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Ionic Liquid Matrix for Direct UV-MALDI-TOF-MS Analysis of Dermatan Sulfate and Chondroitin Sulfate Oligosaccharides

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Polyanionic oligosaccharides such as dermatan sulfate (DS) and chondroitin sulfate (CS) exhibit poor ionization efficiencies and tend to undergo thermal fragmentation through the loss of SO_3 under conventional ultraviolet matrix-assisted laser desorption/ionization (UV-MALDI) conditions. A new ionic liquid matrix (ILM), a guanidinium salt of α-cyano-4-hydroxycinnamic acid, facilitates direct UV-MALDI mass spectrometric (MS) analysis of underivatized DS and CS oligosaccharides up to a decasaccharide in their common form as sodium salts. The resulting mass spectra show very low extent of fragmentation through an SO_3 loss. The new ILM is suitable for MALDI-MS analysis of mixtures containing oligosaccharides with different numbers of sulfo groups.

The use of room-temperature ionic liquids as matrices is a fast growing area of matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) method development. Ionic liquid matrices (ILMs) have a number of advantages over conventional crystalline matrices including the homogeneity of the analyte−matrix mixture and high vacuum stability of the matrix.[1,2] ILMs have been shown effective in MALDI-MS analysis of several classes of biological molecules including peptides and proteins,[3] phospholipids,[4] oligonucleotides,[5] and neutral oligosaccharides.[6] The goal of this work was to develop a facile MALDI-time-of-flight (TOF)-MS method for the characterization of glycosaminoglycan (GAG)-derived oligosaccharides using an ILM.

When analyzed by mass spectrometry, GAG-derived oligosaccharides have a tendency to fragment through the loss of SO_3 (Δ −102 m/z). In electrospray ionization (ESI) mass spectrometry, these polysulfated, polycarboxylated oligosaccharides often result in several peaks, corresponding to intact oligosaccharide and fragments due to the SO_3 loss,[7] each of which can be sodium-cationized, protonated, or both and have various charge states.[8] The advantage of MALDI is that multiply charged species are not observed in the reflector mass spectra,[9] simplifying data interpretation as compared to the ESI mass spectra. However, due to their thermal lability, poor ionization efficiency, and a tendency to undergo extensive Na/H exchange, polyanions such as GAG-derived oligosaccharides are rarely analyzed by MALDI-MS, and methods for such analysis are scarce.

To date, a very few methods have been reported for MALDI-MS analysis of underivatized GAG-derived oligosaccharides.[10−12] A peptide complexation method described by Juhasz and Biemann[13,14] allows the detection of polysulfated oligosaccharides by MALDI-MS in a form of noncovalent complexes with basic peptides, $(Gly\text{-}Arg)_n$, where n exceeds the number of sulfo groups in the oligosaccharide by one. While this method is sensitive and affords MALDI mass spectra in which SO_3 loss is suppressed,[15] it is limited by the availability of synthetic peptides. Recently, we reported a successful MALDI-MS analysis of sodium salts of an octasulfated disaccharide and an octasulfated pentasaccharide in their uncomplexed, underivatized form using 1-methylimidazolium α-cyano-4-hydroxycinnamate (ImCHCA)[16] and butylammonium 2,5-dihydroxybenzoate (DHBB)[6] as matrices.[17] We demonstrated that it is possible to achieve ionization of uncomplexed, underivatized, polysulfated oligosaccharides under the UV-MALDI conditions using ILMs, but the problem of analyte fragmentation through a loss of SO_3 remained to be addressed. The present work introduces a new ionic liquid matrix, a 1,1,3,3-tetramethylguanidinium salt of α-cyano-4-hydroxycinnamic acid and demonstrates

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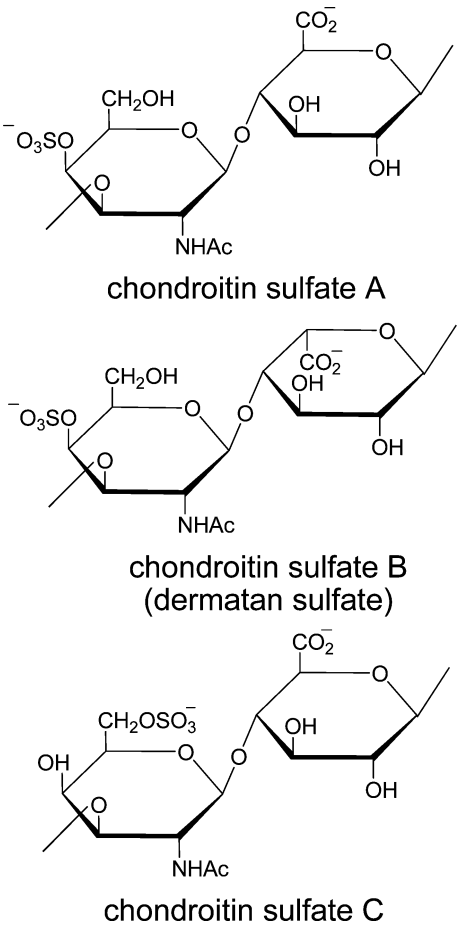

Figure 1. Disaccharide repeating units of chondroitin sulfate A, chondroitin sulfate B (dermatan sulfate), and chondroitin sulfate C.

its utility for direct MALDI-MS analysis of the dermatan sulfate and chondroitin sulfate oligosaccharides.

Chondroitin sulfate (CS) and dermatan sulfate (DS) are linear, sulfated polysaccharides, members of the GAG family.[18] The major disaccharide repeating unit of dermatan sulfate, which is also known as chondroitin sulfate B, is composed of *N*-acetyl-D-galactopyranose, which is most commonly sulfated on the 4-position, and occasionally on the 6-position, and L-idopyranosyluronic acid, which is sometimes sulfated on the 2-position or replaced by D-glucopyranosyluronic acid. The disaccharide repeating unit of chondroitin sulfate A (CSA, or chondroitin 4-sulfate) is GlcA-(α1−3)GalNAc4S(β1−4) and is a stereoisomer of the major disaccharide repeating unit of DS, IdoA(α1−3)GalNAc4S(β1−4). Chondroitin sulfate C (CSC), also known as chondroitin 6-sulfate, differs from CSA in that it is sulfated on the 6-position (Figure 1).

GAG-derived therapeutic agents have well-established antithrombotic activity.[19] A heparin pentasaccharide Arixtra (Fondaparinux Sodium) is a drug administered for prophylaxis of deep vein thrombosis in surgical patients.[20] Danaparoid, a mixture of low-molecular-weight dermatan sulfate, chondroitin sulfate, and heparan sulfate, is also used as the anticoagulant and does not have a side effect of heparin-induced thrombocytopenia.[21] In addition to their anticoagulant properties, GAGs mediate a number of cell signaling events through their interactions with proteins.[19,22] The number of disaccharide repeating units and number and position of functional groups in GAG oligosaccharides determine their primary and secondary structures and control the specificity of the GAG−protein binding.[22] Preparation and characterization of pure GAG oligosaccharides with the defined degree of polymerization (dp) and sulfation pattern is the key step in the structure−activity relationship studies involving GAG−protein interactions. The process of preparation of DS and CS oligosaccharides usually involves partial enzymatic depolymerization of a corresponding glycosaminoglycan by a specific lyase.[23,24] Chondroitin sulfate lyases cleave glycosidic linkages at the 4-position of uronic acid residues through an eliminase mechanism, which results in the formation of a C4−C5 double bond at the nonreducing end of the product. The unsaturated oligosaccharides have absorption maxima at 232 nm, and the extent of depolymerization can be estimated by measuring the UV absorbance of the digest.

In this report, a new ILM, bis-1,1,3,3-tetramethylguanidinium α-cyano-4-hydroxycinnamate (G_2CHCA) suitable for the direct analysis of purified polysulfated oligosaccharides up to a decasaccharide, as well as oligosaccharide mixtures was prepared. G_2-CHCA shows a high tolerance to the presence of buffer salts, facilitating the direct monitoring of partial enzymatic depolymerization of chondroitin 4-sulfate by MALDI-TOF-MS requiring submicroliter digest samples. The MALDI mass spectra, acquired using positive-ion detection, are of better quality than the negative-ion spectra. Mass spectra of DS and CS oligosaccharides are dominated by sodium-cationized molecular species and are virtually free of fragment peaks due to cleavage of O−SO_3Na bonds.

MATERIALS AND METHODS

Chemicals. Dermatan sulfate from porcine intestinal mucosa was purchased from Celsus Laboratories (Cincinnati, OH). Bovine tracheal chondroitin sulfate A, 70% (balance chondroitin sulfate C) and α-cyano-4-hydroxycinnamic acid (CHCA) were from Sigma (St. Louis, MO). Chondroitinase ABC (chondroitin ABC lyase, EC 4.2.2.4) from *Proteus vulgaris* was purchased from Seikagaku. 1,1,3,3-Tetramethylguanidine was from Acros. Gel permeation chromatography (GPC) was performed using Bio-Gel P2 and P10 (superfine) resins from Bio-Rad (Richmond, CA). All other reagents were analytical grade; all solvents used were HPLC grade.

Dermatan Sulfate Oligosaccharides. Dermatan sulfate (20 mg/mL) in 50 mM Tris-HCl/60 mM sodium acetate buffer, pH 8.0, was treated with chondroitin ABC lyase at 37 °C. When the absorbance at 232 nm indicated the digestion was 50% completed, the digestion mixture was heated at 100 °C for 3 min. High-molecular-weight products and protein were removed by ultrafiltration using a 5000 MWCO membrane. The resulting oligosaccharide mixture was concentrated by rotary evaporation and

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fractionated by low-pressure GPC on a Bio-Gel P10 (superfine) column (4.8 × 100 cm) using a 100 mM sodium chloride mobile phase at a flow rate of 1.5 mL/min. Fractions containing disaccharides, tetrasaccharides, hexasaccharides, octasaccharides, and decasaccharides were concentrated, desalted by GPC on a Bio-Gel P2 column (4.8 × 70 cm) using water as the mobile phase, and freeze-dried. The resulting size-fractionated oligosaccharide mixtures were characterized by PAGE, ESI-MS, and high-field nuclear magnetic resonance (NMR) spectroscopy. Further purification of DS oligosaccharides was achieved using strong anion-exchange high-pressure liquid chromatography (SAX-HPLC) on a semipreparative SAX S5 Spherisorb column (Waters) with a 0.1−2 M sodium chloride (pH 3.5) linear gradient elution. SAX-HPLC fractions containing purified oligosaccharides were collected, desalted by GPC, and freeze-dried.

Concentrations of oligosaccharide solutions used for MALDI-MS were determined by measuring the absorbance at 232 nm ($\epsilon = 3800\ M^{-1}$).[25] Samples used for MALDI-MS analyses were 30−100 μM.

Ionic Liquid Matrix. ILM was prepared by adding 2 equiv of 1,1,3,3-tetramethylguanidine to 1 equiv of CHCA dissolved in methanol followed by evaporating the solvent under vacuum overnight. The resulting solid was washed several times with acetone and dried at room temperature and atmospheric pressure. The matrix solution for MALDI-MS was prepared by dissolving 70−90 mg of G_2CHCA in 100% methanol. In our experience, the matrix concentration should be optimized for the particular instrument configuration, i.e., the ion source geometry, laser power, and laser frequency.

Mass Spectrometry. MALDI-TOF mass spectra were acquired on a Bruker Autoflex II instrument (Bruker Daltonics, Billerica, MA) equipped with a 337-nm nitrogen laser. Samples for MALDI analyses were spotted onto a ground steel target plate (Bruker part no. 209519) and analyzed in the reflector mode using factory-configured instrument parameters suitable for a 0−2 kDa m/z range: source 19 kV, extraction 17 kV, and reflector 20 kV. Time delay between laser pulse and ion extraction was set to 80 ns, and the laser frequency was 25 Hz. Laser power was adjusted to result in a strong analyte signal with minimal matrix interference and usually did not exceed 30 μJ.

ESI-MS was used as a complementary technique to characterize oligosaccharides or their mixtures prior to MALDI-MS analysis. ESI mass spectra were acquired on an Agilent 1100 LC/MSD instrument equipped with an ion trap mass analyzer. Samples were introduced through direct infusion through an autosampler. A 50% methanol/50% water mobile phase was delivered by an Agilent 1100 series binary pump at a flow rate of 0.2 mL/min. The drying gas flow was 5−10 L/min, the drying gas temperature was 350 °C, and the nebulizer gas pressure was 15−20 psi. The spray voltage was adjusted to result in minimum analyte fragmentation and typically was in a range of 3−3.5 kV.

Partially Depolymerized Chondroitin Sulfate Samples. Chondroitin ABC lyase was reconstituted with 0.1% BSA solution in a 50 mM Tris/60 mM sodium acetate buffer, pH 8 adjusted with HCl. A 5-μL aliquot of the enzyme solution (~0.5 mU) was combined with 250 μL of a 20 mg/mL CSA solution in the Tris-Cl/sodium acetate buffer. The reaction was placed in a dry block at 37 °C. Samples for MALDI-MS analysis were prepared by withdrawing 0.25-μL aliquots at 10-min intervals, mixing them with an equal volume of matrix solution, and applying this mixture to a stainless steel MALDI target.

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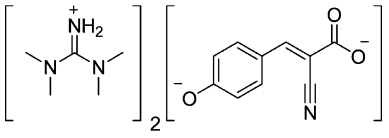

Figure 2. Structure of ionic liquid matrix G_2CHCA.

RESULTS AND DISCUSSION

Using MALDI-TOF-MS, we examined purified DS oligosaccharides as well as several DS and CS oligosaccharide mixtures: (1) mixtures containing equal amounts of purified DS oligosaccharides with increasing degree of polymerization from trisaccharide to decasaccharide (even-numbered oligosaccharides predominate in these mixtures with odd-numbered oligosaccharides derived only from the GAG chain ends), (2) size-fractionated mixtures containing DS oligosaccharides with narrow molecular-weight distribution, and (3) the CS partial enzymatic depolymerization samples containing, in addition to CS oligosaccharides, the undigested GAG, proteins, and buffer salts. Initial MALDI-TOF-MS experiments were performed in both negative-ion and positive-ion reflector modes. However, using the positive-ion detection permitted acquisition of mass spectra with less extensive Na/H exchange. Thus, all MALDI-MS data discussed below were acquired using positive-ion reflector mode.

Ionic Liquid Matrix. After our first report describing the use of ILMs for the MALDI-TOF-MS analysis of polysulfated oligosaccharides, we continued searching for ways to improve the quality of MALDI mass spectra acquired with these polyanions. We noticed that ILMs prepared using CHCA generally afford better detection sensitivity than those prepared using DHB. ILMs prepared using stronger organic bases generally resulted in a reduced fragmentation through a loss of SO_3 in the positive-ion detection mode. CHCA has two acidic protons in its structure, and it seemed reasonable to prepare two ILMs in which one or both acidic protons are replaced by 1,1,3,3-tetramethylguanidinium cation ($pK_a > 12$). Comparison of the resulting ILMs, GCHCA and G_2CHCA, showed that the latter affords higher sensitivity than any ILMs we tested for the analysis of sulfated oligosaccharides.

The new ILM, G_2CHCA, was solid at room temperature with a melting point of 169−170 °C, measured using an Electrothermal melting point apparatus. The structure of the new ILM (Figure 2) was confirmed by proton NMR spectroscopy. UV absorbance profiles of CHCA and G_2CHCA recorded using equimolar solutions of these compounds in methanol were identical in the range of wavelengths between 260 and 400 nm; and a 10 mg/mL aqueous solution of G_2CHCA had a pH of 9.

G_2CHCA was effective as a MALDI matrix in the analysis of compounds with various molecular weights and hydrophilicities and permitted acquisition of up to 400-shot single spectra from one physical spot without an observable deterioration of the analyte signal. Protein and peptide standards were routinely used with G_2CHCA for the instrument calibration. Comparison of ImCHCA and DHBB, which we had previously found effective in

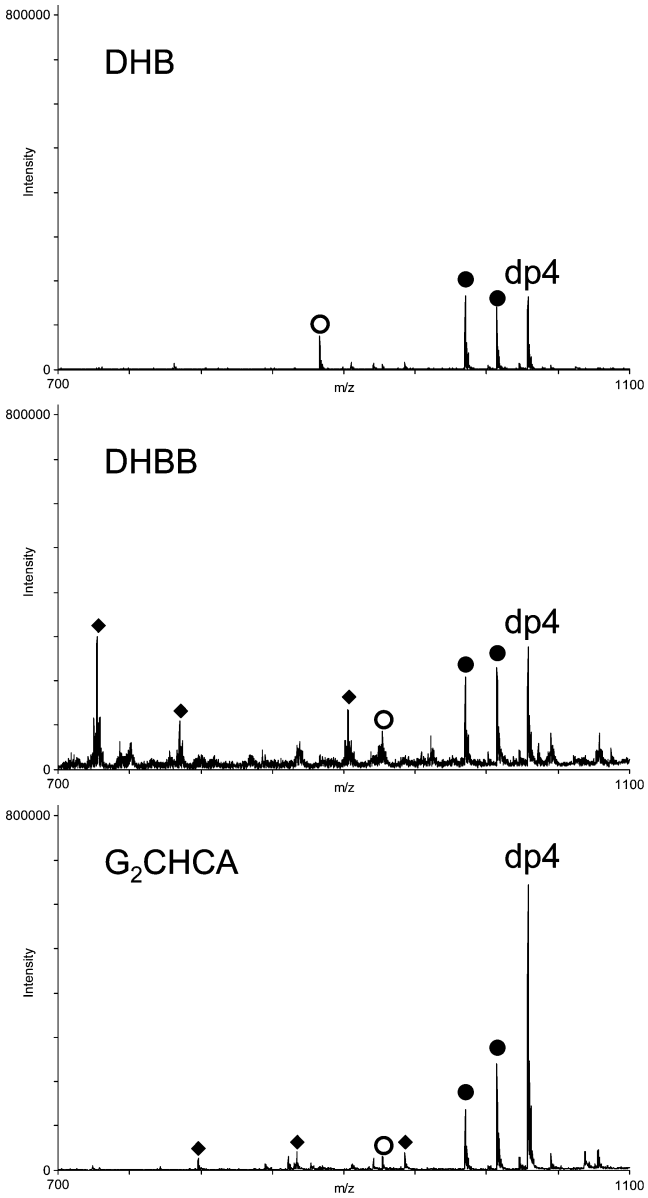

Figure 3. Positive-ion reflector MALDI mass spectra of purified dermatan sulfate tetrasaccharide (DS dp4) acquired with DHB (upper panel), DHBB (middle ,panel) and G_2CHCA (lower panel) under the same experimental conditions. The peak, corresponding to the DS dp4, sodium salt ($[M + Na]^+$, *m*/*z* 1029) is accompanied by Na/H exchange peaks, denoted by closed circles. Peaks corresponding to the SO_3 loss fragments are denoted by open circles; matrix clusters are denoted by diamonds.

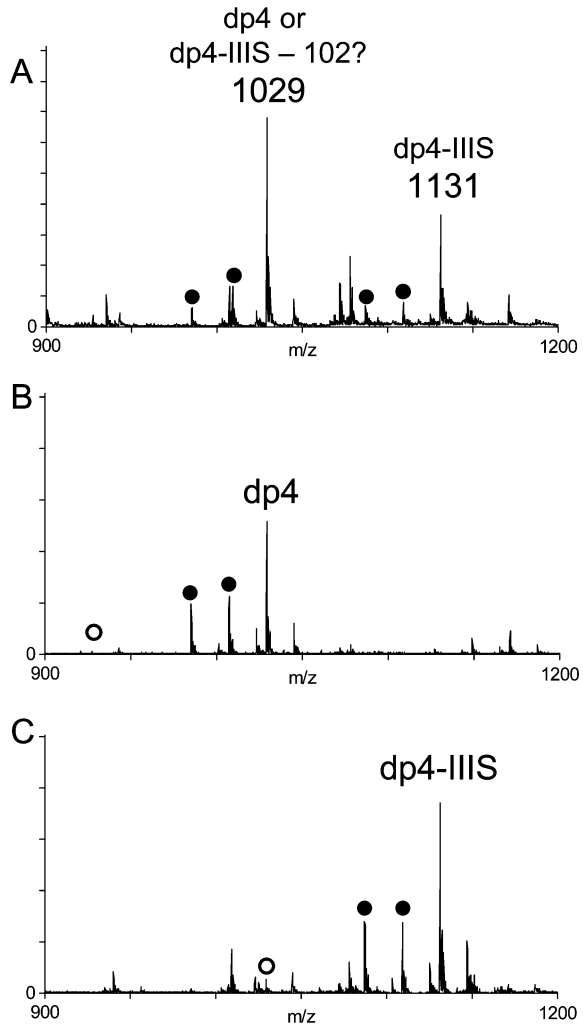

Figure 4. MALDI mass spectra of (A) an equimolar mixture of the purified DS dp4 and DS dp4-III*S* (trisulfated tetrasaccharide), (B) purified DS dp4, and (C) purified DS dp4-III*S*, acquired with G_2CHCA. Peaks corresponding to Na/H exchange are denoted by closed circles; SO_3 loss peaks are denoted by open circles. Unlabeled peaks are due to the matrix clusters.

the analysis of polysulfated oligosaccharides, showed that G_2-CHCA afforded a significant improvement in sensitivity. The improvement in the quality of the analyte signal observed in MALDI mass spectra acquired with G_2CHCA was due to both the increase in absolute signal intensity of the $[M + Na]^+$ peak and the increase in a signal intensity ratio of the $[M + Na]^+$ peak over the $[M + H - SO_3]^+$ peak. The use of DHBB or crystalline DHB for MALDI-MS analysis of DS oligosaccharides generated mass spectra containing more intense Na/H exchange peaks and the SO_3 loss peaks than observed with G_2CHCA (Figure 3).

The appearance of SO_3 loss fragments in mass spectra acquired with mixtures of oligosaccharides with different numbers of sulfo groups is undesirable because it creates ambiguity in the data interpretation. Using G_2CHCA for the analysis of DS and CS oligosaccharides afforded MALDI mass spectra with very low extent of thermal fragmentation through a loss of SO_3. MALDI mass spectra of the purified dp4 (Figure 4B) and dp4-III*S* (Figure 4C) suggest that a contribution from the signal corresponding to a dp4-III*S* sulfate loss fragment to the intensity of the dp4 peak is small. Thus, a higher signal intensity of the 1029 *m*/*z* peak compared to that of the 1131 *m*/*z* peak in the MALDI mass spectrum of the dp4/dp4-III*S* equimolar mixture (Figure 4A) apparently reflects the difference between MALDI efficiencies of the two oligosaccharides. The MALDI mass spectra of the dp4/dp4-III*S* equimolar mixture obtained using a conventional DHB preparation showed no assignable peaks.

Analysis of Purified DS Oligosaccharides. We first analyzed individual, purified DS oligosaccharides to establish how MALDI efficiency using G_2CHCA varies with increasing degree of polymerization. The purification avoids competing ionization of minor oligosaccharide components that are usually present in the size-fractionated mixtures and allows a more accurate assessment of MALDI efficiency of the major component.

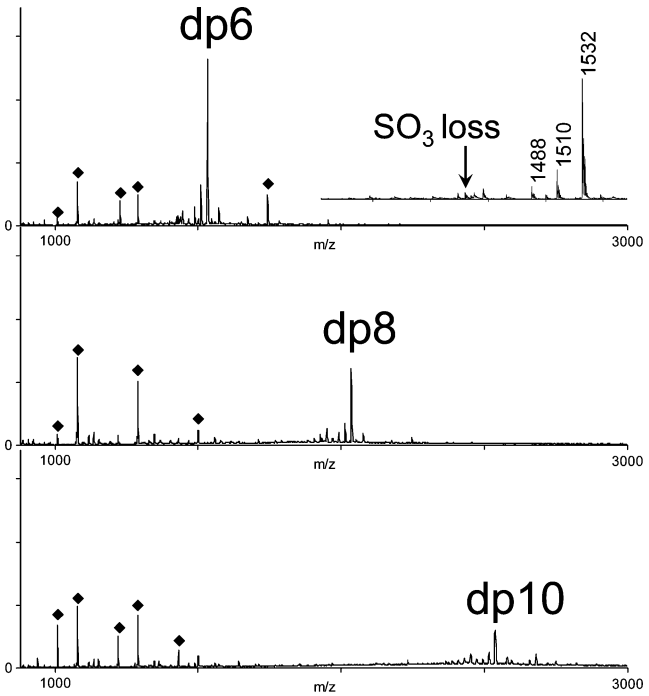

Figure 5. Positive-ion MALDI mass spectra of sodium salts of the DS hexasaccharide (dp6, *m*/*z* 1532), octasaccharide (dp8, *m*/*z* 2035), and decasaccharide (dp10, *m*/*z* 2537) acquired with G_2CHCA. Expansion of the DS dp6 mass spectrum shows Na/H exchange peaks ($\Delta = -22$ *m*/*z*) and a small peak corresponding to a loss of SO_3. A similar pattern was observed in the DS dp8 and DS dp10 MALDI mass spectra. Matrix peaks are denoted by diamonds.

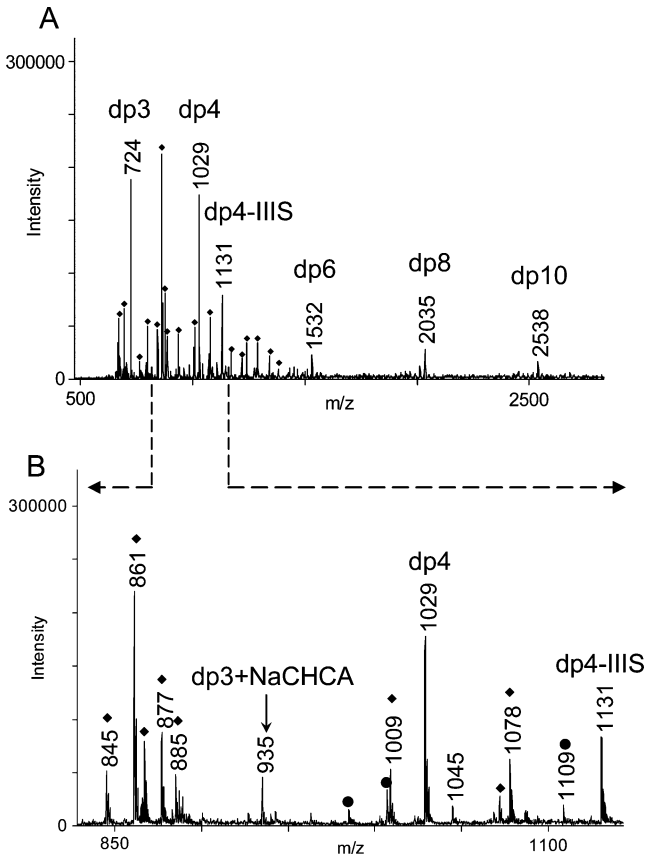

Figure 6. Positive-ion MALDI mass spectrum of a mixture of six purified DS oligosaccharides, 30 μM each, acquired with G_2CHCA (A) and an expansion of the same mass spectrum (B). Peaks marked with diamonds correspond to matrix clusters, $CHCA^-{}_nH_xNa_yK_z$, where $x + y + z = n + 1$. Analyte peaks corresponding to the Na/H exchange products are denoted by closed circles ($\Delta = -22$ *m*/*z*).

Under the same experimental conditions, MALDI efficiency of DS oligosaccharides in positive-ion and negative-ion modes decreased with increasing molecular weight and number of negative charges (Figure 5). This trend was particularly apparent for octasaccharide (dp8) and decasaccharide (dp10). We were able to acquire a mass spectrum of 0.2 mM solution of hexasulfated decasaccharide (dp10-VI*S*, *m*/*z* 2639), but at a 0.05 mM concentration, the isotope resolution of the $[M + Na]^+$ peak was too low for assigning the signal with confidence. Thus, at a 30−100 μM range of oligosaccharide concentrations, the detection of DS dp10 was the upper *m*/*z* limit achieved using G_2CHCA.

The evaluation of MALDI efficiency of DS disaccharide (dp2, *m*/*z* 526) was complicated by strong matrix interference in the low *m*/*z* region of the mass spectra. The matrix interference was observed not only with G_2CHCA and DHBB but also with crystalline DHB and seemed to arise due to the anionic nature of the analyte rather than the ionic nature of matrix. In our experience, the appearance of matrix cluster peaks and matrix adducts can be reduced by optimizing matrix-to-analyte ratio and matrix solvent system as well as using a suitable additive. For example, we found that Na/H exchange can be reduced by the addition of 0.5 μL of a 1−2 mM NaCl, NaOH, or both to 1 μL of the G_2CHCA/analyte mixture, although the addition of salt to the sample reduced the sensitivity of analysis.

Analysis of DS and CS Oligosaccharide Mixtures. (a) Mixtures of Purified DS Oligosaccharides. We prepared and analyzed several equimolar mixtures having different combinations of purified DS oligosaccharides. The trend in MALDI signal for the oligosaccharide mixtures was similar to that observed for the single-component oligosaccharide samples: smaller oligosaccharides with fewer sulfo groups exhibited higher signal intensities than larger oligosaccharides with a greater number of sulfo groups. DHB and G_2CHCA showed similar sensitivity in the analysis of mixtures containing dp3 (ΔUA (α1→3) GalNAc4S (β1→4) IdoA, *m*/*z* 724), dp4, and dp4-III*S*. However, DHB was ineffective in the analysis of mixtures containing oligosaccharides with dp>4. G_2CHCA was effective over a broad *m*/*z* range, facilitating the analysis of an equimolar mixture of six DS oligosaccharides from dp3 to dp10 at as low as 30 μM concentration of each individual component (Figure 6). MALDI-MS analysis of an equimolar mixture containing dp10 and dp10-VI*S* (a hexasulfated decasaccharide, *m*/*z* 2639) using G_2CHCA did not result in the detection of dp10-VI*S*; only the dp10 signal was detected.

(b) Size-Fractionated Mixtures of DS Oligosaccharides. Size-fractionated mixtures of DS oligosaccharides were obtained by GPC separation of the partially enzymatically depolymerized DS. GPC fractions corresponding to disaccharide, tetrasaccharide, hexasaccharide, octasaccharide, and decasaccharide were desalted and analyzed by SAX-HPLC and MALDI-MS using G_2CHCA (Figure 7).

The SAX-HPLC analysis indicated that GPC fractions were 50−70% pure. Based of the number of chromatographic peaks detected by UV absorbance at 232 nm, each size-fractionated mixture contained 3−4 major components (>10%) and a number of minor components. MALDI-MS analysis of the size-fractionated mixtures

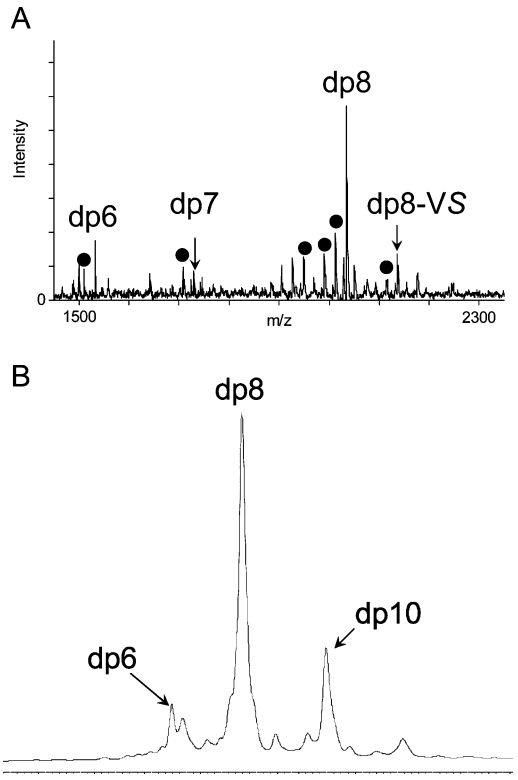

Figure 7. MALDI mass spectrum (A) and a SAX-HPLC trace (B) of a GPC fraction corresponding to dp8. The mass spectrum was acquired with G_2CHCA using a 30 μM total oligosaccharide concentration ($Abs^{232} = 0.1$). Peaks corresponding to Na/H exchange are denoted by closed circles.

permitted the detection of fewer components in a mixture but afforded higher analytical specificity and time efficiency. It should be noted that oligosaccharides having the same molecular weight but sulfated on different positions are indistinguishable by mass spectrometry but can be separated by chromatography.

The MALDI mass spectrum of a DS dp8 sizing fraction (Figure 7A) contained peaks corresponding to dp6, dp7 (a trisulfated unsaturated heptasaccharide), dp8, and dp8-V*S* (a pentasulfated octasaccharide). Three largest chromatographic peaks detected during the SAX-HPLC separation of the dp8 size-fractionated mixture (Figure 7B) were collected and analyzed by MS. The third chromatographic peak, corresponding to dp10, was not detected by MALDI-MS analysis of the size-fractionated mixture, even though it constituted more than 10% of the mixture.

Comparing the data obtained by SAX-HPLC and MALDI-MS suggests that the two techniques can be successfully used in tandem for the characterization of partial depolymerization mixtures. Using G_2CHCA as matrix affords sufficient sensitivity for the method to be useful for the routine analyses of oligosaccharide mixtures. The absence of SO_3 loss fragments and multiply charged species from the mass spectra make MALDI-MS detection an attractive alternative to the ESI-MS.

(c) CSA/CSC Digestion Samples. Glycomics experiments involving protein−GAG interactions are often carried out with partially depolymerized GAGs. Usually, the extent of a GAG depolymerization reaction is estimated using UV absorbance at 232 nm. This is often an empirical process and is both time-consuming and material-consuming. MALDI-MS cannot compete with UV spectroscopy in terms of cost efficiency, but it allows

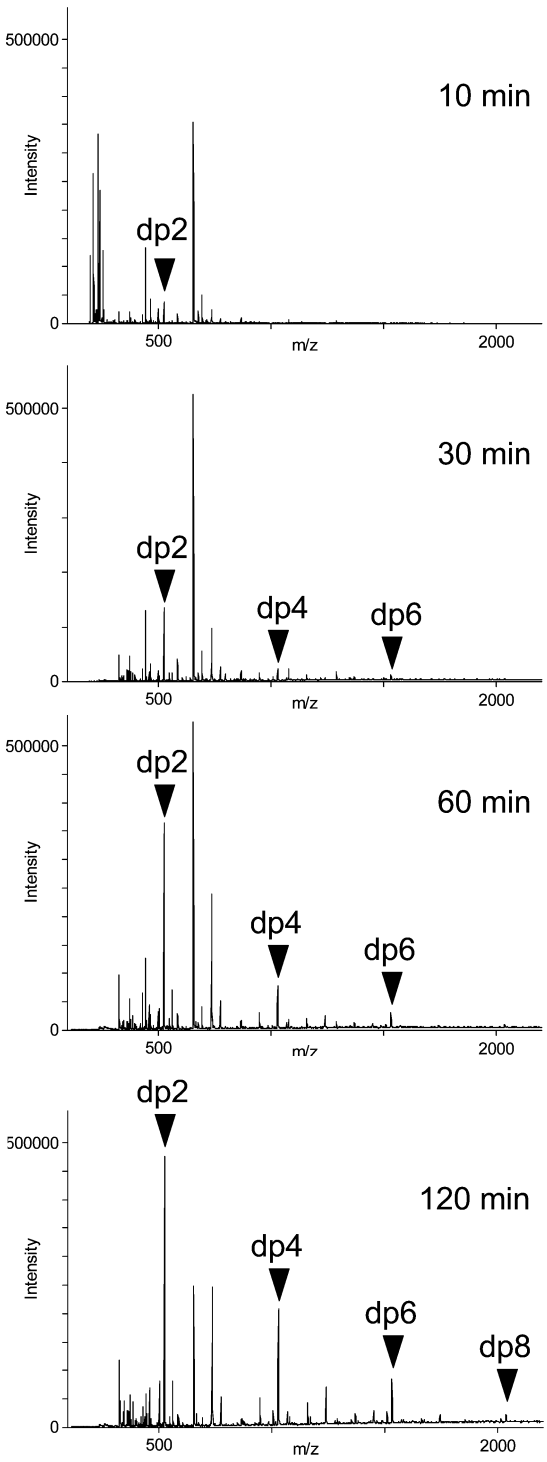

Figure 8. MALDI mass spectra of CS depolymerization reaction mixture acquired at several time points. Unlabeled peaks are matrix clusters.

acquiring specific information from submicroliter sample volumes in a time-efficient manner.

To demonstrate the utility of the G_2CHCA-based MALDI-MS method in monitoring the partial enzymatic depolymerization of GAGs, we carried out a model reaction on a microliter scale with the lyase and substrate concentrations that can be used for the gram-scale preparation. The reaction progress during the first 2 h was monitored by withdrawing 0.25-μL aliquots of the reaction mixture at 10-min intervals, mixing them with a G_2CHCA solution,

and applying this mixture to a target plate, followed by MALDI-MS analysis (Figure 8). The most prominent feature in the resulting mass spectra was the increase in signal corresponding to the CS disaccharide (526 m/z) as a function of reaction time, in accordance with the data obtained by analytical SAX-HPLC. Chromatographic traces of the CS digest obtained at timed intervals during the first 2 h showed an increase in the area of the two peaks attributed to CSA and CSC disaccharides, which served as an indication of the reaction progress. However, using the chromatography alone afforded very little if any information about composition of the reaction mixture, unless the appropriate standards were used for determining the retention times of oligosaccharides of interest. Based on the results presented here, MALDI-MS could be used as an alternative approach for determining the extent of CS depolymerization reaction with a greater time-efficiency than SAX-HPLC and a greater specificity than UV spectroscopy.

CONCLUSIONS

A new ILM, G_2CHCA, was introduced, having a number of practical advantages in carbohydrate research. This matrix facilitates the detection of uncomplexed, underivatized polysulfated, polycarboxylated oligosaccharides by MALDI-MS in their common form as sodium salts. Both purified single-component samples and mixtures of several DS and CS oligosaccharides can be successfully analyzed using G_2CHCA with reasonable sensitivity.

Thermal fragmentation through a loss of SO_3 is suppressed in the MALDI mass spectra of polysulfated, polycarboxylated oligosaccharides using the new ILM described here. The presence of Na/H exchange peaks in the mass spectra is apparently unavoidable under the experimental conditions and presents a minor drawback by comparison to SO_3 loss. Suppression of the Na/H exchange can be achieved by the addition of small amounts of salt to the MALDI sample but at the expense of sensitivity.

Future studies will be directed toward the characterization of more complex mixtures of GAG-derived oligosaccharides, such as heparin oligosaccharides, using the described MALDI-MS method. We will also further explore application of the method in the direct monitoring of enzymatic depolymerization reactions by MALDI-MS.

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