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Sequence analysis of the pyruvylated galactan sulfate-derived oligosaccharides by negative-ion electrospray tandem mass spectrometry

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1-Phenyl-3-methyl-5-pyrazolone (PubChem CID: 90474051)
Potassium bromide (PubChem CID: 253877)
Sodium hydroxide (PubChem CID: 14798)
Sulfuric acid (PubChem CID: 1118)
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ABSTRACT

Five sulfated oligosaccharide fragments, F1–F5, were prepared from a pyruvylated galactan sulfate from the green alga *Codium divaricatum*, by partial depolymerization using mild acid hydrolysis and purification with gel-permeation chromatography. Negative-ion electrospray tandem mass spectrometry with collision-induced dissociation (ES-CID-MS/MS) is attempted for sequence determination of the sulfated oligosaccharides. The sequence of F1 with homogeneous disaccharide composition was first characterized to be Gal*p*-(4SO_4)-(1 → 3)-Gal*p* by detailed nuclear magnetic resonance spectroscopic analyses. The fragmentation pattern of F1 in the product ion spectra was established on the basis of negative-ion ES-CID MS/MS, which was then applied to sequence analysis of other sulfated oligosaccharides. The sequences of F2 and F3 were deduced to be Gal*p*-(4SO_4)-(1 → 3)-Gal*p*-(1 → 3)-Gal*p*-(1 → 3)-Gal*p* and 3,4-*O*-(1-carboxyethylidene)-Gal*p*-(6SO_4)-(1 → 3)-Gal*p*, respectively. The sequences of major fragments in F4 and F5 were also deduced. The investigation demonstrated that negative-ion ES-CID-MS/MS was an efficient method for the sequence analysis of the pyruvylated galactan sulfate-derived oligosaccharides which revealed the patterns of substitution and glycosidic linkages. The pyruvylated galactan sulfate-derived oligosaccharides were novel sulfated oligosaccharides different from other algal polysaccharide-derived oligosaccharides.

1. Introduction

Pyruvylated galactan sulfates are often found in red algal polysaccharides, which generally contain 3-substituted 4,6-*O*-(1-carboxyethylidene)-D-galactopyranose residues [1–3]. Novel pyruvylated galactan sulfates with different structural characterizations have been also found from marine green seaweeds recently. These pyruvylated galactan sulfates consist of (1 → 3)-β-D-galactopyranose residues connected by (1 → 6) linkages, sulfate groups are mainly at C-4 and in minor amounts at C-6 of the β-D-galactose residues, pyruvate is forming mainly five-membered ketals with C-3 and C-4 of nonreducing terminal galactose residues, minor part of pyruvate forms six-membered cyclic ketals with C-4 and C-6 [4–6]. The pyruvylated galactan sulfates exhibit various activities and hold a great potential

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application in biology and pharmacology [7–9].

Polyanionic oligosaccharides such as those derived from glycosaminoglycans have attracted considerable interests recently because of the increased awareness of their functional properties. The functional properties of pyruvylated galactan sulfates and their oligosaccharide fragments are intimately linked to their composition and sequences of the sugar residues. Methods for identification of oligosaccharides derived from the pyruvylated galactan sulfates, and their sequence determination, are important for better understanding the structure-activity relationships of pyruvylated galactan sulfates. Nuclear magnetic resonance (NMR) spectroscopy have been the primary method for structural analysis of the sulfated polysaccharide and derived oligosaccharides [4,6,10]. But, NMR requires relatively large amounts of materials, while mass spectrometric techniques have provided alternatives for composition analysis of sulfated polysaccharide by molecular mass determination [11,12]. Recently, sequence analysis of oligosaccharides by mass spectrometry has been of intense interest [13–15]. Negative-ion electrospray tandem mass spectrometry with collision-induced dissociation (ES-CID-MS/MS) in the negative-ion mode is recognized as a powerful and highly sensitive analytical method for the characterization of sulfated oligosaccharides [16–18]. Unique fragmentation can arise at certain monosaccharide residues with specific linkages under CID MS/MS conditions, and these provide important information on sequence and linkages. However, sequence analysis of the pyruvylated galactan sulfate-derived oligosaccharides by ES-CID MS/MS has not been reported.

In the present paper, the pyruvylated galactan sulfate from the green alga *Codium divaricatum* was used for preparation of oligosaccharides. The pyruvylated galactan sulfate contains (1 → 3)-β-D-galactopyranose residues without (1 → 6) linkages, branches are attached to the main chain at C-4 not C-6 positions, sulfate groups are at C-4 of (1 → 3)-linked β-D-galactopyranose and C-6 of non-reducing terminal galactose residues, pyruvate forms cyclic ketals with C-3 and C-4 of the non-reducing terminal galactose residues [10]. After mild acid hydrolysis of the pyruvylated galactan sulfate and fractionation with gel-permeation chromatography, five sulfated oligosaccharide fragments were obtained. The sequence of the pyruvylated galactan sulfate-derived oligosaccharides were determined by negative-ion ES-CID MS/MS.

2. Results and discussion

2.1. Preparation of the pyruvylated galactan sulfate-derived oligosaccharides

The pyruvylated galactan sulfate from the green alga *C. divaricatum* was partially depolymerized by mild acid hydrolysis. Reversed-phase HPLC analysis demonstrated that the oligomeric mixture mainly consisted of galactose. The sulfate ester content of the oligomeric mixture was about 23.4%. IR spectrum of the oligomeric mixture (Fig. 1A) was similar to that of the parent pyruvylated galactan sulfate and showed the similar absorption intensity of sulfate groups at 843 and 1254 cm^{-1} [10]. The signal at 927 cm^{-1} was due to the absorbance of β-D-galactopyranose and the band at 1604 cm^{-1} was characteristic of the pyruvic acid. The bands at 1648 and 1453 cm^{-1} would correspond to the carboxylic group of carboxylate groups. The results indicated that the method of the acid hydrolysis used in the experiment could effectively break glycosidic linkages in the pyruvylated galactan sulfate without destroying the basic chemical structure of the sulfated polysaccharide.

The oligomeric mixture was isolated by gel-filtration chromatography on the Bio-Gel P-4 column (Fig. 1B). Fractions were pooled according to phenol-sulfuric acid detection curve and further purified on the Bio-Gel P-4 column. F0 was a monosaccharide. Finally, five oligosaccharide fractions (F1–F5) were obtained. Reversed-phase HPLC analysis demonstrated that F1–F5 were composed of galactose, and the absolute configuration of the galactose was D-configuration. The purity of the oligosaccharides was determined by the negative-ion ES-MS spectrum. The molecular mass information of the oligosaccharides can be obtained from the dominant $[M\text{-}nH]^{n-}$ ions in the negative-ion ES-MS spectrum. The oligosaccharide F1 was deduced to be a predominant monosulfated galacto-disaccharide (Fig. 2a), the oligosaccharide F2 was a monosulfated galacto-tetrasaccharide (Fig. 2b), and the oligosaccharide F3 was a monosulfated and pyruvylated galacto-disaccharide (Fig. 2c). In the fraction F4, major peaks in the high mass ranged from three to six galactose units containing two sulfates and one pyruvate at m/z 366–609 for $[Gal_{3-6}(SO_4)_2Pyr\text{-}2H]^{2-}$ (Fig. 2d). For the fraction F5, major peaks in the high mass ranged from four to eight galactose units at m/z 324–644 for $[Gal_{4-8}(SO_4)_3Pyr\text{-}3H]^{3-}$ and $[Gal_6(SO_4)_2Pyr_2\text{-}2H]^{2-}$ (Fig. 2e).

2.2. NMR spectroscopy of the oligosaccharide F1

In the anomeric region, ^1H NMR spectrum of F1 (Fig. 3a) showed major proton signals at 5.32, 4.71 and 4.66 ppm. The signal at 5.32 ppm was assigned to α-galactopyranose residue and the other anomeric proton signals were assigned to β-galactopyranose residues [19]. The proton signals located at 3.59–4.35 ppm were assigned to H2–H6 of the sugar residues. In the ^{13}C NMR spectrum of F1 (Fig. 3b), the main anomeric carbon signals appeared at 105.8, 97.7 and 93.7 ppm. The signal at 105.8 ppm was deduced to be C-1 of non-reducing galactopyranose units. The signals at 97.7 and 93.7 ppm were attributed to C-1 of reducing galactopyranose units

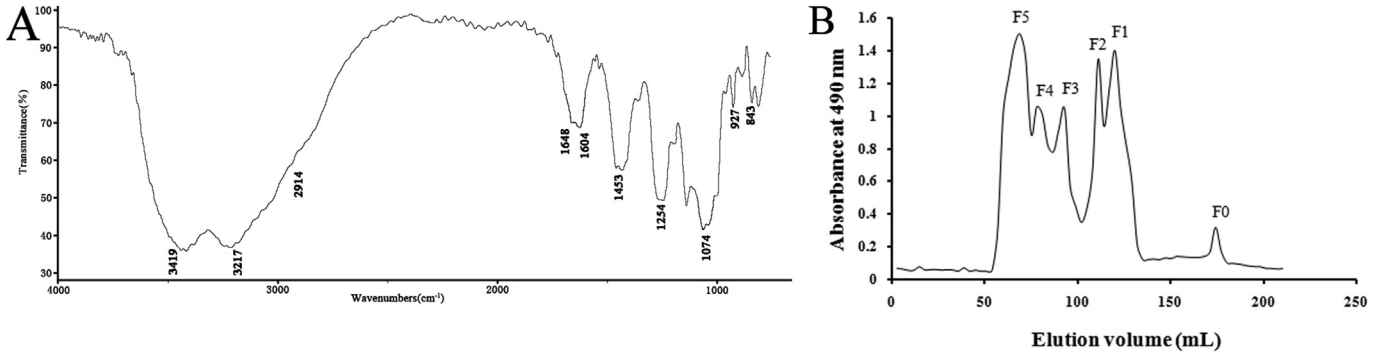

Fig. 1. FT-IR spectrum and isolation of the oligomeric mixture obtained from mild acid hydrolysis of the pyruvylated galactan sulfate. (**A**) FT-IR spectrum; (**B**) isolation of the oligomeric mixture on a Bio-Gel P-4 column.