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Note

Chemical characteristics of a polysaccharide from *Porphyra capensis* (Rhodophyta)

Quanbin Zhang,[a,*] Huimin Qi,[a,b] Tingting Zhao,[a,b] Eric Deslandes,[c] Ninasayaeli Mbise Ismaeli,[d,✠] F. Molloy[d] and Alan T. Critchley[e]

[a]*Institute of Oceanology, Chinese Academy of Sciences, Qingdao 266071, China*
[b]*The Graduate School of the Chinese Academy of Sciences, Beijing 100039, China*
[c]*Lebham European Institute for Marine Studies, University of Western Brittany, 29280 Plouzane, France*
[d]*Coastal Research Centre, University of Namibia, Henties Bay, Namibia*
[e]*Degussa Texturant Systems France SAS, F-50500 Baupte, France*

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Abstract—The structure of a polysaccharide from the red seaweed, *Porphyra capensis*, growing along the coast of Namibia and South Africa was investigated. Algae growing at different sites and collected at different times gave a polysaccharide extract with similar chemical components. FTIR and NMR spectral analysis showed that the polysaccharide from *P. capensis* had a typical porphyran structure. It has the linear backbone of alternating 3-linked β-D-galactose and 4-linked α-L-galactose-6-sulfate or 3,6-anhydro-α-L-galactose units. The ratio of α-L-galactose-6-sulfate and the 3,6-anhydrogalactose is 1.2:1, as reflected by a ^{1}H NMR spectrum. A high degree of methylation occurred at the C-6 position of the D-galactose units. The degree of methylation was 0.64 for the D-galactose residues.

Keywords: *Porphyra capensis*; Polysaccharide; Structure; Methylation

Intertidal red algae belonging to the genus of *Porphyra* occur on rocky shores from the polar to tropical seas. Although this genus is widespread along most temperate oceanic coastlines, the recorded distribution of individual species tends to be more limited, and species are generally considered to be confined to clearly demarcated geographical regions. More than 130 species of Porphyra have been described worldwide. *Porphyra* an important food source in many parts of the world, is nutritious with abundant proteins, polysaccharides, vitamins, and minerals.[1]

Several investigations of the structure and function of the polysaccharides isolated from different *Porphyra* species have been undertaken.[2–8] Porphyrans, the sulfated polysaccharides comprising the hot-water soluble portion of the cell wall and intercellular region, are the main components of *Porphyra*. Agarose, a family of cell-wall polysaccharides extracted from marine red algae, consists of alternating 3-linked β-D-galactose and 4-linked 3,6-anhydro-α-L-galactose units. Porphyran is closely related to agarose in its basic structure, while it is very different in terms of having L-galactose-6-sulfate.[4] But the chemical components and structure of porphyrans isolated from different species showed expected variations.[2]

Porphyra capensis is distributed in the southern part of Africa. *P. capensis* was harvested between 1965 and 1978 in small quantities. South Africa *Porphyra* spp. may also have an application as a food supplement for abalone.[9] However, as an important component, the chemical properties of its polysaccharide extract have not yet been investigated.

In this study, the polysaccharide of *P. capensis* was isolated from hot-water soluble extracts. The

* Corresponding author. Tel./fax: +86 532 82898703; e-mail: qbzhang@ms.qdio.ac.cn
✠ Deceased.

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Table 1. Yield and chemical composition of polysaccharides extracted from *P. capensis* growing at different sites and time (% w/w of dry weight)

Place of collection	Time of collection	Yield	Total sugar	Sulfate	3,6-AG
Swakopmund, Namibia	August 1998	25.4	73.3	11.5	12.3
	November 1998	18.8	78.7	11.5	12.9
	January 1999	23.4	77.4	9.7	12.9
Lüderitz, Namibia	January 1998	17.1	76.6	9.6	11.3
	November 1998	20.5	72.0	12.1	10.1
	January 1999	22.3	70.7	10.1	11.0
Cape Town, South Africa		20.9	72.2	11.7	11.5

polysaccharide obtained by hot-water extraction at 120 °C in an autoclave was a pale-yellow, fibrous material. The yields ranged from 17.1% to 25.4%. The chemical composition of polysaccharides extracted from algae growing at different sites and collected at different times is shown in Table 1. The growing place had no obvious influence on total sugar, sulfate, and 3,6-AG content in the polysaccharide extracts. However, there is a slight seasonal variation in the level of sulfate content, with levels being low in January (9.6%, 9.7%, and 10.1%, respectively), and a little higher in August and November (11.5–12.1%). But because we did not have an adequate number of samples, the seasonal changes in the chemical composition of porphyran from *P. capensis* need further investigation.

Rees and Conway studied the variability in the composition of polysaccharide extracts of *Porphyra* species and found that there was wide variability; ester sulfate may be present in amounts from 6% to 11%, and 3,6-anhydrogalactose from 5% to 19%.[2] The chemical composition of *P. capensis* polysaccharide is in the reported range for other *Porphyra* species.

Infrared spectroscopic analysis indicated that all the polysaccharide extracts showed typical signals of porphyran, including signals at 3420, 1646, 1419, 1225, 1155, 1073, 930, and 817 cm^{-1}. The IR spectrum of the polysaccharide extracted from *P. capensis* collected at Cape Town, South Africa are shown in Figure 1. The signal at 1225 cm^{-1} is assigned to the asymmetric stretching vibration of sulfate group, and the signal at 817 cm^{-1} or so is indicative of a sulfate group attached to a primary hydroxyl group. These data suggest that the sulfate groups occur at C-6 of the galactose residues.[10] The band at 930 cm^{-1} is attributed to the absorption of 3,6-anhydrogalactose.

Treatment of agaroids containing L-galactose-6-sulfate residues (precursor units) with alkali results in intramolecular displacement of the sulfate group and formation of 3,6-anhydro-L-galactose residues. To confirm the presence of such precursor units, the polysaccharide from *P. capensis* was treated with alkali, and its infrared spectrum was recorded. After alkali modification, the band at 1225 and 817 cm^{-1} for a sulfate group disappeared, while the intensity of the absorption band for 3,6-anhydrogalatose at 930 cm^{-1} increased

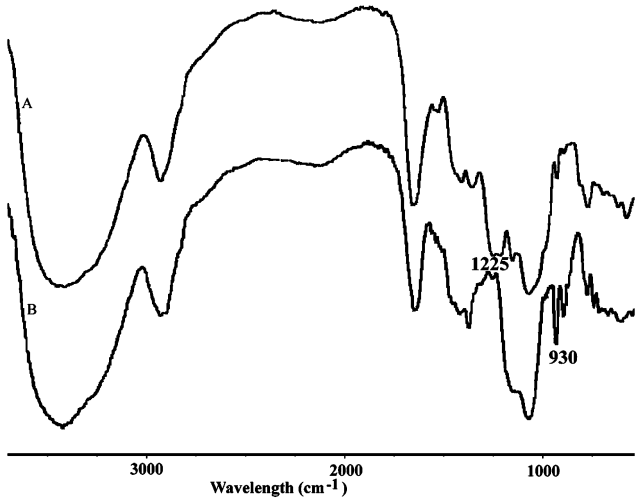

Figure 1. FTIR spectra of native and alkali-treated polysaccharide extracted from *P. capensis* collected at Cape Town, South Africa. A: native polysaccharide; B: alkali-treated polysaccharide.

(Fig. 1). This confirmed that sulfate ester was substituted at the C-6 position of the L-galactose unit, which can be converted to 3,6-anhydrogalactose after alkali treatment.[5]

Considering the similarity in chemical composition of polysaccharides extracted from *P. capensis* growing at different sites and collected at different times, in the following experiment, we selected the polysaccharide extracted from *P. capensis* collected at Cape Town, South Africa, for NMR spectral analysis.

The structure of polysaccharide from *P. capensis* was elucidated with NMR spectroscopy. The ^1H NMR spectrum is shown in Figure 2. In the ^1H NMR spectrum, the anomeric resonances at 5.15–5.28 ppm originated from 4-linked-α-L-galactopyranose units.[11,12] The signal at 5.28 ppm was attributed to the anomeric proton of the agarose precursor 4-*O*-linked α-L-galactose-6-sulfate units. The signal at 5.15 ppm was due to the anomeric proton of the 3,6-anhydro-α-L-galactose units. The ratio of the intensity of signals at 5.28 to the intensity of signal at 5.15 is 1.2:1. It can be calculated from the data listed in Table 1 that the molar ratio of sulfate to L-galactose is approximately 1.3:1, which is in accordance with the data estimated from the NMR spectrum. These results indicated that the precursor 4-*O*-linked

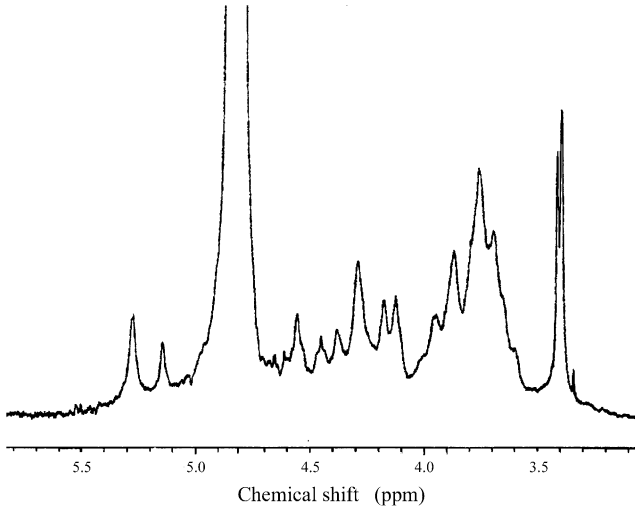

Figure 2. ^{1}H NMR spectrum of polysaccharide from *P. capensis*.

α-L-galactose-6-sulfate units were a little higher than the 3,6-anhydro-α-L-galactose units. The strong signal present at 3.40 ppm was attributed to the methyl group of 6-*O*-methyl-D-galactose.[13,14] The *O*-methyl content was estimated by the ratio between 1/3 of the area of the methyl resonances at 3.40 ppm and the area of the H-1s of 4-linked L-galactose in the region of 5.15–5.29 ppm, assuming a perfect alternating agaroid backbone. The degree of substitution by methyl groups, estimated by the ^{1}H NMR spectrum, was 0.64 for the D-galactose residue. However, the signal at 3.51 ppm due to methylation at the C-2 position of 3,6-anhydro-L-galactose was absent in the ^{1}H NMR spectrum. Signals were not detected for agaroid substituted with pyruvate ketals by ^{1}H NMR spectroscopy at 1.60 ppm.[15]

The ^{13}C NMR spectrum for the polysaccharide is shown in Figure 3. The signal assignment of the polymer was achieved by comparison with previously reported spectra of model compounds, agarose, and other related polysaccharides.[5,16–20] Two groups of signals corresponding to the repeating units of the agarose and the agarose biological precursor, respectively, were detected. The signals of −3-β-D-Gal-(1→4)-linked 3,6-anhydro-α-L-Gal (G-A) units, that is, the agarose repeating units are (G1, 102.4; G2, 70.1; G3, 82.2; G4, 69.1; G5, 75.4; G6, 61.5; A1, 102.4; A2, 70.1; A3, 80.1; A4, 77.3; A5, 75.4; A6, 61.5 ppm). The signals correspond to the -3-β-D-Gal-(1→4)-linked -α-L-Gal-6-sulfate (G-L6S) units, the agarose biological precursor units, include (G′1, 103.6; G′2, 69.7; G′3, 81.0; G′4, 69.1; G′5, 75.9; G′6, 61.7; L1, 101.5; L2, 69.1; L3, 71.0; L4, 78.9; L5, 70.8; L6, 67.9 ppm). Methylation at the C-6 position of β-D-galactose was indicated by the characteristic signals due to 6-*O*-methyl-β-D-galactose (73.6, 71.8, and 59.1 ppm). The signal at 78.5 ppm may be attributed to 2-*O*-methyl-3,6-anhydro-α-L-galactose, but this signal is quite weak and another signal corresponding to A2M (82.5 ppm) was not detected in the ^{13}C NMR spectrum.

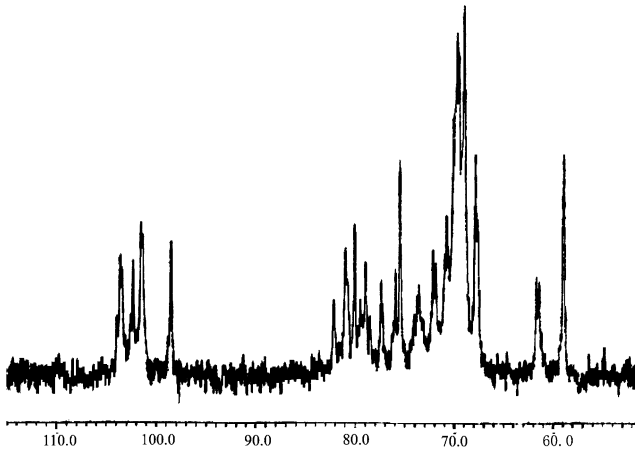

Figure 3. ^{13}C NMR spectrum of polysaccharide from *P. capensis*.

It can be concluded from the above analysis that the polysaccharide from *P. capensis* has a typical porphyran structure, with the linear backbone of alternating 3-linked β-D-galactose units and 4-linked α-L-galactose-6-sulfate and 3,6-anhydro-α-L-galactose units. The ratio of α-L-galactose-6-sulfate units and 3,6-anhydrogalactose units is 1.2:1. A high degree of methylation (DM) occurred at the C-6 position of the D-galactose units (DM = 0.64).

1. Experimental

1.1. Materials

P. capensis, collected on the coast of Swakopmund and Lűderitz, Namibia, and Cape Town, South Africa, at different time, was provided by Dr. Alan Critchley and R. J. Anderson of Seaweed Section, MCM, South Africa, respectively.

1.2. Extraction

The polysaccharide was prepared as described previously.[8] In brief, the alga was autoclaved for 3 h at 120 °C in 40-fold water and successively filtered through gauze and siliceous earth as filter aid. This process was repeated once. The filtrate was combined, dialyzed against running tap water for 24 h and distilled water for 24 h, and then concentrated to one-fourth of the original volume under reduced pressure. The solution was lyophilized to obtain the dry polysaccharide.

1.3. Alkaline modification of polysaccharide

The polysaccharide was treated with $NaBH_4$–NaOH according to Craigie and Leigh.[21] Briefly, 100 mg of

polysaccharide was dissolved in 20 mL distilled water with 0.05% (w/v) $NaBH_4$ and maintained overnight under stirring at 20 °C. NaOH (3 M) containing 0.3% (w/v) $NaBH_4$ was added up to 0.6 M as the final concentration. After being maintained under agitation for 3 h at 80 °C, the solution was neutralized with HCl, dialyzed exhaustively against distilled water, and freeze-dried.

1.4. Analytical methods

1.4.1. General analyses. Total sugar content was analyzed by the phenol–sulfuric acid method using D-galactose as standard.[22] Sulfate content was determined according to the method of Kawai.[23] 3,6-Anhydrogalactose content was determined as described previously.[24]

1.4.2. Spectroscopic methods. Fourier-transformed infrared spectra were recorded from polysaccharide powder in KBr pellets on a Nicolet Avatar 360 FTIR spectrophotometer. For nuclear magnetic resonance (NMR) spectroscopic analysis, the lyophilized sample was dissolved in D_2O and ^1H NMR (600 MHz, acquisition time 2.7 s, delay 4 s) and ^{13}C NMR (150 MHz, acquisition time 0.5 s, delay 1 s) spectra were acquired on a Jeol ECP600 NMR spectrometer at 25 °C. ^1H chemical shifts were measured relative to external DSS, and proton-decoupled ^{13}C NMR chemical shifts were measured in parts per million relative to internal dimethyl sulfoxide at 39.6 ppm.

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