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Note

Isolation and quantification of alginate-derived oligouronic acids by fluorophore-assisted carbohydrate electrophoresis

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Abstract

Alginate-derived oligouronic acids were analyzed by fluorophore-assisted carbohydrate electrophoresis (FACE). Oligoguluronic acids and oligomannuronic acids were labeled with 8-aminonaphthalene-1,3,6-trisulfonic acid (ANTS) and were separated by polyacrylamide gel electrophoresis. The mobilities of oligoguluronic acids and oligomannuronic acids were much higher than those of maltooligosaccharides, and the best resolution of oligouronic acids having DPs lower than 10 was achieved on 40% polyacrylamide gel. Over the range 0.25 to 4 nmol, the fluorescence intensity of an ANTS-labeled oligouronic acid band could be correlated linearly to the quantity of the oligouronic acid. The distribution of DPs of polyguluronic acid and polymannuronic acid also were estimated by using FACE.

Keywords: Fluorophore-assisted carbohydrate electrophoresis; Oligouronic acid; Alginic acid

One of our research programs comprises the elucidation of the substrate specificity of alginate lyase originating from different sources. Since alginates are heterogeneous acidic polysaccharides composed of α-L-guluronic acid (GulA) and β-D-mannuronic acid (ManA) residues [1], various kinds of oligoalginates are produced by the enzymatic degradation of the alginates. For the separation of oligoalginates, a wide variety of techniques has been used, e.g. paper chromatography [2–4], urea-polyacrylamide gel electrophoresis [5–7], ion-pair reversed-phase HPLC [8,9], and anion-exchange HPLC [10]. However, these techniques are not suitable for the separation of oligoalginates with a high degree of polymerization (DP) because of the low resolution and low sensitivity. Therefore, we investigated the possibility of developing a better method for separation and quantification of oligoalginates.

In earlier reports, Jackson described the separation of neutral oligosaccharides with a high DP by fluorophore-assisted carbohydrate electrophoresis (FACE) [11,12], also called polyacrylamide gel electrophoresis of fluorophore-labeled saccharides (PAGEFS) [13]. Moreover, a similar approach involving the use of

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FACE was developed for the structural analysis of acidic polysaccharides such as heparin [14]. So far, studies on the application of FACE to oligoalginates having a high DP are not available. For this reason, we decided to investigate the FACE methodology for the analysis of oligoalginates from low to high DP. This paper describes the separation and quantification of oligoalginates by improved FACE, and it turned out that the method is applicable for the analysis of oligoalginates having a wide DP, such as DP 2–20 or even higher.

First, three kinds of gels, which had different acrylamide concentrations, were tested for the separation of the fluorophore-labeled saccharides. Acid hydrolysates of poly(GulA) and poly(ManA) were labeled with 8-aminonaphthalene-1,3,6-trisulfonic acid (ANTS), and loaded on the gels. Authentic oligouronic acids, purified by gel filtration, were also used for the identification of saccharide bands in the acid hydrolysates.

Fig. 1 shows the effect of polyacrylamide concentration on the electrophoretic mobilities of ANTS-labeled oligouronic acids. Jackson [11,12] reported that a 20–40% acrylamide gradient gel was effective in separating ANTS-labeled neutral oligosaccharides. However, the mobilities of oligo(GulA) and oligo(ManA) were much higher than those of the maltooligosaccharides, and GulA and ManA migrated close to the band of unreacted ANTS on a 20–40% polyacrylamide gradient gel (Fig. 1a). The resolution of the individual oligouronic acids having DPs lower than 10 increased with the concentration of acrylamide, but both di(GulA) and di(ManA) gave a minor band having a mobility slightly greater than the major band on a 40% acrylamide isocratic gel (the position is indicated by an arrow in Fig. 1c). The minor bands had the same color of fluorescence as the major bands had, and the percentage of the fluorescence intensity of these minor bands related to di(GulA) and di(ManA) were 1.5% and 3.5%, respectively. When the volume of the reaction solution and the ratios of reagents (ANTS and sodium cyanoborohydride) to the saccharides were varied, the percentage of the minor bands did not change. These results suggest that the minor bands were contaminants in the diuronic acids.

The electrophoretic mobilities of oligo(GulA) were slightly greater than those of oligo(ManA) having the same DP (DP $\geqslant$ 6) on a 40% acrylamide isocratic gel. However, the mobilities of GulA and low-molecular-mass oligo(GulA) (DP $\leqslant$ 5) were identical to those of ManA and low-molecular-mass oligo(ManA) (Fig. 1c). These results suggest that labeling with ANTS causes a large change in the total negative charge-to-

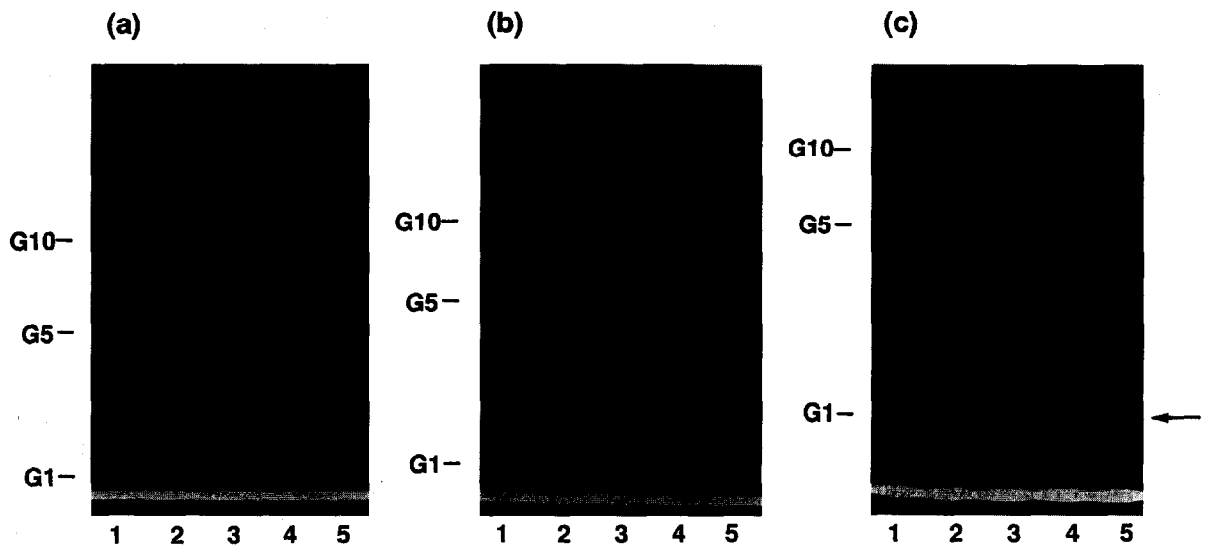

Fig. 1. Effect of polyacrylamide concentration on the electrophoretic mobilities of ANTS-labeled oligouronic acids. Oligoguluronic acids and oligomannuronic acids were labeled with ANTS, and were separated by electrophoresis on the following types of polyacrylamide gels: (a) 20–40% gradient acrylamide gel; (b) 30–40% gradient acrylamide gel; (c) 40% isocratic acrylamide gel. Lane 1, ladder marker of maltooligosaccharides; lane 2, acid hydrolysate of poly(GulA); lane 3, authentic GulA to nona(GulA) from bottom to top; lane 4, acid hydrolysate of poly(ManA); lane 5, authentic ManA to hepta(ManA) from bottom to top; G1, glucose; G5, maltopentaose; G10, maltodecaose. The arrow indicated the position of minor bands.

mass ratio for the smaller oligosaccharides. As oligouronic acids with a broad range of DPs could be resolved on a 30–40% acrylamide gradient gel, this gel was used in further studies.

The fluorescence intensity of the band of ANTS-labeled penta(GulA) was measured by using a BioImage analyzer. Various quantities of penta(GulA) (1.25–20 nmol), each containing maltopentaose (5 nmol) as an internal standard, were labeled with ANTS under standard conditions, and separated on a 30–40% acrylamide gradient gel. Fig. 2 shows the relationship between the intensity and quantity of penta(GulA). Over the range 0.25 to 4 nmol per band, a linear response was obtained and the relation gave a calibration curve; $\log y = 0.68 \log x - 0.62$, where x and y are the quantity of penta(GulA) and the intensity, respectively. As the sensitivity of detection of the labeled saccharide depends on the performance of the imaging system, it may be possible to increase the sensitivity by using low-noise integrating charge-coupled device-based imaging systems.

In Fig. 3 a distribution of DPs of poly(GulA) and poly(ManA) is shown. PAGE of ANTS-labeled poly(GulA) and poly(ManA) indicates that the DPs of poly(GulA) and poly(ManA) were in the range 4 to approx. 30 and in the range 7 to approx. 30, respectively. These results are in good agreement with the results reported by Haug et al. [1]. It is well known that the strength of calcium gels of alginic acids depends not only on the ManA/GulA ratio but also on the length of the homopolymeric block structures. So far, the average length of poly(GulA) has been estimated by measuring the total amount of carbohydrate and the reducing power. In contrast to such a method, using FACE the distribution of DPs can be estimated more exactly.

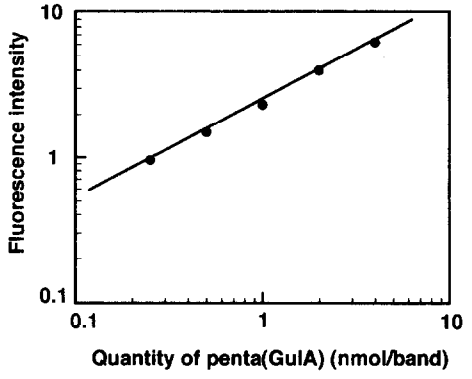

Fig. 2. Relationship between quantity of ANTS-labeled penta(GulA) and fluorescence intensity of its band measured by using the CCD-based imaging system. The double logarithmic plot was used to encompass conveniently all of the data.

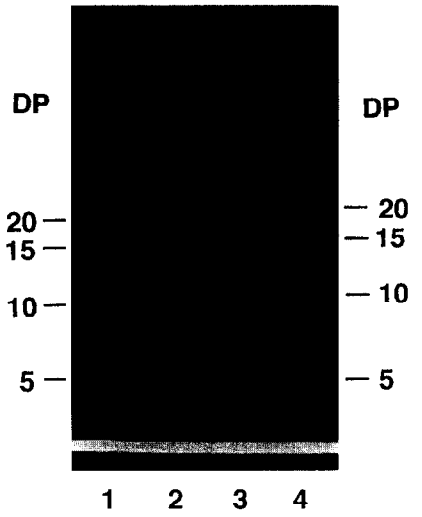

Fig. 3. A distribution of DPs of poly(GulA) and poly(ManA) analyzed by FACE on a 30–40% acrylamide gradient gel. Lane 1, authentic GulA to nona(GulA) from bottom to top; lane 2, poly(GulA); lane 3, authentic ManA to hepta(ManA) from bottom to top; lane 4, poly(ManA).

In conclusion, we achieved the separation of high oligoalginates with high resolution and sensitivity by using the FACE method. The method is more simple and rapid than the chromatographic and electrophoretic methods used so far. Moreover, FACE became a very useful method since it enables the separation, determination of DP and quantification of oligoalginates simultaneously. A study into the substrate specificity of alginate lyase toward high oligoalginates is now in progress in our laboratory.

1. Experimental

Saccharides.—Poly(GulA) and poly(ManA) were prepared from sodium alginate (Duck Algin 350-M; ManA/GulA ratio 0.94, Kibun Food Chemifa, Tokyo) by partial acid hydrolysis [1]. Circular dichroism analysis [15] established that poly(GulA) contained 79% GulA and poly(ManA) contained 92% ManA. Authentic GulA and oligo(GulA) (DP 2–9) were prepared from poly(GulA) as described previously [16]. Authentic ManA and oligo(ManA) (DP 2–7) were prepared from poly(ManA) by the same procedure. A ladder marker of maltooligosaccharides was prepared by partial acid hydrolysis of potato

amylose (type III, Sigma, MO) as described by John et al. [17].

Fluorescent labeling of saccharides.—Oligosaccharides were labeled with 8-aminonaphthalene-1,3,6-trisulfonic acid (ANTS, Molecular Probes, OR). Labeling of saccharides [each 2 nmol of authentic saccharides, 100 μg of acid hydrolysate, or each 150 μg of poly(GulA) and poly(ManA)] was carried out by the standard procedure described by Jackson [12], without any modification. After labeling, the reaction mixture was concentrated in a centrifugal vacuum evaporator, and the residue was dissolved in 50 μL aq. 20% glycerol.

Electrophoresis of ANTS-labeled saccharides.—ANTS-labeled saccharides were separated by polyacrylamide gel electrophoresis using a Tris-glycine/Tris-HCl discontinuous buffer system as described by Laemmli [18] with a slight modification [11,12]. A stock solution containing 51.91% acrylamide and 1.42% methylenebisacrylamide was used for gel preparation. Linear gradient 20–40%, linear gradient 30–40%, and isocratic 40% acrylamide resolving gels (95 mm length) were poured into glass plates (140 mm wide) using 1.0 mm spacers. Stacking gels (5% acrylamide, 15 mm length) were cast on top of the resolving gels. After the samples (10 μL) had been loaded on the gels, the electrophoresis was carried out at 100 V for 1 h, and then at 400 V until the unreacted ANTS reached 10 mm from the bottom of the gel (approximately 1.5–2 h).

Analysis of electrofluorograms.—After electrophoresis, gels were placed on a DT-20 LP UV transilluminator (365 nm, ATTO, Tokyo), and photographed through a Wratten 8 filter (Kodak, NY), using T-Max 400 black-and-white film (Kodak). For quantitative analysis, gels were viewed using a BioImage analyzer (Millipore, MA) based on a cooled charge-coupled device camera system, and band intensities of labeled saccharides were determined using maltopentaose as an internal standard.

Acknowledgements

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