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Note

Detection of cellulose in the cell wall of some red algae by ^{13}C NMR spectroscopy

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The polysaccharides constituting the cell wall of red algae may be grouped as skeletal and matrix components [1]. The skeletal glycans, such as cellulose, mannans, and xylans, account only for a small part of the total algal polysaccharides [2], which are composed mainly of the water-soluble sulphated galactans, agars, and carrageenans [1,3]. In contrast to the matrix materials, skeletal polysaccharides have been less extensively investigated. For example, cellulose, which is a linear homopolymer composed of β-(1 → 4)-linked D-glucopyranosyl residues, is insoluble in water and in the most common organic solvents [4]. Evidence for the presence of this polysaccharide in red algae was obtained by X-ray diffraction studies [5], cytochemical staining reactions [1], GC–MS analysis of derivatives of cell-wall fractions [6], and ^{1}H NMR spectroscopy of permethylated cell-wall material [7]. However, no chemical characterisation of unmodified algal cellulose in solution has so far been reported.

In this work, we describe a method for the detection of algal cellulose in solution by ^{13}C NMR spectroscopy, using lithium chloride–*N*,*N*-dimethylacetamide (LiCl–DMAc), which is a suitable nondegrading mixture for dissolving underivatised cellulose [8].

The ^{13}C NMR spectra of the material solubilised by LiCl–DMAc from the whole thalli of some selected red algae (*Gracilaria longa*, *Gracilaria dura*, and

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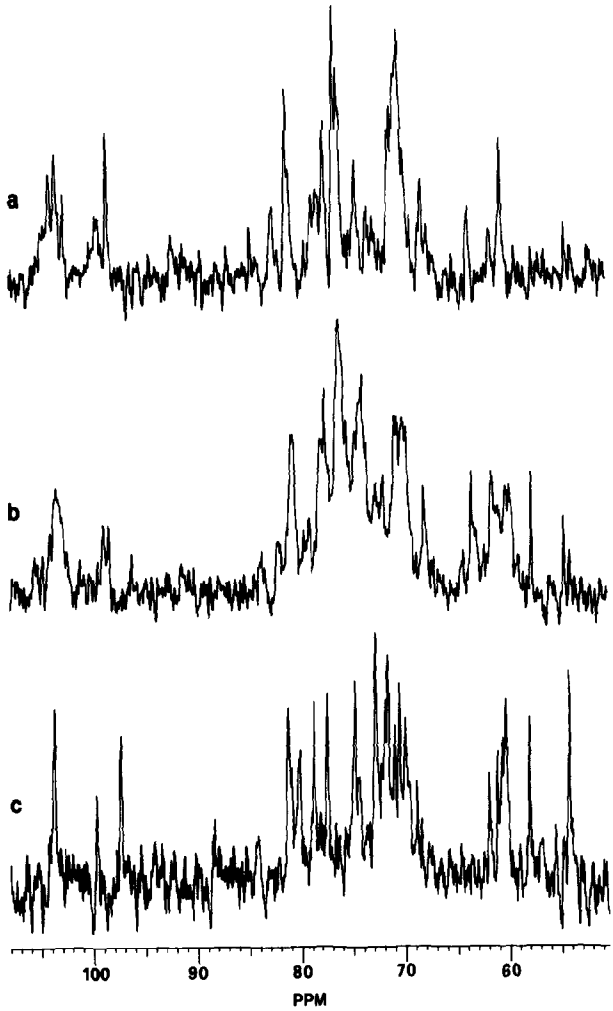

Fig. 1. ^{13}C NMR spectra of whole thalli of the red algae (a) *Gracilaria longa*, (b) *Gracilaria dura*, and (c) *Hypnea musciformis* in LiCl–DMAc.

Hypnea musciformis) (Fig. 1) had major signals similar to those observed in the ^{13}C NMR spectra of the corresponding purified agars or carrageenans in the same solvent (data not shown). This may be explained by the abundance of these galactans in the cell wall compared to the skeletal polysaccharides. Furthermore, the complex nature of the spectra, especially those obtained from the two species of *Gracilaria*, suggested that the treatment in hot LiCl–DMAc may cause some thermal degradation of the matrix polysaccharides.

The LiCl–DMAc ^{13}C NMR spectra of the algal residues after water extraction of the matrix galactans showed five major signals (103.5, 79.5, 78.4, 74.5, and 60.9 ppm) characteristic of β-(1 → 4)-linked D-glucopyranosyl residues [8], similar to

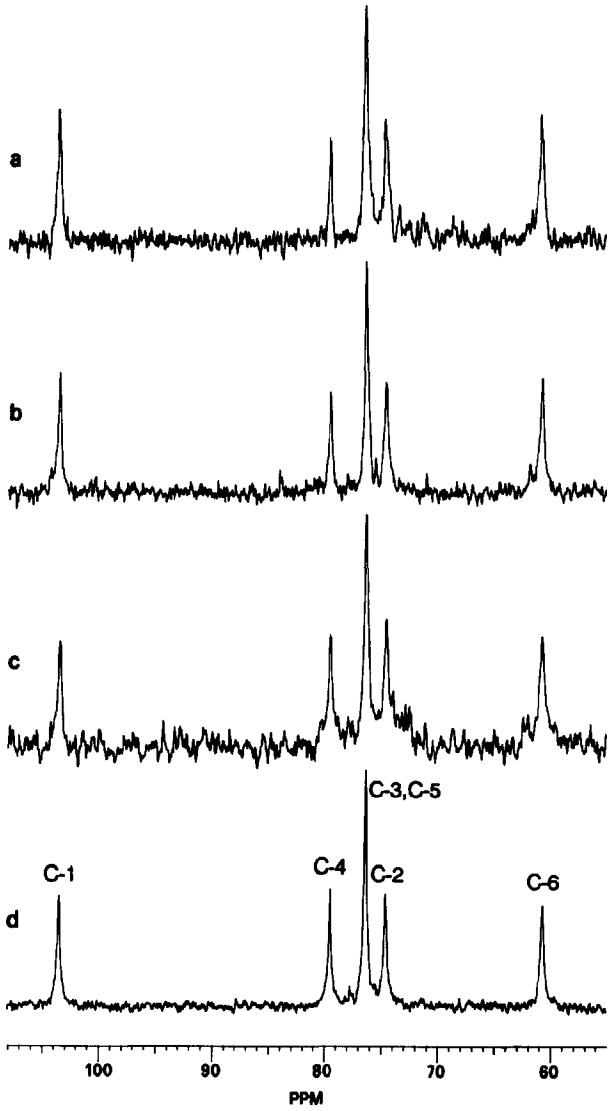

Fig. 2. ^{13}C NMR spectra of algal residues of (a) *Gracilaria longa*, (b) *Gracilaria dura*, and (c) *Hypnea musciformis*, and (d) commercial cellulose in LiCl–DMAc.

those of the commercial cellulose (Fig. 2). Furthermore, GC analysis revealed the presence of glucose, with traces of mannose, in the algal residues, thus confirming that cellulose was the preponderant LiCl–DMAc-soluble polymer left in the red algal thalli after extraction of sulphated galactans. This finding suggested that the unassigned minor signals in the ^{13}C NMR spectra of the algal residues might be due to a mannose-containing polysaccharide. Moreover, comparison of the ^{13}C NMR spectra of the algal residues with those of amylose and xylan, dissolved in

the same solvent, excluded the presence of α-linked glucans and β-linked xylans in these algal residues. ^{13}C NMR data were finally corroborated by FT-IR spectra both of the rhodophycean whole thalli, which showed the typical pattern of the corresponding water-soluble galactans [9], and of the algal residues, which exhibited a pattern closely related to that of commercial cellulose [10].

^{13}C NMR, FT-IR, and GC provide evidence that cellulose is the major skeletal component in the mature cell wall of the red algae *Gracilaria longa*, *Gracilaria dura*, and *Hypnea musciformis*. The NMR procedure described above, which provides a straightforward way for the detection of unmodified algal cellulose in solution, may be proposed as an innovative method to study the cell-wall polysaccharides, in particular the skeletal components, and clarify taxonomical affinities within the red algae.

1. Experimental

Samples (100–150 mg) of algal material (dried thalli, water-soluble extracts, and residues after water extraction [11]) obtained from the red algae *Gracilaria longa*, *Gracilaria dura*, and *Hypnea musciformis*; commercial cellulose and amylose (Aldrich); and (4-*O*-methyl-D-glucurono)-D-xylan [12] were treated with 6 mL of LiCl–DMAc (8.4% w/v) for 4 h at 120°C. All samples were clarified by centrifugation and supernatant solutions subjected to ^{13}C NMR spectroscopy.

^{13}C NMR unlocked experiments were performed overnight at 90°C on a Bruker AC 200 spectrometer, using 5-mm (o.d.) sample tubes. NMR data were acquired with 16k points, using a spectral width of 20 kHz and a 75° flip angle. Chemical shifts were referred to tetramethylsilane. FT-IR analysis of KBr pellets was performed with a Perkin–Elmer 1750 spectrometer. Sugar analysis of algal residues was performed by gas chromatography as previously described [13].

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