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Short communication

Application of high-performance capillary electrophoresis to the purification process of *Escherichia coli* K4 polysaccharide

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Abstract

The high-performance capillary electrophoresis (HPCE) (electrokinetic chromatography with sodium dodecyl sulphate) technique was applied to the extraction and purification process of the K4 polysaccharide from cultured bacteria in several stages. HPCE proved to be a technique with high resolution and sensitivity in analyzing K4 polysaccharide during its purification, in particular by using a strong anion-exchange resin. This is of paramount importance to monitor the product during the extraction and purification process or to test the purity of the final product. Furthermore, HPCE is able to verify that the extraction and purification process adopted is not carried out under drastic conditions capable of inducing fructose removal from the polysaccharide backbone.

Keywords: *Escherichia coli*; Capillary electrophoresis; K4 polysaccharide; Glycosaminoglycans; Chondroitin

1. Introduction

Escherichia coli (*E. coli*) K4 synthesizes a capsular polysaccharide consisting of a chondroitin [→4)-βGlcA-(1,3)-β-GalNAc-(1→]$_n$ backbone to which β-fructofuranose units are linked to C-3 of D-glucuronic acid residues [1]. The defructosylated product of K4 (K4d) is useful to gain detailed insight into the mode of action of several enzymes, such as the C-5 epimerase involved in dermatan sulfate biosynthesis [2], and both the GlcA- and the GalNAc-transferases in chondroitin sulfate formation [3]. Therefore, for this purpose native K4 is defructosylated to produce the polysaccharide possessing the chondroitin backbone.

In a previous paper [4], a rapid, highly sensitive and reproducible high-performance capillary electrophoresis (HPCE) method (electrokinetic chromatography with sodium dodecyl sulfate) was described for the determination of the K4 polysaccharide and its defructosylated product. The two polyanions, K4 and defructosylated K4, were separated and readily determined within 30 min on an uncoated fused-silica capillary using normal polarity at 20 kV and detection at 200 nm. A linear relationship was found for the two polysaccharides over a wide range of concentrations, from approximately 30–210 ng.

K4 polysaccharide, like other *E. coli* bacteria capsular polysaccharides (K antigens), is produced by extraction from the bacteria cultures and purified according to different preparation approaches [1,3]. A rapid, specific and sensitive analytical method to detect this polymer during the extraction and purification procedures is necessary. Furthermore, quantitative responses are important in the light of the modification of the bacteria culture conditions to increase the production of K4 polysaccharide.

In a previous paper [5], HPCE was qualitatively and quantitatively applied to the extraction and purification process of the uropathogenic *E. coli* K5, a natural polysaccharide having the structure of a desulfo-heparin, from cultured bacteria in several stages [5]. K4 and K5 polysaccharides show a very

Abbreviations: HPCE, high-performance capillary electrophoresis; K4d, defructosylated K4; GalNAc, N-acetyl-D-galactosamine; GlcA, D-glucuronic acid

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different structure and, as a consequence, various different bacterial growth conditions and possible extraction, purification and analytical approaches. This rationale stimulated me to investigate on the possible use of HPCE (electrokinetic chromatography with sodium dodecyl sulfate) for the qualitative and quantitative determination of the native K4 polysaccharide during the extraction and purification processes from cultured bacteria.

2. Materials and methods

2.1. Materials

E. coli U1-41 05:K4:H4 was from the Status Serum Institut, Copenhagen, Denmark. Ion-exchange resin QAE Sephadex® A-25 was from Pharmacia Biotech. Dialysis tubes having a cut-off of 5000 Da were from Spectrum Labs. All the other reagents were of analytical grade.

2.2. Purification of K4 polysaccharide

E. coli cells were grown for 12 h at 37 °C in a medium containing per liter 9.7 g K_2HPO_4, 2 g KH_2PO_4, 0.5 g sodium citrate, 20 g casamino acids, 20 g ammonium sulfate, 2 g glucose and the dialysible part of yeast (100 ml from 500 g, in 5 l of deionized water) according to Rodriguez et al. [1], and harvested at 5000 g for 10 min. The medium was further centrifuged at 10,000 × *g* for 30 min and the recovered supernatant was freeze-dried. The cell pellet was washed once in 50 ml of phosphate-buffered saline (pH 7.2), resuspended in 50 ml of extraction buffer (50 mM Tris–Cl, 5 mM EDTA, pH 7.3), and incubated at 37 °C for 30 min. The cells were pelleted by centrifugation and treated three more times with extraction buffer. The supernatants were collected and freeze-dried. The reunited dried material from medium and cells was dissolved (about 100 mg) in 10 ml of 50 mM NaCl. After centrifugation at 10,000 × *g* for 10 min, 2 ml of the supernatant was applied to a column (1 cm × 20 cm) packed with QAE Sephadex® A-25 anion-exchange resin equilibrated with the same NaCl solution. K4 polysaccharide was eluted with a linear gradient of NaCl from 50 mM to 2 M from 0 to 150 min using low-pressure liquid chromatography (LP chromatography system from BioRad) at a flow of 1 ml/min. Fractions of 2 ml were collected. K4 polysaccharide was detected in the collected fractions by HPCE (see below). The collected fractions corresponding to K4 polysaccharide were dialyzed by double distilled water and freeze-dried. Approximately 1 mg of the purified K4 was recovered.

2.3. Capillary electrophoresis

Capillary electrophoresis was performed as previously reported [4,5]. Separation and analysis by electrokinetic chromatography with sodium dodecyl sulfate were carried out on an uncoated fused-silica capillary tube (50 μm i.d., 65 cm total length and 50 cm from the injection point to the detector) at 25 °C by using a Beckman HPCE instrument (P/ACE system 5000) equipped with a UV detector set at 200 nm. The buffer consisted of disodium hydrogen phosphate (40 mM), sodium tetraborate (10 mM) and SDS (40 mM) buffered at pH 9.0 by the addition of 1 M HCl. The buffer was degassed by vacuum filtration through a 0.2 μm membrane filter and shaken in an ultrasonic bath. Before each run, the capillary tube was washed with 0.1 M NaOH for 1 min, double distilled water for 5 min, and then conditioned with the operating buffer for 5 min. The samples to be analyzed were injected automatically, using the pressure injection mode, in which the sample is pressurised for 10 s. The injection volume can be calculated with the Poiseuille equation, as proposed by the manufacturer, giving an estimated volume of 6 nl/s of injection time. Electrophoresis was performed at 20 kV (about 45 μA) using normal polarity. Peak areas were recorded and calculated using the Beckman software system Gold V810.

3. Results and discussion

Fig. 1 shows the HPCE electropherogram of the reunited dried material derived from the medium and from the pooled cellular supernatants after treatment with the extraction buffer. Several non-identified species are separated and detected at 200 nm together with the K4 polysaccharide established at a time of approximately 20.60 min (see also [4]) by adding a known amount of K4 standard.

Due to the presence of many contaminants and of lipopolysaccharide from the outer membrane of *E. coli* extracted with the K4 polysaccharide, this polymer was further purified by using preparative anion-exchange chromatogra-

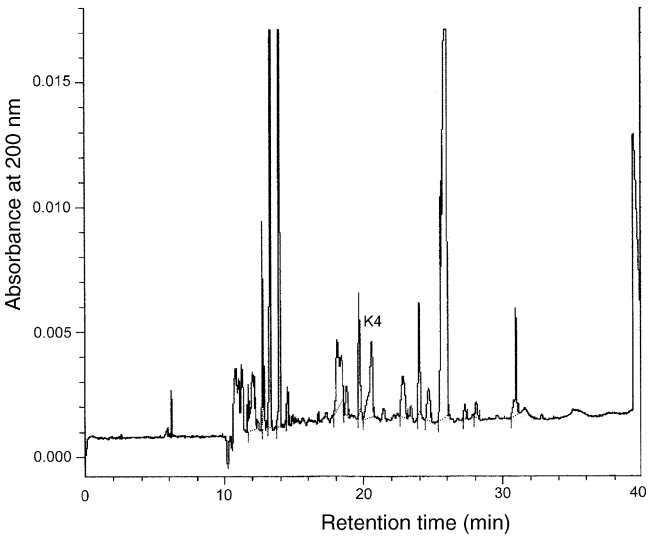

Fig. 1. HPCE electropherogram of the reunited dried material derived from the medium and from the pooled cellular supernatants after treatment with the extraction buffer. K4 indicates the polysaccharide species at a migration time of approximately 20.60 min.

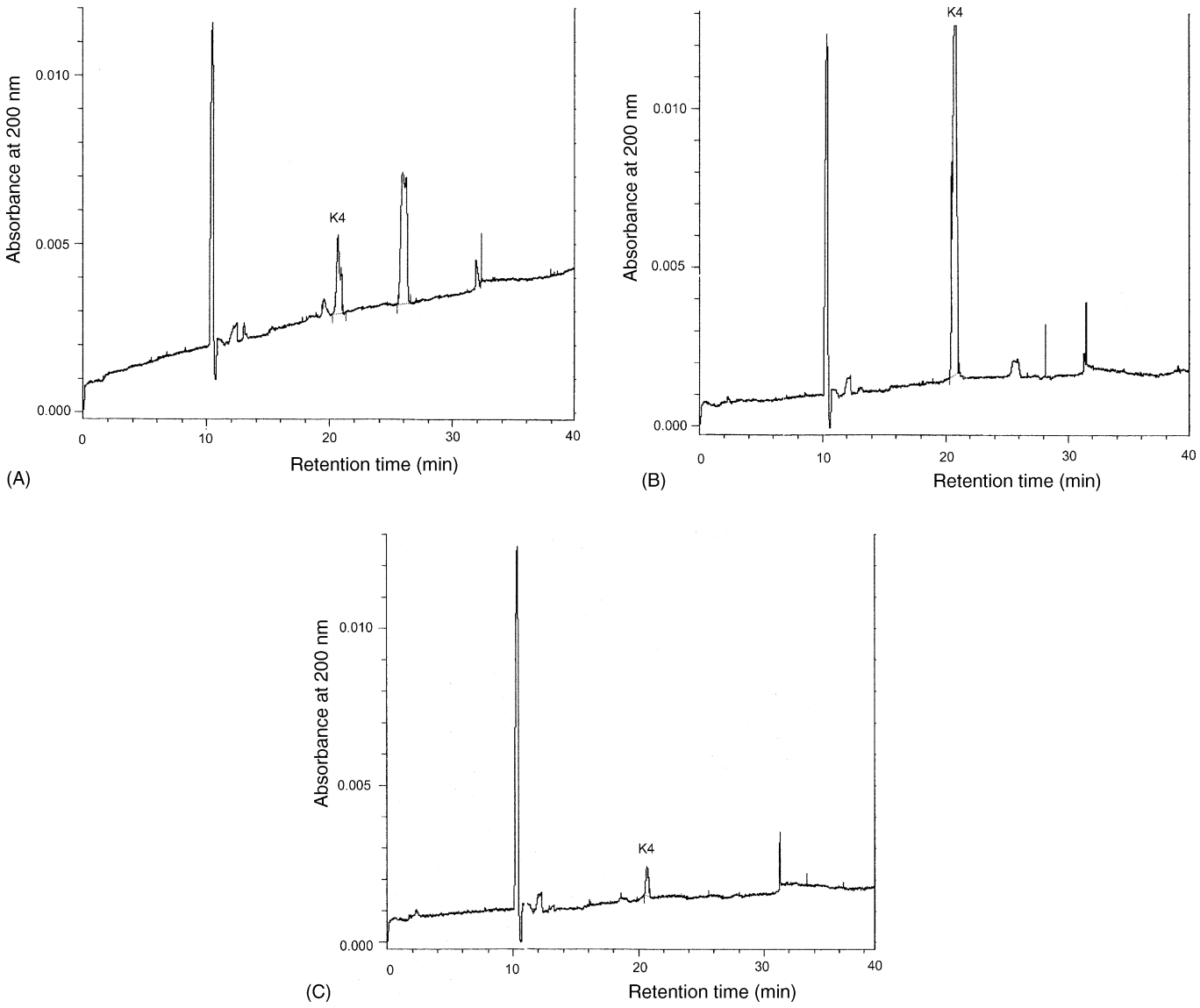

Fig. 2. HPCE electropherogram of anion-exchange K4 polysaccharide fractions eluted from the resin: (A) fraction 33, (B) fraction 35, (C) fraction 40. K4 indicates the polysaccharide species at a migration time of approximately 20.60 min. No presence of the defructosylated product of K4 (normally detected at approximately 22.80–23.00 min [4]) is evident.

phy. Fractions of 2 ml were collected from the resin and HPCE was used for the quantitative evaluation (see [4] for the calibration curves of K4 and K4d from 30 to 210 ng) of the presence of K4 in each single fraction and also to assess its purity. As illustrated in Fig. 2 (from A to C corresponding to the fractions 33, 35 and 40, respectively), HPCE was useful for the determination of the K4 polymer in each anion-exchange fraction also in the presence of a high concentration of sodium salt necessary to elute the polysaccharide from the resin. Furthermore, no defructosylated product of K4 (normally detected at approximately 22.80–23.00 min, see [4]) was evident, showing that the preparative protocol adopted does not result in the loss of the fructose from the polysaccharide backbone.

The HPCE separation of the K4 polysaccharide purified by means of anion-exchange chromatography and further dialysed and freeze-dried is illustrated in Fig. 3 showing a polysaccharide free of the contaminants present in the medium and cell extract (see Fig. 1) and devoid of the defructosylated product.

The purification process of K4 from the bacteria extract generally requires treatment with quaternary ammonium salts, anion-exchange resins and precipitation with organic solvents [1]. HPCE proved to be a technique with high resolution and sensitivity in analyzing K4 polysaccharide during the purification process by using a strong anion-exchange resin enabling a detection limit lower than 30 ng (see [4]). This is of paramount importance to monitor the product dur-

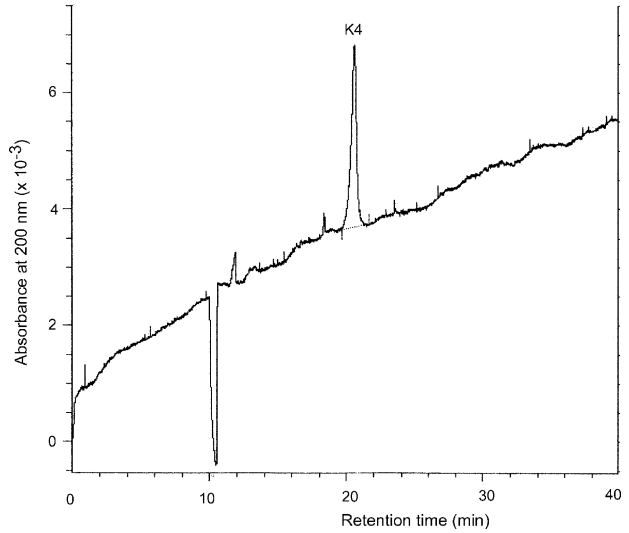

Fig. 3. HPCE electropherogram of the purified *E. coli* K4 polysaccharide. The sample at a concentration of 2 μg/μl distilled water was injected for 20 s for a total of 240 ng. K4 indicates the polysaccharide species at a migration time of approximately 20.60 min.

ing the extraction and purification process, as verified in this study, or to test the purity of the final product. Furthermore, as HPCE is able to detect at the same time the presence of K4 and its defructosylated product K4d [4], it is possible to verify that the extraction and purification process is not carried out under drastic conditions capable of inducing fructose removal from the polysaccharide backbone.

4. Conclusions

In this paper *E. coli* K4 polysaccharide produced by bacteria cultures has been purified and qualitatively and quantitatively analyzed by HPCE (electrokinetic chromatography with sodium dodecyl sulphate) showing high sensitivity and capacity for the processing of many samples during routine screening. Furthermore, HPCE is able to verify that the extraction and purification process adopted is not carried out under drastic conditions capable of inducing fructose removal from the K4 polysaccharide backbone.

References

[1] M.L. Rodriguez, B. Jann, K. Jann, Eur. J. Biochem. 177 (1988) 117.
[2] H.H. Hannesson, A. Hagner McWhirter, K. Tiedemann, U. Lindahl, A. Malmstrom, Biochem. J. 313 (1996) 589.
[3] K. Lidholt, M. Fjelstad, J. Biol. Chem. 272 (1997) 2682.
[4] N. Volpi, Electrophoresis 25 (2004) 692.
[5] N. Volpi, Electrophoresis, in press.