Enhanced electrospray ionization Fourier transform ion cyclotron resonance mass spectrometry of long-chain polysaccharides

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A novel strategy was developed to extend the application of electrospray ionization (ESI) Fourier transform ion cyclotron resonance (FTICR) mass spectrometry (MS) to the analysis of long-chain polysaccharides. High molecular weight polydisperse maltodextrins (poly- α (1-4) glucose) and dextrans (poly- α (1-6) glucose) were chosen as model compounds in the present study. Increased ionization efficiency of these mixtures in the positive ion mode was achieved upon modification of their reducing end with nitrogen-containing groups. The derivatization method is based on the formation of a new C–N bond between 1,6-hexamethylenediamine (HMD) and the reducing end of the polysaccharide, which exists in solution as an equilibrium between the hemiacetal and the open-ring aldehyde form. To achieve the chemical modification of the reducing end, two synthetic pathways were developed: (i) coupling of HMD by reductive amination and (ii) oxidation of the hemiacetal to lactone, followed by ring opening by HMD to yield the maltodextrin lactonamide of 1,6-hexanediamine (HMMD). Amino-functionalized polysaccharides were analyzed by electrospray ionization Fourier transform ion cyclotron resonance mass spectrometry (ESI FTICR-MS) in the positive ion mode by direct flow injection. The hexamethylenediamine (HMD) and maltodextrin lactonamide of 1,6-hexanediamine (HMMD) moieties provide increased proton affinities which dramatically improve the detection of the long-chain polysaccharides by FTICR-MS. The present approach allowed for identification of single components in mixtures with prominent heterogeneity in the degree of polymerization (DP), without the need for chromatographic separation prior to MS. The high mass accuracy was essential for the unambiguous characterization of the species observed in the analyzed mixtures. Furthermore, molecular components containing up to 42 glucose residues were detected, representing the largest polysaccharide chains analyzed so far by ESI FTICR-MS.

Carbohydrates represent an important class of biopolymers exhibiting a high degree of structural complexity and functions.¹ Most secreted proteins are ubiquitously modified by covalent attachment of carbohydrates at specific sites²

and, thus, are involved in recognition by other binding molecules, prevention of aggregation, proteolytic stability, cellular adhesion of bacteria and viruses, and cell signaling.^{3–6} Specific structures have been identified as biomarkers of several diseases,^{7,8} while others play an essential role in fertilization, embryogenesis, blood clotting and cell cycle regulation.⁹ The structural diversity of naturally occurring carbohydrates is derived from the existence of a large number of monosaccharide building blocks with multiple linkage possibilities in their stereoisomers, enabling storage of a large amount of biological information.

In nature, carbohydrates are present as either polysaccharides or glycoconjugates containing the oligosaccharide chain covalently attached to an aglycon.¹⁰ Polysaccharides serve as either structural components or energy-storage molecules.

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Usually, polysaccharides form linear or branched homopolymers and heteropolymers bearing a large number of monosaccharide units joined by glycosidic bonds. Various natural carbohydrates, in particular polysaccharides, are readily available for chemical modification or as building blocks for the synthesis of hybrid materials. Recently, chemical surface modification of nonpolar polymer matrices with hydrophilic carbohydrates has been pursued in an effort to obtain block copolymers with amphiphilic properties which enable biochemical or biological reactions at the polymer surface.^{11,12} One example is the derivatization of poly(dimethylsiloxanes) with oligoglycans,¹³ in which the carbohydrate was covalently attached to the hydrophobic polymer backbone via C–N or C–O bonds at the reducing end. The classic approach to building a new C–N bond between a diamine and a glycan chain is aminolysis of the lactone ring created by oxidation of the polysaccharide reducing end. Lactone ring opening has been widely employed in formation of the C–N bond between an oligosaccharide and amines, such as: *N*-allylamine,^{14–16} 1,4-diaminobutane,¹⁷ *N*-hexylamine,¹² amino-poly(dimethylsiloxanes),¹³ monoamino-functionalized polystyrene (M_n 6300),¹⁸ and lactolactone,¹⁷ maltolactone¹² and maltoheptaonolactone.^{14–16,18} In this context, we have recently introduced a novel strategy for the chemical modification of maltodextrins and high molecular weight dextran by hexamethylenediamine (HMD) using reductive amination, which allows detection by ESI quadrupole time-of-flight (QTOF) mass spectrometry of chains containing over 40 monomer repeats.¹⁹ A rigorous analytical characterization is a prerequisite for the determination of the product distribution and molecular heterogeneity in such polymeric mixtures and represents a key step in the development of materials with well-defined physical and chemical properties.

In recent years, FTICR-MS using electrospray²⁰ and matrix-assisted laser desorption/ionization²¹ has become an invaluable tool for structural analysis of carbohydrates, as it offers precise results, high sensitivity and analytical versatility.^{22,23} Determination of the sugar composition is greatly enhanced by the high resolution and mass accuracy of FTICR-MS.^{24,25} Carbohydrate, and in particular, polysaccharide analysis by MS, however, represents a challenge due to the physicochemical properties exhibited by this class of compounds,²² which are usually overcome by derivatization of the sample.²⁶ Furthermore, decreasing ionization efficiency is correlated with increasing chain length and inversely with the degree of branching,^{22,23} hence the detection of large structures in complex mixtures becomes very difficult. Lately, several groups have developed strategies for the analysis of carbohydrates by MS, involving the use of nano-liquid chromatography on graphitized chips²⁷ or on amide columns²⁸ to achieve oligosaccharide separation prior to MS. A recent study²⁹ reported the ESI FTICR-MS characterization of *O*-glycopeptides bearing up to 51 monosaccharide residues at a single *O*-glycosylation site, which demonstrated that the presence of a moiety with high proton affinity, such as the peptide backbone, dramatically enhances the ESI FTICR-MS analysis of long carbohydrate chains.

We report herein the development of a novel protocol for the ESI FTICR-MS structural investigation of linear polysaccharides based on the modification of the reducing end with a functional spacer arm. Thus, 1,6-hexamethylenediamine (HMD) was coupled to maltodextrins and dextrans by: (i) classic aminolysis of the lactone ring yielding maltodextrin lactonamide of 1,6-hexanediamine (HMMD) and (ii) reductive amination. In this derivatization procedure the same linker – NH-(CH₂)₆-NH₂ – is attached to the hemiacetal carbon via a C–N bond (HMD) or a CO–NH bond (HMMD). Positive ion ESI FTICR-MS analysis was performed on the synthesis products and demonstrated a significant increase in the ionization efficiency for both product mixtures. These derivatization procedures allowed the identification of high molecular weight polydisperse maltodextrins and dextrans and the first detection by ESI FTICR-MS of an intact linear polysaccharide chain containing 42 monosaccharide repeat units.

EXPERIMENTAL

Materials and standards

Methanol and formic acid (98%) were purchased from Merck (Darmstadt, Germany) and used without further purification. Distilled and deionized water from a Milli-Q water system (Millipore, Bedford, MA, USA) was used for sample solution preparation. Sample solutions were dried in an model 5301 concentrator (Eppendorf, Wesseling-Berzdorf, Germany). Prior to MS analysis, the samples were centrifuged for about 30 min in a Biofuge 13 centrifuge (Heraeus Sepatech, Osterode/Harz, Germany). Dextran (MW 6000 Da, denoted D6000, from Fluka, Seelze, Germany), maltodextrins (Paselli MD20, denoted M1000, and Paselli MD6, denoted M3000, from AVEBE, Veendam, The Netherlands), 1,6-hexamethylenediamine (HMD), sodium cyanoborohydride, dimethylformamide (DMF), dimethyl sulphoxide (DMSO) (all purchased from Aldrich Chemical Co., Milwaukee, WI, USA) were used without further purification. Dialysis membranes (molecular weight cut-off (MWCO) 1000 and 3500) were purchased from Spectrum Europe B.V. (Breda, The Netherlands). The cation-exchange resin (Amberlite IR-120) and the 3 Å molecular sieve were from Sigma (Steinheim, Germany).

Sample preparation

Synthesis of the HMMD-linked polysaccharides

HMMD-linked polysaccharides were prepared following the strategy described by von Braunmühl and Stadler¹⁴ (Fig. 1).

In the first step a 50-mL round-bottomed flask equipped with a Teflon-coated magnetic stirrer and refrigerant was loaded with 3 g (1 mmol) of maltodextrin Paselli MD6 (M3000) dissolved in 50 mL of water. Then 100 mL of a 0.05 M solution of I₂ (in KI) and 50 mL 0.1 M NaOH were added dropwise over 48 h. The solution was concentrated to 50 mL under reduced pressure in a rotary evaporator (bath temperature <35°C) and then dialyzed against water (MWCO 1000). The resulting solution was passed through a strong cation-exchange column (2 cm i.d. × 50 cm) packed

Figure 1. Synthesis strategy of HMD-modified maltodextrins: iodine oxidation of the reducing end (step 1), followed by subsequent ring opening with HMD (step 2).

with Amberlite IR-120. The eluted fraction was lyophilized (yield: 98%).

In the second step to 5 mL of DMSO (dried over MS 4 Å molecular sieve) containing 1 g of maltodextrin lactone (0.33 mmol), 1,6-hexanediamine (1.2 g, 0.01 mol) (freshly distilled over BaO) was added. The reaction solution was stirred at 80°C for 3 days. The resulting mixture was then dialyzed against water (MWCO 1000), concentrated and precipitated with 100 mL absolute ethanol. The resulting powder was dissolved in 25 mL deionized water and purified through a strong cation-exchange resin (IR-120). The unreacted lactone polysaccharide was washed off with water and reused. The maltodextrin lactone amide of 1,6-hexanediamine (HMD) (Fig. 1) was desorbed with 10% ammonia solution. The eluate was concentrated to 50 mL under vacuum pressure (bath temperature <35°C) and dialyzed against water. Finally, the resulting solution was lyophilized to give 798 mg of a white powder of HMD-linked maltodextrin (yield: 80%).

Synthesis of the HMD-linked polysaccharides

HMD-linked maltodextrins and dextran were prepared following the strategy shown in Fig. 2, as previously described.¹⁹ A 50-mL round-bottomed flask equipped with a Teflon-coated magnetic stirrer and refrigerant was loaded with HMD (1.44 g, 12.0 mmol), sodium cyanoborohydride (0.6 g, 8.3 mmol), glacial AcOH (0.35 mL) and DMF (10 mL). The mixture was heated up to 85°C and maltodextrin Paselli MD 20 (M1000) or dextran D6000 (1.02 g, 1.2 mmol) was

added in small portions. The reaction mixture was stirred at 85°C for 24 h and then cooled down to room temperature. Subsequently, *p*-xylene (100 mL) was added and concentrated by vacuum distillation. The product obtained was dissolved in 50 mL deionized water and the polysaccharide mixture was precipitated with 200 mL absolute ethanol, filtered and washed with absolute ethanol and acetone. The resulting powder was dissolved in deionized water and passed through a strong cation-exchange column packed with Amberlite IR-120. The unreacted polysaccharide was washed off with water and reused. The HMD-linked polysaccharide was desorbed with 10% ammonia solution and concentrated under vacuum pressure to give 910 mg of a light yellow HMD-linked maltodextrin (HMD-M1000) powder or HMD-linked dextran (HMD-D6000).

ESI FTICR-MS

Mass spectrometry was performed on a Bruker Apex II Fourier transform ion cyclotron resonance mass spectrometer (Bruker Daltonik, Bremen, Germany) equipped with a 7.0 T superconducting actively shielded magnet (Magnex Scientific Ltd., Oxford, UK) and an Infinity™ cell. Gas-phase ions were generated from solution by micro-electrospray ionization (ESI) in the positive ion mode using an Apollo ion source (Bruker Daltonik), by applying a voltage of −3600 V between the metal-coated capillary entrance and the grounded needle. The voltage applied on the end cap was −3800 V. Nitrogen gas was used as a nebulizer at a pressure of 2 psi. The voltage at the capillary

Figure 2. Synthesis strategy of HMD-modified maltodextrins ($\alpha(1-4)$ glycosidic linkage) and dextran ($\alpha(1-6)$ glycosidic linkage), by reductive amination with HMD in the presence of NaBH_3CN as the reducing agent.

exit was 50–60 V. In this configuration the modified polysaccharide mixture, dissolved to a concentration of about $10 \text{ pmol}/\mu\text{L}$ (calculated for an average MW) in $\text{MeOH}/\text{H}_2\text{O}/\text{HCOOH}$ (50:49:1, v/v/v; pH 5.0) was infused by ESI into the FTICR mass spectrometer using a syringe pump operating at a flow rate of $3 \mu\text{L}/\text{min}$. The mass spectra were obtained in the positive ion mode by accumulation of 32–64 scans. The instrumental parameters were carefully optimized to ensure optimal ionization, desolvation, ion transport and trapping and to minimize the in-source ion decomposition. The electrospray-generated ions were accumulated for 0.5–1 s in the hexapole located after the second skimmer of the ion source, and then transferred into the ICR cell. External calibration was carried out using the monoisotopic masses of angiotensin I product ions formed by in-source fragmentation at a declustering potential of 150 V.

RESULTS AND DISCUSSION

In this study, two highly heterogeneous mixtures of linear glucose polymers, containing either $\alpha(1-4)$ (maltodextrin) or $\alpha(1-6)$ glycosidic linkages (dextran), were chosen as model compounds. These are characterized by the dextrose equivalent (DE) given by: $M_n = (100/\text{DE}) \times 162$, where M_n represents the average molecular weight of the polysaccharide mixture. Consequently, maltodextrins with low M_n have high DE values (the mixture Paselli MD20 has a $\text{DE} = 20$ and an M_n of 850), while mixtures with high M_n have low DE values (Paselli MD6 has a $\text{DE} = 6$ and an M_n of 2800). The average degree of polymerization (DP) represents an indication of the average number of monomeric glucose units contained in a polysaccharide chain and is obtained by dividing the M_n by the mass of the glucose (162.048 Da).

(+) ESI FTICR-MS of the HMMD-linked polysaccharides

The (+) ESI FTICR-MS analysis of the HMMD-modified M1000 maltodextrin mixture is presented in Fig. 3. The spectrum, acquired by applying an electrospray voltage of -3.6 kV , was summed over 32 scans which, at the flow rate of $3 \mu\text{L}/\text{min}$, is equivalent to approximately 30 pmol of analyte consumption for a mass-screening experiment. The expected DP of 5 for this dispersion was calculated by dividing the average MW of 850 Da, specified by the producers of Paselli MD20, by the mass of a glucose repeat unit (162.048 Da). The high signal-to-noise (S/N) ratio of this complex FTICR mass spectrum indicates that, following the modification of the reducing end with the HMMD linker, superior ESI efficiency could be achieved. The spectrum contains four major series of ions from various HMMD-M1000-related molecular species, detected as singly and doubly protonated molecules within an m/z range of 400–1300. An average mass accuracy of 8 ppm was observed. The most abundant series is represented by a singly charged envelope of six protonated molecules (m/z 457.2390, 619.2939, 781.3510, 943.4095, 1105.4608 and 1267.4913) separated by a mass interval of 162.05, the mass of the glucose repeat unit. These ions were assigned, based on the exact mass calculation, to HMMD-modified maltodextrin species containing from two to seven glucose building blocks, in addition to the modifying reducing end moiety. Molecular components with a DP ranging from 6 to 15 were observed as doubly protonated species forming a less abundant distribution indicated with an asterisk (*) in Fig. 3. The ions of the remaining (+2)-charge envelopes were assigned to monosodiated monoprotonated adducts (depicted with #) and to monodehydrated doubly protonated molecules (depicted with •), respectively, of the same DP as the doubly

Figure 3. Positive ESI FTICR-MS of the HMMD-M1000 mixture. Solvent system: MeOH/H₂O/HCOOH, (50:49:1), pH 5.0; sample concentration 10 pmol/μL (calculated for an average molecular weight of 850); capillary voltage: −3.6 kV; capillary exit: 50–60 V; flow rate: 3 μL/min; acquisition: 32 scans. Inset: structure of the HMMD-maltodextrin chain.

Table 1. Assignment of the major ions observed in the HMMD-M1000 sample

m/z (exp)	m/z (theor)	Species	Error (ppm)	Proposed structure
457.2390	457.2397	$[\text{M}+\text{H}]^+$	2	$\text{Glc}_2\text{-HMMD}$
553.2298	553.2294	$[\text{M}+2\text{H}]^{2+}$	1	$\text{Glc}_6\text{-HMMD}$
564.2214	564.2204	$[\text{M}+\text{H}+\text{Na}]^{2+}$	2	$\text{Glc}_6\text{-HMMD}$
619.2939	619.2925	$[\text{M}+\text{H}]^+$	2	$\text{Glc}_3\text{-HMMD}$
634.2579	634.2558	$[\text{M}+2\text{H}]^{2+}$	3	$\text{Glc}_7\text{-HMMD}$
645.2488	645.2467	$[\text{M}+\text{H}+\text{Na}]^{2+}$	3	$\text{Glc}_7\text{-HMMD}$
715.2866	715.2822	$[\text{M}+2\text{H}]^{2+}$	6	$\text{Glc}_8\text{-HMMD}$
726.2777	726.2732	$[\text{M}+\text{H}+\text{Na}]^{2+}$	6	$\text{Glc}_8\text{-HMMD}$
781.3510	781.3453	$[\text{M}+\text{H}]^+$	7	$\text{Glc}_4\text{-HMMD}$
796.3148	796.3086	$[\text{M}+2\text{H}]^{2+}$	8	$\text{Glc}_9\text{-HMMD}$
807.3065	807.2996	$[\text{M}+\text{H}+\text{Na}]^{2+}$	9	$\text{Glc}_9\text{-HMMD}$
877.3432	877.3350	$[\text{M}+2\text{H}]^{2+}$	9	$\text{Glc}_{10}\text{-HMMD}$
888.3350	888.3260	$[\text{M}+\text{H}+\text{Na}]^{2+}$	10	$\text{Glc}_{10}\text{-HMMD}$
943.4095	943.3981	$[\text{M}+\text{H}]^+$	12	$\text{Glc}_5\text{-HMMD}$
958.3718	958.3614	$[\text{M}+2\text{H}]^{2+}$	11	$\text{Glc}_{11}\text{-HMMD}$
969.3622	969.3524	$[\text{M}+\text{H}+\text{Na}]^{2+}$	10	$\text{Glc}_{11}\text{-HMMD}$
1039.4028	1039.3878	$[\text{M}+2\text{H}]^{2+}$	14	$\text{Glc}_{12}\text{-HMMD}$
1050.3922	1050.3788	$[\text{M}+\text{H}+\text{Na}]^{2+}$	13	$\text{Glc}_{12}\text{-HMMD}$
1105.4608	1105.4509	$[\text{M}+\text{H}]^+$	9	$\text{Glc}_6\text{-HMMD}$
1120.4264	1120.4142	$[\text{M}+2\text{H}]^{2+}$	11	$\text{Glc}_{13}\text{-HMMD}$
1131.4149	1131.4052	$[\text{M}+\text{H}+\text{Na}]^{2+}$	9	$\text{Glc}_{13}\text{-HMMD}$
1201.4588	1201.4404	$[\text{M}+2\text{H}]^{2+}$	15	$\text{Glc}_{14}\text{-HMMD}$
1267.4913	1267.5037	$[\text{M}+\text{H}]^+$	10	$\text{Glc}_7\text{-HMMD}$
1282.4828	1282.4630	$[\text{M}+2\text{H}]^{2+}$	15	$\text{Glc}_{15}\text{-HMMD}$

Figure 4. Positive ion ESI FTICR-MS of the HMMD-M3000 mixture; acquisition: 64 scans. Solvent system: MeOH/H₂O/HCOOH, (50:49:1), pH 5.0; sample concentration 10 pmol/μL (calculated for an average molecular weight of 2800); capillary voltage: −3.6 kV; capillary exit: 50–60 V; flow rate: 3 μL/min. Inset: structure of the HMMD-maltodextrin chain.

protonated species discussed above (Fig. 3). Under these conditions, the maximum DP observed by (+) ESI FTICR-MS analysis of the HMMD-1000 mixture was 15. The most abundant ion in Fig. 3 was assigned to a molecule with a composition of Glc₃-HMMD while the ion exhibiting the lowest abundance was assigned to Glc₁₅-HMMD. The major molecular species detected in the HMMD-M1000 mixture are presented in Table 1. Hence, the overall distribution of the ionic species and their abundances is in agreement with the M_n of 850 Da specified by the manufacturer of the Paselli

M20 product, which demonstrates that no or little variation in the ionization efficiency of individual components occurred.

The reaction product HMMD-M3000 was analyzed by (+) ESI FTICR-MS under identical solution and instrumental settings as the HMMD-M1000 mixture. Because of the increased complexity of this mixture and the spray stability, signal was accumulated for 64 scans. Under these conditions, about 60 pmol of polysaccharide mixture were used, which still places the measurement sensitivity in the low- to

Table 2. Assignment of the major ions observed in the HMMD-M3000 sample

<i>m/z</i> (exp)	<i>m/z</i> (theor)	Species	Error (ppm)	Proposed structure
796.3085	796.3086	[M+2H] ²⁺	0	Glc ₉ -HMMD
877.3366	877.3350	[M+2H] ²⁺	2	Glc ₁₀ -HMMD
958.3647	958.3614	[M+2H] ²⁺	3	Glc ₁₁ -HMMD
1039.3931	1039.3878	[M+2H] ²⁺	5	Glc ₁₂ -HMMD
1120.4223	1120.4142	[M+2H] ²⁺	7	Glc ₁₃ -HMMD
1201.4538	1201.4406	[M+2H] ²⁺	11	Glc ₁₄ -HMMD
1282.4820	1282.4670	[M+2H] ²⁺	12	Glc ₁₅ -HMMD
1363.5126	1363.4934	[M+2H] ²⁺	14	Glc ₁₆ -HMMD
1444.5431	1444.5198	[M+2H] ²⁺	16	Glc ₁₇ -HMMD
1525.5676	1525.5462	[M+2H] ²⁺	14	Glc ₁₈ -HMMD
1606.6017	1606.5726	[M+2H] ²⁺	18	Glc ₁₉ -HMMD
1687.6247	1687.5990	[M+2H] ²⁺	15	Glc ₂₀ -HMMD
1768.6578	1768.6254	[M+2H] ²⁺	18	Glc ₂₁ -HMMD

middle-picomole range. The resulting FTICR mass spectrum is shown in Fig. 4. The expected average DP is 18, calculated by dividing the M_n of 3000 Da by 162.05 – the mass of the monomeric glucose. The spectrum contains doubly protonated species comprising two envelopes associated with two major series of HMMD-M3000 molecular species. The first series, indicated with asterisks in Fig. 4, encompasses thirteen highly abundant doubly protonated molecules separated by an m/z interval of 81.02, which equals one-half of the mass of the Glc repeat unit. The ions in this series were assigned with an average mass accuracy of 10 ppm over the range m/z 800–1800 to HMMD-M3000 species with DPs ranging from 9 to 21, which is in agreement with the estimated mass dispersion for maltodextrin M3000. The maltodextrin chains associated with these ions are presented in Table 2. The most abundant molecular components observed were Glc_{13} , Glc_{14} , Glc_{15} and Glc_{16} -HMMD, around which the charge envelope is centered. The second series includes eight dehydrated species as $[\text{M}+\text{H}+\text{Na}]^{2+}$. According to mass calculation, these ions correspond to the HMMD-M3000 chains containing from 9 to 18 Glc residues.

Notably, maltodextrin species with DP higher than 7 were detected as doubly protonated species, while those with DPs

below 5 were observed only as singly protonated species (Figs. 3 and 4), indicating that the number of the monomeric units in a linear chain is important for the degree of protonation in addition to the basic group at the reducing end. It is probable that short chains do not accommodate more than one charge, due to electrostatic repulsion. Cationization by sodium was also observed in both spectra. However, only the doubly charged species were observed as sodium adducts and their abundance was comparable with that of the doubly protonated species. No sodiated adducts were observed for chain lengths ranging from 2 to 5, suggesting that the HMMD moiety, as a result of the enhanced proton affinity, is the charge carrier in these ionic species. In addition to the major ion series unambiguously attributed to HMMD-M1000 and M3000 chains, both spectra (Figs. 3 and 4) exhibit an accompanying sequence of several singly charged ions of lower abundance. According to their m/z values, these ions are also due to polysaccharides and have a composition Glc_n , with n ranging from 3 to 8 in the case of the HMMD-M1000 sample and from 5 to 9 in the case of HMMD-M3000. These could either represent species derived from unreacted starting material or arise from unspecific in-source fragmentation during the transfer of the analyte ions into the mass spectrometer.

Figure 5. Positive ion ESI FTICR-MS of the HMMD-M1000 mixture. Solvent system: MeOH/H₂O/HCOOH, (50:49:1), pH 5.0; sample concentration 10 pmol/μL (calculated for an average molecular weight of 850); capillary voltage: −3.6 kV; capillary exit: 50–60 V; flow rate: 3 μL/min; acquisition: 32 scans. Insets: structure of the HMD-maltodextrin chain and *N*-formylation as side reaction.

Figure 6. Positive ion ESI FTICR-MS of the HMD-D6000 mixture. Solvent system: MeOH/H₂O/HCOOH, (50:49:1), pH 5.0; sample concentration 10 pmol/ μ L (calculated for an average molecular weight of 5800); capillary voltage: -3.6 kV; capillary exit: 50–60 V; flow rate: 3 μ L/min; acquisition: 64 scans. Insets: structure of the HMD-dextran chain.

(+)ESI FTICR-MS of the HMD-linked polysaccharides

The ESI FTICR-MS conditions optimized for the detection of intact HMMD-modified species were applied without retuning to screen the HMD-linked polysaccharides. These complex mixtures contain the HMD-derivatized maltodextrin (HMD-M1000, MW 850) and dextran (HMD-D6000, MW 6000), with an expected average DP of 5 (HMD-M1000) and 36 (HMD-D6000). The FTICR mass spectra are presented in Fig. 5 (HMD-M1000) and Fig. 6 (HMD-D6000). Each spectrum represents the sum of 64 scans which, at the sample concentration of 10 pmol/ μ L (calculated for M_n values of 850 and 5800, respectively), is equivalent to a sample consumption of 60 pmol. The spectrum (Fig. 5) is more complex than that observed for the corresponding HMMD mixture, and it contains singly and doubly protonated species belonging to the major HMD-M1000 maltodextrin chains. The doubly protonated series is significantly more abundant for this derivative than for the HMMD derivative. The ions in both series were assigned with an average mass accuracy of 5 ppm over the m/z 400–1300 range to Glc_{*n*}-HMD species with *n* ranging from 2 to 14, which corresponds to a mass dispersion within the mass range 444–2388 Da. Two additional series of ions were observed in this experiment (Fig. 5): a singly charged envelope (indicated with black triangle) and a doubly

charged envelope (indicated with #), both indicating a mass increase of 28 Da compared with the Glc_{*n*}-HMD components detected as the singly and doubly protonated molecules. These ions are probably attributable to side products resulting from *N*-formylation of the Glc_{*n*}-HMD products by DMF (side reaction, inset Fig. 5) during the synthetic processes. This probably arises by addition of a formyl group at the terminal amino group, hence explaining the effective mass increase of 28 Da; the molecular composition derived from the accurate mass measurement is consistent with the theoretical composition of the *N*-formyl side products. Overall, the doubly protonated molecule assigned to Glc₆-HMD represents the most abundant ion of the mixture, followed by the species Glc₇-HMD and Glc₈-HMD.

The mass spectrum of HMD-D6000 (Fig. 6) also reflects an increased proton affinity for the HMD vs. HMMD derivative as evidenced by the high abundance of triply protonated molecules. This observation can obviously be of great utility in the FTICR-MS analysis of long carbohydrate chains. The eighteen different HMD-dextran species and their molecular compositions, reliably assigned with an average mass accuracy of 12 ppm within the m/z 1200–2500 range, are presented in Table 3. In this m/z range 36 different triply charged species were detected, forming two distinct (+3) charge envelopes: 18 triply protonated (indicated with § in Fig. 6) and their 18 doubly protonated, monosodiated

Table 3. Assignment of the major ions observed in the HMD-D6000 sample

<i>m/z</i> (exp)	<i>m/z</i> (theor)	Species	Error (ppm)	Proposed structure
1390.8399	1390.8302	[M+3H] ³⁺	7	Glc ₂₅ -HMD
1444.8622	1444.8478	[M+3H] ³⁺	10	Glc ₂₆ -HMD
1498.8848	1498.8654	[M+3H] ³⁺	13	Glc ₂₇ -HMD
1552.8690	1552.8830	[M+3H] ³⁺	9	Glc ₂₈ -HMD
1606.9182	1606.9006	[M+3H] ³⁺	11	Glc ₂₉ -HMD
1661.8983	1660.9182	[M+3H] ³⁺	12	Glc ₃₀ -HMD
1715.9615	1714.9358	[M+3H] ³⁺	15	Glc ₃₁ -HMD
1769.9711	1768.9534	[M+3H] ³⁺	10	Glc ₃₂ -HMD
1822.9874	1822.9710	[M+3H] ³⁺	9	Glc ₃₃ -HMD
1877.0049	1876.9886	[M+3H] ³⁺	9	Glc ₃₄ -HMD
1931.0370	1931.0062	[M+3H] ³⁺	16	Glc ₃₅ -HMD
1985.0516	1985.0238	[M+3H] ³⁺	14	Glc ₃₆ -HMD
2039.0169	2039.0414	[M+3H] ³⁺	12	Glc ₃₇ -HMD
2093.0814	2093.0590	[M+3H] ³⁺	11	Glc ₃₈ -HMD
2147.0988	2147.0766	[M+3H] ³⁺	10	Glc ₃₉ -HMD
2201.1236	2201.0942	[M+3H] ³⁺	13	Glc ₄₀ -HMD
2255.1509	2255.1118	[M+3H] ³⁺	17	Glc ₄₁ -HMD
2309.1648	2309.1294	[M+3H] ³⁺	15	Glc ₄₂ -HMD

counterparts (depicted with black circle). The *m/z* interval between the ions of these series is 54.01, which is consistent with one-third the mass of the glucose repeat. The compositions, based on mass measurements, correspond to the triply protonated and the doubly protonated monosodiated adducts of the Glc_n-HMD with *n* = 25–42. It can be seen that even after thorough instrument calibration a large number of ions could be assigned to their corresponding structures only at a mass accuracy above 10 ppm. This mass deviation can be rationalized by space-charge effects which, according to previous findings,^{30–32} occur when a high number/type of ions are present within narrow *m/z* ranges.

In the spectrum shown in Fig. 6 no *N*-formyl side products were observed, as DMSO was used as the solvent instead of DMF for the synthesis of HMD-D6000. Dextran species with DP ranging from 29 to 38 were observed with similar abundance in our experiment and this pattern is in agreement with the calculated average DP of 37 for the D6000 mixture. It is worthwhile mentioning that the FTICR-MS detection of such large linear polysaccharide chains was accompanied by minimal in-source decomposition and this aspect is essential for the estimation of the real distribution of polysaccharide molecular species in complex mixtures. Remarkably, for large carbohydrate molecules, the HMD linker appears to favor multi-charging, as no singly protonated or sodiated species were detected in the spectrum shown in Fig. 6. A beneficial consequence of this experimental result is the expansion of the detection limit to the 42-mer (detected as [M+3H]³⁺ at *m/z* 2309.2349), which represents the longest polysaccharide chain confirmed so far by ESI FTICR-MS.

CONCLUSIONS

In this study, we demonstrated the applicability of ESI FTICR-MS to the analysis of large linear polysaccharides by introducing a chemical procedure based on derivatization with hexamethylenediamine and reductive amination. The protocol, consisting of reducing end alteration through a new

C–N bond between hexamethylenediamine and the terminal monosaccharide, was applied to high molecular weight polydisperse maltodextrins/dextrans using either a reductive amination reaction or aminolysis of the lactone ring.

Amino-derivatized polysaccharides exhibiting degrees of polymerization ranging from 2 to 42 could be analyzed by ESI FTICR-MS in the positive ion mode due to the high proton affinities of the attached HMD and HMMD groups, which significantly increased the ionization efficiency. Hence, the first detailed investigation of long-chain polysaccharides at high resolution and mass accuracy was accomplished. Optimized ESI FTICR-MS was able to provide a reproducible compositional mapping of HMMD-M1000 and M3000, as well as HMD-M1000 and D6000 polysaccharide mixtures, without the need for chromatographic and/or electrophoretic separation off- or on-line MS. In the HMD-D6000 mixture we were able to identify, with an average mass accuracy of 10 ppm, chains containing up to 42 Glc residues, which represent the largest polysaccharide detected so far by ESI FTICR-MS.

Compared with the classical derivatization method, permethylation, the protocol developed here renders a similar increase in the ionization efficiency being, however, simpler and more easily applicable to long carbohydrate chains. Therefore, it is recommended for the characterization of large polysaccharides lacking easily ionizable groups, which, in their native form, can be analyzed by electrospray ultrahigh resolution mass spectrometry only with significantly more difficulty.

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