Anal. Chem. **2001,** *73,* 3556−3561

Identification and Structural Elucidation of Lectin-Binding Oligosaccharides by Bioaffinity Matrix-Assisted Laser Desorption/Ionization Fourier Transform Mass Spectrometry

Ken Tseng,[†] Hao Wang,[†] Carlito B. Lebrilla,*[,†] Barry Bonnell,[‡] and Jerry Hedrick*[,‡]

Department of Chemistry and Section of Molecular and Cellular Biology, University of California, Davis, California 95616

Cortical granule lectin (CGL) is released by the egg of the South African toad *Xenopus laevis* upon fertilization. The lectin binds to oligosaccharides in the extracellular matrix of the egg to form a physical block to prevent additional sperm penetration or polyspermy. To identify the oligosaccharides that bind to CGL, the lectin was immobilized on the surface of a matrix-assisted laser desorption/ionization probe. This bioaffinity probe was used to determine oligosaccharides that bind preferentially to CGL. Structural analyses based on collision-induced dissociation was used to determine that oligosaccharides with the sulfate esters at the nonreducing ends preferentially bind to the lectin.

The extracellular matrix surrounding most animal eggs plays essential roles. One of those, mediating sperm−egg binding, is critical for fertilization. However, the block of further sperm penetration (polyspermy), after fertilization has occurred, is similarly important for creating viable offspring. The cortical granule lectin (CGL) is released by the egg of the South African toad *Xenopus laevis* upon fertilization to prevent secondary sperm penetration.[1−3] The extracellular matrix of *X. laevis* contains a vitelline envelope and a jelly coat composed of three morphologically distinct regions designated J1, J2, and J3. The lectin interacts with the innermost layer (J1) of the jelly coat to form a fertilization layer, which acts as an impenetrable barrier for additional sperm penetration. Sialic acid and sulfate residues are known constituents of the glycan moieties, but most, if not all, sulfate sugar esters are believed to reside in the J1 layer. Indeed, binding studies indicate that the lectin attaches specifically to two highly anionic glycoproteins, with apparent molecular masses of 630 (gp630) and 450 kDa (gp450).[4]

Identification of the oligosaccharide components that bind to the CGL is important for elucidating the nature of the interaction. However, traditional methods for studying lectin−carbohydrate binding demand considerable effort in the isolation and identification of the substrates, requiring a larger amount of material than can be obtained from biological sources. In this report, a bioaffinity probe composed of immobilized CGL in conjunction with matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) was used to isolate oligosaccharides that have affinity for the lectin. The procedure outlined in this report for producing the affinity MALDI probe has been used in this laboratory to prepare avidin-immobilized bioaffinity probes.[5] The method is fast, requires only minutes, and is highly effective. Furthermore, we have shown that avidin immobilized on the polymer surface remains active, although some of the binding sites are blocked by the surface. In the free state, the avidin−biotin ratio is four while on the polymer it is 1.2. Collision-induced dissociation (CID) was also used to provide structural information on strongly binding and weakly binding oligosaccharides.

The use of bioaffinity surfaces in conjunction with MALDI-MS has recently received considerable attention. Agarose beads with bioactive species immobilized on the surface, such as biotin, have been used to isolate specific substrates. The beads are then removed and analyzed by MALDI-MS.[6,7] Microchips, particularly those used in biomolecular interaction analysis (BIA) technology, have been used to immobilize proteins and extract specific substrates. The chips were then directly analyzed by MALDI-MS.[8−10] Chemical modification of MALDI-MS surfaces by the covalent attachment of bioactive species has been employed.[11−14] The use of polymer surfaces to immobilize proteins noncovalently has also been reported.[5,15] Only one other report has been

[†] Department of Chemistry.

[‡] Section of Molecular and Cellular Biology.

(1) Grey, R. D.; Wolf, D. P.; Hedrick, J. L. *Dev. Biol.* **1974**, *36*, 44−61.
(2) Wyrick, R. E.; Nishihara, T.; Hedrick, J. L. *Proc. Natl. Acad. Sci. U.S.A.* **1974**, *71*, 2067−2071.
(3) Greve, L. C.; Hedrick, J. L. *Gamete Res.* **1978**, *1*, 13−18.
(4) Bonnell, B. S.; Reinhart, D.; Chandler, D. E. *Dev. Biol.* **1996**, *174*, 32−42.
(5) Wang, H.; Tseng, K.; Lebrilla, C. B. *Anal. Chem.* **1999**, *71*, 2014−2020.
(6) Zhao, Y.; Muir, T. W.; Kent, S. B. H.; Tischer, E.; Scardina, J. M.; Chait, B. T. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 4020−4024.
(7) Schriemer, D. S.; Li, L. *Anal. Chem.* **1996**, *68*, 3382−3387.
(8) Krone, J. R.; Nelson, R. W.; Dogruel, D.; Williams, P.; Granzow, R. *Anal. Biochem.* **1997**, *244*, 124−132.
(9) Yu, L.; Gaskell, S. J.; Brookman, J. L. *J. Am. Soc. Mass Spetrom.* **1998**, *9*, 208−215.
(10) Sönksen, C. P.; Nordhoff, E.; Jansson, Ö.; Malmqvist, M.; Roepstorff, P. *Anal. Chem.* **1998**, *70*, 2731−2736.
(11) Ching, J.; Voivodov, K. I.; Hutchens, T. W. *Bioconjugate Chem.* **1996**, *7*, 525−528.
(12) Hutchens, T. W.; Yip, T. T. *Rapid Commun. Mass Spectrom.* **1993**, *7*, 576−580.
(13) Brockman, A. H.; Orlando, R. *Anal. Chem.* **1995**, *67*, 4581−4585.
(14) Brockman, A. H.; Orlando, R. *Rapid Commun. Mass Spectrom.* **1996**, *10*, 1688−1692.
(15) Liang, X.; Lubman, D. M.; Rossi, D. T.; Nordblom, G. D.; Barksdale, C. M. *Anal. Chem.* **1998**, *70*, 498−503.

10.1021/ac010182v CCC: $20.00 © 2001 American Chemical Society
Published on Web 06/26/2001

published using a lectin-based bioaffinity probe. Bundy and Fenselau employed these probes to immobilize an oligosaccharide and identify a binding epitope in a bacterium.[16]

EXPERIMENTAL SECTION

Release and Isolation of Oligosaccharides. Release of the eggs from *X. laevis* and isolation of the glycoproteins (gp630 and gp450) were performed by a method described in earlier publications.[17,18] O-Linked oligosaccharides were released from egg jelly glycoproteins by reacting them in a solution containing 1.0 M $NaBH_4$ and 0.1 M NaOH. The reaction was quenched after 19 h at 48 °C when Dowex (H^+ form) was added into the solution. The solution was filtered and adjusted to pH 6.5 with 0.1 N NaOH or 0.1 N formic acid. The resulting mixture of oligosaccharides was methanol-distilled in a rotary evaporator up to six times to remove boron. A P2 column (1.5 × 40 cm) was used for desalting the oligosaccharide mixture. The fractions from the P2 column were collected and tested for the presence of oligosaccharides by mass spectrometry. It was determined that mass spectrometry provided a more precise selection of fractions containing oligosaccharides than the more traditional phenol−sulfuric acid test.

The corticle granule lectin (CGL) was obtained and isolated using the procedure outlined in an earlier publication.[19] The concentration of the lectin was approximately 1−2 mg/mL.

Mass Spectrometry. MALDI-FTMS of neutral oligosaccharides is described in detail in earlier publications.[17,20] For the MALDI-FTMS analysis of singly charged anionic oligosaccharides, an NH_4^+ resin was used on the probe tip to further remove other positively charged contaminants in the sample. The addition of the NH_4^+ resin significantly improved the signal of sulfated oligosaccharides. A 2.0-μL aliquot of saturated 2,5-dihydroxyacetophenone (DHAP) in ethanol was then added on the probe tip for the MALDI matrix. The MALDI crystals were formed under vacuum without removal of the resin, and the sample was analyzed directly. For the analysis of doubly sulfated oligosaccharides, the NH_4^+ resin was first added to the sample solution in the vial. The sample was centrifuged, and a small aliquot of the solution (1−5 μL) was removed and placed on the probe tip. Matrix was added as before. The ambient concentration of sodium ions in the matrix and solution was sufficient to produce a singly charged species in the negative mode with one sodium coordinated to the doubly sulfated oligosaccharide.

To perform the MALDI affinity experiments on the mixtures, lectins were immobilized on microporous polyester by the procedure described earlier for avidin-immobilized affinity probes.[5] The polymer was affixed to the MALDI probe head using cellophane tape. The surface of the polymer was washed with ethanol and a 1-μL CGL solution was placed on the surface and dried with forced air. The solution containing the oligosaccharides released from the whole egg jelly was placed on the surface. The sample was allowed to incubate for 5 min and washed with Nanopure water (3 × 40 μL). The solution surface was allowed to dry and a 2-μL solution of saturated DHAP in ethanol was added for the MALDI matrix. Mass analyses were performed on an Ionspec HiRES MALDI Fourier transform mass spectrometer with a 4.7-T superconducting magnet. The mass scale was externally.

Collision-Induced Dissociation. For experiments involving collision-induced dissociation, the desired ion was first isolated in the analyzer with the use of an arbitrary-waveform generator or a frequency synthesizer. All CID experiments were performed at +1000 Hz off-resonance to the cyclotron frequency of the isolated ion. The CID excitation time was 1000 ms. Two pulses of argon were introduced into the analyzer chamber at 0 and 500 ms as collision gas. The excitation voltages ranged from 2.5 to 8.0 V depending on the desired level of fragmentation and the size of the oligosaccharide.

RESULTS AND DISCUSSION

Affinity MALDI Probes Employing Known Lectin−Substrate Systems. In a previous publication, we immobilized neutrAvidin on a polymer film surface and illustrated the affinity of the treated surface for biotinylated oligosaccharides.[5] Because of the high binding affinity of biotin for avidin—it is the strongest noncovalent interaction known—the native oligosaccharides were readily washed away so that only biotinylated oligosaccharides were observed in the MALDI-FTMS spectra. Lectin−oligosaccharide interactions exhibit neither the same strength of binding nor the specificity. For this reason, the lectin probes were not expected to be highly selective. For this study, several model lectins were examined to determine whether selectivity would be detected in the MALDI-FTMS spectra. The affinity study of two lectins are described below and validate the construction of the oligosaccharide affinity probes.

The lectin *Ulex europaeus* agglutinin (UEA) preferentially retains α-L-fucose. A mixture containing the trisaccharides Fucα1−2Galβ1−4Glc (**1**) and GlcNacβ1−4GlcNAcβ1−4GlcNAc (**2**) was examined to determine whether the fucosylated compound **1** would be preferentially retained. An approximately equal amount was placed on the polymer probe (without lectin) to observe the ionization behavior of the two compounds. With the addition of the matrix and the appropriate sodium dopant, the MALDI-FTMS spectrum of the sample was obtained (Figure 1a). The sodium adduct of the fucosylated compound **1** is ∼50% of the nonfucosylated compound, the base peak, suggesting a slightly better ionization efficiency for the nonfucosylated compound **2**. A polymer film was then treated with the UEA as described in the Experimental Section to produce the bioaffinity probe. The same sample mixture was placed on the lectin-immobilized probe and incubated for 5 min. Water was added when needed to prevent drying. After the incubation period, the sample was washed with Nanopure water. Matrix and sodium dopant were then added to the probe, and the sample was analyzed by MALDI-FTMS. The resulting spectrum is shown in Figure 1b. Note that the relative intensities are reversed with the nonfucosylated substrate **2** now 60% of the fucosylated compound **1**, the base peak, suggesting that indeed **1** was preferentially retained. As a control, the polymer film without lectin was pretreated and used in the same manner as that outlined in the previous experiment. Upon washing and MALDI-FTMS analysis, no oligosaccharide was observed in the mass spectra (Figure 1c), confirming the binding of the oligosaccharides to the lectin.

(16) Bundy, J.; Fenselau, C. *Anal. Chem.* **1999**, *71*, 1460−1463.
(17) Tseng, K.; Lindsay, L. L.; Penn, S. G.; Hedrick, J. L.; Lebrilla, C. B. *Anal. Biochem.* **1997**, *250*, 18−28.
(18) Tseng, K.; Hedrick, J. L.; Lebrilla, C. B. *Anal. Chem.* **1999**, *71*, 3747−3754.
(19) Nishihara, T.; Wyrick, R. E.; Working, P. K.; Chen, Y.-H.; Hedrick, J. L. *Biochemistry* **1986**, *25*, 6013−6020.
(20) Cancilla, M. T.; Wong, A. W.; Voss, L. R.; Lebrilla, C. B. *Anal. Chem.* **1999**, *71*, 3206−3218.

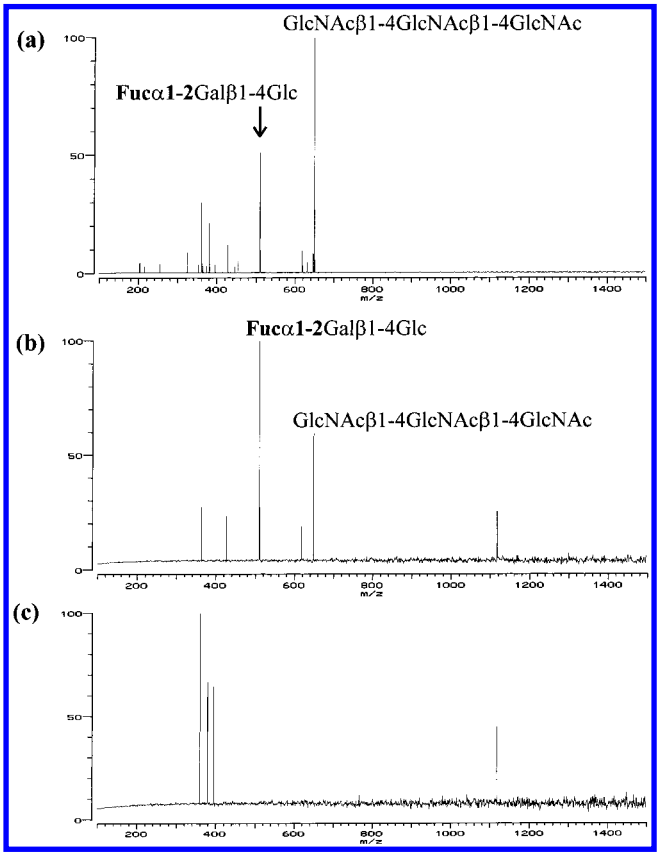


Figure 1. MALDI-FTMS spectra of a mixture containing approximately equimolar concentrations of Fucα1−2Galβ1−4Glc and GlcNAcβ1−4GlcNAcβ1−4GlcNAc on (a) a polymer film probe, (b) a polymer film probe treated with lectin UEA followed with washings to remove unbound oligosaccharides, and (c) a polymer film probe not treated with lectin followed with washings. The lectin is specific for α-fucose.

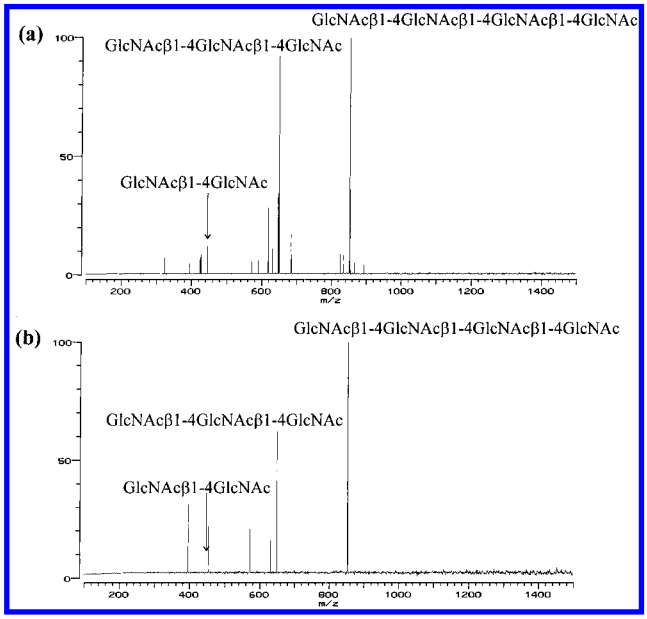


Figure 2. MALDI-FTMS spectra of mixture containing GlcNAc dimer, trimer, and tetramer in approximately equal concentrations from (a) a polymer film probe and (b) a polymer film probe treated with WCA lectin followed with washings to remove unbound oligosaccharides. The binding of this lectin favors tetramer > trimer > dimer.

These experiments were repeated in triplicate with the same results obtained for all experiments. There were slight variations in the relative intensities of the quasimolecular ions of the test compounds, but the relative variations were less than 10% and the overall features of the spectra were maintained. Note also the weak abundances of matrix peaks in the region below m/z 400. The lack of a significant matrix interference is characteristic of MALDI-FTMS and makes it superior to MALDI time-of-flight instruments for analyzing small compounds. The highly reproducible nature of the relative intensities of quasimolecular ions in mixtures is another hallmark of FTMS. We have shown in an earlier publication that quantification of various mixtures may be performed on FTMS if the specific ionization efficiencies are normalized.[21]

As a further test of the efficacy of the lectin probe, wheat germ agglutinin (WGA), which specifically retains β-GlcNAc, was examined by employing a mixture of three GlcNAc oligomers (GlcNAcβ1−4GlcNAc (**3**), GlcNAcβ1−4GlcNAcβ1−4GlcNAc (**4**), GlcNAcβ1−4GlcNAcβ1−GlcNAcβ1−4GlcNAc (**5**)). Binding studies in solution have shown that the preferences for the oligomers followed the order **5** > **4** > **3**.[22,23] Equimolar mixtures of the compound applied and analyzed on the bare polymer film show that the tetramer and the trimer have similar signal responses while the dimer has significantly less (Figure 2a). The quasimolecular ion is the sodium-coordinated species in the positive mode. We have previously shown that small oligomers, particularly disaccharides, bind sodium cations more weakly than larger oligomers causing a decrease in abundance in these species.[24] Furthermore, the instrument has been optimized for masses corresponding to the tri- and tetrasaccharides rather than the disaccharide. The same mixture applied to the lectin-immobilized probe followed by an incubation and washing yielded the spectrum shown in Figure 2b. The disaccharide **3** is totally absent while the trisaccharide **4** is diminished relative to the tetrasaccharide **5**. These experiment were repeated again in triplicate with highly reproducible results.

By comparing MALDI-FTMS spectra of the mixture on the untreated polymer probe and the lectin-immobilized polymer probe, we observe that the natural affinity of the lectin is duplicated in the relative abundances in the mass spectra. We conclude that the lectin remains active on the probe surface, and the comparison of the relative intensity of the quasimolecular ion in both bare film and lectin-immobilized film provides a method for observing the affinity of the lectin.

Determination of Anionic Oligosaccharides Bound to CGL MALDI Probe. The oligosaccharides obtained from gp630 contain neutral, sialylated, sulfated, and doubly sulfated oligosaccharides. The neutral and the anionic components were released and determined using standard MALDI−FTMS procedures. The results are summarized in Tables 1−3 along with the composition of the oligosaccharides according to their m/z. Although the neutral components of the whole egg jelly have recently been

(21) Wong, A. W.; Wang, H.; Lebrilla, C. B. *Anal. Chem.* **2000**, *72*, 1419−1425.
(22) Allen, A. K.; Neuberger, A. *Biochem. J.* **1973**, *135*, 307−314.
(23) Rice, R. H.; Etzler, M. E. *Biochemistry* **1975**, *14*, 4093−4099.
(24) Cancilla, M. T.; Penn, S. G.; Carroll, J. A.; Lebrilla, C. B. *J. Am. Chem. Soc.* **1996**, *118*, 6736−6745.

Table 1. Neutral Oligosaccharides from Gp630 Determined by Positive Ion Mass Spectrometry

	neutral oligosaccharides in positive mode $[M + Na]^+$				
no.	theor *m*/*z*	exp *m*/*z*	Hex	Fuc	HexNAc
1	611.23	611.22	1	0	2
2	716.26	716.27	2	1	1
3	757.28	757.27	1	1	2
4	773.28	773.29	2	0	2
5	862.32	862.33	2	2	1
6	903.34	903.35	1	2	2
7	919.34	919.35	2	1	2
8	935.33	935.34	3	0	2
9	960.36	960.38	1	1	3
10	1065.40	1065.40	2	2	2
11	1227.45	1227.46	3	2	2
12	1373.51	1373.53	3	3	2
13	1389.50	1389.53	4	2	2

Table 2. Anionic Oligosaccharides with One Sulfate Ester from Gp630 Determined by Negative Ion Mass Spectrometry

	singly sulfated $[M - H]^-$					
no.	theor *m*/*z*	exp *m*/*z*	Hex	Fuc	HexNAc	sulfate
1	505.13	505.13	0	0	2	1
2	667.19	667.18	1	0	2	1
3	813.24	813.23	1	1	2	1
4	829.24	829.23	2	0	2	1
5	975.30	975.29	2	1	2	1
6	1032.32	1032.31	2	0	3	1
7	1073.35	1073.32	1	0	4	1
8	1219.40	1219.38	1	1	4	1
9	1235.40	1235.48	2	0	4	1
10	1381.46	1381.43	2	1	4	1
11	1486.49	1486.46	3	2	3	1
12	1527.51	1527.48	2	2	4	1
13	1673.57	1673.54	2	3	4	1
14	1933.67	1933.60	2	2	6	1

Table 3. Anionic Oligosaccharides with Two Sulfate Esters from Gp630 Determined by Negative Ion Mass Spectrometry

	doubly sulfated $[M + Na - H]^-$						
no.	theor *m*/*z*	exp *m*/*z*	Hex	Fuc	HexNAc	sulfate	Na^+
1	1175.41	1175.37	1	0	4	2	1
2	1321.34	1321.39	1	1	4	2	1
3	1483.40	1483.36	2	1	4	2	1

characterized,[17,25] there is relatively little structural information on the anionic components. A small number of sialylated oligosaccharides have been elucidated, and there are a few reported structures of sulfated oligosaccharides.[26] From the results in the tables, we concluded that sulfated oligosaccharides make up a large component of the anionic oligosaccharides. They are also the major reason for the anionic character of the protein. The presence of sulfate esters has long been known. The sulfated oligosaccharides are particularly important in the J1 layer, the source of the 630 protein.[27] Unfortunately, the limited amount of material precluded the lectin binding experiments with this mixture. The lectin binding experiments were performed with the whole egg jelly of which the oligosaccharides in gp630 form a subset.

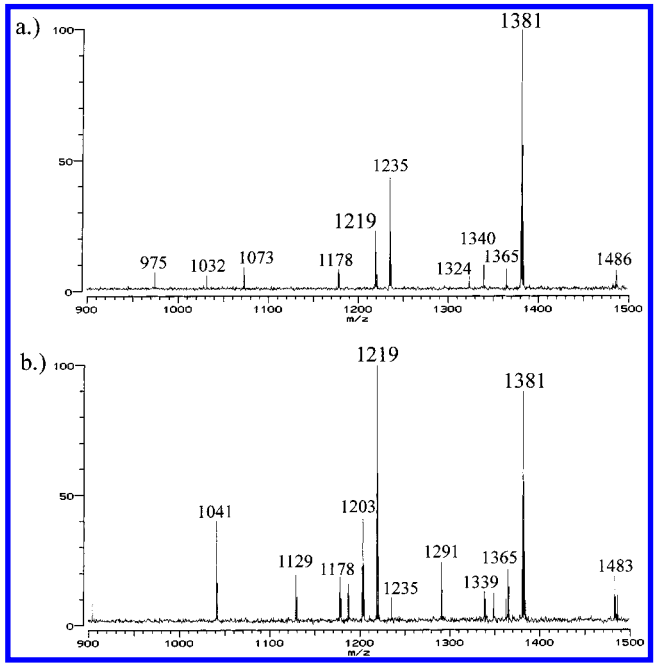


Figure 3. MALDI-FTMS spectra in the negative mode (*m*/*z* 900−1500) of an O-linked oligosaccharide mixture released from *X. laevis* egg jelly glycoproteins on (a) a polymer film probe and (b) a bioaffinity probe with immobilized CGL followed with washings. The species corresponding to *m*/*z* 1381 is not as strongly retained as the species corresponding to *m*/*z* 1219.

Figures 3a and 4a show representative MALDI-FTMS spectra, in the negative mode, of the whole egg jelly mixture on a polymer film probe (with no washings). The spectra in the same mass ranges of the mixture in the affinity MALDI probes are shown in Figure 3b and 4b. These measurements were similarly repeated three times. While the signal-to-noise ratios varied between spectra, the relative intensities of the peaks remained essentially constant with less than 10% variations. A comparison of Figure 3a and 3b and Figure 4a and 4b shows specific intensities that are attenuated and signals that are enhanced. For example, *m*/*z* 1381 is diminished in the CGL probe while *m*/*z* 1219 is enhanced (Figure 3b). Similarly, in Figure 4b, *m*/*z* 1934 is enhanced in the CGL probe. Some minor components disappear while others appear. For example, *m*/*z* 1073, with composition corresponding to one hexose, four *N*-acetylhexoses, and one sulfate group, is not observed in the CGL probe. The majority of the signals in the CGL probe correspond to sulfated oligosaccharides. There are similarly signals observed in the CGL bound probe that are not found in the untreated polymer film probe; some including *m*/*z* 1129 and 1291 correspond to sialylated oligosaccharides. The presence of sialic acid in these compounds was confirmed by the use of sialidase and the presence of the appropriate mass shift in the respective mass spectra (data not shown). In our experience, signals of sialylated oligosaccharides are typically suppressed by sulfated oligosaccharides during MALDI-MS and are therefore

(25) Strecker, G.; Wieruszeski, J.-M.; Plancke, Y.; Boilly, B. *Glycobiology* **1995**, *5*, 137−146.

(26) Plancke, Y.; Wieruszeski, J. M.; Alonso, C.; Boilly, B.; Strecker, G. *Eur. J. Biochem.* **1995**, *231*, 434−439.

(27) Hedrick, J. L.; Smith, A. J.; Yurewicz, E. C.; Oliphant, G.; Wolf, D. P. *Bio. Reproduction* **1974**, *11*, 534−542.

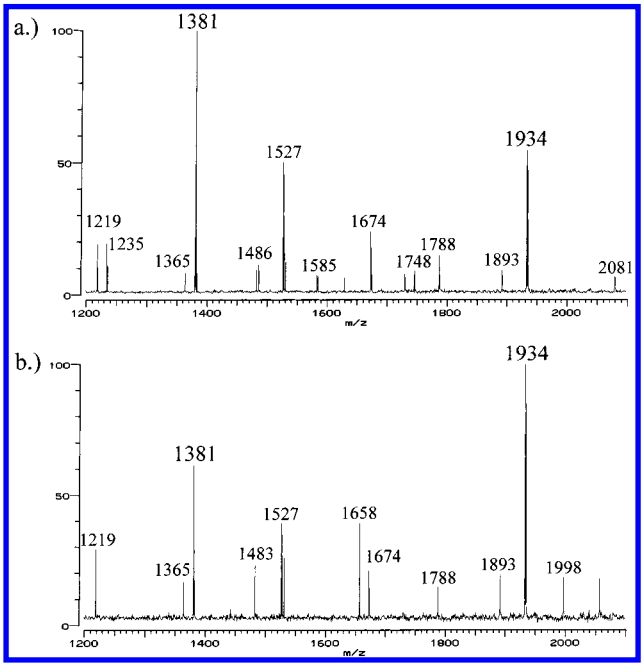


Figure 4. MALDI-FTMS spectra in the negative mode (*m*/*z* 1200−2100) of an O-linked oligosaccharide mixture released from *X. laevis* egg jelly glycoproteins on (a) a polymer film probe and (b) a bioaffinity probe with immobilized CGL followed with washings. The species corresponding to *m*/*z* 1934 is strongly retained.

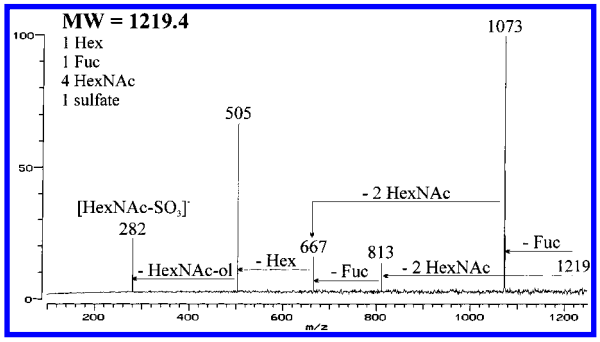
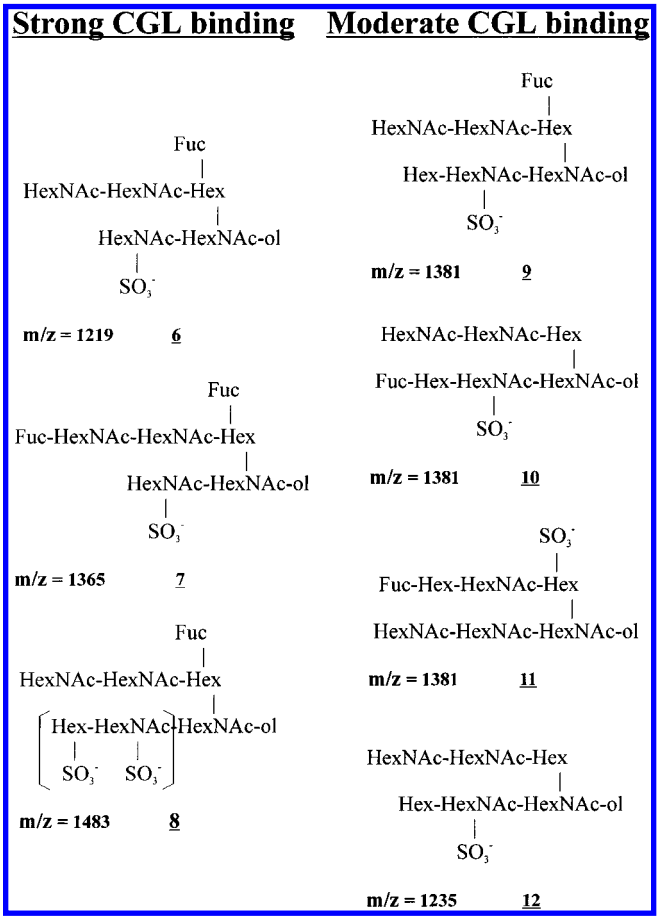


Figure 5. Tandem MS (MS/MS) spectrum of *m*/*z* 1219, a species retained on the CGL probe. The fragmentation pattern is consistent with the structure proposed in Chart 1.

Chart 1



not strongly observed in the whole egg mixtures with the polymer film probe. Other signals observed only in the lectin probe correspond to neutral oligosaccharides. For example, *m*/*z* 1041 and 1203 in the negative mode correspond to *m*/*z* 1065 (M + Na^+) and 1227, respectively, in the positive mode. The structures of these compounds are known and both have galactose in the nonreducing ends.[18]

Structural Elucidation of Sulfated Oligosaccharides by CID. The most noticeable changes in intensities between the polymer film probe and the lectin-treated probe in Figures 3 and 4 occurred for three abundant ions, *m*/*z* 1219, 1381, and 1934. The ion *m*/*z* 1381 was clearly not as strongly retained as *m*/*z* 1219 and 1934. These species and a few others were further investigated using CID. CID was performed using off-resonance excitation of compounds according to a procedure described in earlier publications.[17,18] Each ionic species was mass-selected prior to the CID.

The CID spectrum of *m*/*z* 1219, a retained oligosaccharide, shown in Figure 5, is consistent with a pure compound whose proposed structure is shown (**6**, Chart 1). The most abundant peak corresponded to the loss of fucose (Fuc). Fucose loss was a common fragmentation of oligosaccharides in both the positive and negative ion modes. The loss of two *N*-acetylhexoses (HexNAc) from the quasimolecular ion to give *m*/*z* 813 indicated that the two HexNAc were at the nonreducing end. The loss of fucose from *m*/*z* 813 to yield *m*/*z* 667 and the subsequent loss of a hexose (Hex) determined the position of the fucose. The loss of HexNAc-ol, the alditol-converted reducing end, and the presence of *m*/*z* 282 corresponding to a sulfated *N*-acetylhexose (HexNAc-SO_3^-) yielded the biantennary nature of the compound. Moreover, the position of the sulfate was on one of the nonreducing termini. A similar analysis of the CID spectrum of *m*/*z* 1934 (spectrum not shown), another retained oligosaccharide, yielded an apparently pure compound, but sufficient fragmentation could not be obtained to sequence this compound.

Two other retained species were examined in a similar manner, *m*/*z* 1365 and 1483. Based on the CID spectrum, the proposed structure of *m*/*z* 1365 is provided (**7**). This species is another oligosaccharide with the sulfate ester on a HexNAc positioned on a nonreducing terminus. The species *m*/*z* 1483 yielded a composition that contained two sulfate ester groups. CID of *m*/*z* 1483 indicated that two adjacent residues on the reducing end contain the sulfate ester **8**. It could not be determined which of the two resides on the terminal position.

The CID of *m*/*z* 1381 yielded a mixture of compounds. Separation with HPLC on an amine column was performed,

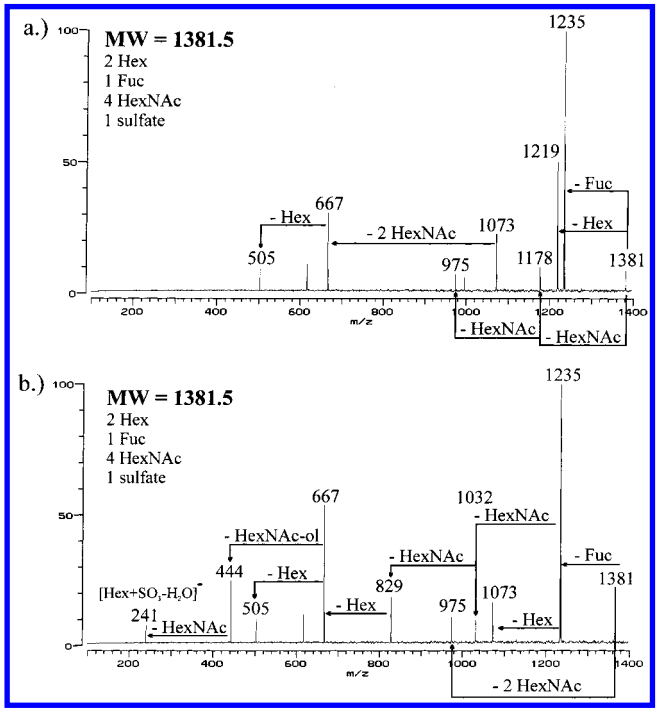


Figure 6. Tandem MS (MS/MS) spectra of three species of *m*/*z* 1381. (a) Mass spectrum of a single component. (b) Mass spectrum of a two-component mixture. These compounds are not strongly retained on the CGL probe. The fragmentation patterns are consistent with the structures proposed in Chart 1.

resulting in two peaks. One peak yielded the CID spectrum in Figure 6a. The fragmentation pattern was consistent with a single dominant species whose structure is proposed as **9**. The losses of Hex and HexNAc from the quasimolecular ion yielded a biantennary structure with a Hex and a HexNAc on the reducing end. The loss of two HexNAc from the nonreducing end revealed similarities with the preceding structure. The ion with *m*/*z* 1073 corresponded to a loss of one Fuc and one Hex, and the loss of two HexNAc to yield *m*/*z* 667 further confirmed the two HexNAc moieties. The loss of Hex from *m*/*z* 667 leaves the HexNAc-ol with the HexNAc-SO_3^-. The important structural feature here is that the sulfated residue is not on the nonreducing end.

The second HPLC peak produced the CID presented in Figure 6b. The presence of *m*/*z* 241 is consistent with a sulfated Hex unit while *m*/*z* 505 is consistent with two HexNAc, one of which is sulfated. Thus, this sample was likely composed of two species with the sulfate on different residues. Figure 6b is a spectrum of a mixture with structures proposed as **10** and **11**, based on the similar analyses performed on Figure 6a. The important feature of these two oligosaccharides is that the sulfate is again on an internal residue. A similar analysis of another unretained oligosaccharide, *m*/*z* 1235, yielded another sulfated oligosaccharide with the sulfate ester also in the internal residue **12**.

CONCLUSIONS

The use of bioaffinity MALDI probes to identify specific lectin substrates provides a rapid tool for screening protein−substrate binding. The oligosaccharides that are preferentially bound are separated from the other oligosaccharides in Chart 1. The structures of seven sulfated oligosaccharides were determined using CID.

These results are consistent with the notion that the nonreducing ends of the oligosaccharide moieties represent the recognition components of the glycoprotein. The oligosaccharides that were preferentially retained all had sulfate esters on the terminal (nonreducing) position. The oligosaccharides that were not preferentially retained had sulfate esters in the internal residues. The position of the sulfate esters is therefore important, and the sulfate esters must be intimately involved in the binding of CGL to the egg jelly. The lectins themselves are highly glycosylated so that the interactions between the lectin and the egg jelly may occur through oligosaccharide−oligosaccharide interactions via the sulfate groups. This notion is supported by the behavior of oligosaccharides, which are known to be strong chelators of sulfate groups.[28] The binding of the lectin to oligosaccharides may also involve ionic interactions because the binding is calcium dependent.[29]

ACKNOWLEDGMENT

Funding provided by the National Institutes of Health and the National Science Foundation is gratefully acknowledged.

Received for review February 14, 2001. Accepted May 15, 2001.

AC010182V

(28) Wong, A. W.; Cancilla, M. T.; Voss, L. R.; Lebrilla, C. B. *Anal. Chem.* **1999**, *71*, 205−211.
(29) Quill, T. A.; Hedrick, J. L. *Arch. Biochem. Biophys.* **1996**, *333*, 326−332.