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Chemical modifications of biopolymers: quaternization of the extracellular polysaccharide of the red microalga *Porphyridium* sp.

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Abstract

Sulfated polysaccharides that encapsulate the cells of red microalgae are high-molecular weight polymers (about 7×10^6 Da) with complex chemical structures. To broaden their potential industrial applications, a study was initiated to tailor additional characteristics by means of chemical modifications. Here, the quaternization of the extracellular polysaccharide derived from the red microalga *Porphyridium* sp. was performed. The quaternization reaction was carried out with 3-chloro-2-hydroxypropyltrimethyl ammonium chloride under basic conditions. A higher degree of substitution was obtained in lower volumes of water in which the polysaccharide solution had a gel-like form. The product was analyzed by infrared and vacptppm-^{13}C spectroscopy. Good correlation was found between the nitrogen content of the quaternized polysaccharide and the IR peak ratio of the relevant C–N stretching vibrations. The solid-like (elastic) response (G') to imposed small deformation of the quaternized polysaccharide was higher than that of the unmodified biopolymer. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: Red microalgae; Extracellular polysaccharide; Quaternization

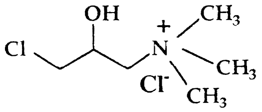
1. Introduction

Polysaccharides and their derivatives have found numerous applications in a variety of fields including the paper and textile, food, cosmetics, and chemical and pharmaceutical industries. The high potential for exploiting these natural biopolymers with their broad range of structural, functional and physicochemical properties, in various applications has provided the stimulus for the search for new or modified polysaccharides.

Various modifications have been performed on cellulose and other algal and bacterial polysaccharides (Scheme 1) to produce new properties; for example, methylation improved thickening, binding and stabilizing properties (Hiatt & Rebel, 1971), conversion to the phosphate ester introduced flame-resistant properties and ion-exchange capability (Isogai, Ishizu & Nakano, 1986), and quaternization (introduction of quaternary ammonium groups) conferred adhesive properties (Ebringerova, Hromadkova, Kacurakova & Antal, 1994).

Among the modifications cited above, quaternization often constitutes an efficient means of conferring properties of commercial value on the existing polysaccharides. This type of modification changes the functionality, while maintaining most of the other properties of the polysaccharide intact. Quaternization of hemicelluloses has previously been performed by Pulkkinen, Reintjes and Starr (1973). Ebringerova et al. (1994) prepared quaternized xylans.

In this work, we describe the quaternization of the extracellular polysaccharide derived from the red microalga *Porphyridium* sp.; 3-chloro-2-hydroxypropyltrimethylammonium chloride (CHMAC) served as the quaternizing agent.



The characterization of chemical and physical properties of the *Porphyridium* sp. polysaccharide and the delineation of its potential applications constitute an important aspect in the research and development program of our laboratory. The polysaccharide was found by Geresh, Lupescu and Arad (1992) to be a heteropolymer with a high molecular weight ($\sim7\times10^6$ Da). It is composed of about 10 different sugars, with xylose (40%), galactose (18%), and glucose (15%) being the main monosugars. The polysaccharide is anionic due to the presence of glucuronic acid and half ester sulfate groups as reported by Geresh, Dubinsky, Christiaen and Arad (1990) and Geresh, Lupescu, Arad, Bernstein and

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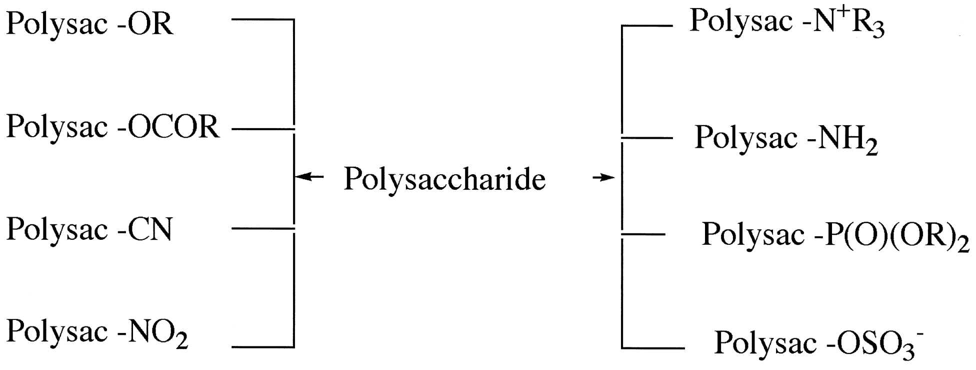

Scheme 1.

Glazer (1991). This property has been applied in the preparation of ion exchangers for the removal of toxic ions from industrial wastes, not only with this polysaccharide, but also with the polysaccharide of another red microalga—*Rhodella reticulata* (Geresh, Arad & Shefer, 1997). The polysaccharide exhibits non-Newtonian rheological behavior in aqueous solutions (Geresh & Arad, 1991) and has surfactant properties.

2. Experimental

2.1. General

Analytical grade CHMAC was purchased from Aldrich Chemical Co. Nitrogen content was measured by the Kjeldahl method. IR spectra (KBr pellets) were obtained with a Nicolet FT-IR spectrophotometer. Total sugars were determined by the phenol sulfuric acid method by means of an LKB Novaspec spectrophotometer. Quaternization of the polysaccharide was confirmed by ^{1}H and ^{13}C NMR spectroscopies with a 500 MHz Brucker spectrometer. Solid state ^{13}C NMR of the quaternized polysaccharide was also performed with variable amplitude cross polarization spectroscopy with two-pulse phase modulation decoupling (vacptppm) and vacptppmnqs non-quaternary carbon suppression (vacptppmnqs) with a 50 ms total delay except for that of the quaternary carbon atoms. For NMR measurements, hydrolyzates of the native and modified polysaccharides were dissolved in DMSO-d_6.

2.2. Quaternization reaction

The extracellular polysaccharide of *Porphyridium* sp. was isolated as previously reported by Geresh et al. (1992). Usually, about 500 mg of polysaccharide was dispersed in sodium hydroxide solution, and the mixture was stirred continuously for 30 min at room temperature. Various amounts of CHMAC (in aqueous solution) were added, and stirring was continued for 20 h at room temperature. One volume of reaction product was poured into four volumes of acidified (1% HCl) ethanol, and the precipitate was washed with four volumes of 80% ethanol. The precipitate was then dispersed in doubly distilled water and dialyzed until free of chloride ions. The dialyzed product was then dried by lyophilization

Since the native polysaccharides of the red microalgae also have a protein moiety, blanks (without CHMAC) were run for different conditions of the reaction, and the nitrogen contents were determined. The reported nitrogen content of a particular product is thus the difference between the nitrogen content of the product and the nitrogen content of the corresponding blank. The IR spectrum and the nitrogen content of the products were compared with the findings for the native polysaccharide and with those obtained from the blank reaction (without CHMAC), and the extent of quaternization was thus ascertained. Experiments were performed under different reaction conditions with the aim of optimizing the reaction parameters.

The phenol sulfuric acid method developed by Dubois, Gilles, Hamilton, Rebes and Smith (1956) was used to determine the total sugar content of the native, the blank and the modified polysaccharides. A calibration curve was constructed with galactose, and the total sugar content of the polysaccharide was reported in terms of D-galactose.

2.3. Preparation of hydrolyzates

The modified product and the native polysaccharide were hydrolyzed in 2 M trifluoroacetic acid at 100°C for 2 h. After work-up, the hydrolyzates were dissolved in 1 ml DMSO-d_6, and NMR spectra were recorded. For the reagent CHMAC, NMR spectra were measured in D_2O as well as in NaOD.

2.4. NMR spectroscopy

Solid-state ^{13}C NMR spectra (125 MHz) were measured in 4-mm zirconia rotors at 299 K on a Bruker DMX-500 NMR Fourier transform spectrometer equipped with a CP-MAS accessory/BL-4 probe head. Vacptppm and Vacptppmnqs (50 ms delay) Bruker Library pulse programs were used for the data acquisition at a spinning rate of

Table 1
Effect of different amounts of CHMAC on the quaternization of the polysaccharide of *Porphyridium* sp (polysaccharide: 500 mg, CHMAC/NaOH: 1.2, total volume: 60 ml)

Ratio by weight CHMAC/polysaccharide	Nitrogen content[a] (%)	IR peak ratio 1476 cm^{-1}/983 cm^{-1}
36.50	2.80	0.91
16.40	2.70	0.90
13.68	3.00	0.93
11.72	2.60	0.92
10.24	3.00	0.94
9.10	2.80	0.93
8.20	2.80	0.71
7.40	1.6	0.45
6.32	0.8	0.41

[a] Kjeldahl method.

10,000 Hz. The carbonyl signal of glycine (176.03 ppm) was used as the external spectral reference.

2.5. FT-IR spectroscopy

As a result of substitution, the absorbance peak for C–N stretching vibration appears on the IR spectrum at 1476 cm^{-1}. This peak is absent in the native polysaccharide and in the product obtained from the blank reaction (with NaOH but without CHMAC). Absorbance readings starting from 1528 cm^{-1} (for the 1476 cm^{-1} peak) and from 800 cm^{-1} (for the 983 cm^{-1} peak) were used as the base lines for the measurement of the height of the peaks.

2.6. Rheology

Rheological studies were conducted in a controlled stress rheometer (Carri-Med CSL 50, TA Instrument, Surrey, England) operated in a cone–plate mode (cone angle 1°, diameter 60 mm). The frequency range examined was 0.01–30 Hz. The frequency sweeps were carried out within the linear viscoelastic region.

3. Results and discussion

3.1. Preparation and characterization of the quaternized polysaccharide

Introduction of cationic groups into the microalgal anionic polysaccharide of *Porphyridium* sp. was performed according to the general method described in the literature (Ebringerova et al., 1994). The proof for quaternization was obtained from IR and NMR spectra and from the nitrogen content of the products.

To activate the polysaccharide before the reaction, a strong aqueous solution of sodium hydroxide was used. Since sodium hydroxide also hydrolyzes CHMAC, an excess of CHMAC was usually added. Under these circumstances, it was important to determine the exact quantity of the two reagents required to give an optimal reaction.

In keeping with the strategy of Ebringerova et al. (1994), a series of experiments were conducted to determine the exact quantity of CHMAC and sodium hydroxide needed for an optimal reaction as well as the molar ratios of the reagents NaOH and CHMAC to a given weight of polysaccharide (Table 1). Fig. 1 shows that there is indeed a maximum ratio (1.2) of reagents that affords the highest degree of quaternization: the degree of substitution decreased at values above and below that ratio. Further experiments were thus performed with a molar ratio of CHMAC/NaOH of 1.2, while changing the amount of CHMAC per unit weight of polysaccharide (Table 1). As the amount of CHMAC increased per unit weight of polysaccharide, the extent of quarternization also increased. However, even when the amount of CHMAC was more than doubled, there was no further increase in the degree of substitution,

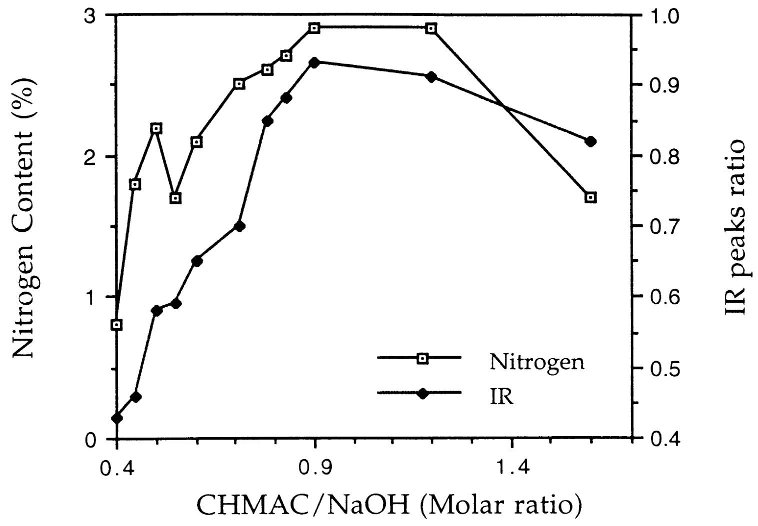

Fig. 1. The extent of quaternization expressed as a function of nitrogen content (wt%) and IR peak ratio as a function of the molar ratio of CHMAC/NaOH.

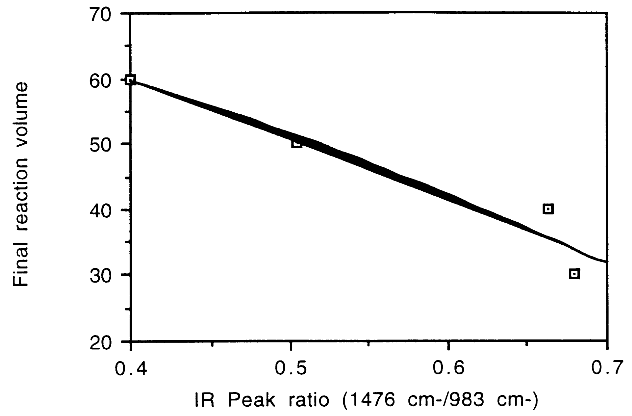

Fig. 2. Correlation between the final reaction volume and the IR peak ratio (1476 cm^{-1}/983 cm^{-1}).

possibly indicating the absence of sites still available for quaternization (Table 1, Entries 1 and 2). The optimum ratio of CHMAC/ polysaccharide was found to be between 9 and 10.

The degree of substitution on the polysaccharide as a result of quaternization could be determined in terms of the nitrogen content of the product. Increasing the amount of CHMAC resulted, as expected, in a higher nitrogen content. There was also a gradual increase in the IR peak ratio (1476 cm^{-1}/983 cm^{-1}) as the molar ratio of CHMAC/ NaOH increased, indicating an increase in substitution. As was previously shown by Ebringerova et al. (1994), there was rather good agreement between the content of nitrogen in the product and the ratio of the IR peaks at 1476 and 983 cm^{-1}. The highest nitrogen content (about 3%) was obtained when the molar ratio of CHMAC/NaOH was about 1.2.

When the effect of dilution of the reaction mixture on the degree of substitution was investigated, it was found that more substitution was obtained when the reaction was carried out in lower volumes of water, in a gel-like phase (Fig. 2).

Determination of the total sugar content of the product, the blank obtained after activation with sodium hydroxide and the native polysaccharide showed that there was no change in the total sugar content of the product and blank compared with that of the native polysaccharide; this finding indicates that there was no degradation of the polysaccharide during the quaternization reaction. Total sugar content for all the three polysaccharides (native, blank and product) was found to be about 85% on a dry weight basis, the remainder of the material being made up of sulfate, ash and protein.

3.2. NMR analysis

Quaternization was confirmed at first by recording ^{13}C NMR spectra of CHMAC, the hydrolyzate of the native polysaccharide, and that of the quaternized product. The

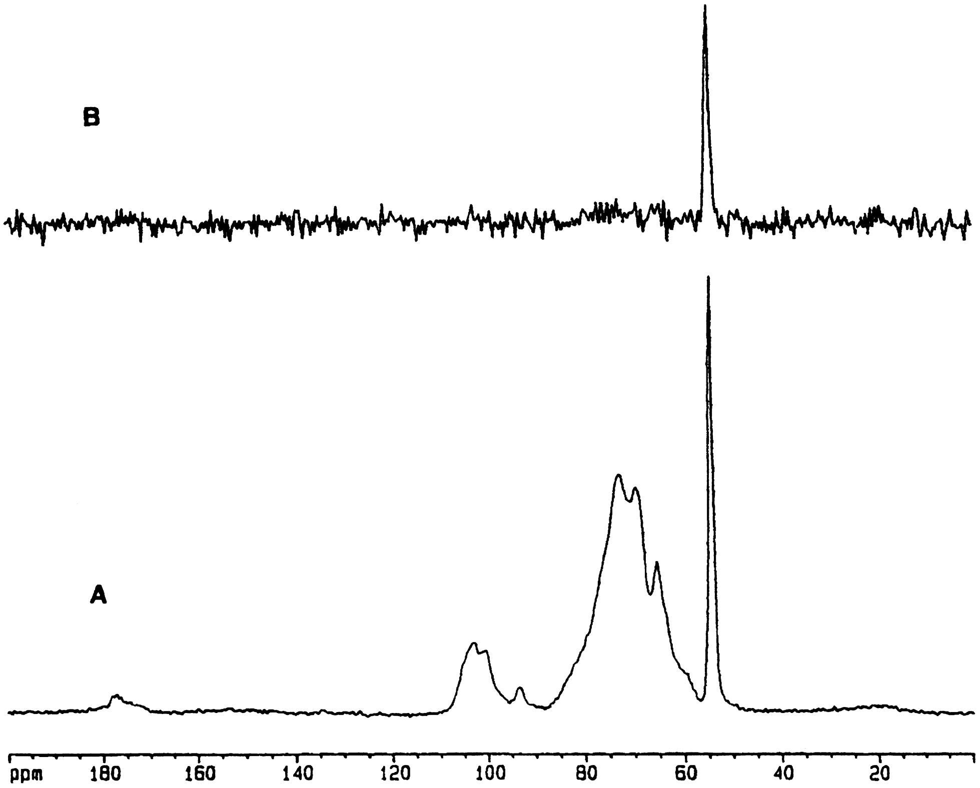

Fig. 3. Solid state CP-MAS ^{13}C NMR spectra of quaternized polysaccharide of *Porphyridium* sp. (A) Vacptppm spectrum; and (B) Vacptppmnqs spectrum with a 50 ms total delay except for that of quaternary carbon atoms.

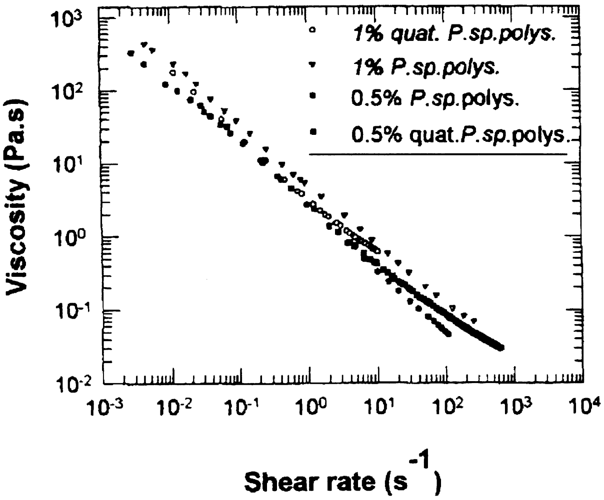

Fig. 4. Viscosity of aqueous preparations of native and quaternized polysaccharide of *Porphyridium* sp. at 25°C as a function of shear rate and sample concentration.

^{13}C NMR spectrum of the reagent showed a prominent peak at 56.8 ppm for the three methyl carbons attached to nitrogen. In the hydrolyzate of the modified product the same peak appeared around 54.9 ppm. The chemical shift of the carbon attached to hydroxyl group was 68 ppm in the case of the reagent, while the corresponding carbon in the modified product appeared at 68.1 ppm.

Solid-state CP-MAS ^{13}C NMR spectroscopy was used to compare polysaccharide samples with and without the attachment of cationic-$CHCH_2N^+(CH_3)_3$ groups. Similar spectra were observed in both cases with the exception of a relatively sharp high intensity signal at 54.50 ppm in the spectrum of the modified cationic polysaccharide (Fig. 3A). The chemical shift value of this new signal was consistent with that expected from a quaternary ammonium *N*-methyl carbon nucleus. A vacptppm (variable amplitude cross polarization with two pulse phase modulation proton decoupling) pulse sequence was used for data acquisition. A vacptppmnqs (nqs—non-quaternary carbon suppression) dipolar dephasing spectrum using a 50 ms delay period prior to data acquisition, showed only the 54.50 ppm signal, and confirmed its $N^+(CH_3)_3$ assignment (Fig. 3B). In this spectral editing experiment, the signals from less efficiency relaxed quaternary and methyl carbon nuclei remained after the relaxation delay, while zero or near-zero intensities were found for signals from more efficiency relaxed methylene and methine carbon nuclei.

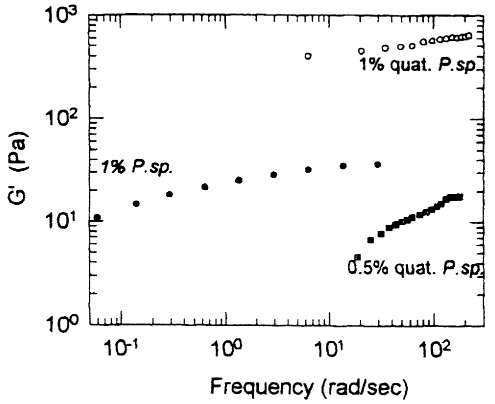

Fig. 5. Dependence of the elastic modulus G' on the frequency for the native (1.0 wt%) and the quaternized (0.5 and 1.0 wt%) polysaccharides of *Porphyridium* sp.

3.3. Rheological characterization

The relevance of studying the effect of chemical modifications on the functional properties of polysaccharides lies in the design of new characteristics. Physicochemical studies of modified polysaccharides may be used to explain how changes in the structure affect changes in the functionality and properties. In our case, one of the functions of the polysaccharide of *Porphyridium* sp. as shown by Geresh and Arad (1991), is its ability to modify the rheology of aqueous solutions. Thus, to compare the rheological behavior of the modified polymer with that of the native one, the steady shear viscosity was measured and dynamic small deformation oscillatory measurements were performed. The viscosity of the quaternized polysaccharide was found to be similar to that of the native polysaccharide at 0.5 and 1.0 wt% (Fig. 4). Small deformation mechanical studies of an aqueous preparation (1.0% (w/v)) of the quaternized polysaccharide revealed elastic behavior, indicative of a weak, gel-like network of highly associated biopolymer chain molecules (Fig. 5). The solid-like (elastic) response (G') to an imposed small deformation of the quaternized polysaccharide was higher than that of the unmodified biopolymer (Fig. 5). Thus, the chemical modification of the polysaccharide expressed itself in the macroproperties, as revealed by its solution rheology.

4. Conclusion

Optimal conditions for the quaternization of polysaccharides of the red microalga *Porphyridium* sp. are defined. The successful quaternization of the extracellular polysaccharide can be followed either by nitrogen analysis or by IR spectroscopy. The quaternization reaction occurs without degradation of the polysaccharide as found from sugar analysis. Although the viscosities of the native and modified polysaccharides were similar, the elasticity of the quaternized polysaccharide was higher than that of the native material. The quaternization of this type of biopolymers opens new possibilities for commercial applications. For example, for hair coloring, quaternary ammonium groups are required for electrostatic interactions with the keratin of the hair. It thus seems that such a quaternized polymer may find application as a naturally derived ingredient in cosmetic formulations.

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