m*/*z*)	Molar ratio			Sugar (alditol acetates)
	III-Du	III-Ds	III	
43, 73, 101, 117, 129, 161, 205	9.31	9.36	9.35	2,3,4-Me_3Xyl*p*
43, 73, 101, 117, 129, 161, 189, 233	31.86	32.44	32.97	3,4-Me_2Xyl*p* or 2,3-Me_2Xyl*p*
43, 45, 101, 117, 129, 145, 161, 205	3.88	3.97	3.98	2,3,4,6-Me_4Gal*p*
43, 87, 101, 117, 129, 161, 173, 233	17.25	18.53	18.98	2,4,6-Me_3Gal*p*
43, 87, 101, 117, 129, 161, 173, 233	8.28	19.58	3.37	2,4,6-Me_3Glc*p*
43, 87, 117, 129, 159, 189, 233	25.42	10.70	25.74	2,4-Me_2Glc*p*

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