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Application of high-performance anion-exchange chromatography with pulsed amperometric detection and statistical analysis to study oligosaccharide distributions – a complementary method to investigate the structure and some properties of alginates

Simon Ballance [a,*], Synnøve Holtan [a], Olav Andreas Aarstad [a], Pawel Sikorski [b], Gudmund Skjåk-Bræk [a], Bjørn E. Christensen [a]

[a] *Norwegian Biopolymer Laboratory (NOBIPOL), Department of Biotechnology, Norwegian University of Science and Technology (NTNU), Sem Saelands vei 6/8, N-7491 Trondheim, Norway*
[b] *NOBIPOL, Department of Physics, Norwegian University of Science and Technology (NTNU), N-7491 Trondheim, Norway*

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Abstract

Alginates comprised of essentially alternating units of mannuronic (M) acid-guluronic (G) acid (MG-alginate), and G-blocks isolated from a seaweed where subjected to partial acid hydrolysis at pH 3.5 The chain-length distribution of oligosaccharides in the hydrolysate were investigated by statistical analysis after their separation with high-performance anion-exchange chromatography and pulsed amperometric detection (HPAEC–PAD). Simulated depolymerisation of the MG-alginate provided an estimate of the ratio between two acid hydrolysis rate constants ($p = 8.3 \pm 1$) and the average distribution of the MM linkages in the original sample of polysaccharide chains. In conclusion, we found HPAEC–PAD together with statistical analysis was a useful method to investigate the fine structure and some properties of binary polysaccharides.

Keywords: Acid hydrolysis; Carbohydrate; Chain-length distribution; Degree of polymerisation

1. Introduction

Statistical analysis of the chain-length distribution of oligosaccharides obtained from the partial depolymerisation of polysaccharides can yield information on the mechanism of their formation, and the structure of the polysaccharide they originated from [1]. Our current understanding of the complex three-dimensional structure of amylopectin in starch granules is supported by analysis of the chain-length distribution of its oligosaccharides generated by enzymatic hydrolysis [2]. A similar approach, but using acid rather than enzymatic hydrolysis, also confirmed that the glycosidic linkages in cellulose were most probably identical [3], and that alginate contained some block sequences comprised of alternating mannuronic (M) and guluronic (G) acid [4]. The conclusions of this latter study were reached by using a combination of gel-filtration chromatography and a kinetic theory to analyse the products of the partial mild acid hydrolysis of a fragment of alginate. Oligosaccharides were fractionated according to their chain-length and the yield of each fraction used in computer simulation. This enabled the statistical determination of the nearest neighbour frequencies in the starting polymer and ultimately a description of its sequential structure [4]. The success of this approach contributed to the understanding that the depolymerisation of natural alginates

* Corresponding author. Tel.: +47 73598225; fax: +47 73593337.
E-mail address: simon.ballance@biotech.ntnu.no (S. Ballance).

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in weakly acidic media was due largely to intramolecular catalysis of glycosidic cleavage by the carboxyl group in the respective aglycone units [5].

In a more recent study [6], we showed that high-performance anion-exchange chromatography (HPAEC) on an analytical column of IonPac AS4A pellicular resin, combined with pulsed amperometric detection, was a useful and sensitive tool for the quantitative chain-length analysis of a partially degraded de-acetylated bacterial (1 → 4)-β-D-mannuronan, referred to as mannuronan hereon (Fig. 1A). Statistical analysis of this distribution confirmed its structure and revealed that hydrolysis of the glycosidic linkage in the polymer chain occurred at random positions. This implied that the process of acid hydrolysis of mannuronan under mildly acidic conditions could be adequately described by one rate constant. It proved to be a useful complementary method to NMR spectroscopy [6].

The advantage of HPAEC using pellicular resins over other methods, including other forms of chromatography (such as that used by Larsen et al. [4]), is its superior ability to achieve baseline separation of defined populations of oligosaccharides from polydisperse preparations. Oligosaccharides, with a chain-length of up to 30–40 monomer units, have previously been routinely separated without extensive optimisation of the chromatographic conditions [7]. Even trace constituents are captured in the analysis. It is therefore not a surprise that numerous other studies have used this technique to assess the chain-length distribution of various preparations of polysaccharide hydrolysates and then used this information to comment on the properties of the original polymer (see review by Zhang and Lee [7]). The most intensively studied of these originate from starch (see review by Wong and Jane [8]).

Despite the potential, to our knowledge there have been few instances where HPAEC with pulsed amperometric detection (PAD) and statistical analysis have been applied to study the chain-length distribution of oligosaccharides originating from glycuronans, especially binary ones, such as most alginates. A major reason for this is often the difficulty in obtaining appropriate oligosaccharide standards of required purity. These are needed firstly to calibrate the molar response of the PAD as a function of chain-length and secondly, in the case of heteropolysaccharides, they are needed to assist in the assignment of peaks in the resulting chromatograms. However, these obstacles are not insurmountable. In an investigation of the distribution of methyl esters in poly-galacturonic acid, a lack of oligosaccharide standards was circumvented by indirectly calculating the PAD response, via UV detection of oligosaccharides tagged with a chromaphore at their reducing-end [9]. Other workers have used post-column enzyme reactors to convert eluting oligosaccharides into equivalent amounts of easily quantifiable monomers [10,11].

In this study, we have utilised and extended the previous application of HPAEC–PAD and statistical analysis that investigated the chain-length distribution of oligosaccharides from mannuronan [6]. We now analyse two different alginates whose basic properties have both been prior investigated by NMR. The first is an engineered linear binary hetropolysaccharide with a predominantly alternating [4)-β-D-Man*p*A-(1 → 4)-α-L-Gul*p*A-(1→]$_n$ structure referred to as MG-alginate hereon (Fig. 1B). This alginate was engi-

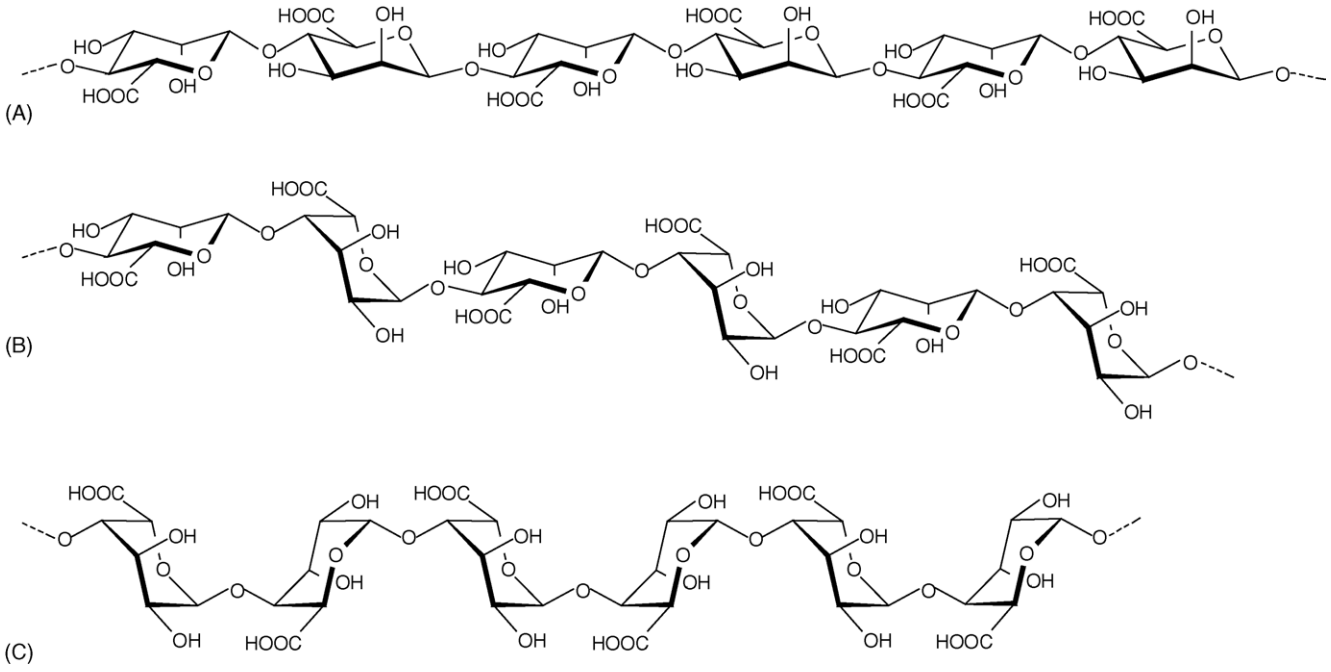

Fig. 1. Conformational representation of segments of mannuronan (A), MG-alginate (B) and G-blocks (C).