A straightforward electrospray ionization high resolution mass spectrometry method for underivatized long chain polysaccharides

Abstract: Structural analysis of long chain polysaccharides by electrospray ionization mass spectrometry (ESI-MS) is challenging since these molecules do not contain readily ionizable groups. Their mass spectra are dominated by singly charged ions, limiting the detection of high molecular weight species. Derivatization can enhance ionization, but analyte loss on purification decreases sensitivity. We report a method based on nanoESI-MS and MS/MS by collision induced dissociation (CID) for underivatized long chain polysaccharides. The procedure was tested on underivatized polydisperse dextrans (average molecular weight 4,000) at 2.6 kV ESI voltage and CID MS/MS at energies between 30-60 eV. 113 ions corresponding to species from Glc\textsubscript{2} to Glc\textsubscript{35} were detected. Ions at \textit{m/z} 1,409.48, 1,107.35 and 1,438.47, assigned to \(\text{[G}_{17}+2\text{Na}]^{2+}\), \(\text{[G}_{20}+\text{H}+\text{Na}+\text{K}]^{3+}\) and \(\text{[G}_{35}+2\text{H}+\text{Na}+\text{K}]^{4+}\), were sequenced and characterized by MS/MS. The component containing 35 Glc repeats is the longest polysaccharide chain detected by ESI-MS and structurally analyzed by MS/MS without prior derivatization and/or separation.

Keywords: polysaccharide sequence, nanoelectrospray, QTOF MS, MS/MS

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1 Introduction

Dextrans are linear bacterial and yeast polysaccharides of repeating (α1-6)-linked poly-D-glucose; all have (α1-3) branches and some also contain (α1-2) or (α1-4) branches [1]. The chain lengths vary exhibiting molecular weights from 1,000 Da to 2,000 kDa.

Dextrans have clinical and pharmaceutical applications. High molecular weight dextrans of 40 and 70 kDa (Dex 40, Dex 70) have antithrombotic effects. They are also used as osmotic agents in hypovolemia and to improve blood circulation. Dextrans are drug delivery systems [2] and lubricants in eye drops [3]. Low molecular weight dextrans such as dextran 1,000 are used as predictors for dextran-induced anaphylaxis [4].

Mass spectrometric (MS) structural analysis of long chain polysaccharides is challenging. Their poor ionization, yielding mainly singly charged ions, hampers the detection of high molecular weight species. To increase ionization efficiency, signal intensity and mass range, long chain polysaccharides have been derivatized for electrospray (ESI) MS analysis. The most popular techniques are methylation, permethylation and benzimidazole derivatization [5], acetylation, and reductive amination [6,7]. Although successful, derivatization requires laborious and time consuming synthesis and purification, causing loss of sample and sensitivity reduction.

Native long chain polysaccharides have been structurally analyzed using soft ionization techniques such as MALDI and ESI. MALDI-TOF structural analysis of dextrans provided reduced mass accuracy and resolution and low sensitivity [8,9]: sample solutions of 1 mg mL\textsuperscript{-1} were necessary for dextrans of average molecular weight 5,000 [10]. Negative or positive ion mode ESI MS of 1,000 [11], 5,000 [10] and 12,000 [10] molecular weight dextrans gave low resolution and mass accuracy as well as poor detection of long chains. For dextran of average molecular weight 5,000, chains of no more than 26 Glc repeats,
corresponding to a mass of only 4,212, were detected by direct syringe pump electrospray injection.

We report here a novel and straightforward method for structural analysis of underivatized long chain dextrans based on high resolution mass spectrometry using a hybrid quadrupole time-of-flight instrument (QTOF MS) with direct sample injection by nanoelectrospray ionization (nanoESI). An underivatized polydisperse dextran with an average molecular weight of 4,000 (Dex 4) was exhaustively characterized by positive ion mode nanoESI-MS and tandem MS (MS/MS) using collision induced dissociation (CID) at low energies. Water was the solvent and spraying agent. We detected species containing up to 35 Glc repeats, having the mass of 5,670 Da. Glc\textsubscript{35} represents the largest polysaccharide chain detected by ESI-MS and sequenced using MS/MS without prior derivatization, alkali treatment and/or separation.

2 Experimental procedure

2.1 Materials

Dex 4 (99% purity) was purchased from Sigma (Germany). Deionized water from a SG Water (Germany) system was used. Water gave the best solubility and ionization efficiency. NanoESI experiments were carried out using self-pulled omega glass capillaries (Analytik Vertrieb, Germany) produced on a model 720 vertical pipette puller (David Kopf Instruments, Tujunga, CA, USA) by an “omega” shape filament.

2.2 Preparation of sample solutions for MS

Dextran solution was prepared by dissolving dry Dex 4 in deionized water to a concentration of 5 pmol μL\textsuperscript{-1} calculated for an average molecular weight of 4,000. The solution was centrifuged (10 minutes at 6,000 rpm) in a ROTH (Germany) mini-centrifuge and the supernatant was analyzed by MS.

2.3 QTOF MS and MS/MS

MS and MS/MS were performed on a hybrid QTOF micro instrument (Micromass/Waters, Manchester, UK) with direct nanoESI injection in the Micromass Z-spray geometry. A PC running MassLynx 4.1 software controlled the instrument and performed data acquisition and processed MS data. Signal was acquired at 1 scan s\textsuperscript{-1} for about 5 minutes per sample. All spectra were acquired in the positive ion mode, known to be best for dextran [12-15]. For efficient ionization and minimal in-source fragmentation the cone voltage was varied within 30–50 V. No signal was observed below 30 V. The source block was set/kept at 80°C. MS/MS was performed at low ion acceleration energies using argon as the collision gas at 12 psi. For MS/MS, the ions were isolated by setting both LM and HM parameters to 10. The resulting spectrum is a sum of scans over the total ion current (TIC) acquired at 30-80 eV collision energy to provide the full set of diagnostic fragment ions.

The m/z scale was calibrated by using a polyethylene glycol (PEG) “tuning mix” external calibration standard from Waters (Manchester, UK). This provided a high coverage of the m/z scan range in both MS and MS/MS experiments. The obtained average mass accuracy of 27 ppm is situated within the normal range for a QTOF MS instrument.

The ions were assigned following the nomenclature of Domon and Costello [16].

3 Results and discussion

3.1 Screening of the Dex 4 mixture

Aqueous Dex 4 solution (10 μL) was loaded into the nanoelectrospray capillary and injected into the quadrupole time-of-flight mass spectrometer. The parameters were optimized to minimize in-source fragmentation and to produce a spectrum with high s/n ratio [17,18], shown in Fig. 1. Corresponding structures are listed in Table 1.

The spectrum contains four main peak series, shaped as envelopes typical for polydisperse mixtures [15]. These series include singly, doubly, triply and quadruply charged molecules. The observed multiple charging is typical for electrospray, and beneficial: i) indicates that if the charges were preserved, the instrumental parameteres are set at values that also preserve “in source fragmentation”; ii) shows that the analyte is a carbohydrate, since artifacts or contaminants do not undergo multiple charging; iii) greatly improves the upper m/z detection limit, as high molecular weight species may not be detected as singly charged molecules.

All carbohydrate ions are formed as alkali adducts. Typical for ESI with water as the spraying agent, this behavior favors ionization of species that lack readily ionizable groups such as dextrans [19]. Interestingly, cationization by alkali adducts was induced solely...
Figure 1: Positive nanoESI QTOF MS of Dex 4. Cone voltage: 30 V. Capillary voltage: 2.4 kV. Acquisition: 300 scans. Argon pressure: 12 p.s.i.

Table 1: Assignment of the major ions in Dex 4.

| No. peak | m/z (experimental) | m/z (theoretical) | Mass accuracy (ppm) | Structure |
|----------|--------------------|-------------------|---------------------|-----------|
| 1.       | 345.20             | 345.19            | 14                  | \([G_4+H+Na]^+\) |
| 2.       | 353.19             | 353.18            | 28                  | \([G_4+H+K]^+\) |
| 3.       | 365.26             | 365.27            | 27                  | \([G_4+Na]^+\) |
| 4.       | 381.24             | 381.25            | 26                  | \([G_4+H+Na]^+\) |
| 5.       | 426.24             | 426.22            | 46                  | \([G_5+H+K]^+\) |
| 6.       | 434.19             | 434.20            | 23                  | \([G_5+H+K]^+\) |
| 7.       | 497.23             | 497.21            | 40                  | \([G_6+2Na]^2+\cdot H_2O\) |
| 8.       | 507.26             | 507.24            | 39                  | \([G_6+H+Na]^2+\) |
| 9.       | 515.25             | 515.23            | 38                  | \([G_7+H+K]^+\) |
| 10.      | 518.25             | 518.23            | 38                  | \([G_7+2Na]^2+\) |
| 11.      | 527.31             | 527.32            | 18                  | \([G_7+Na]^+\) |
| 12.      | 543.28             | 543.30            | 36                  | \([G_7+K]^+\) |
| 13.      | 547.21             | 547.19            | 36                  | \([G_7+2Na]^2+\cdot H_2O\) |
| 14.      | 551.22             | 551.20            | 36                  | \([G_7+2H+2K]^4+\) |
| 15.      | 579.27             | 579.25            | 34                  | \([G_8+H+K]^2+\cdot H_2O\) |
| 16.      | 588.20             | 588.18            | 34                  | \([G_8+H+Na]^+\) |
| 17.      | 591.73             | 591.71            | 33                  | \([G_8+2H+2K]^4+\) |
| 18.      | 596.27             | 596.26            | 16                  | \([G_8+H+K]^+\) |
| 19.      | 599.27             | 599.26            | 16                  | \([G_8+2Na]^2+\) |
| 20.      | 632.24             | 632.22            | 31                  | \([G_9+2H+2K]^4+\) |
| 21.      | 669.32             | 669.30            | 29                  | \([G_{10}+H+Na]^+\) |
| No. peak | m/z (experimental) | m/z (theoretical) | Mass accuracy (ppm) | Structure |
|----------|-------------------|-------------------|---------------------|-----------|
| 22.      | 677.29            | 677.28            | 14                  | [G₈₊H⁺⁺K⁺]²⁺ |
| 23.      | 680.31            | 680.29            | 29                  | [G₈₊2Na⁺]²⁺ |
| 24.      | 689.36            | 689.37            | 14                  | [G₈₊Na⁺]⁺   |
| 25.      | 705.36            | 705.38            | 28                  | [G₈₊K⁺]⁺    |
| 26.      | 709.27            | 709.25            | 28                  | [G₉₊2H⁺Na⁺K⁺]⁴⁺ |
| 27.      | 713.32            | 713.31            | 14                  | [G₉₊2H⁺⁺K⁺]⁴⁺ |
| 28.      | 750.33            | 750.32            | 13                  | [G₉₊H⁺Na⁺]²⁺ |
| 29.      | 753.75            | 753.72            | 39                  | [G₉₊2H⁺2K⁺]⁴⁺ |
| 30.      | 758.33            | 758.31            | 26                  | [G₉₊3H⁺⁺K⁺]⁴⁺ |
| 31.      | 761.32            | 761.31            | 13                  | [G₉₊2Na⁺]²⁺ |
| 32.      | 769.33            | 769.30            | 26                  | [G₉₊Na⁺K⁺]²⁺ |
| 33.      | 783.32            | 783.29            | 38                  | [G₉₊H⁺Na⁺K⁺]²⁺ |
| 34.      | 790.31            | 790.28            | 37                  | [G₉₊2H⁺Na⁺K⁺]⁴⁺ |
| 35.      | 794.31            | 794.28            | 37                  | [G₉₊2H⁺⁺K⁺]⁴⁺ |
| 36.      | 831.36            | 831.35            | 12                  | [G₉₊H⁺Na⁺]²⁺ |
| 37.      | 837.32            | 837.30            | 23                  | [G₉₊Na⁺Na⁺K⁺]²⁺ |
| 38.      | 839.33            | 839.34            | 11                  | [G₉₊H⁺⁺K⁺]²⁺ |
| 39.      | 842.36            | 842.34            | 23                  | [G₉₊2Na⁺]²⁺ |
| 40.      | 851.40            | 851.42            | 23                  | [G₉₊Na⁺]⁺   |
| 41.      | 867.39            | 867.41            | 23                  | [G₉₊K⁺]⁺    |
| 42.      | 871.32            | 871.31            | 11                  | [G₉₊2H⁺Na⁺K⁺]⁴⁺ |
| 43.      | 875.34            | 875.30            | 45                  | [G₉₊2H⁺⁺K⁺]⁴⁺ |
| 44.      | 886.34            | 886.30            | 45                  | [G₉₊2Na⁺⁺K⁺]⁴⁺ |
| 45.      | 891.35            | 891.32            | 33                  | [G₉₊H⁺Na⁺K⁺]²⁺ |
| 46.      | 893.34            | 893.32            | 22                  | [G₉₊3Na⁺]³⁺ |
| 47.      | 912.36            | 912.38            | 21                  | [G₉₊H⁺Na⁺]²⁺ |
| 48.      | 920.34            | 920.36            | 21                  | [G₉₊H⁺⁺K⁺]²⁺ |
| 49.      | 923.35            | 923.37            | 21                  | [G₉₊2Na⁺]²⁺ |
| 50.      | 940.05            | 940.02            | 31                  | [G₉₊H⁺⁺Na⁺K⁺]⁴⁺ |
| 51.      | 945.36            | 945.34            | 21                  | [G₉₊H⁺Na⁺K⁺]²⁺ |
| 52.      | 947.37            | 947.34            | 31                  | [G₉₊3Na⁺]³⁺ |
| 53.      | 993.37            | 993.40            | 30                  | [G₉₊H⁺Na⁺]²⁺ |
| 54.      | 999.38            | 999.36            | 20                  | [G₉₊Na⁺Na⁺K⁺]²⁺ |
| 55.      | 1001.37           | 1001.39           | 19                  | [G₉₊H⁺⁺K⁺]²⁺ |
| 56.      | 1004.36           | 1004.39           | 29                  | [G₉₊2Na⁺]²⁺ |
| 57.      | 1013.45           | 1013.48           | 29                  | [G₉₊Na⁺]⁺   |
| 58.      | 1029.43           | 1029.46           | 29                  | [G₉₊K⁺]⁺    |
| 59.      | 1048.01           | 1048.05           | 38                  | [G₉₊H⁺Na⁺K⁺]²⁺ |
| 60.      | 1053.38           | 1053.37           | 9                   | [G₉₊Na⁺Na⁺K⁺]²⁺ |
| 61.      | 1055.33           | 1055.37           | 37                  | [G₉₊3Na⁺]³⁺ |
| 62.      | 1074.40           | 1074.43           | 27                  | [G₉₊H⁺Na⁺]²⁺ |
| 63.      | 1082.38           | 1082.42           | 36                  | [G₉₊H⁺⁺K⁺]²⁺ |
| 64.      | 1085.39           | 1085.42           | 27                  | [G₉₊2Na⁺]²⁺ |
| 65.      | 1093.37           | 1093.41           | 36                  | [G₉₊Na⁺Na⁺K⁺]²⁺ |
| 66.      | 1107.36           | 1107.39           | 27                  | [G₉₊Na⁺Na⁺K⁺]²⁺ |
| 67.      | 1109.36           | 1109.39           | 27                  | [G₉₊3Na⁺]³⁺ |
| 68.      | 1114.38           | 1114.39           | 8                   | [G₉₊2H⁺Na⁺K⁺]⁴⁺ |
Table 1: Assignment of the major ions in Dex 4.

| No. peak | m/z (experimental) | m/z (theoretical) | Mass accuracy (ppm) | Structure |
|----------|---------------------|-------------------|---------------------|-----------|
| 69.      | 1118.34             | 1118.38           | 35                  | [G₁₀⁺₂Na⁺₂K]⁺⁺ |
| 70.      | 1148.40             | 1148.43           | 26                  | [G₁₀⁺₂Na]⁺⁻H₂O  |
| 71.      | 1155.42             | 1155.45           | 25                  | [G₁₀⁺Na⁺Na⁺]⁺⁻ |
| 72.      | 1161.70             | 1161.73           | 25                  | [G₁₀⁺Na⁺Na⁺K]⁺⁻ |
| 73.      | 1163.40             | 1163.44           | 34                  | [G₁₀⁺Na⁺K]⁺⁻   |
| 74.      | 1166.42             | 1166.45           | 25                  | [G₁₀⁺Na⁺Na⁺]⁺⁻ |
| 75.      | 1175.50             | 1175.53           | 25                  | [G₁⁺Na⁺]⁻      |
| 76.      | 1191.34             | 1191.37           | 25                  | [G₁₀⁺₂H⁺₂Na]⁺⁺ |
| 77.      | 1195.40             | 1195.36           | 33                  | [G₁₀⁺₂H⁺Na⁺Na⁺K]⁺⁻ |
| 78.      | 1199.37             | 1199.41           | 33                  | [G₁₀⁺₂H⁺₂K]⁺⁻   |
| 79.      | 1210.31             | 1210.35           | 33                  | [G₁₀⁺₂Na⁺₂K]⁺⁻ |
| 80.      | 1215.39             | 1215.43           | 32                  | [G₁₀⁺Na⁺Na⁺K]⁺⁻ |
| 81.      | 1217.39             | 1217.42           | 24                  | [G₁₀⁺₃Na]⁺⁻     |
| 82.      | 1236.45             | 1236.48           | 24                  | [G₁⁺⁺Na⁺Na⁺]⁻   |
| 83.      | 1244.45             | 1244.47           | 16                  | [G₁⁺⁺Na⁺K]⁻     |
| 84.      | 1247.43             | 1247.47           | 32                  | [G₁⁺⁺₂Na⁺]⁻     |
| 85.      | 1255.43             | 1255.46           | 23                  | [G₁⁺⁺Na⁺Na⁺K]⁻   |
| 86.      | 1269.42             | 1269.44           | 15                  | [G₁⁺⁺Na⁺Na⁺K]⁺⁻ |
| 87.      | 1271.40             | 1271.44           | 31                  | [G₁⁺⁺₃Na]⁺⁻     |
| 88.      | 1276.39             | 1276.39           | 31                  | [G₁⁺⁺₂H⁺Na⁺Na⁺K]⁺⁻ |
| 89.      | 1280.46             | 1280.43           | 23                  | [G₁⁺⁺₂H⁺₂K]⁺⁻   |
| 90.      | 1281.90             | 1281.93           | 23                  | [G₁⁺⁺₂Na⁺₂K]⁺⁻   |
| 91.      | 1317.47             | 1317.51           | 30                  | [G₁⁺⁺Na⁺Na⁺K]⁻   |
| 92.      | 1323.44             | 1323.46           | 15                  | [G₁⁺⁺Na⁺Na⁺K]⁺⁻ |
| 93.      | 1325.45             | 1325.49           | 30                  | [G₁⁺⁺Na⁺K]⁻     |
| 94.      | 1328.46             | 1328.50           | 30                  | [G₁⁺⁺₂Na⁺]⁻     |
| 95.      | 1337.38             | 1337.40           | 14                  | [G⁺⁺Na⁺]⁻     |
| 96.      | 1353.44             | 1353.47           | 22                  | [G₁⁺⁺₂H⁺₂Na⁺]⁺⁻ |
| 97.      | 1357.25             | 1357.21           | 29                  | [G₁⁺⁺₂H⁺Na⁺Na⁺K]⁺⁻ |
| 98.      | 1372.41             | 1372.45           | 29                  | [G₁⁺⁺₂Na⁺⁺₂K]⁺⁻ |
| 99.      | 1398.48             | 1398.53           | 35                  | [G⁺⁺Na⁺Na⁺]⁻     |
| 100.     | 1406.48             | 1406.52           | 28                  | [G⁺⁺Na⁺K]⁻     |
| 101.     | 1409.48             | 1409.52           | 28                  | [G⁺⁺₂Na⁺]⁻     |
| 102.     | 1417.47             | 1417.51           | 28                  | [G⁺⁺Na⁺Na⁺K]⁻   |
| 103.     | 1432.95             | 1433.00           | 34                  | [G⁺⁺₃H⁺⁺K]⁺⁻   |
| 104.     | 1438.45             | 1438.49           | 27                  | [G⁺⁺₂H⁺Na⁺Na⁺K]⁺⁻ |
| 105.     | 1480.15             | 1480.19           | 27                  | [G⁺⁺₂H⁺₂Na⁺]⁻   |
| 106.     | 1487.51             | 1487.55           | 26                  | [G⁺⁺Na⁺K]⁻     |
| 107.     | 1490.51             | 1490.55           | 26                  | [G⁺⁺₂Na⁺]⁻     |
| 108.     | 1499.58             | 1499.63           | 33                  | [G⁺⁺Na⁺]⁻     |
| 109.     | 1568.53             | 1568.57           | 25                  | [G⁺⁺H⁺⁺K]⁺⁻   |
| 110.     | 1571.44             | 1571.49           | 31                  | [G⁺⁺₂Na⁺]⁻     |
| 111.     | 1661.46             | 1661.51           | 30                  | [G⁺⁺Na⁺]⁻     |
| 112.     | 1733.49             | 1733.54           | 28                  | [G⁺⁺₂Na⁺]⁻     |
| 113.     | 1865.54             | 1865.58           | 21                  | [G⁺⁺₃Na⁺]⁻   |
A straightforward electrospray ionization high resolution mass spectrometry method by sample spraying in pure water without deliberate addition of alkali metal compounds. This is important since without cationization the analysis of undervatized polysaccharides in pure water would not be feasible.

In the screening mass spectrum of Fig. 1 the major distribution corresponds to doubly charged molecules of $[G_n^\pm + H^+ Na^\pm]^{2+}$, $[G_n^\pm + H^+ K^\pm]^{2+}$ or $[G_n^\pm + 2Na^\pm]^{2+}$, where $n$ represents the number of glucose repeats. Also, a small number (four) of $[G_n^\pm + Na^\pm K^\pm]^{2+}$ ions are present as signals of fair intensity at $m/z$ 769.33, 1,093.37, 1,255.43 and 1,417.47 corresponding to $n$ = 9, 13, 15, and 17. In addition, a series of signals attributed to lower and higher molecular weight triply and quadruply charged oligomers are observed. The highest molecular weight ions are two quadruply charged species containing 35 glucose residues (Table 1). Ions at $m/z$ 1,432.95 and 1,438.45 were assigned to $[G_{35}^\pm + 3H^+ K^\pm]^{4+}$ and $[G_{35}^\pm + 2H^+ Na^\pm K^\pm]^{4+}$. These correspond to the maximum molecular weight of 5,690 Da detected in Dex 4.

Previously, a Dex 5 mixture dissolved in water/methanol has been screened by direct infusion using a LCQ ion trap with electrospray injection. The longest chain detected contained 26 glucose residues [10]. As compared to earlier studies, in this work nanoESI using pure water allowed the formation of multiply charged ions, improving the ionization and detection of longer polysaccharides. Moreover, the sensitivity represents another major advantage of nanoESI. The flow rate was about 300 nLmin$^{-1}$, requiring only 1.5 pmol of Dex 4 for a full scan mass spectrum.

### 3.2 Fragmentation analysis by MS/MS

To confirm the polysaccharide structures in Dex 4 the doubly sodiated $[G_n^\pm + 2Na^\pm]^{2+}$ at $m/z$ 1,409.48 was isolated within a window with LM 10 and HM 10 and fragmented by MS/MS. The spectrum generated by varying the collision energy within 30-60 eV is depicted in Fig. 2. The high number of sequence ions observed show that the ionization and fragmentation conditions were optimized to generate high sequence coverage and complete structural data.

Because of the aglycon absence, symmetry, and repetitive structure of the molecule, discrimination between the reducing (Z ions) and non-reducing ends (B ions) is not feasible. The spectrum contains the whole series of singly charged B- and/or Z-fragments [20],
from B/Z at m/z 163.27 to B/Z at m/z 1,805.55. The 162 Da intervals correspond to the repeating glucose unit. Alongside the B/Z series, a large number of C and/or Y fragment ions from C/Y at m/z 203.27 up to C/Y at m/z 1,823.57 were formed. Sodiated singly charged fragment ions of higher masses, such as B/Z, B/Z, B/Z and C/Y, and C/Y at m/z 1,481.40, 1,643.45, 1,805.55, 1,661.46 and 1,823.57, are also observed at lower intensity, still detectable by the high sensitivity nanoelectrospray QTOF MS.

The fragmentation of [G_17+2Na]^2+ at m/z 1,409.47 produced doubly charged fragments such as C/Y, C/Y, and C/Y at m/z 761.33, 1,247.45 and 1,328.48. The spectrum also shows the whole series of monoprotonated monopotassiated doubly charged fragments from B/Z to B/Z at m/z 182.26, 263.30, 344.20, 425.23, 506.30, 58749 and their C/Y to C/Y counterparts at m/z 272.31, 353.20, 434.21.

The triply charged [G_20+H+Na+K]^3+ at m/z 1,107.35 was examined at 50 V cone voltage, 2.6 kV capillary voltage and 30-60 eV collision energy. Fig. 3 shows that [G_20+H+Na+K]^3+ followed the same fragmentation pathway as [G_17+2Na]^2+. These conditions generated fairly intense signals for the entire sodiated B/Z series together with the counterpart C/Y series. MS/MS of the triply charged ion generated a series of monoprotonated monopotassiated doubly charged ions from m/z 182.22 to 749.42 for the B/Z series, including the two most abundant ions at m/z 263.28 (B/Z) and 344.21 (B/Z) and from m/z 272.29 to 920.35 for the C/Y series. The interval between these doubly charged ions is 81, corresponding to half of the repeating glucose unit mass. The doubly sodiated dehydrated ion at m/z 497.21 corresponding to C/Y and a monoprotonated monosodiated ion at m/z 912.36 corresponding to C/Y also were formed. Interestingly, a series of B/Z and C/Y sodiated ions could be detected between m/z 1300-2200.

The most important aspect of the fragmentation process is the formation of triply charged ions at m/z 783.30, 893.33, 1,048.02 and 1,053.39 corresponding to [C/Y+H+Na+K]^3+, [C/Y+3Na]^3+, [C/Y+H+2Na]^3+, and [C/Y+H+Na+K]^3+, since these ions unambiguously show the presence of 20 Glc repeats.

To test the method’s feasibility and sensitivity we characterized the longest polysaccharide chain detected in the MS screen of Dex 4.
Figure 4: Positive nanoESI QTOF MS/MS of the m/z 1,438.47 ion, corresponding to \([G_{35}+2H+Na+K]^+\) in Dex 4. Cone voltage 50 V. Capillary voltage 2.6 kV. Acquisition 1000 scans. MS/MS at 40-80 eV collision energy. Argon pressure: 12 p.s.i. a). zoomed area 100-1050; b). zoomed area 1050-2230.
According to an exact mass calculation, the ion with low abundance at \( m/z \) 1,438.47 corresponds to \([\text{Glc}_{17}^4 + 2\text{H} + \text{Na} + \text{K}]^{4+}\). For MS/MS the ion was isolated within the narrow window of LM 10 and HM 10. Optimal fragmentation occurred at 40-80 eV collision energies. In view of the low signal intensity the MS/MS was accumulated for 1000 scans. Since the long chain fragmented to over 80 ions of different charges, the spectrum is presented in Figs. 4a (zoomed area \( m/z \) 100-1,050) and 4b (zoomed area \( m/z \) 1,050-2,230).

Fragmentation of the \( G_n \) precursor followed the same pathway as the ions described above. It is characterized by the series from \( B/Y \) to \( B/Y \) and from \( C/Y \) to \( C/Y \). Most important, a number of \( C/Y \) type fragments originating from the doubly protonated, monosodiated and monopotassiated parent molecule exhibit the same feature, supporting the \([\text{Glc}_{17}^4 + 2\text{H} + \text{Na} + \text{K}]^{4+}\) structure at \( m/z \) 1,438.47. The sequence ions: \([\text{C}_{15}/\text{Y}_{15} + 2\text{H} + \text{Na} + \text{K}]^{4+}\) at \( m/z \) 709.27, \([\text{C}_{15}/\text{Y}_{15} + 2\text{H} + \text{Na} + \text{K}]^{4+}\) at \( m/z \) 790.30, \([\text{C}_{15}/\text{Y}_{15} + 2\text{H} + \text{Na} + \text{K}]^{4+}\) at \( m/z \) 871.33, \([\text{C}_{15}/\text{Y}_{15} + 2\text{H} + \text{Na} + \text{K}]^{4+}\) at \( m/z \) 952.35, \([\text{C}_{15}/\text{Y}_{15} + 2\text{H} + \text{Na} + \text{K}]^{4+}\) at \( m/z \) 1,114.40, \([\text{C}_{15}/\text{Y}_{15} + 2\text{H} + \text{Na} + \text{K}]^{4+}\) at \( m/z \) 1,195.39, \([\text{C}_{15}/\text{Y}_{15} + 2\text{H} + \text{Na} + \text{K}]^{4+}\) at \( m/z \) 1,276.37, \([\text{C}_{15}/\text{Y}_{15} + 2\text{H} + \text{Na} + \text{K}]^{4+}\) at \( m/z \) 1,357.23, \([\text{C}_{15}/\text{Y}_{15} + 2\text{H} + \text{Na} + \text{K}]^{4+}\) at \( m/z \) 1,397.94 containing from 17 to 34 Glc repeats clearly document the precursor chain length. The remaining quadruply, triply and doubly charged ions containing from 1 to 34 Glc residues also support the \( G_{35} \) structure.

4 Conclusions

We report the first high resolution method for screening and sequencing underivatized long chain polysaccharides. Its feasibility and advantages were demonstrated on a polydisperse mixture of glucose chains, \( i.e., \) dextran of average molecular weight 4,000.

The combination of the high nanoESI sensitivity with the high resolution and mass accuracy of the QTOF analyzer allowed an efficient ionization of long chain underivatized linear polysaccharides for the first time. Using pure water as solvent singly through quadruply charged ions were formed related to chains containing up to 35 Glc repeats. \( \text{Glc}_{35} \) represents the longest chain ionized and detected by electrospray without previous derivatization and/or separation.

MS/MS structural confirmation of three chains containing 17, 20 and 35 repeating Glc residues was achieved by varying the collision energy, inducing efficient ion formation with high sequence coverage and ions diagnostic for the chain length. MS/MS was successfully applied to fragmentation analysis of an underivatized chain containing 35 glucose repeats for the first time.

The simple and straightforward protocol developed here may have applications to analysis of even longer linear polysaccharides and branched glycans in their native state.

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Abbreviations

Dex 4: dextran with average molecular weight 4,000; Glc: glucose; \( G_n \): oligo- and polysaccharides; \( n=2 \div 35 \); CID: collision-induced dissociation; ESI: electrospray ionization; MS/MS: tandem mass spectrometry; TIC: total ion chromatogram; QTOF MS: quadrupole time-of-flight mass spectrometer/spectrometry; LM: low mass resolution; HM: high mass resolution.

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