Short Communication

Gas chromatography–mass spectrometry method for the determination of the reducing end of oligo- and polysaccharides

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ABSTRACT

A gas chromatography–mass spectrometry (GC–MS) method for determining oligo- and polysaccharide reducing end-groups has been developed. This method, which is based on the monitoring of ions unique to the fragmentation of deuterated alditol acetates in mixtures also containing sugar acetates, can be used to identify the end-group and the degree of polymerization (DP). For the first time, GC–MS can be used in these types of analyses for polysaccharides of DP 100 or greater.

INTRODUCTION

An exopolysaccharide (EPS)-degrading enzyme isolated in our laboratory has the unusual property of not degrading the polymer to its basic subunit but to a lower-molecular-mass polysaccharide. In order to determine the specificity of this enzyme, and to confirm that it is indeed a polysaccharide depolymerase, it was necessary to determine the release of reducing end-groups in the depolymerization. End-groups have been determined by enzymatic, chromatographic and NMR methods, usually as a means of determining the degree of polymerization (DP) of polysaccharides. Use of enzymes is limited to those cases for which appropriate enzymes are available [1,2]. The chromatographic methods ultimately involve gas chromatographic (GC) separation of the alditol acetate (derived from $NaBH_4$ reduction of the reducing end) from a derivative, such as the aldononitrile acetate, of the internal sugars released on hydrolysis [3]. A GC–mass spectrometry (MS) method has been described that determines end-group by the sequence of reactions: sodium borodeuteride reduction, hydrolysis and sodium borohydride reduction [4]; the end-group/internal sugar ratio is then determined by the ratio of the deuterated ion, m/z 146, to the corresponding undeuterated ion, m/z 145, and retention time is used to determine the sugar. An NMR method [5] measures $NaBD_4$-reduced sugars before and after hydrolysis to get this ratio.

These analyses are often inaccurate when analyzing heteropolysaccharides, particularly when the relative concentration of end-groups to in-chain sugars is very small, which was the case for the polysaccharide we wished to analyze. The product of the

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enzymatic treatment was still a relatively high-molecular-mass polymer (*ca.* 200 000), therefore it was necessary to characterize an end-group that was a minor fraction (*ca.* 1%) of the total mixture. The method described in ref. 3 could not be applied because it was impossible to resolve and observe the alditol peaks in the presence of the large amounts of glucose and galactose aldononitrile acetates; the small amount of alditols also precluded using a method that has been described for separating alditols by ion exchange prior to analysis [6]. The GC–MS method presented similar problems in that the intensity of the ion used to determine end groups, *i.e.*, 146, has a major contribution from the isotope peak of the ion at m/z 145. The method [4] is considered to be only reliable for oligosaccharides and therefore not suitable for our problem. For similar reasons, the NMR method was not applicable to our problem.

To overcome the drawbacks of the methods described, we have developed an end-group analysis that is based on GC–MS selective ion monitoring, but in contrast to the previously described GC–MS method, is not restricted by sugar composition and can be applied to polysaccharides with DP of *ca.* 100 or greater.

EXPERIMENTAL[a]

Materials

The isolation and characterization of *Pseudomonas marginalis* strain HT041B EPS has been described [7]. The EPS was depolymerized with an enzyme, to be described in a later publication, isolated from the same organism. The enzymatic depolymerization was monitored by measuring the change in viscosity of a solution of the EPS in aqueous 20 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffer, pH 7.2.

Analysis

Polysaccharide solutions (*ca.* 1 mg/ml) were initially reduced with sodium borodeuteride (2 h at room temperature), neutralized with cation-exchange resin (Dowex 50W-X4) and then hydrolyzed

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[a] Mention of brand or firm names does not constitute an endorsement by the US Department of Agriculture over others of a similar nature not mentioned.

for 1.5 h at 100°C with 0.5 M H_2SO_4 and then neutralized with $BaCO_3$ as previously described [7]. The hydrolysate was acetylated by reacting with pyridine–acetic anhydride (1:1, v/v) at 70°C for 30 min. The resultant deuterated alditol acetates were analyzed on a Hewlett-Packard 5995B GC–MS system, fitted with an SP-2330 (Supelco) capillary column (15 m × 0.25 mm I.D.), temperature programming the column for 150 to 250°C at 4°C/min. The ions at m/z 188 and m/z 218 in the mass spectrum were scanned to determine the presence of deuterated alditols.

RESULTS AND DISCUSSION

The ions m/z 188 and m/z 218 are of relatively low intensity in the mass spectra of the deuterated alditol acetates (Table I) however they are not observed in the spectra of the corresponding aldose acetates. This feature makes it possible to detect very low concentrations of alditols in the presence of many fold excess of aldose acetates which is not possible with published methods, including the previously reported GC–MS method, for the reasons discussed in the introduction.

We have successfully used this method to determine the specificity of the enzymatic hydrolysis of an EPS, isolated from *P. marginalis* strain HT041B, which contains alternating glucose and galactose residues. The single-ion, co-chromatography GC–MS (Fig. 1a) unequivocally shows that enzymatic hydrolysis is at C-1 of glucose; *i.e.*, only deuterated glucitol acetate is present in the mixture after the reduction and derivatization of the hydrolysate as determined by the presence of m/z 188 and 218 at the retention time of glucitol acetate (end-group

TABLE I

ION INTENSITY (RELATIVE TO THE BASE PEAK = 100) OF DIAGNOSTIC IONS FOR DEUTERATED ALDITOL ACETATES

Deuterated alditol acetate	Relative ion intensity	
	m/z 188	m/z 218
Rhamnitol	12	8
Arabinitol	19	10
Glucitol	13	9
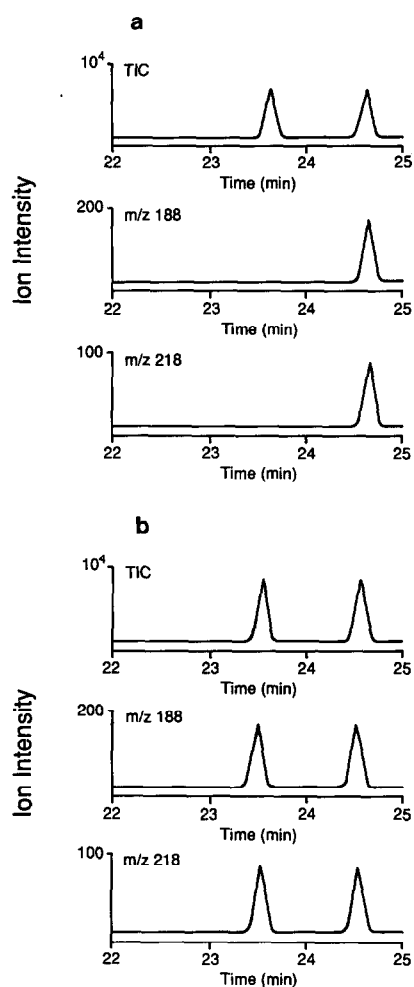
Fig. 1. GC–MS of (a) enzymatically and (b) chemically depolymerized *P. marginalis* EPS. Glucitol acetate retention time = 23.7 min, galactitol retention time = 24.6 min. TIC = Total ion current.

analysis for a partial chemical hydrolysis which yield, ultimately, deuterated glucitol and galactitol acetates is shown in Fig. 1b).

To determine the applicability of this method for determining molecular mass, a dextran molecular mass standard, (M_r = 20 000) was subjected to the analysis using a methyl silicone column for GC so that the separation of the sugar acetate anomers was eliminated, *i.e.*, the amount of internal sugar was determined by the measurement of only one peak (although one of the furanose anomers separated, it represents less than 1% of total sugar acetate mixture). The ratio of internal sugar to end-group was determined to be 127 which corresponds to a number-average molecular mass (M_n) of 20 592.

In conclusion, this relatively simple method can be used to characterize the reducing end of a polysaccharide without interference from the sugars within the polymer chain. The method is only limited in the determination of degree of polymerization by how accurately the deuterated alditol acetate/aldoze acetate ratio can be measured. For all the common sugars found in polysaccharides, identity of the alditol can be made unambiguously, on the basis of chromatographic retention time.

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