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Characterization of Neutral Oligosaccharide-Alditols from *Xenopus laevis* Egg Jelly Coats by Matrix-Assisted Laser Desorption Fourier Transform Mass Spectrometry

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Neutral oligosaccharides were released by alkaline sodium borohydride reduction of the jelly coating from the South African clawed toad, *Xenopus laevis.* The oligosaccharides were isolated by HPLC and analyzed by matrix-assisted laser desorption ionization (MALDI)-Fourier transform mass spectrometry (FTMS). The mass spectrometry analysis allowed confirmation of 12 structures first proposed by Strecker *et al.* using nuclear magnetic resonance. In addition, seven new oligosaccharides with weak abundances were found and characterized by mass spectrometry. A method for discriminating metastable fragments from quasimolecular ions is described. It involves doping the sample with cesium chloride. Cesium-coordinated oligosaccharides do not fragment as readily as those coordinated to sodium. Tandem MS experiments are performed on an unknown oligosaccharide illustrating the potential of MALDI-collision-induced dissociation-FTMS. © 1997 Academic Press

Mature unfertilized eggs of the South African clawed toad, *Xenopus laevis,* contain three transparent jelly coat layers (1). Similar coatings but varying in number and composition are found in many amphibian species (2, 3). The layers are known to have high carbohydrate contents, as much as 50% for some species (1, 4). Recent studies of the jelly coating of *X. laevis* have yielded the primary structures of several neutral and acidic oligosaccharides. In two key reports, neutral and acidic oligosaccharides were released by alkaline sodium borohydride reduction to the corresponding free alditol (5, 6). The oligosaccharides, purified and separated by high-performance liquid chromatography, were analyzed using NMR spectroscopy. In the study of the isolated neutral oligosaccharides by Strecker *et al.* (5), up to 12 oligosaccharide-alditols were isolated and structurally elucidated. The compounds ranged in size from a trisaccharide to a heptasaccharide. Several of the oligosaccharides contained structures that were unique to this species. Additionally, there were other minor components present in the HPLC separation that could not be further characterized due to small amounts of material.

Mass spectrometry (MS),[2] particularly when coupled with matrix-assisted laser desorption ionization (MALDI), has been shown increasingly to be a highly sensitive method for the analysis of oligosaccharides (7–14). MALDI-MS provides detection limits typically between picomole and femtomole, which is three to six orders of magnitude better than NMR. In addition, MS analysis is fast, lasting typically less than a few minutes for a measurement. At the present, MS can not provide the linkage and stereochemical information obtained by NMR. However, it can provide complementary information, such as the molecular weight, which can be used to confirm the assigned structure. In systems that have been well characterized by NMR, MS can also be used for accurate quantification. Because the limit of detection is significantly better than that for NMR, it can further provide information on minor components of mixtures that would otherwise be unobtainable.

In this paper, we perform mass spectrometry on the fractions separated by HPLC and liberated by alkaline borohydride reduction of the jelly coats of *Xenopus laevis.* Fourier transform mass spectrometry (FTMS) is

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[2] Abbreviations used: MS, mass spectrometry; MALDI, matrix-assisted laser desorption ionization; FTMS, Fourier transform mass spectrometry; DHB, dihydroxybenzoic acid; CID, collision-induced dissociation; MS^3, tandem MS.

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used to provide supporting evidence for the previously assigned structure (5) and to gain information on minor components not characterized by NMR. FTMS is a relatively new tool in the analysis of oligosaccharides. When combined with MALDI, FTMS provides several useful features, including ultra-high-resolution, accurate mass determination, and facile tandem MS capabilities (15–17). This report illustrates the first application of MALDI-FTMS to oligosaccharides of unknown structure.

MATERIALS AND METHODS

The oligosaccharides were released by using published methods (5). Briefly, the eggs were obtained by injecting human chorionic gontrophin in the frog. All three jelly coat layers were separated from the egg by adding mercaptoethanol. Dialysis of the jelly solution was performed in deionized water overnight. The jelly was then lyophilized resulting in 348 mg of dried material. This entire amount was reacted with a solution of 100 mM NaOH and 1.0 M $NaBH_4$ at 57°C for 23 h. Hydrogen-form Dowex 50 (40 g) was added to the mixture to quench the reaction. The solution was filtered and adjusted to pH 6.5 with a 0.1 M NaOH solution and lyophilized. Methanol was added and the solution distilled to remove boric acid as the methyl ester. This process was repeated four times. The resulting solution was purified on a Bio-Gel P2 column and the oligosaccharides were collected. A phenol–sulfuric acid assay (18) was used to determine the concentration of total carbohydrate, using galactose as a standard. The combined fractions was treated with a COO^--form Dowex 1 to remove acidic oligosaccharide alditols. MALDI/FTMS was performed on the mixture and it was found that some of the oligosaccharides coordinated with boron. The neutral oligosaccharides were again subjected to MeOH distillation six more times to remove residual amounts of boric acid. The final mixture of the neutral oligosaccharides was separated by HPLC on a primary amine-bonded silica (25.0 cm; Supelco, Bellefonte, PA) using a 75:25 acetonitrile:water mixture. A total of 70 fractions was collected and subjected to MALDI.

MALDI-FTMS was performed on a commercial instrument (IonSpec Corp., Irvine, CA). Operational and technical details of a similar instrument have been provided earlier (15–17). The important feature of the instrument includes the external MALDI source and a 4.7 T superconducting magnet. The samples were prepared by applying 20 μL, in 3-μL aliquots followed by evaporation, on the sample probe. A 1-μL solution of 0.01 M NaCl was added followed by 1 μL of 0.4 M 2,5-dihydroxybenzoic acid (DHB). The NaCl solution was used to enrich the Na^+ concentration and produce primarily sodiated species. DHB was used as matrix. A nitrogen laser operating at 337 nm was used for desorption.

RESULTS

The HPLC trace of the oligosaccharide mixture is shown in Fig. 1. For organization purposes, the peaks are grouped and labeled A–H. The HPLC trace obtained in this work closely matches that of Strecker *et al.* with the peaks labeled C in this work corresponding to peaks 1 and 2 in Strecker's. The group of peaks which elute between 6 and 9 min (A peaks) is composed of small molecules with molecular weight between 300 and 600 u and were not discussed by Strecker. Based on the MS data, we conclude that these compounds are not oligosaccharides and they were not further analyzed. The second group of peaks, B, is high-molecular-weight oligosaccharides complexed to boron, as indicated by the mass spectra. The short retention times are unusual for such high-molecular-weight oligosaccharides observed in the spectra, but consistent with a tightly folded structures resulting from the complexion of the oligosaccharide to boron. Consequently, it is extremely difficult to remove trace boron from the sample. We found further evidence that the complexes might dissociate during HPLC, providing a trace background of oligosaccharide contaminants throughout the separation.

The MALDI-FTMS spectrum of the mixture before the HPLC separation is shown in Fig. 2. The 12 oligosaccharide structures proposed in the paper by Strecker *et al.* are shown in Chart 1. The designation in this paper, the designation by Strecker *et al.,* and the corresponding theoretical and experimental masses of the all ^{12}C sodiated species, the "quasimolecular ion," for each compound are provided in Table 1. For neutral oligosaccharides in the cation mode, the MALDI-MS spectra often yield the sodiated as well as some potasiated parents as quasimolecular ions. Alkali metals are present from several sources including the sample, the glass containers, and even the matrix. In this work, the sample was purposely doped with NaCl to favor a single alkali metal-coordinated species. The corresponding masses of nearly all 12 structures, with the exception of structures **III, VI,** and **IX,** are found in the MALDI-FTMS of the mixtures. However, the presence of the signals at specified masses does not by itself indicate that the corresponding structure is present. Some of the signals may be due to fragments of larger compounds. Additionally, the absence of some signals at other specified masses does not immediately indicate the absence of the corresponding structure. Suppression of some signals in MALDI may occur, particularly in mixtures as complex as this one. Analysis of the components separated by HPLC produces mass spectra that show masses corresponding to those observed in the mixture, in addition to those not observed in the mixture. There are also some signals that clearly do not correspond to oligosaccharide components, based

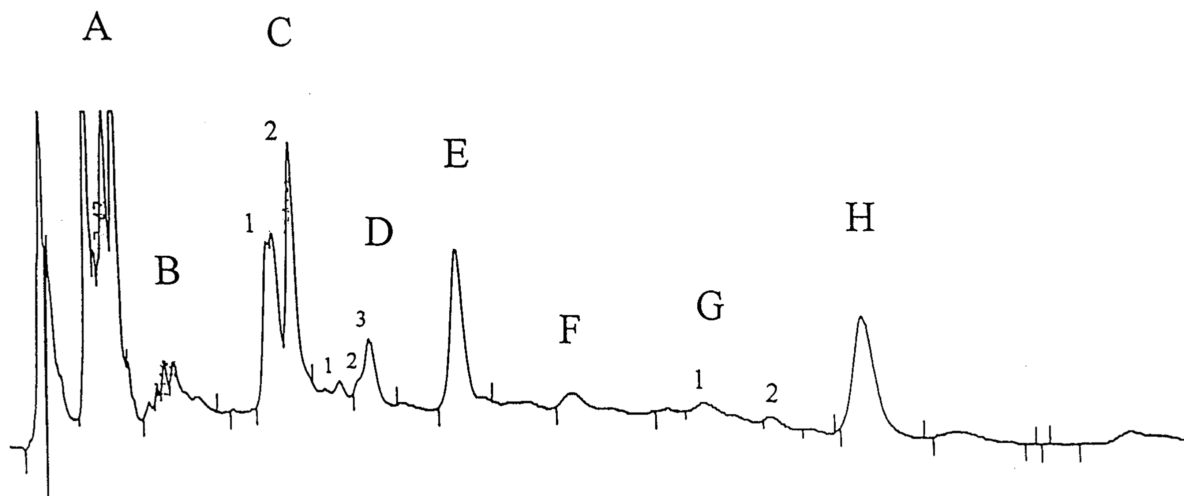


FIG. 1. HPLC chromatogram of oligosaccharide-alditols liberated from the eggs of *X. laevis* by alkaline borohydride reduction. The groups of peaks are labeled for reference.

on the masses. They are not matrix peaks. As shown in subsequent spectra, matrix interference is minimal in MALDI-FTMS even at low mass because of the long time scale of detection. The matrix interference observed in time-of-flight instruments are metastable peaks that do not persist in FTMS. Some of the signals appear to have isotopic pattern consistent with the presence of boron as discussed with the HPLC above. Boron complexed to oligosaccharide is observed even after several (at least five) distillations with methanol. This result should serve as a caveat when boron reagents, such as sodium borohydrate, are used with oligosaccharides. The boron is difficult to eliminate totally. These complexes and the other unknown peaks are eliminated, however, during HPLC.

The peaks on the HPLC trace labeled C correspond

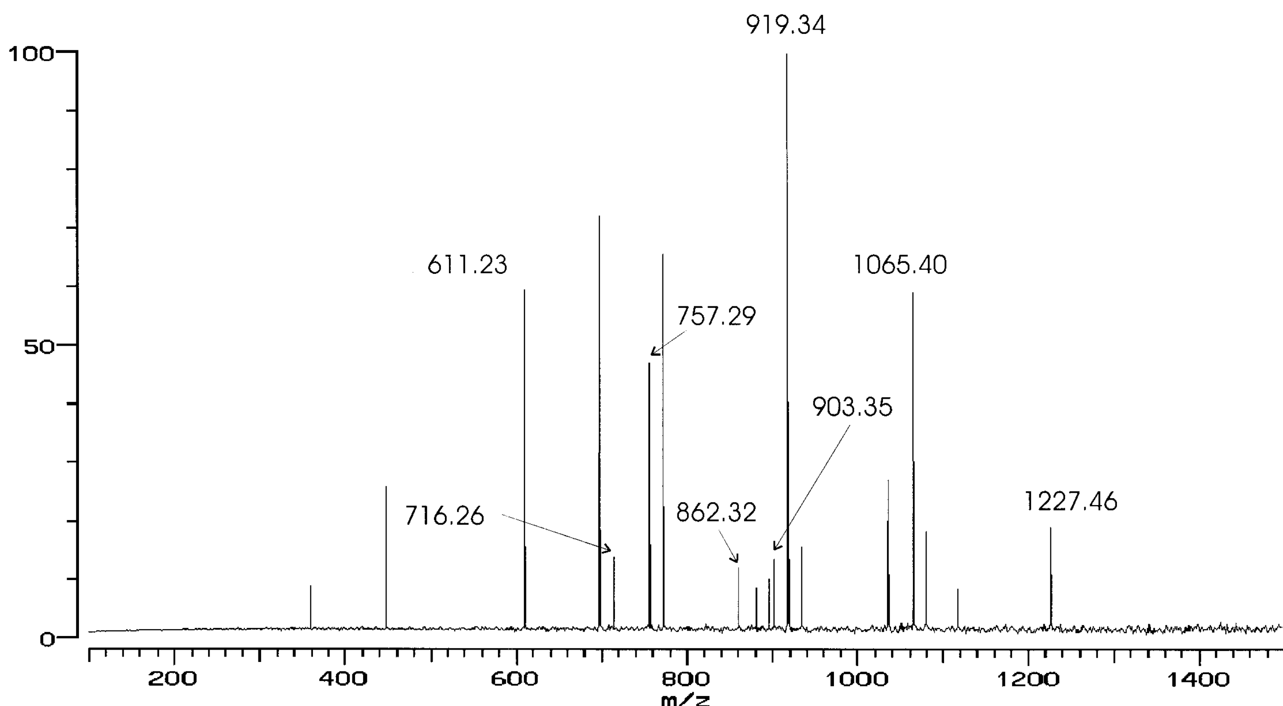


FIG. 2. The MALDI-FTMS spectrum of the mixture after treatment and purification and before separation with HPLC.

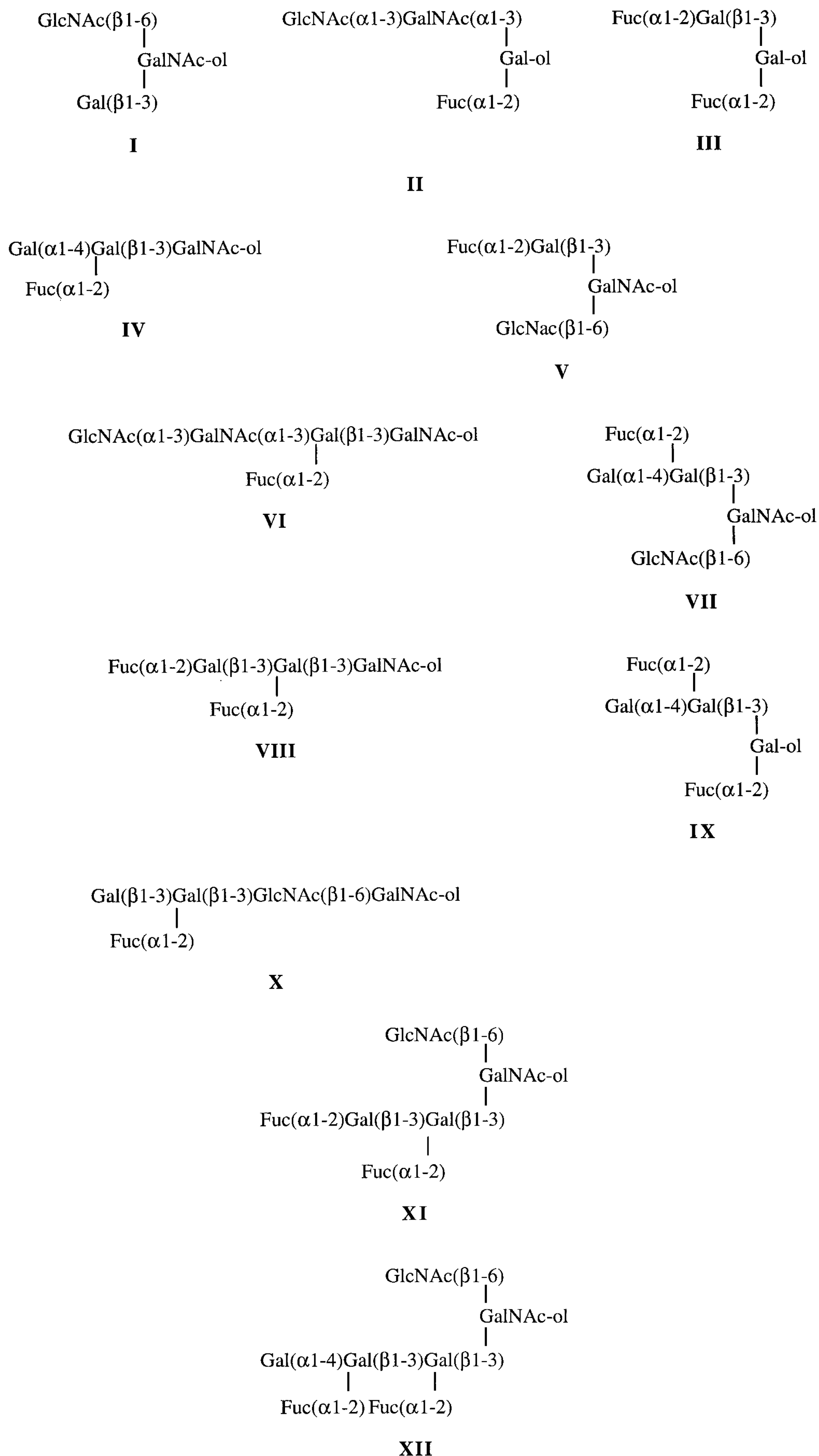


CHART 1. Structure of 12 oligosaccharides proposed by Strecker *et al.* from NMR data.

TABLE 1

Proposed Structures of Oligosaccharides Isolated by Strecker *et al.* and Elucidated by NMR

Structure	Strecker *et al.* HPLC label	Fig. 1 HPLC label	Theoretical $[M + Na]^+$ *m/z*	Experimental $[M + Na]^+$ *m/z*
I	1A	C1	611.23	611.23
II	1B-A	C1	757.29	757.29
III	1B-B	C1	659.24	659.24
IV	1C	C1	716.26	716.26
V	2	C2	757.29	757.29
VI	3	D2	960.37	960.37
VII	4A	E	919.34	919.34
VIII	4B	E	862.32	862.32
IX	4C	E	821.29	821.30
X	5	F	919.34	919.35
XI	7	H	1065.40	1065.40
XII	8	Mixture	1227.50	1227.46

Note. The theoretical mass of the all ^{12}C quasimolecular ions is provided.

to a group of at least four oligosaccharides assigned by Strecker *et al.* with the structures **I–IV.** Peaks C is the combination of at least two peaks (C1 and C2) referred to by Strecker *et al.* as 1 and 2. The spectrum of the onset of C1 (not shown) exhibit three major signals corresponding to *m/z* 611.23 (100%, base peak), 716.26 (25%), 757.29 (80%). These masses correspond directly to those expected for structures **I, IV,** and **II,** respectively. The MALDI-FTMS spectrum of sample collected at the top of peak C1 (not shown) still shows major intensities at *m/z* 611 (63%) and 757 (100%). In addition, a new signal with *m/z* 659.24 (33%) corresponding to the expected mass of structure **III** appears while *m/z* 716 disappears. The intensity of *m/z* 659 (**III**) is relatively weak, about 1/3 that of *m/z* 757 (**II**), which matches well with proposed ratio of 1 (**III**):4 (**II**) by Strecker *et al.* At the maximum of peak C2 the only remaining signal is *m/z* 611 and 757, while at the tail end of peak C2 only *m/z* 757 is observed (Fig. 3). The signal at *m/z* 757 corresponds to the proposed structure **V** and verifies this structural assignment. The intensities at *m/z* 757 in both C1 and C2 peaks indicate the presence of two isomers with a nominal mass of *m/z* 757. The isomer with the shorter retention time likely corresponds to structure **II,** while the one with the longer retention time corresponds to **V.**

Because of fragmentation during MALDI, there is some difficulty in differentiating quasimolecular ions and fragments. For example, the intensity at *m/z* 611 in the spectrum of C2 does not necessarily signify the presence of **I** or its isomer in this fraction. We performed Cs^+ cationization experiments which decreased the amount of fragment ions and concluded that this signal corresponded to a fragment of *m/z* 757, resulting in a loss of a fucose. The spectrum of the cesium-doped sample shows only *m/z* 867, a cessiated quasimolecular ion (Fig. 4). There is an absence of the cessiated ion at *m/z* 721 that corresponds to the shifted *m/z* 611 ion. This indicates that *m/z* 611 is a fragment of *m/z* 757 in the MALDI-FTMS of fractions containing peak C2.

The peaks at D (peak 3 of Strecker *et al.*) is a combination of at least three peaks. The first, D1, yields an MS spectrum that has only a single component at *m/z* 903.35 (100%) (MALDI-FTMS not shown; MS of isolated ion is shown in Fig. 5). This mass has no corresponding structure based on the 12 and is designated as Unknown **1.** The other two HPLC peaks are larger and not well resolved, D2 and D3. The MALDI spectrum of the fraction that contains D2 and D3 yields at least two major ions with *m/z* 903.35 (100%) and 960.37 (25%) (spectrum not shown). The ion with *m/z* 960 corresponds correctly with the expected mass for structure **VI.** The signal at *m/z* 903.35 is due to a another oligosaccharide, Unknown **2,** that was also not reported by Strecker and is a likely isomer of Unknown **1.** Interestingly, its abundance is greater than that of *m/z* 960, the quasimolecular ion for compound **VI.** The smaller oligosaccharide is not a likely fragment of the larger one as a loss of 57 u from *m/z* 960 is not a common oligosaccharide fragmentation.

The peak E (Peak 4 in Strecker *et al.*) was proposed by Strecker *et al.* to be composed of three oligosaccharides with structures **VII, VIII,** and **IX.** The expected masses for the three sodiated compounds match perfectly with *m/z* 919.34, 862.32, and 821.30, respectively, obtained from the mass spectrum of peak E (spectrum not shown). Three fractions were obtained and analyzed for this peak corresponding to the beginning of the peak, the maximum, and the end. At the beginning of the peak all three masses, *m/z* 821 (100%), *m/z* 862 (38%), and *m/z* 919 (42%), are observed in the mass spectrum (not shown). At the peak maximum, *m/z* 862 (100%) becomes the most abundant signal, followed by *m/z* 919 (50%) and *m/z* 821 (<5%). At the end of the

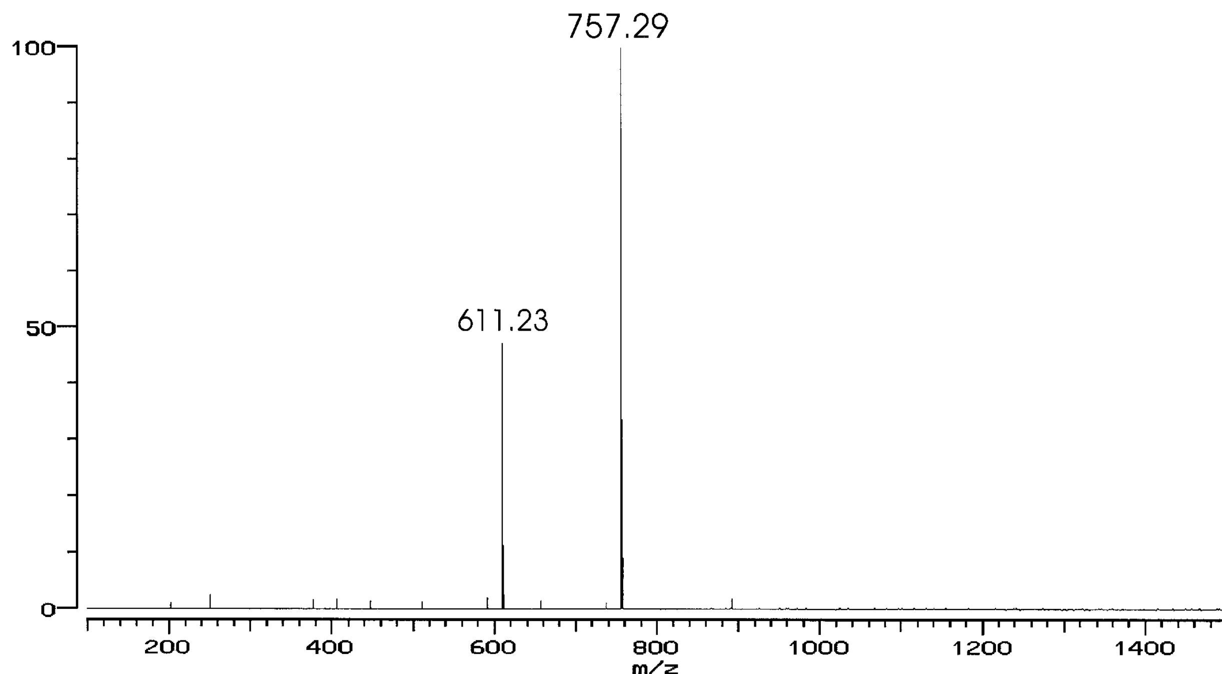


FIG. 3. The MALDI-FTMS of the C2 peak. The intensities at *m/z* 757.29 and 611.23 correspond to oligosaccharide masses. See text for further description.

peak both *m/z* 862 (90%) and 919 (100%) become the dominant peaks (spectrum not shown). The trend suggests the elution order of the three compounds as **IX**, **VIII**, and **VII.**

The fraction containing peak F yields a mass spectrum with two major intensities, *m/z* 919.34 (100%) and *m/z* 1065.40 (50%) (spectrum not shown). The component is minor in this HPLC trace and in Strecker *et*

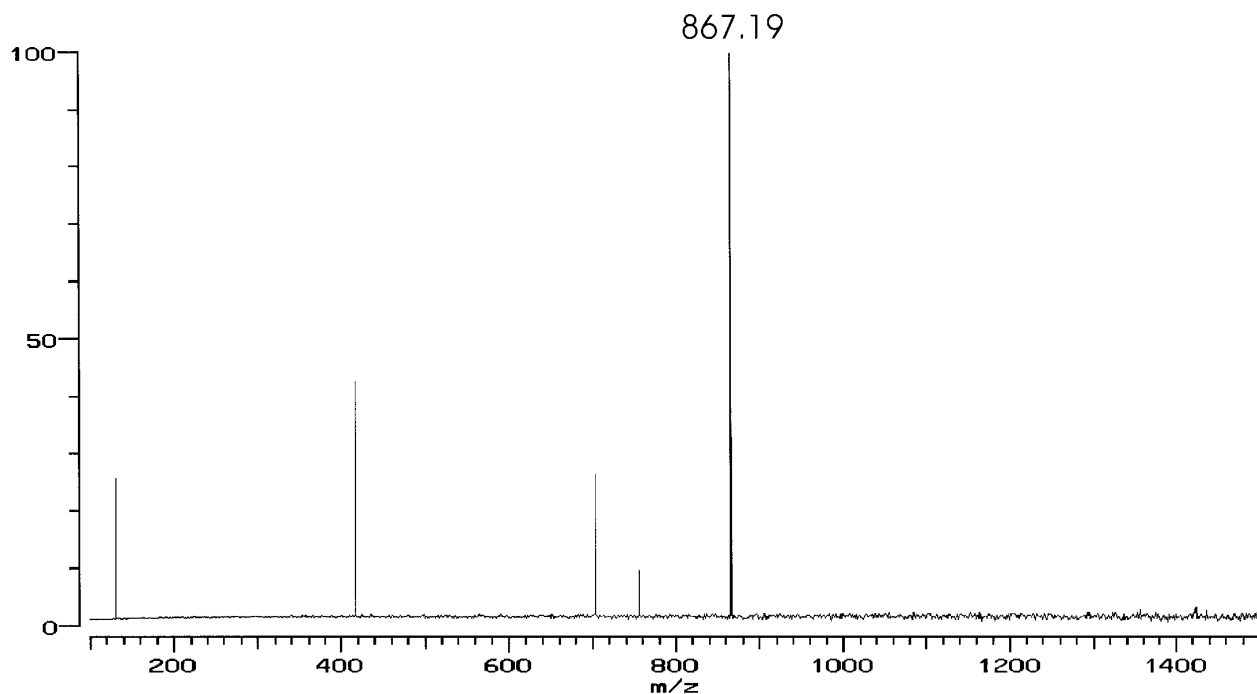


FIG. 4. The MALDI-FTMS spectrum of same fraction measured in Fig. 3 but doped with CsCl. The intensity at *m/z* 867 is the sodium analog of *m/z* 757 in Fig. 3. The mass corresponds to the quasimolecular ion at **V.**

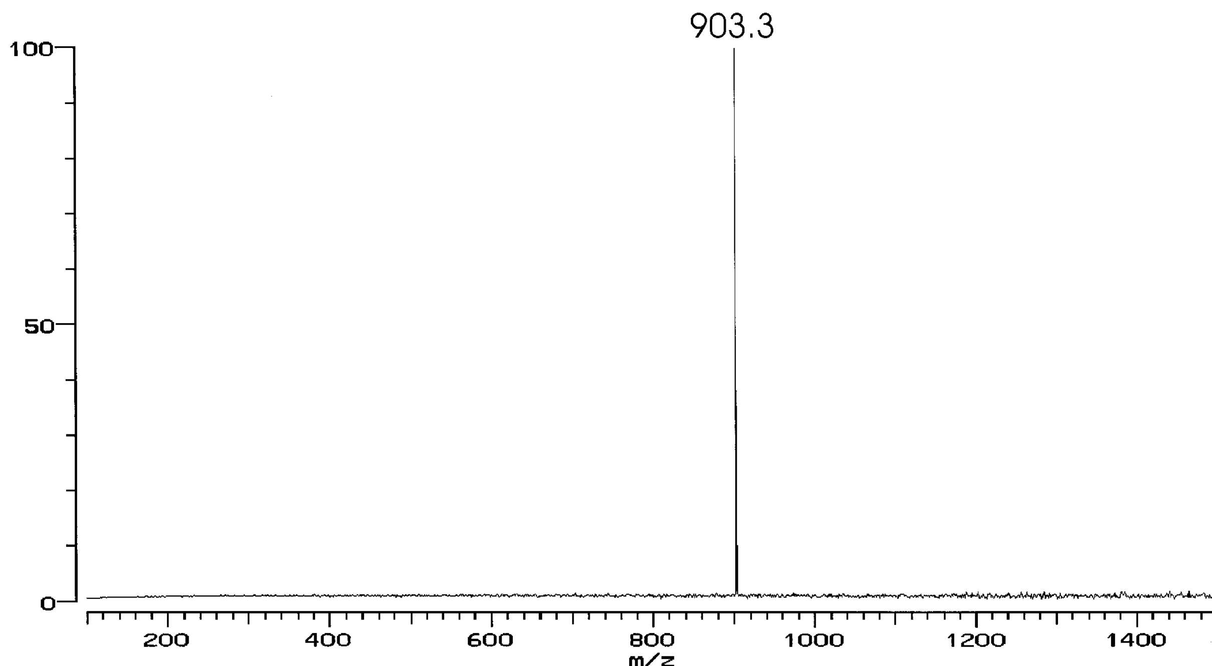


FIG. 5. Isolation of *m/z* 903 from MALDI-FTMS of fractions collected from Peak D. Isolation was performed by injection of all other ions from the analyzer cell of the FTMS.

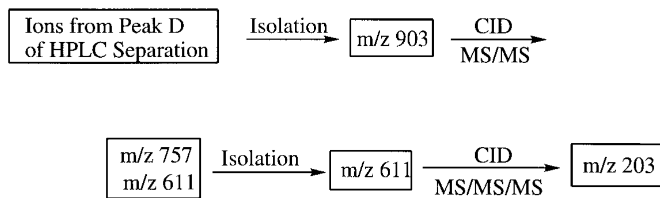
al. The smaller oligosaccharide corresponds to that for structure **X,** while the larger species corresponds to that of **XI.** However, **XI** is expected and found in Peak H below. The mass *m/z* 1065 therefore corresponds to a new oligosaccharide—Unknown **3,** an isomer of **XI,** which was not observed by NMR.

The peaks at G were observed by Strecker *et al.* (Peak 6) but were not analyzed, probably due to the small amounts of material. This group of peaks is the least abundant and is composed of at least two components. The mass spectrum of the fraction containing the larger peak G1 shows three intense signals at *m/z* 919.34 (40%), *m/z* 1024.36 (100%), and *m/z* 1389.48 (25%) (spectrum not shown). The masses all correspond to those of oligosaccharides. Subsequent doping with Cs^+ revealed that all three were quasimolecular ions representing three different oligosaccharides (*vide supra*). The three signals are given the designation Unknown **4** (*m/z* 919), Unknown **5** (*m/z* 1024), and Unknown **6** (*m/z* 1389). A signal with nominal *m/z* 919 is observed in the earlier peaks E and F. Those have been assigned with the corresponding structures **VII** and **X,** respectively. The signal at *m/z* 919 obtained from peak G is another likely isomer of the previous two isobaric compounds. Both *m/z* 1389 and *m/z* 1024 are unique masses and are not isomers of the oligosaccharides **I–IX.** These masses represent two new oligosaccharides also not observed by Strecker *et al.* The peak G2 was also analyzed and found to contain an oligosaccharide with *m/z* 1065 (Unknown **7**). This compound is an isomer of a known oligosaccharide found in peak H (structure **XI**).

The peak at H is a major component in the HPLC trace, and the mass spectrum shows only two ionic species with *m/z* 1065.40 (100%) and *m/z* 919.35 (20%) (spectrum not shown). Doping the sample with Cs^+ shows the complimentary *m/z* for the larger species but the not the smaller one, indicating the smaller is the fragment, due to a loss of fucose, while the larger oligosaccharide corresponds in mass to **XI.**

Elucidation of Mixtures by Addition of Cs^+

Doping the sample with Cs^+ prior to MALDI analysis provides a rapid and general method for differentiating quasimolecular ions from fragments. The large alkali metal ion coordinates with the parent and produces a complex that does not readily fragment. These findings are based on an earlier studies of several branched oligosaccharides and appear to be general (16). Fundamental studies on unimolecular dissociation of oligosaccharides cationized by various alkali metals further show an inverse correlation between alkali metal size and fragment ion yield during collisional activation of MALDI produced ions (17). Coordination with Cs^+ ions decreases the fragmentation significantly compared to smaller ions such as Na^+. This behavior is useful in determining fragments from quasimolecular ions in the MALDI-FTMS spectra. As previously discussed, the *m/z* 611.23 peak in the MS of C2 turns out to be the



SCHEME 1

fragment of the quasimolecular ion at 757.29 (Fig. 3). This was evident when doping with CsCl produced a mass shifted intensity for the larger ion but not the smaller. The mass difference between the two (*m/z* 757 and *m/z* 611) is equivalent to a loss of fucose, which is a common fragmentation pathway for fucose-containing sugars. To achieve this result, a 10-μl portion of the HPLC eluent was placed on a probe tip, dried, and mixed with a 2-μl aliquot of a 0.01 M CsCl solution.

We use this method to differentiate fragments from quasimolecular ions in some HPLC fractions with ambiguous compositions. The same Cs^+ doping method was used to determine that the oligosaccharide signals in peaks G1 correspond to three distinct oligosaccharides. The sodium coordinated species produced signals with *m/z* 919.34, 1024.36, 1323.84, and 1389.44. The Cs^+-doped sample produced spectrum with *m/z* 1029.24, 1134.28, and 1499.39, indicating that *m/z* 919, 1024, and 1389 in the former spectrum were due to distinct oligosaccharides, while *m/z* 1324, which did not have the corresponding *m/z* 1434 in the Cs^+-doped sample, was a fragment of *m/z* 1389 from loss of a fucose residue.

*Tandem MS (MS^3) of Unknown **1** (m/z 903)*

Collision-induced dissociation (CID) is an additional structural probe effectively used in MALDI-FTMS to obtain structural information (17). The abundance of *m/z* 903 from the D peaks was sufficiently strong to allow tandem MS experiments (Scheme 1). First, isolation was performed by resonance ejection of all other masses in the spectrum using standard FTMS procedures. The spectrum of isolated *m/z* 903.3 is shown in Fig. 5. Isolation was performed very effectively with no other ions observed in the mass spectrum. Second, the isolated ion was translational excited and allowed to collide with Ar gas pulsed into the chamber after the excitation event. Collision of the ion with Ar produced fragments constituting an MS/MS experiment (Fig. 6). The major fragments were due to the loss of fucose; a one unit loss yields *m/z* 757.3 and two yields *m/z* 611.2. No other fragmentation such as cross ring cleavages was observed. Fucose, along with sialic acids, is the most labile residue in oligosaccharides and is the first to dissociate from this compound. Further isolation of *m/z* 611.2 and CID again produced several products including a loss of one and two *N*-acetylhexosamine (*m/z* 408.1 and *m/z* 205.1, respectively). There are also losses of H_2O from the isolated ion and from the product, resulting from the loss of one *N*-acetylhexosamine (Fig. 7). The smallest fragment (*m/z* 205.1) corresponds to an alditol coordinated with Na^+.

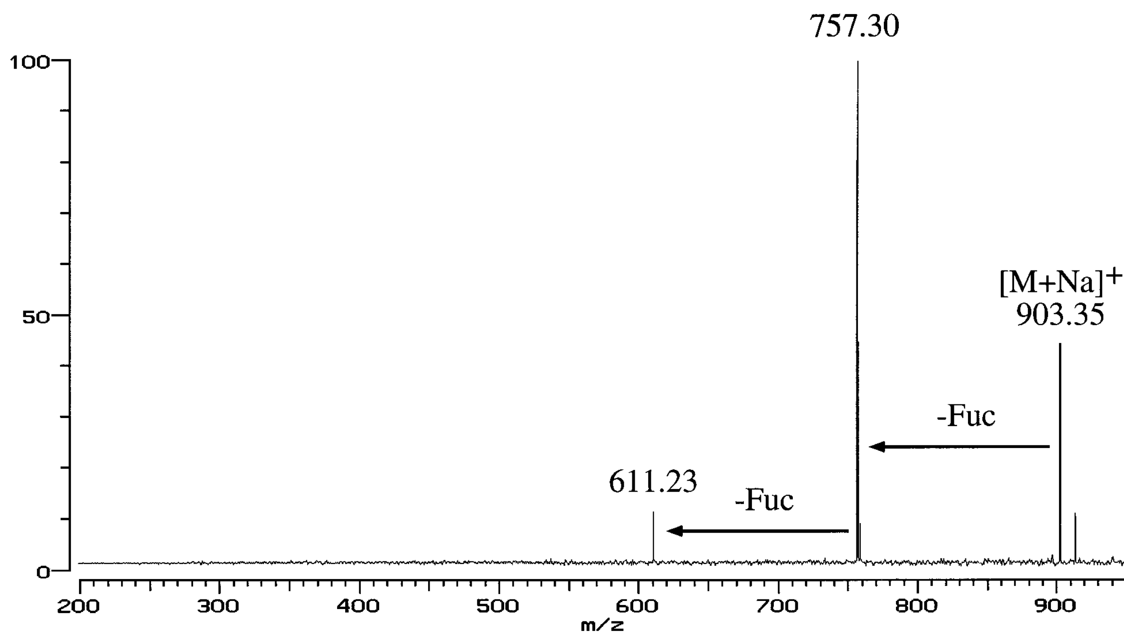


FIG. 6. Tandem MS (MS^2) of *m/z* 903 from isolation in Fig. 5. The intensity at *m/z* 757 corresponds to a loss of a fucose. The intensity at *m/z* 611 is from the loss of two fucoses.

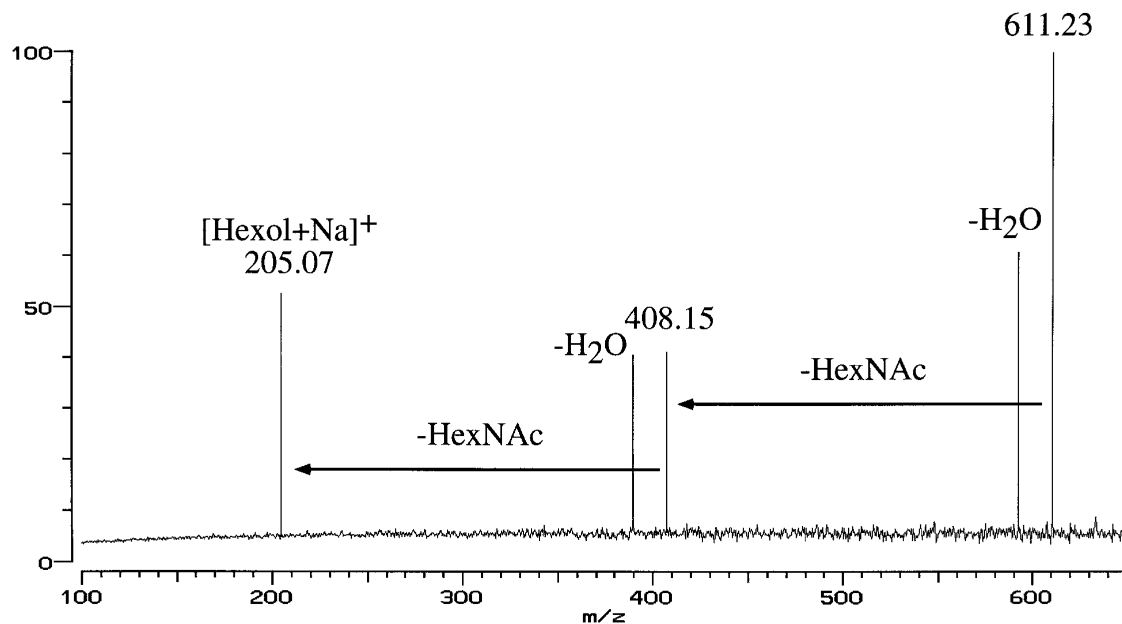
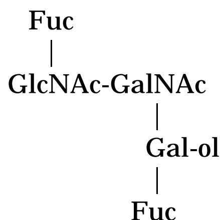


FIG. 7. MS/MS/MS beginning with *m/z* 903, followed by isolation and fragmentation of *m/z* 611. The intensity at *m/z* 408 and *m/z* 205 correspond to loses of one and two *N*-acetylhexosamine, respectively, from *m/z* 611.

Based on the CID data, we know that *m/z* 903 contains two fucose groups, two N-acetylhexosamine and one hexose, consistent with the exact mass. The data also indicate that the reducing end, or the alditol sugar, is a hexose and it is linked to two N-acetylhexosamine. This compound is similar to the known compound **II** with an addition of a fucose. By analogy to structure **II,** a probable structure for the Unknown **1** compound is shown.



Unfortunately, cross-ring cleavages which could be used to elucidate linkage are not observed. CID of oligosaccharides containing intact pyranose on the reducing end typically yield cross-ring cleavages and thus linkage information (17). We observed no cross-ring cleavages with these alditol oligosaccharides. This behavior may be a consequence of the alditol in the reducing end which somehow prohibits cross ring cleavages. Unfortunately, CID studies of alditol oligosaccharides formed by MALDI are not currently available. A more thorough investigation is required but is beyond the scope of this paper. For this reason, tandem MS of the other unknowns was not performed. Nonetheless, the experiments illustrate the potential utility of tandem MS for structural elucidation of oligosaccharides. Furthermore, tandem MS of ions produced by MALDI-FTMS is relatively simple and can be performed even with minute amounts (nano- to picomoles) of material. In this particular case, a complicated experiment such as MS^3 was performed and could be repeated routinely with no special treatment or chemical pretreatment. Work in our group is currently underway to obtain cross-ring cleavages in CID mass spectra.

DISCUSSION

The MALDI-FTMS analysis of individual HPLC fractions supports all the assignments made by Strecker *et al.* based on NMR. The expected masses correspond to the proposed structures for oligosaccharides **I–XI.** The predicted masses are found in the same corresponding peaks of both HPLC traces. The excellent detection limits of MALDI-FTMS is also illustrated in this study. The HPLC separation was performed on approximately 0.1 mg of oligosaccharides. This produced 70 × 1-ml fractions. To produce a MALDI-FTMS spectrum, 20 μl of a fraction was concentrated on the sample probe surface. Each mass spectrum, therefore, corresponds to submicrograms to nanograms of oligosaccharides. There were sufficient amounts of material from the HPLC separation to perform numerous analyses on each fraction. The MS analysis and the tandem MS experiments (MS^3) involving the unknown oligosac-

TABLE 2

Mass, Location on the HPLC Trace, and the Composition of Seven Oligosaccharides Found Using MALDI-FTMS

Unknown	Fig. 1 HPLC peak	Theoretical mass (m/z)	Experimental mass (m/z)	Composition
1	D1	903.34	903.35	1 Hex, 2 Fuc, 2 HexNAc
2	D3	903.34	903.35	1 Hex, 2 Fuc, 2 HexNAc
3	F	1065.40	1065.40	2 Hex, 2 Fuc, 2 HexNAc
4	G1	919.34	919.34	2 Hex, 1 Fuc, 2 HexNAc
5	G1	1024.34	1024.36	3 Hex, 2 Fuc, 1 HexNAc
6	G1	1389.50	1389.48	4 Hex, 2 Fuc, 2 HexNAc
7	G2	1065.40	1065.40	2 Hex, 2 Fuc, 2 HexNAc

charide with m/z 903 (HPLC Peak D1) were performed with the same fraction of a single HPLC separation.

The MALDI-FTMS study of nearly all collected fractions indicate seven oligosaccharides that were not identified in the earlier NMR paper. These oligosaccharides have quasimolecular ions with m/z 903, 1065, 1389, 1024, and 919. The smallest of the unknown oligosaccharides, **1** and **2,** m/z 903, were obtained from the fractions D1 and D2. Other unknown oligosaccharides, **3** and **7,** m/z 1065, were obtained from peak F and G2, respectively. The remaining three (unknown oligosaccharides **4, 5,** and **6**) were found in the G1 peak. The number of hexose, *N*-acetylhexosamines, and fucose for each unknown oligosaccharides are obtained based on the exact masses and are provided in Table 2. Since fucose can not be at the reducing end, the alditol is either an hexose or an *N*-acetylhexosamine.

There are expected similarities between the unknown oligosaccharides and those already elucidated by NMR. The unknown hexose of the seven oligosaccharides is probably the same galactose residue found in the known oligosaccharides. Similarly, *N*-acetylhexosamine is either of the form GlcNAc or GalNAc. Unknowns **1** and **2,** with m/z 903, differ in mass from **II** (m/z 757) by a fucose group. We may speculate that all three have the same base structure. Indeed, the MS/MS spectrum of m/z 903, unknown **1,** shows the loss of two fucose groups while the MS^3 spectrum shows the additional loss of *N*-acetylhexosamine, consistent with a general structure similar to **II.**

Two of the new oligosaccharides are positional isomers of those already assigned in the earlier paper. Unknown **3,** with m/z 1065, found in Peak F, is an isomer of **XI** found later in Peak H. Both contain two fucose, two hexose, and two *N*-acetylhexosamine. Unknown **4** with m/z 919, found in peak G, is an isomer of the two oligosaccharides **VII** (Peak E) and **X** (Peak F). It similarly contains one fucose, two hexoses, and two *N*-acetylhexosamines. Unknowns **1, 2, 5, 6,** and **7** are not isomers of those found in the earlier study. Both **1** and **2** contains two fucose, one hexose, and two N-acetyl hexose. Unknown **5** contains two fucose, three hexose, and one *N*-acetylhexosamine, while Unknown **6** contains two fucose, four hexose, and two *N*-acetyl hexose. Unknown **6** is also the largest observed oligosaccharide thus far derived from *X. laevis* jelly coat glycoproteins. Unknown **7** is composed of two fucose, two hexose, and two *N*-acetylhexosamine.

CONCLUSION

MALDI-FTMS of HPLC separated fractions support the mass assignment by Strecker *et al.* on the 12 most abundant oligosaccharides in the egg jelly coat of *X. laevis.* Seven additional oligosaccharides were found by MALDI-FTMS that were not observed with NMR. MALDI-FTMS provides confirmation of the structural assignments of the oligosaccharides. There are several important features of FTMS that facilitate the analysis. The absences of matrix peaks even at low masses provide mass spectra that are easily interpretable. The high mass accuracy-high resolution feature is also important allowing rapid identification of peaks that are due to oligosaccharides. The mass assignments in this work have been conservatively given to two decimal points, corresponding to a typical error of 10 ppm. In many of the samples, the peak assignments are less than 10 ppm, but they rarely exceed 20 ppm. The tandem MS capabilities, illustrated here in MS^3, is also invaluable for providing some sequence information. Work is currently underway to optimize tandem FTMS and to provide more structural information.

MALDI-FTMS could already be an important quantitative tool, especially in systems where structures are already known and the aim is to observe the effects of external stimuli on the absolute abundances of individual oligosaccharides. It can similarly guide the search for new structures of other low abundant oligosaccharides. Ideally, it would be best to obtain all structural information with mass spectroscopy. The current limitation, such as the lack of cross-ring cleavages during tandem MS, requires additional fundamental studies on ion dissociation. The further development of FTMS is similarly critical for the rapid analyses of naturally occurring oligosaccharides.

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