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Short communication

Analysis of oligo- and poly-N-acetylneuraminic acids and their lactones by capillary electrophoresis

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Abstract

The present work establishes the separation procedure of fragments from colominic acid, a mixture of homopolymers of N-acetylneuraminic acid (NeuAc), and their lactones by capillary electrophoresis. Using borate buffer containing sodium dodecyl sulfate and methyl cellulose, colominic acid can be separated up to triacontamer of NeuAc by capillary electrophoresis. The method can also separate lactones and enzymic degradation products of NeuAc oligomers.

Keywords: Colominic acid; Neuraminic acid; Acetylneuraminic acid; Lactones; Polyacetylneuraminic acid

1. Introduction

Colominic acid prepared from *E. coli* is a mixture of linear α-(2→8)-linked homopolymers of NeuAc with different degrees of polymerization (DP) and is a useful source of a wide variety of NeuAc oligo/polymers and their derivatives [1–3]. This acid can be separated preparatively into each component polymer by an anion-exchange chromatography [4].

Thin-layer chromatography (TLC) is a suitable tool for the analysis of poly-NeuAc [5,6], but there is a limit to this method in the resolution of their lactones.

Capillary electrophoresis (CE) [7,8] is a useful technique for the separation of carbohydrates with various DP. However, as far as we know, few applications of CE to analysis of NeuAc oligo/polymer and their lactones have been reported. In this paper we describe a direct and rapid method for estimation of fragments from colominic acid and their lactones by CE.

2. Experimental

2.1. Chemicals and materials

Boric acid and disodium tetraborate were purchased from E. Merck (Darmstadt, Germany). Di-, tri- and tetramers of NeuAc, and methyl cellulose (MC; Nos. 4000 and 8000) were obtained from Nacalai Tesque (Kyoto, Japan). Sodium dodecyl sulfate (SDS) and other analytical grade chemicals were obtained from Wako (Osaka, Japan). Colominic acid, NeuAc and neuraminidase (from *Cl. perfrin-*

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gens) are products from Sigma (St. Louis, MO, USA).

Other oligomers and polymers of NeuAc were prepared from colominic acid with DEAE-Sephadex A-25 column chromatography [4]. The purity of each polymer up to the DP of 15 was proved to be about 100% by CE and TLC.

2.2. Instrumentation

CE was carried out on a Quanta 4000 system of Waters (Milford, MA, USA) equipped with a UV filter detector adjusted to 185 or 214 nm and a capillary cassette, fitted with 60~120 cm×50 μm I.D. fused-silica capillary tube from Waters. A Waters Model 740 data module integrator was used to record electropherograms and to calculate the peak area of oligomers and polymers of NeuAc.

2.3. Procedures

2.3.1. Capillary electrophoresis

A fresh capillary was hydrated by washing with 1.0 *M* NaOH for 10 min, followed by rinsing with running buffer without MC (5 min), and finally replacing with running buffer (10 min). The running buffer was 25 m*M* disodium tetraborate–75 m*M* boric acid (pH 8.8) containing 0.3% MC 8000 and 100 m*M* SDS. On analysis of lactones of NeuAc oligomers, pH of the running buffer was adjusted to 6.9 with 6 *M* HCl. Before use, all solutions were degassed with a vacuum pump, filtered through a filter with 0.45 μm pore size (Millipore, Bedford, MA, USA) or centrifuged at 3000 rpm for 15 min to avoid capillary clogging. All experiments were carried out at about 30°C, and the samples were injected in the hydrostatic mode at 10 cm of differential height for 30~120 s according to the concentration of the samples.

2.3.2. Lactonization

Each oligomer of NeuAc was lactonized in glacial acetic acid at room temperature overnight [9].

2.3.3. Digestion

Degradation of the oligomers and their lactones by neuraminidase (EC 3.2.1.18, S:E 50:1) were performed in 50 m*M* acetate buffer (pH 5.1) at room temperature [10,11]. The samples for electrophoresis were taken from the reaction mixture at various intervals.

3. Results and discussion

Since colominic acid has a negatively charged carboxyl group in the NeuAc residue, separation of colominic acid to each fragment by CE was performed at relatively high pH (pH 9). The use of a borate buffer consisted of 25 m*M* disodium tetraborate and 75 m*M* boric acid rather than conventional 100 m*M* tetraborate solution resulted in good separation because of the resulting high voltage and low current. Addition of SDS in concentration of more than 75 m*M* improved in the resolution of higher fragments (Fig. 1) and is necessary to dissolve MC into the buffer.

In order to separate higher polymers, we utilized SDS–non-gel-sieving electrophoresis [12] using crosslink-free polymer, MC 4000 (3500–5600 cps) or 8000 (7000–10 000 cps) [13] as a support. The effect of MC became recognizable at the concentrations more than 0.5% and 0.25% of MC 4000 and MC 8000, respectively.

Based on the results described above, the best resolution was obtained under the conditions described in the method section. As shown in Fig. 2, CE enabled a baseline separation up to pentadecamers with distinct peaks as far as triacontamer of NeuAc.

Fig. 3 shows CE pherograms during the enzymic hydrolysis of NeuAc polymers. By neuraminidase in 50 m*M* acetate buffer (pH 5.1) at room temperature, the polymer (DP 15) was successively degraded to shorter polymers and finally to the monomer. The capillary electropherograms suggest that this method can be applied to the kinetic study on the enzymic degradation of NeuAc polymers and characterization of enzymic action such as endo- or exo-degradation.

Oligomers of NeuAc were lactonized in higher yield (more than 95%) at room temperature in glacial acetic acid than in hydrochloric acid solution at pH 2. It was confirmed by CE that the lactones reverted to the parent oligomers after a mild alkali treatment such as at pH 9 at 40°C for 10 min or at 4°C overnight. CE analysis of lactones of NeuAc oligo-

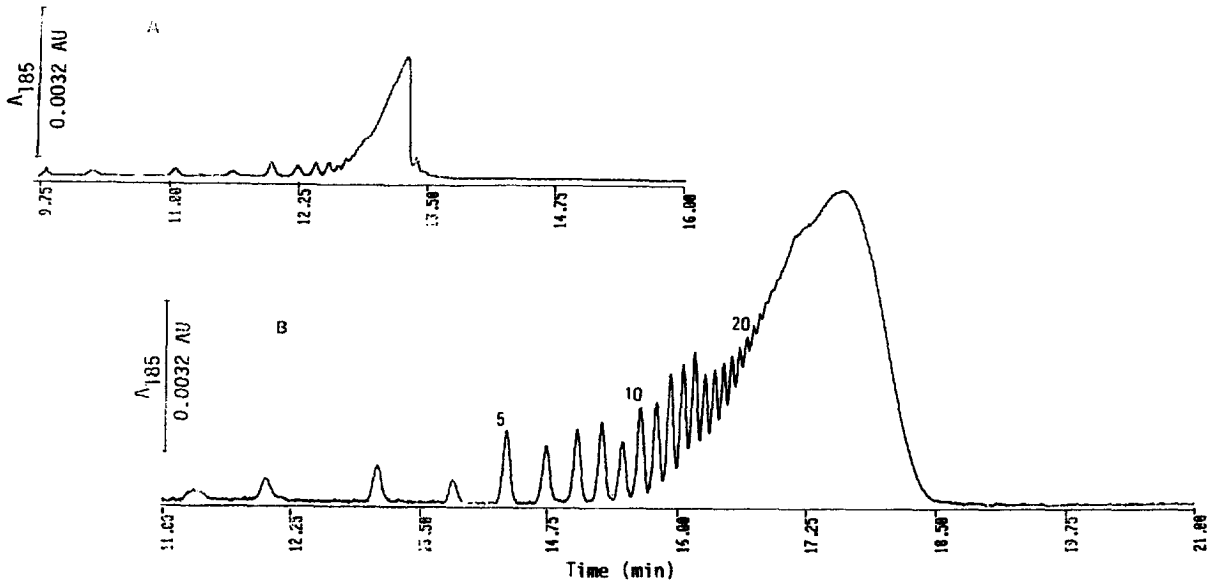

Fig. 1. Effect of SDS on separation of colominic acid. CE was carried out in 25 m*M* disodium tetraborate–75 m*M* boric acid (pH 8.8) without (A) or with (B) 100 m*M* SDS at 29°C and 15 kV. Column: 60 cm×50 μm I.D. On-capillary detection was at 185 nm. Injection was carried out by hydrostatic mode at 10 cm of differential height for 60 s (about 3 nl). Each peak number corresponds to the number of DP of NeuAc.

mers was carried out at pH 6.9 to avoid their degradation during the analysis. As shown in Fig. 4, the major peaks (2La, 3La, 4La, and 5La) represent completely lactonized oligomers, while the minor peaks (3Lb,c, 4Lb,c, and 5Lb,c) arise from incomplete ones. The major peaks migrated faster than the NeuAc monomer. The minor peaks were characterized by CE before and after neuraminidase degradation. The lactone species (3La,b, 4La,b, 5La,b) which the carboxyl group in non-reducing terminal residue was lactonized were not cleaved by neuraminidase. The lactones with free carboxyl group in non-reducing terminal residue were cleaved to the species lactonized in the residue. Thus, 2La, 3La and 4La were produced from 3Lc, 4Lc and 5Lc, respectively.

4. Conclusion

The optimum conditions for the separation of fragments from colominic acid and their lactones by

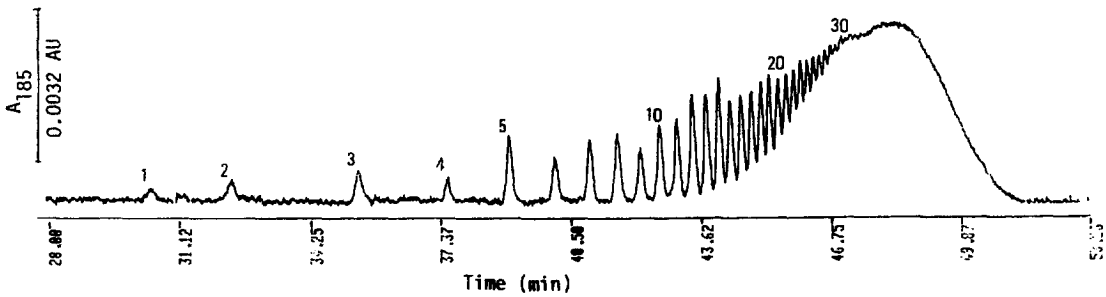

Fig. 2. CE of colominic acid. CE was carried out in 25 m*M* disodium tetraborate–75 m*M* boric acid (pH 8.8) containing 0.3% MC 8000 and 100 m*M* SDS at 20 kV. Column: 120 cm×50 μm I.D. Other conditions and symbols are same as in Fig. 1.

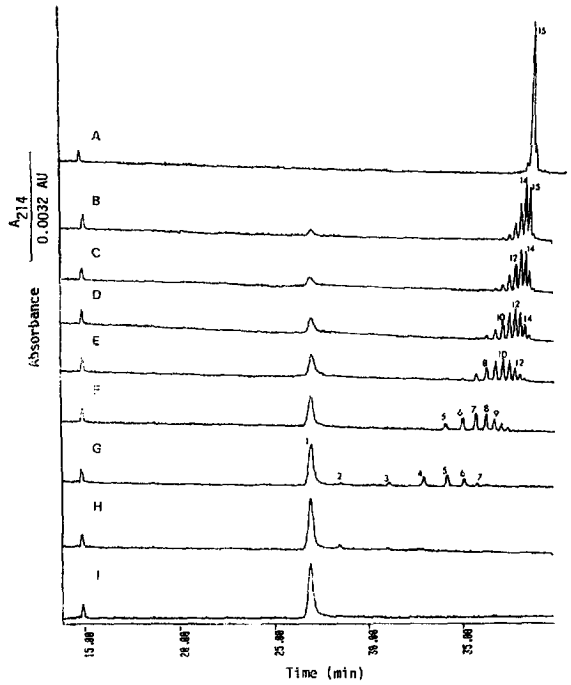

Fig. 3. Degradation of a polymer (DP 15) by N-acetylneuraminidase. CE was carried out as in Fig. 1, except with 100 cm×50 μm I.D. column and at 214 nm. Each peak number corresponds to the number of DP of NeuAc. A–I correspond to a degradation time of 0, 5, 10, 20, 40, 60, 120, 180, 240 min, respectively.

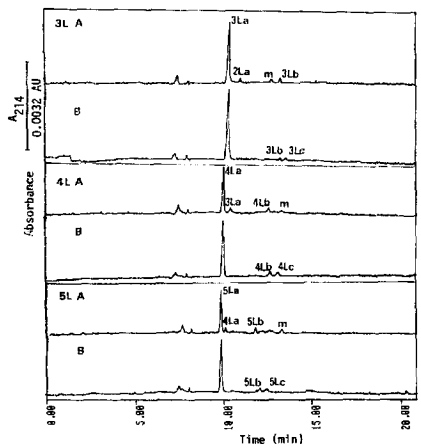

Fig. 4. CE of lactones of NeuAc oligomers. CE was carried out as in Fig. 1, except pH of running buffer (6.9) and monitoring wavelength (214 nm). Abbreviations: A and B, after and before neuraminidase treatment; 2L, 3L, 4L and 5L, lactones of di-, tri-, tetra- and pentamers; M, monomer; a, full lactonized species; b and c, partially lactonized species.

CE were established. It is also demonstrated that CE is a powerful tool for kinetic study of enzymic degradation of NeuAc oligomers and their derivatives.

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