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Analysis of 8-aminonaphthalene-1,3,6-trisulfonate-derivatized oligosaccharides by capillary electrophoresis–electrospray ionization quadrupole ion trap mass spectrometry

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Abstract

Dextran was partially hydrolyzed with 0.1 mol/l HCl and the hydrolysate was derivatized with 8-aminonaphthalene-1,3,6-trisulfonate (ANTS) by reductive amination. The derivatized-oligosaccharide mixture was separated by capillary electrophoresis (CE) in a buffer of 1% HAc–NH_4OH, pH 3.4, and the separated components were detected on-line by electrospray ionization quadrupole ion trap mass spectrometry (ESI-QIT-MS) in the negative ion mode. A mass accuracy lower than 0.01% could be achieved and as low as 1.6 pmol of detxran octaose could be detected. ANTS-derivatized dextran oligosaccharide with a degree of polymerization (DP) lower than 6 produced both $[M-H]^-$ and $[M-2H]^{2-}$ ions, whereas those with a DP of 6 or higher than 6 produced only $[M-2H]^{2-}$ ion. As $1 \leq DP \leq 6$, the percentage of $[M-2H]^{2-}$ ion in the total ions of $[M-H]^-$ and $[M-2H]^{2-}$ was found to be a linear function of the logarithmic DP. Molecular mass determination with ESI-QIT-MS strengthens the power of CE analysis of oligosaccharides. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Mass spectrometry; Oligosaccharides; Aminonaphthalene trisulfonate; Dextran

1. Introduction

In recent years, the important and diverse roles played by oligosaccharides in the biological function of proteins urged many researchers to develop new methods for the analysis of oligosaccharides. Glycoprotein-derived oligosaccharides often form a heterogeneous mixture called a glycan pool. A successful analysis of such a complicated carbohydrate mixture demands high resolving separation methods. Among the oligosaccharide separation techniques, capillary electrophoresis (CE) has shown powerful resolution and the highest sensitivity [1–8]. However, the detectors generally used with CE, UV, laser-induced fluorescence (LIF), electrochemical and thermooptical absorbance (TOA) detectors, merely trace the presence or not, but cannot give information features, such as molecular mass or even structure of the underivatized or derivatized oligosaccharide separated by CE. The strategy of preliminary identification of individual components is commonly the comparison of migration times with those of the oligosaccharide underivatized or derivatized standards, of which structures are known

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[2,5,9–11]. Therefore, a definite identification of an oligosaccharide can not be performed only by a separation method. The emergence of ionization techniques, electrospray ionization (ESI) [12] and matrix-assisted laser desorption/ionization (MALDI) [13] has opened up broad and promising areas for the application of mass spectrometry (MS) to the characterization of biopolymers, peptides, proteins, oligonucleotides and oligosaccharides. Since the analyte determined by a mass spectrometer is required to be surface-active in a viscous matrix and the free oligosaccharides are exceedingly hydrophilic [14], and although MALDI-TOF (time-of-flight) MS can be used to analyze underivatized oligosaccharides, the sensitivity is not high because picomole analysis can not be achieved by these instruments [15]; derivatization for oligosaccharides is highly recommended to enhance hydrophobicity [14]. Derivatization methods include permethylation or peracetylation of all labile hydrogen, and reductive amination with a hydrophobic chromophore. Reductive amination has improved the sensitivity 1000–5000-fold in ESI [16,17] and MALDI [18] experiments over the free oligosaccharides. Currently, on-line CE coupling with MALDI-MS remains problematic, while off-line combination of CE and MALDI-MS is possible. Suzuki et al. [19] reported the use of a high-resolution automated CE fraction collector to isolate 1-aminopyrene-3,6,8-trisulfonate (APTS)-derivatized oligosaccharides, followed by analysis with MALDI-TOF-MS. On-line CE coupling with ESI-MS such as ESI-triple quadrupole (TQ) and ESI-quadrupole ion trap (QIT) mass spectrometers has already shown excellent performances for high sensitivity for identification of proteins [20–23]. More recently, electrospray ionization quadrupole ion trap mass spectrometry (ESI-QIT-MS) has been introduced to the analysis of oligosaccharides [24–27]. However, analysis of oligosaccharides by using on-line CE–ESI-QIT-MS has not been reported before.

In this paper we describe the first use of ESI-QIT-MS as an on-line molecular mass detector for the analysis of 8-aminonaphthalene-1,3,6-trisulfonate (ANTS)-derivatized oligosaccharides separated by CE. ANTS was originally introduced by Jackson [28] on fluorophore-assisted carbohydrate electrophoresis (FACE) at the beginning of the 1990s, soon after that it has been widely used in CE for the analysis of saccharides [1–3,29]. The derivatization with ANTS not only presents a hydrophobic chromophore but also puts three negative charges on oligosaccharides, which facilitate the neutral oligosaccharides to migrate in an electric field and to be separated. In the CE–ESI-QIT-MS study here, CE separation was performed using an acetic acid–ammonium hydroxide acidic buffer and the mass detection was carried out in the negative ion mode. A mixture of partial hydrolysates of dextran was studied as a model of oligosaccharides.

2. Experimental

2.1. Materials

Dextran (M_r=523 000) was purchased from Pharmacia. ANTS was a product of Molecular Probes (Eugene, OR, USA). Sodium cyanoborohydride ($NaCNBH_3$), acetic acid, ammonium hydroxide and formic acid were bought from Sigma. Dimethyl sulfoxide (DMSO) and trifluoroacetic acid (TFA) were from Fluka (Buchs, Switzerland). Two synthetic peptides Tyr–Asp–Asp–Asp–Asp–Lys and Asp–Ala–Ala–Ala were gifts of Professor Da-Fu Cui (Shanghai Institute of Biochemistry, China). All other chemicals were of analytical-reagent grade. Milli-Q water was used.

2.2. CE–UV absorbance detection

CE separations were performed on a P/ACE 5500 system (Beckman, Fullerton, CA, USA) equipped with a diode array detection (DAD) system. Samples were introduced into the 57 cm (50 cm anode to detector)×75 μm I.D. fused-silica capillary. The separation voltage was −20 kV (negative polarity at inlet vial) and the scanning wavelength range was 190–450 nm with an interval of 1 nm. The temperature of capillary was controlled at 25°C. Electropherograms were obtained by using the Beckman Gold System soft version 8.2 controlled by an IBM PS/VP Model. The capillary was sequentially rinsed prior to each run with 0.1 *M* NaOH and water, followed by reconditioning with an electrophoretic buffer, 50 m*M* NaH_2PO_4–H_3PO_4, pH 2.5.

2.3. CE–ESI-QIT-MS

On-line CE–ESI-QIT-MS analysis was carried out using a Beckman CE system P/ACE 5500 coupled to a LCQ mass spectrometer (Finnigan) equipped with an electrospray ionization source. The CE–MS adapter for an ESI source of a LCQ instrument was from Finnigan. This adapter belongs to a sheath flow (coaxial) interface. The tuning and the calibration were performed in the negative ion mode by peptide Tyr–Asp–Asp–Asp–Asp–Lys ion signal (*m/z* 768.7), peptide Asp–Ala–Ala–Ala (*m/z* 345.3) and the TFA anion (*m/z* 113). Mass spectrometer source conditions were as follows: capillary temperature, 200°C; sheath gas (nitrogen) flow-rate, 50 units; spray voltage, 4.5 kV; CE–ESI sheath liquid, ethanol–water (60:40) with 1% formic acid at a 1 μl/min flow-rate. CE conditions were as follows: fused-silica capillary, 80 cm×50 μm I.D.; running buffer was 1% HAc–NH_4OH, pH 3.4; separation voltage was −20 kV. MS data were processed on a Gateway 2000 computer (Gateway) using the BioExplore software package (Finnigan).

2.4. Preparation of the hydrolysate of dextran and derivatization with ANTS

A quantity of 10 mg dextran was added to the bottom of a net ampere vial, followed by addition of 0.5 ml of 0.1 *M* HCl, flushing with nitrogen for 1 min and sealing the vial. Hydrolysis was carried out at 100°C for 4 h, then the reaction mixture was lyophilized and redissolved in 1.0 ml of water, and lyophilized again to remove the HCl. The mixture of dextran oligosaccharides was dissolved in 150 μl of 0.2 *M* ANTS in HAc–water (3:17) and 150 μl of 1.0 *M* $NaCNBH_3$ in DMSO. This reaction mixture was heated in a water bath at 40°C for 15 h, then 20 μl of the mixture was diluted to a final volume of 200 μl and the diluted sample was directly subjected to the analysis by CE–UV or CE–ESI-QIT-MS.

3. Results and discussion

3.1. CE–UV analysis of ANTS-derivatized dextran oligosaccharides

Fig. 1 illustrates the general structure of ANTS-derivatized dextran oligosaccharides. The three negatively charged sulfonic acid groups provide a high electrophoretic mobility for each dextran oligosaccharide and make rapid separation. Fig. 2 shows the electropherogram of ANTS-derivatized dextran oligosaccharides separated by CE in a buffer of 50 m*M* NaH_2PO_4–H_3PO_4, pH 2.5. About 13 peaks could be observed. Since ANTS peak and peak 1 were preliminarily identified as ANTS and ANTS-Glc by coinjection of the mixture of ANTS-derivatized dextran oligosaccharides with ANTS and ANTS-Glc, respectively, it can be theoretically deduced that the other peaks are sequentially the ANTS-derivatized oligosaccharides with a degree of polymerization (DP) of 2, 3, 4, ..., and 13, respectively. The small ANTS peak indicates the majority of ANTS was coupled to oligosaccharides. The UV–visible spectrum of each peak can be recorded by CE–DAD in a single run. Fig. 3 shows the UV–visible spectra of ANTS and of ANTS-derivatized dextran oligosaccharides, respectively. ANTS has the highest molar absorption at 233 nm in a pH 2.5 phosphate buffer (see Fig. 3a). The absorption maximum wavelength shifts to 223 nm when ANTS is coupled to a carbohydrate (see Fig. 3b–d) and the absorptivity of ANTS drops about 24%, compared to that at 233 nm. Although the specific UV–visible

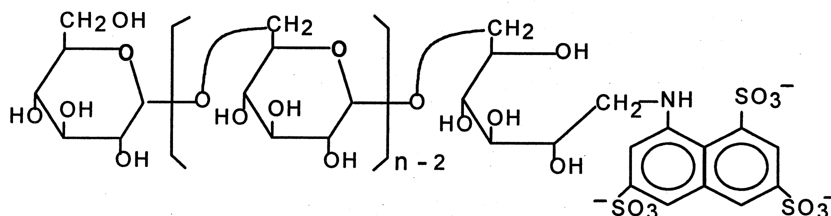

Fig. 1. General structure of ANTS-derivatized dextran oligosaccharides (*n* is the number of glucose residues in the molecule, *n*=DP).

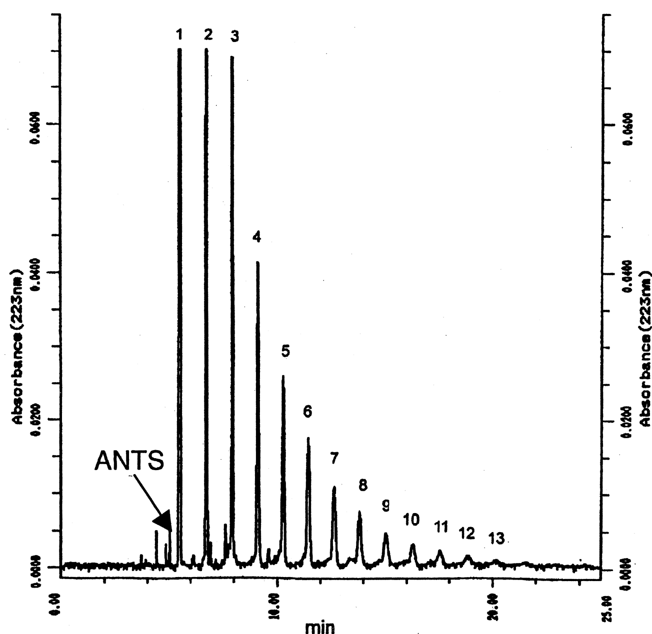

Fig. 2. CE–UV analysis of the mixture of ANTS-derivatized dextran oligosaccharides. Vacuum injection for 3 s (about 5 nl), other conditions are described in Section 2.2.

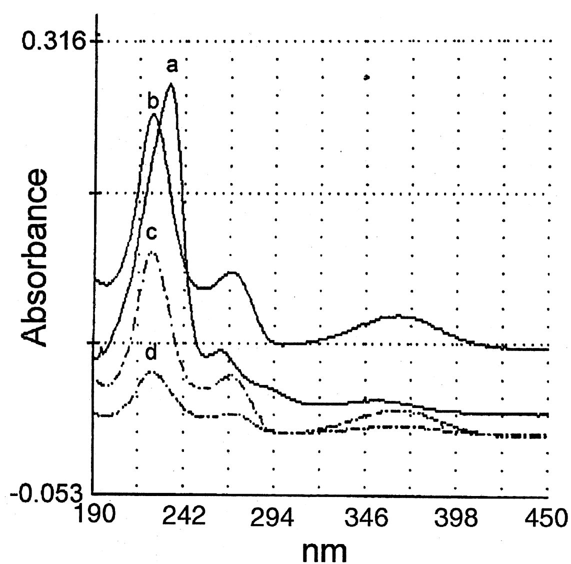

Fig. 3. UV–visible spectra of ANTS and ANTS-derivatized dextran oligosaccharides obtained by using DAD UV detection during CE separation. (a) ANTS, (b) ANTS-Glc, (c) ANTS-Glc_2, (d) ANTS-Glc_3. Those of other peaks have similar profiles with ANTS-Glc_3. Other conditions are described in Section 2.2.

spectra can also be used to characterize the derivatized oligosaccharides, it is impossible to distinguish one from the other among the derivatized oligosaccharides through their very similar UV–visible spectra, let alone discriminate the differences in their molecular masses and structures.

3.2. CE–ESI-QIT-MS analysis of ANTS-derivatized dextran oligosaccharides

Since ANTS-derivatized carbohydrates are negatively charged even at low pH, for MS detection the negative ion mode was selected. Fig. 4 demonstrates the total ion current electropherogram during CE separation. Fig. 5A–H show full-scan mass spectra of the derivatized-oligosaccharides obtained by ESI-QIT-MS as they migrated out off the CE capillary. Table 1 lists the predicted and the observed mass of ANTS-derivatized oligosaccharides. The best mass accuracy lower than 0.01% and the worst mass accuracy of 0.07% were obtained. The average mass accuracy of ANTS-derivatized oligosaccharide ion mass assignment was 0.03%, which was reasonable.

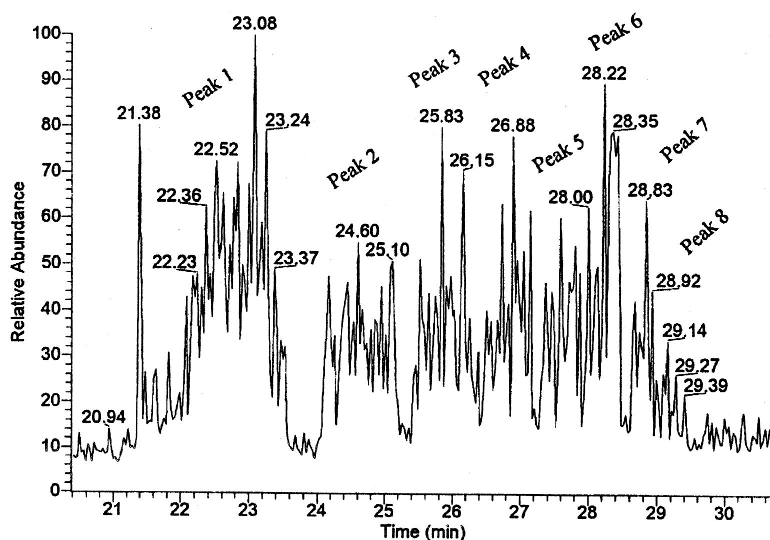

Fig. 4. CE–ESI-QIT-MS total ion current electropherogram of the ANTS-derivatized dextran oligosaccharide mixture from m/z 100 to 2000.

A molecular mass with that accuracy can allow determination of the composition of oligosaccharide comprised of different monosaccharides with unique incremental masses (e.g., fucose, M_r 146; hexose, 162; *N*-acetylhexosamine, 203; *N*-acetylneuraminic acid, 219; *N*-glycolylneuraminic acid, 235) [30]. Based on the data of accurate molecular mass in Table 1, it is easy to achieve a definite identification of these dextran oligosaccharides. The DP of each dextran oligosaccharide in the hydrolysate mixture is in accord with the value obtained by theoretical deducing (see Fig. 2). The difference in molecular mass, 162, between the two immediately neighboring oligosaccharides indicates the monomer of these polymers is a hexose. According to the data (not given) of peak areas obtained by CE–UV analysis in Fig. 2, the ratio of quantity of dextran octaose to the total quantity of preparation dextran oligosaccharides is calculated as 3.5%. Therefore, it can be estimated that approximate as low as 2 ng (1.6 pmol) of dextran octaose was detected by CE–ESI-QIT-MS. Those oligosaccharides with DPs higher than 8 were not detected by CE–ESI-QIT-MS because the quantities were lower and the electrospray efficiency for a longer carbohydrate chain is lower also. The majority of CE–ESI-MS studies, reported before, are concerned with positively charged ions. The detection of negatively charged ions by CE–MS is still a major challenge because of relative low signal-to-noise ratios (e.g., as in Fig. 4 and Fig. 5 here) compared to that in positive ion detection, which might be due to higher chemical noise in negative mode. Hence, the sensitivity in negative mode may be lower than that in the positive mode.

It is interesting that the ANTS-derivatized oligosaccharide with a DP lower than 5 produced both $[M-H]^-$ and $[M-2H]^{2-}$ ions, whereas those with a DP of 6 or higher produced only $[M-2H]^{2-}$ ion. Furthermore, with the increasing of DP from 1 to 6, the percentage of $[M-2H]^{2-}$ ion in the total ions of $[M-H]^-$ and $[M-2H]^{2-}$ increases from 10.3% to 100% (see Fig. 6). This phenomenon indicates that the larger the size of ANTS-derivatized oligosaccharide is, the higher its charge capacity (capability of holding charges) is. When the DP is 6 or larger than 6, the ANTS-derivatized oligosaccharide has a charge capacity high enough to accommodate two charges. Fig. 6 shows, as DP$\leq$6, plots of the percentage of $[M-2H]^{2-}$ ion in $([M-H]^-+[M-2H]^{2-})$ ions against log DP is linear and the linear correlation coefficient r is 0.9987. Hence, the relationship between the percentage of $[M-2H]^{2-}$ ion in the total ions of $[M-H]^-$ and $[M-2H]^{2-}$ and degree of DP can be described as $P=116.6$

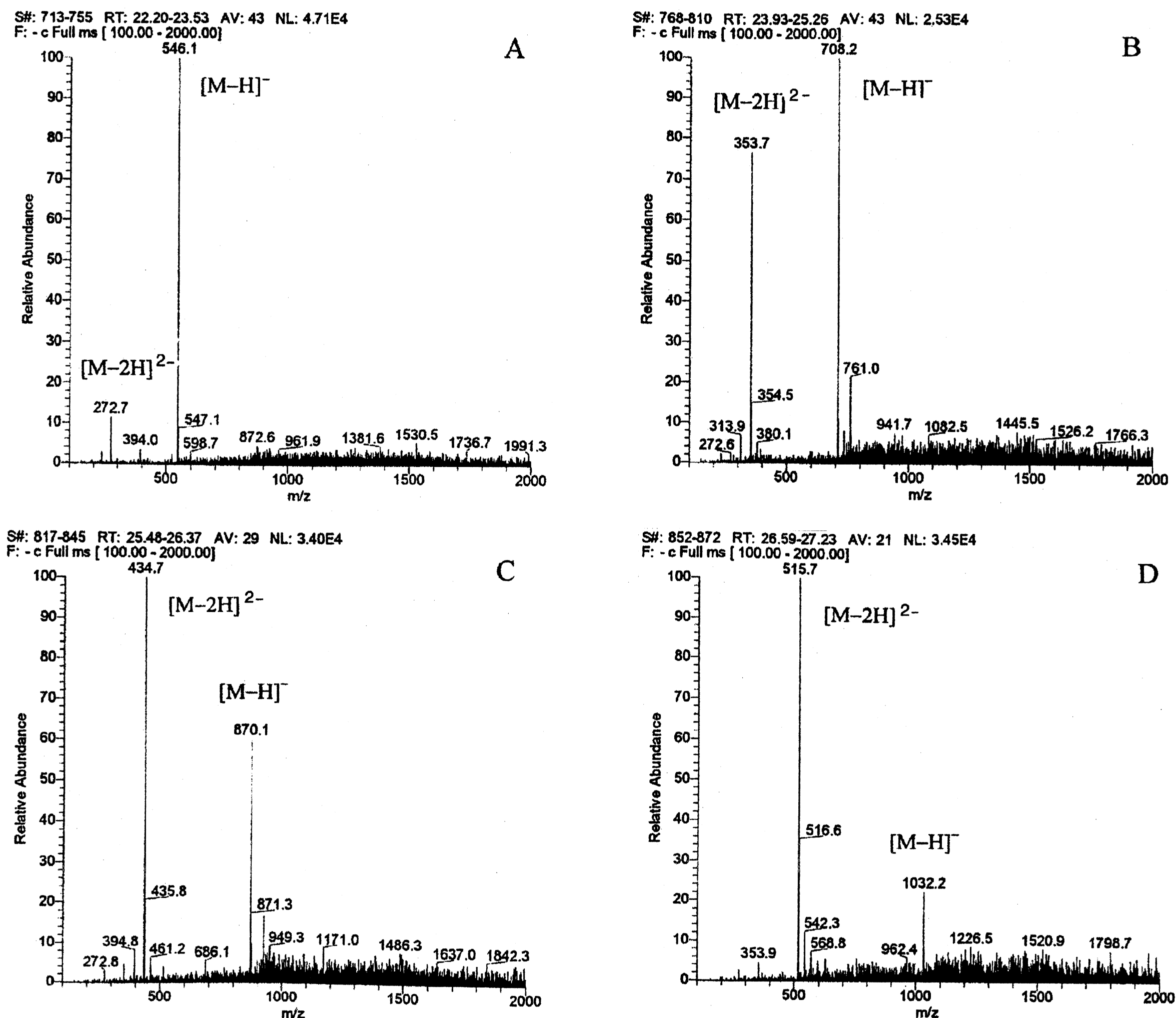

Fig. 5. ESI-QIT-MS mass spectra from same on-line CE–ESI-QIT-MS run as shown in Fig. 4. Conditions, sample injection for 15 s (about 17 nl), other conditions are described in Section 2.3. (A) Mass spectrum of ANTS-Glc from 22.2 to 23.53 min (peak 1); (B) mass spectrum of ANTS-Glc_2 from 23.93 to 25.26 min (peak 2); (C) mass spectrum of ANTS-Glc_3 from 25.48 to 26.37 min (peak 3); (D) mass spectrum of ANTS-Glc_4 from 26.59 to 27.23 min (peak 4); (E) mass spectrum of ANTS-Glc_5 from 27.36 to 28.00 min (peak 5); (F) mass spectrum of ANTS-Glc_6 from 27.71 to 28.54 min (peak 6); (G) mass spectrum of ANTS-Glc_7 from 28.54 to 29.17 min (peak 7); (H) mass spectrum of ANTS-Glc_8 from 29.05 to 29.58 min (peak 8).

log(DP)+9.223 (DP=1, 2,....., 6) and $P=100$ (DP=6, 7, 8).

It can be speculated that ANTS-derivatized oligosaccharide with a DP larger than 8 may take two negative charges or three for a DP large enough. In future, a theoretical explanation for the empirical relationship above will certainly benefit further understanding of the influence of oligosaccharide size and structure on the charge state and stability of ANTS-derivatized oligosaccharide during ESI.

In the total ion current electropherogram (Fig. 4), all the peaks of ANTS-derivatized oligosaccharides are much wider than those in CE–UV analysis (Fig. 2). This might result from two aspects. On the CE–MS interface aspect, the dead volume between the outlet of the CE capillary and the MS system is

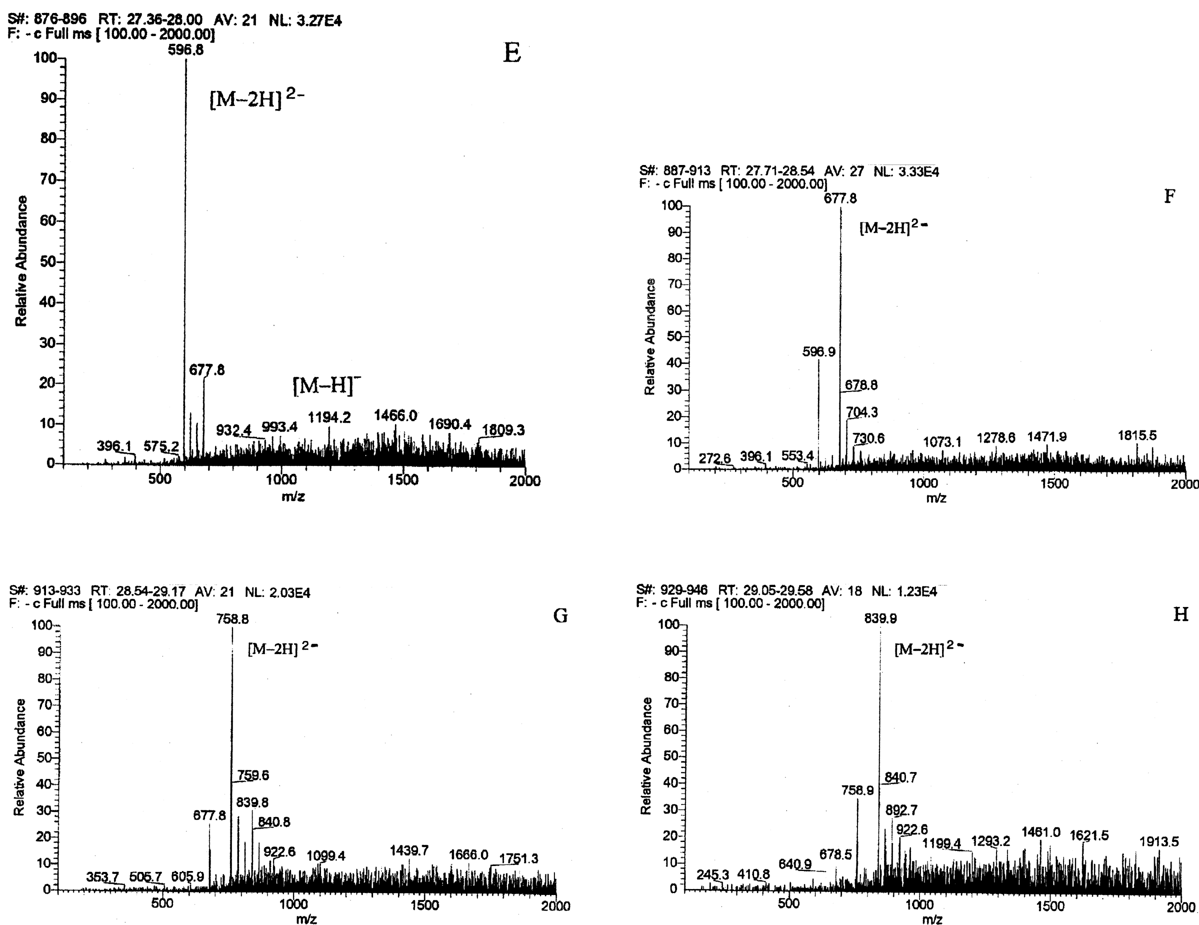

Fig. 5 (*continued*).

the first reason which should be considered. A larger dead volume results in extra peak broadening as well as in sensitivity decrease. So dead volume needs to be avoided or minimized. Sheath liquid flow provides the solvent flow necessary for enhancing the stability of electrospray ionization, but it also dilutes

Table 1
Predicted and observed mass of ANTS-derivatized dextran oligosaccharides

DP	M_r			$\pm\Delta M_r$	Mass accuracy (%)
	$[M-H]^-$ calculated	$[M-H]^-$ observed	$[M-2H]^{2-}$ observed		
1	546.49	546.1	272.7	0.39	0.07
2	708.49	708.2	353.7	0.29	0.04
3	870.49	870.1	434.7	0.39	0.04
4	1032.49	1032.2	515.7	0.29	0.03
5	1194.49	1194.2	596.8	0.29	0.02
6	1356.49		677.8	0.11	0.01
7	1518.49		758.8	0.11	0.01
8	1680.49		839.9	0.31	0.02

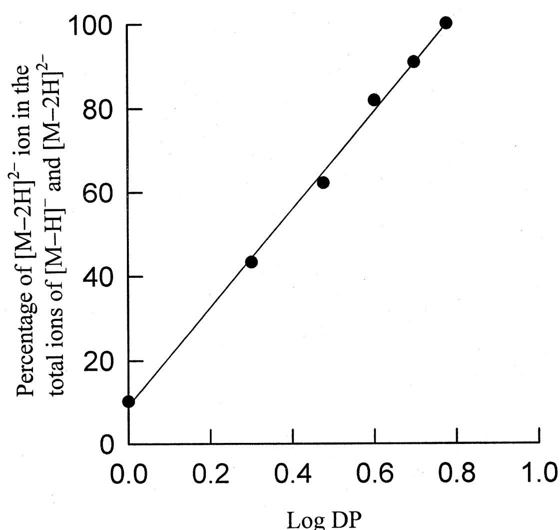

Fig. 6. Relationship between the percentage of $[M-2H]^{-}$ ion in the total ions of $[M-H]^{-}$ and $[M-2H]^{2-}$ and degree of polymerization (DP), DP=1, 2, ..., 6.

the analyte and disturbs CE separation efficiency to some extent, thus brings about peak broadening and sensitivity reducing. It is necessary to minimize the sheath liquid flow while still maintaining a stable spray. As regards the aspect of CE separation conditions, one reason is that the same voltage (20 kV) as in CE–UV analysis applied on a longer capillary (80 cm total length) produced a weaker electric field intensity, hence a slower ion migration rate was obtained. The second is Joule heat generated from applied field, resulted in peak widening because three fourth of the CE capillary stretched out of the capillary cartridge, exposed to the open air, and could not be cooled by the coolant. The next reason is electrodispersion due to mismatched sample and buffer conductivities. In general, the electrophoretic buffer composition used for CE–MS analysis should be of high volatility and relatively low ionic strength, such as acetic acid–ammonium hydroxide, formic acid. Here, the ionic strength of the sample directly subjected to CE–QIT-MS analysis was relatively higher than the running buffer of 1% HAc–NH_4OH, pH 3.4, since the sample contained 57 m*M* $NaCNBH_3$ and three negatively charged ANTS-derivatized oligosaccharides, which has a higher mobility than that of the running buffer. Finally, a longer sample injection time such as 15 s had a larger volume of sample injected into capillary and caused wider solute zones.

The noise-like "spikes" of each peak indicate the unstability of electrospray ionization. It resulted with great possibilities from the voltage fluctuation occurring at the electrospray needle [31]. Sample with high ionic strength might bring about this kind of voltage fluctuation. Another cause might be the intrinsic unstability of electrospray of carbohydrate because of its very high hydrophilicity.

Consequently, sample desalting prior to CE–ESI-QIT-MS analysis is recommended if necessary in order to enhance the resolution of CE separation and the sensitivity of QIT-MS system.

TQ and QIT mass spectrometers are known as the closest rivals. Both of them have the advantage of being easily interfaced on-line with CE. Theoretically, the sensitivity of QIT-MS appears to be higher than that of TQ-MS since the latter is inherently limited for it is a flow-through system while QIT-MS is an ion storage system. Namely, TQ-MS selects, at any time point, ions of a specific mass–charge ratio (m/z) while eliminating all the other ions from analysis [23], and QIT-MS can trap and accumulate ions, followed by a rapid swept ejection/detection [32]. Actually, excellent comparison between CE–ESI-TQ-MS and CE–ESI-QIT-MS performance on the identification of proteins by Figeys and Aebersold [23], showed two important points. One is that the absolute and the concentration limit of detection in MS mode on both systems were comparable. The reason was considered as detection of ions in both instruments is primarily limited by chemical noise. Another is the limit of detection obtained in MS–MS mode on the QIT was better by at least a factor of 10 compared to the value obtained on the TQ. This is mainly due to that on the QIT one can mass-select an ion of interest for storage in the trap and that then after collision-induced dissociation, all the product ions stay in the trap, resulting in 1–2 orders better sensitivity for the product ion spectra. Mid-femtomole to low-picomole levels of sample (protein or peptide) are typically used to obtain both MS and MS–MS spectra by QIT-MS [33]. Our data here show low-picomole levels of ANTS-derivatized oligosaccharides eluted from CE can be generally detected by QIT-MS in the negative mode. Unfortunately, currently there are no data

available on the analysis of derivatized oligosaccharides by CE–ESI-TQ, which may be used to give a comparison between the two type of mass spectrometers. Other advantages of QIT-MS over TQ-MS are that QIT-MS is simple, easy to handle and cheaper.

MALDI-TOF-MS is known to have advantages over other MS methods in terms of sensitivity, efficiency and simplicity [15]. However, the results obtained by Suzuki et al. [19] show that the mass accuracy for APTS-derivatized oligosaccharides using MALDI-TOF-MS was 0.1–0.3%, which is about 10-times lower than that obtained by us using CE–QIT-MS for ANTS-derivatized oligosaccharides. The low resolution (normally only a few hundred) of MALDI-TOF-MS often causes peak broadening and a less accurate mass measurement [34]. These drawbacks could be alleviated by using other types of expensive mass spectrometers such as TOF instruments fitted with reflectrons, ion cyclotron resonance (ICR) and magnetic sector instruments with an array detector [34]. Resolutions in the order of several thousands have been obtained by these techniques. MALDI-TOF-MS is not easily interfaced on-line with CE and this is another limitation.

4. Conclusion

This work opens up the possibility of utilizing on-line CE–QIT-MS for the identification and characterization of derivatized oligosaccharides. Relative low-level detection (1.6 pmol) for dextran octaose in an undesalted derivatized mixture was obtained in the negative ion mode. Only about 22 nl of derivatized sample with 73 ng total of oligosaccharides was consumed to complete both CE–UV and CE–ESI-QIT-MS analysis. This technique combines the merits of CE and QIT-MS mass accuracy and sensitivity, and preliminarily shows a promising tool for the precise identification of much complex carbohydrates. Of course, further work will exploit the most important advantage of MS^n of QIT-MS in CE–MS analysis because this advantage is increasingly important as a carbohydrate gets more complex. Generally, CE–MS is limited by poor concentration detection limits. Many investigations have been carried out to overcome this problem. Recently, micro- and nanoelectrospray ion sources have been introduced to CE–MS and resulted in remarkable sensitivities [20–24,35–37]. With either of the two newly developed ion sources, no sheath liquid is needed and analytes are directly sprayed and ionized without sheath liquid dilution. Hence, it appears necessary to apply micro- or nanoelectrospray ion sources to CE–QIT-MS for the analysis of oligosaccharides, especially, for the analysis of glycoprotein-derived oligosaccharides.

Although the approach described in this paper was demonstrated feasible with ANTS-derivatized dextran oligosacchrides, it may prove useful for other carbohydrates and derivatization regents of aromatic sulfonic acids, such as APTS [6,10,11,19,38] or 7-aminonaphthalene-1,3-disulfonate (ANDS) [39].

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References

[1] M. Stefansson, M. Novotny, Anal. Chem. 66 (1994) 1134.
[2] A. Klockow, R. Amado, H.M. Widmer, A. Paulus, J. Chromatogr. A 716 (1995) 241.
[3] C. Chiesa, C. Horvath, J. Chromatogr. 645 (1993) 337.
[4] S. Suzuki, K. Kakehi, S. Honda, Anal. Biochem. 205 (1992) 227.
[5] P. Hermentin, R. Doenges, R. Witzel, C.H. Hokke, J.F.G. Vliegenthart, J.P. Kamerling, H.S. Conradt, M. Nimtz, D. Brazel, Anal. Biochem. 221 (1994) 29.
[6] F.-T.A. Chen, R.A. Evangelista, Electrophoresis 19 (1998) 2639.
[7] W. Nashabeh, Z. El Rassi, J. Chromatogr. 600 (1992) 279.
[8] J. Liu, O. Shirota, M. Novotny, Anal. Chem. 64 (1992) 973.
[9] S. Honda, A. Makino, S. Suzuki, K. Kaheki, B. Stahl, Anal. Biochem. 191 (1991) 228.
[10] A. Guttman, T. Pritchett, Electrophoresis 16 (1995) 1906.
[11] A. Guttman, F.-T.A. Chen, R.A. Evangelista, Electrophoresis 17 (1996) 412.
[12] J.B. Fenn, M. Mann, C.K. Meng, S.F. Wong, C.M. Whitehouse, Science 246 (1989) 64.
[13] M. Karas, F. Hillenkamp, Anal. Chem. 60 (1988) 2299.
[14] A.L. Burlingame, Curr. Opin. Biotechnol. 7 (1996) 4.

[15] M. Okamoto, K. Takahashi, T. Doi, Y. Takimoto, Anal. Chem. 69 (1997) 2919.
[16] M. Okamoto, K. Takahashi, T. Doi, Rapid Commun. Mass Spectrom. 9 (1995) 641.
[17] K. Yoshino, T. Takao, H. Murata, Y. Shimonshi, Anal. Chem. 67 (1995) 4028.
[18] T. Takao, Y. Tambara, A. Nakamura, K. Yoshino, H. Fukuda, M. Fukuda, Y. Shimonshi, Rapid Commun. Mass Spectrom. 10 (1996) 2073.
[19] H. Suzuki, O. Muller, A. Guttman, B.L. Karger, Anal. Chem. 69 (1997) 4554.
[20] D. Figeys, I. Oostveen, A. Ducret, R. Aebersold, Anal. Chem. 68 (1996) 1822.
[21] D. Figeys, A. Ducret, J.R. Yates III, R. Aebersold, Nat. Biotechnol. 14 (1996) 1579.
[22] D. Figeys, Y. Ning, R. Aebersold, Anal. Chem. 69 (1997) 3153.
[23] D. Figeys, R. Aebersold, Electrophoresis 18 (1997) 360.
[24] U. Bahr, A. Pfenninger, M. Karas, Anal. Chem. 69 (1997) 4530.
[25] A.S. Welskopf, P. Vouros, D.J. Harvery, Anal. Chem. 70 (1998) 4441.
[26] D.M. Sheeley, V.N. Reinhold, Anal. Chem. 70 (1998) 3053.
[27] N. Viseux, E. Hoffmann, B. Domon, Anal. Chem. 70 (1998) 4951.
[28] P. Jackson, Biochem. J. 270 (1990) 705.
[29] F.Y. Che, Z.Y. Liu, K.Y. Wang, Q.C. Xia, Acta Biochim. Biophys. Sinica 30 (1998) 500.
[30] D.I. Papac, A. Wong, A.J.S. Jones, Anal. Chem. 68 (1996) 3215.
[31] R.B. Cole, J. Varghese, R.M. McCormick, D. Kadlecek, J. Chromatogr. A 680 (1994) 363.
[32] R.D. Smith, H.R. Udseth, J.H. Wahl, D.R. Goodlett, S.A. Hofstadler, Methods Enzymol. 271 (1996) 448.
[33] K.R. Jonscher, J.R. Yates III, Anal. Biochem. 244 (1997) 1.
[34] D.J. Harvey, J. Chromatogr. A 720 (1996) 429.
[35] J.F. Kelly, L. Ramaley, P. Thibault, Anal. Chem. 69 (1997) 51.
[36] K.P. Bateman, R.L. White, P. Thibault, Rapid Commun. Mass Spectrom. 11 (1997) 307.
[37] K.P. Batemm, R.L. White, M. Yaguchi, P. Thibault, J. Chromatogr. A 794 (1998) 327.
[38] F.-T.A. Chen, R.A. Evangelista, Anal. Biochem. 230 (1995) 273.
[39] K.B. Lee, S.Y. Kim, R.L. Linhardt, Electrophoresis 273 (1991) 636.