
On the Use of DHB/Aniline and DHB/*N,N*-Dimethylaniline Matrices for Improved Detection of Carbohydrates: Automated Identification of Oligosaccharides and Quantitative Analysis of Sialylated Glycans by MALDI-TOF Mass Spectrometry

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This study demonstrates the application of 2,5-dihydroxybenzoic acid/aniline (DHB/An) and 2,5-dihydroxybenzoic acid/*N,N*-dimethylaniline (DHB/DMA) matrices for automated identification and quantitative analysis of native oligosaccharides by matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS). Both matrices are shown to be superior to pure DHB for native glycans in terms of signal intensities of analytes and homogeneity of sample distribution throughout the crystal layer. On-target formation of stable aniline Schiff base derivatives of glycans in DHB/An and the complete absence of such products in the mass spectra acquired in DHB/DMA matrix provide a platform for automated identification of reducing oligosaccharides in the MALDI mass spectra of complex samples. The study also shows how enhanced sensitivity is achieved with the use of these matrices and how the homogeneity of deposited sample material may be exploited for quick and accurate quantitative analysis of native glycan mixtures containing neutral and sialylated oligosaccharides in the low-nanogram to mid-picogram range. (J Am Soc Mass Spectrom 2008, 19, 1138–1146)   2008 American Society for Mass Spectrometry

Carbohydrates are widely recognized for their vital roles in the biology of organisms [1–4]. Protein glycosylation is ubiquitous, and it is estimated that a significant proportion of all proteins, including viral and bacterial proteins, bear this type of modification [5, 6]. Because changes in glycosylation patterns may be used as biomarkers, detection of such modifications may be used as predictive or diagnostic tools for the identification of biochemical abnormalities, which may be correlated to any pathology observed in a given organism [1, 7, 8]. As a result, there is an increasing need for developing efficient methods for detection and structural characterization of oligosaccharides and glycoproteins. Among many complementary technologies available for these purposes, mass spectrometry (MS) provides an efficient and sensitive means for studying carbohydrates in low-concentration clinical-scale samples and may also be effectively coupled to various separation techniques, both on- and off-line [9, 10].

Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) has proven

itself to be an extremely useful technique for the analysis of various biomolecules [11, 12]. MALDI offers several advantages over other ionization techniques, mainly in terms of modest sample size requirements, relative simplicity of spectral interpretation, and the possibility of preserving the samples for later use. There still exist some challenges in the analysis of oligosaccharides by MALDI-MS, which generally stem from the inherently low ionization efficiency of carbohydrates relative to peptides and proteins [9]. One solution to this problem is to operate an instrument at higher laser power, thus increasing the number of ions produced per MALDI event. This, however, often leads to more extensive fragmentation of the analytes, especially if labile groups such as sulfates or sialic acids are present. A decrease in the signal-to-noise ratio arising from increased formation of the matrix cluster ions [13] would also be observed as a result. Hence the mass spectra obtained do not provide a true representation of the sample constituents and the signal quality is generally diminished. It is therefore difficult to obtain sensitive analysis of native oligosaccharides compared to other compounds such as peptides, for example.

Oligosaccharides are often derivatized before MALDI MS to remedy this problem. Most derivatization proce-

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dures involve chemical modification of the glycans by permethylation, reductive amination, or stable Schiff base formation to render the analytes more susceptible to ionization [9, 14–16]. Although derivatized forms bring substantial improvements in sensitivity relative to native forms, these methods are often susceptible to sample loss and contamination.

Recent publications by this group [17–19] have demonstrated how the incorporation of aniline (An) and *N,N*-dimethylaniline (DMA) into a conventional 2,5-dihydroxybenzoic acid (DHB) matrix solution results in significant improvement in sensitivity for native oligosaccharides when analyzed by MALDI-TOF MS. The present work describes how the two matrices may be used in tandem for automated identification of glycans in mass spectra of complex mixtures. The discussion is also extended to the application of DHB/DMA matrix for quantitative analysis of native and sialylated glycans in the positive-ion extraction mode.

Experimental

Materials and Reagents

Ovalbumin (chicken egg white), α_1 -acid glycoprotein (human), maltohexaose, maltoheptaose, trifluoroacetic acid (TFA), ammonium bicarbonate, and DHB were obtained from Sigma (St. Louis, MO, USA). HPLC grade acetonitrile (ACN), reagent grade aniline (An), and DMA were purchased from Fisher Scientific (Fair Lawn, NJ, USA). PNGase F endoglycosidase enzyme was purchased from ProZyme (San Leandro, CA, USA). Milli-Q water (Millipore, Bedford, MA, USA) was used in preparation of all solutions.

Sample Preparation

Standard solutions were obtained by dissolving 2 mg of each standard (maltohexaose and maltoheptaose) in 1 mL of water. Each solution was mixed using an agitator for 30 s to ensure complete dissolution. A series of thirteen 1-in-2 dilutions were carried out by mixing 0.5 mL of the initial standard solution with 0.5 mL of water, and repeating this step with the new solution. Thus, a total of 14 standard solutions, ranging in concentration from about 0.2 ng/ μ L to 2 μ g/ μ L of the material, were obtained for each set.

Ovalbumin and α_1 -acid glycoprotein oligosaccharides were obtained by first dissolving 2 mg of protein in 200 μ L of 25 mM ammonium bicarbonate buffer at pH 7.8 in a 0.6 mL microcentrifuge tube. PNGase enzyme solution (2 μ L, as provided by the manufacturer) was added to the tube and the contents were mixed using an agitator for about 1 min. The mixture was incubated at 37 °C for 20 h, with additional mixing after the first 10 h. Upon digestion, the mixture was lyophilized and resuspended in 200 μ L of water. Detached glycans were isolated by loading 50 μ L of the digestion mixture onto a C₁₈ reversed-phase column,

prepared by a previously described procedure [20], and flushing with 200 μ L of 0.5% acetic acid aqueous solution to elute the sugars. The eluted sugar solution was then incubated at 37 °C for 2 h to fully deaminate the glycans. It was then lyophilized, resuspended in 200 μ L of water, and mixed to ensure homogeneity.

The solution containing the glycans detached from α_1 -acid glycoprotein was divided into three portions of 50 μ L each. One sample was left untouched (untreated). To another sample (partially desialylated), 50 μ L of 0.5% TFA in water was added and the resulting solution was heated at 75 °C for 15 min to partially remove sialic acid residues from the glycans. The third sample (fully desialylated) was also treated with TFA as above, although the solution was incubated at 75 °C for 1 h to ensure complete removal of sialic acid from the oligosaccharides in the sample. Solutions treated with TFA were evaporated on a SpeedVac system (Thermo Scientific, Milford, MA, USA) and the samples were resuspended in 50 μ L of water.

MALDI Matrix Preparation

DHB matrix solution was prepared by dissolving 100 mg of DHB in 1 mL of a 1:1 solution of water and ACN. The DHB/DMA matrix solution was prepared by adding 20 μ L of neat An or DMA to the DHB matrix solution. Thus, the molar ratio of DHB to An or DMA was about 3:1. Both An and DMA were purified by simple distillation before use. Samples were deposited onto a polished steel MALDI target by mixing the analyte and matrix solutions (1 μ L each) on-target and allowing the mixture to dry by evaporation under ambient conditions.

Mass Spectrometric Measurements

All mass spectra were acquired on a Bruker Biflex IV MALDI-TOF, an axial TOF mass spectrometer (Bruker Daltonics, Billerica, MA, USA) equipped with a 337 nm laser. Mass spectra were obtained in the positive-ion extraction mode with the following voltage settings: ion source 1 (19.0 kV), ion source 2 (15.9 kV), and lens (9.3 kV). The reflector voltage was set to 20 kV. The laser was pulsed at 7 Hz and the pulsed ion extraction time was set to 400 ns. The laser power was kept in the 20–25% range, at a level only slightly above the threshold to obtain a good intensity signal without significant shifts in the baseline or introduction of extensive noise into the mass spectra. This is explained further in Results and Discussion. For analyses of the samples using DHB/An and DHB/DMA matrices, the spots of the deposited material were sampled at five random locations (50 laser pulses each) and the signals were added together. In the cases where DHB was used as the matrix, mass spectra were collected at “sweet spots” and taken as the average of 250 laser shots. Where it was not possible to manually identify a “sweet spot,” a “five random locations” approach, as described earlier,

was adopted. All oligosaccharides observed in the mass spectra corresponded to $[M + Na]^+$ ions.

Results and Discussion

Because 2,5-dihydroxybenzoic acid (DHB) is the most widely used MALDI matrix in the literature for analysis of oligosaccharides by MALDI-MS, it was used here as a benchmark for comparing the performance of the DHB/An and DHB/DMA mixtures as MALDI matrices. As reported earlier [18, 19], both DHB/An and DHB/DMA offered impressive improvements in sensitivity for native glycans observed as sodiated ions compared to unmodified DHB as a matrix.

Morphological differences in the crystal layers resulting from sample deposition in DHB and DHB/DMA by the dried droplet technique are seen in Figure 1a and b. DHB produces irregular needle-shaped crystals, whereas the use of DHB/DMA (and DHB/An also) leads to formation of a more uniform, fine-crystal layer. It was possible to detect signals of native glycans as $[M + Na]^+$ ions in the low-picogram range using these matrices, as seen in Figure 1c. The relationship between morphology of the matrix crystal layer and analyte sensitivity has been well established: generally, more uniform crystal layers result in more homogeneous sample distribution within a spot, thus making detec-

tion of the analyte more even throughout the spot [21, 22]. A common technique used to improve sensitivity with DHB as well as other matrices yielding irregular crystal morphologies is to redissolve the spot on-target in cold ethanol or acetone. In this study, redissolving DHB sample spots in cold ethanol did enhance crystal homogeneity and intensity of signals observed, although significantly less than with the DHB/An or DHB/DMA preparation method [18, 19]. Moreover, the redissolving procedure appeared useful only if a spot already contained ~ 10 pmol (~ 20 ng) of sample material.

Automated Identification of Glycans

The use of DHB/An matrix results in the formation of an aniline Schiff base at the reducing end of the native oligosaccharides, as indicated by the presence of the peaks labeled with asterisks in Figure 2b (glycans detached from chicken ovalbumin). This on-target derivatization reaction does not go to completion, and strong signals corresponding to the underivatized native glycans are also observed. Although there was a certain time dependence on the extent of derivatization (the Schiff base signal does become stronger with time) [17], about 5 h after depositing the sample on the target, signal intensities of the native and Schiff base forms of the glycans in low-picogram (nanograms) quantities were comparable. Thus, the only differences in the mass spectra of oligosaccharides in DHB/An and DHB/DMA matrices are the peaks labeled with asterisks that correspond to the Schiff base derivatives when DHB/An was used. There was no significant improvement in the ionization efficiency observed for the Schiff bases relative to the native glycans. DHB/An thus appears as a less useful MALDI matrix than DHB/DMA, mainly because the signal attributed to a particular glycan is split into two peaks. This affects the sensitivity and yields mass spectra that are more complex for interpretation.

The formation of the Schiff base in DHB/An, however, may be exploited for the automated identification of glycans if mass spectra are acquired consecutively using DHB/An and DHB/DMA. The workflow presented in Scheme 1 has been implemented to accomplish this task.

In the initial step, one is required to obtain mass spectra of the same sample in both matrices. A separate peak list is then generated for each spectrum. In the mass spectrum acquired in DHB/An, peak pairs with a mass difference of 75 m/z units (ascribed to the aniline Schiff base) are identified. Each peak pair tentatively corresponds to a native glycan (lower mass) and a Schiff base derivative peak (+75 m/z). In the next step, these pair selections are verified by searching for the lower mass peaks in the list generated for the sample run in DHB/DMA. If peaks correspond, the following step is to confirm the absence of +75 m/z peaks in the DHB/

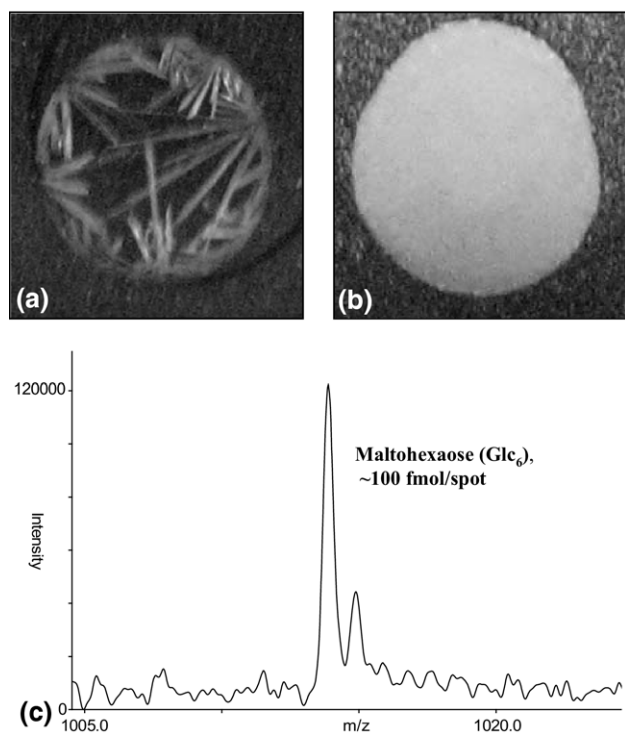


Figure 1. Matrix crystal morphologies: favorable properties of DHB/An and DHB/DMA MALDI matrices for the analysis of oligosaccharide mixtures. Sample spots on a polished steel surface, DHB (a) and DHB/DMA (b) obtained by the dried droplet deposition method. (c) Signal attributed to approximately 100 pg (~ 100 fmol) of maltohexaose as $[M + Na]^+$ ions in DHB/DMA in reflector mode MALDI.

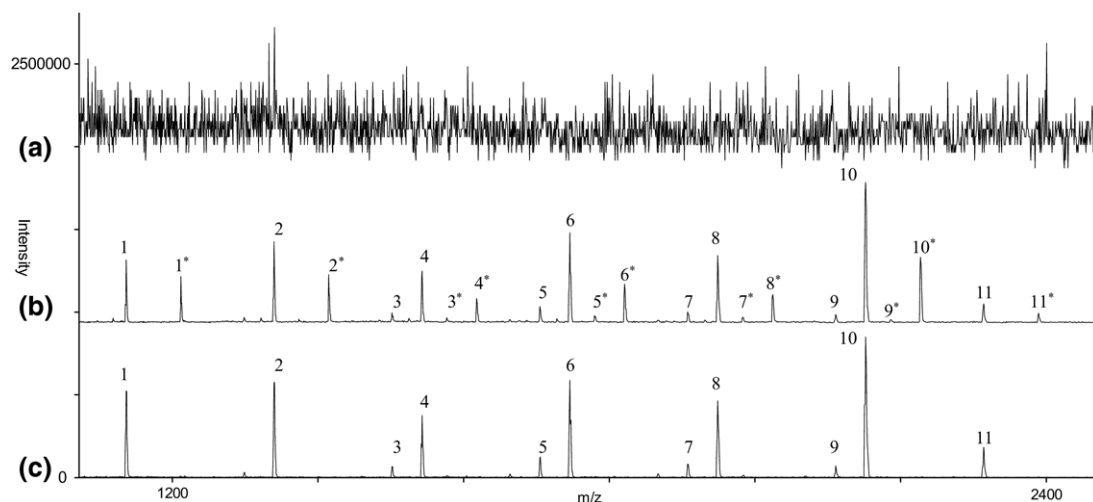


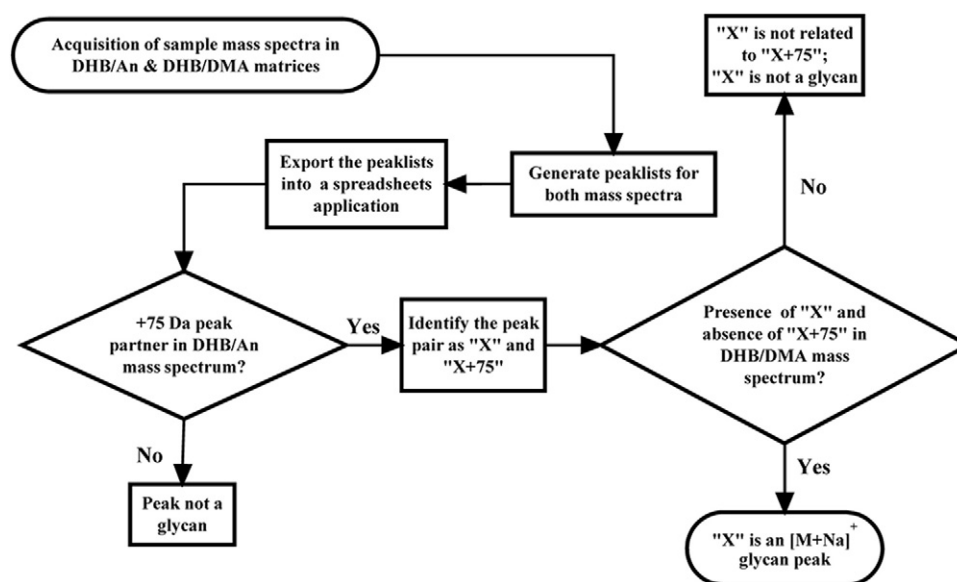
Figure 2. Reflector mode MALDI-TOF mass spectra of oligosaccharides released from chicken ovalbumin by PNGase F ($\sim 5 \mu\text{g}$ digest solution) in DHB (a), DHB/An (b), and DHB/DMA (c). Prominent glycan peaks are labeled and aniline Schiff base derivatives are denoted by an asterisk. All glycans were observed as $[M + \text{Na}]^+$ ions. Note that no signal was detected in the DHB sample spot.

DMA mass spectrum because there should be no aniline Schiff base derivatives formed in this matrix. This step also eliminates the occurrence of false positives resulting from unrelated peaks, even if separated by 75 m/z units. If the cited criteria are met for a particular peak pair, the lower mass peak is then identified as a sodiated glycan, $[M + \text{Na}]^+$.

At first, this procedure was very useful for manual identification of oligosaccharide peaks, and the idea was further tested with a simple algorithm written as an Excel-based macro (Microsoft, Redland, WA, USA). The mass spectra, processed using Bruker flexAnalysis software, were imported as Excel files before application of the algorithm. In Figure 2, all labeled glycan peaks

(from chicken ovalbumin) were identified when the algorithm was applied to the corresponding mass spectral data.

To conduct further identification tests, two additional sets of samples were prepared. The first sample consisted of an LC fraction of a tryptic digest of bovine α_1 -acid glycoprotein containing a desialylated glycopeptide and several small peptides [18–20]. The sample was treated with PNGase F to detach the glycans from the glycopeptide. Mass spectra of this mixture in DHB, DHB/An, and DHB/DMA are given in Figure 3. The lower m/z region of the mass spectra displaying peptide $[M + \text{Na}]^+$ ions is identical for DHB/An and DHB/DMA matrices. Interpretation of this region is compli-



Scheme 1. A workflow for an algorithm designed to perform automated glycan detection in MALDI mass spectra using DHB/An and DHB/DMA matrices.

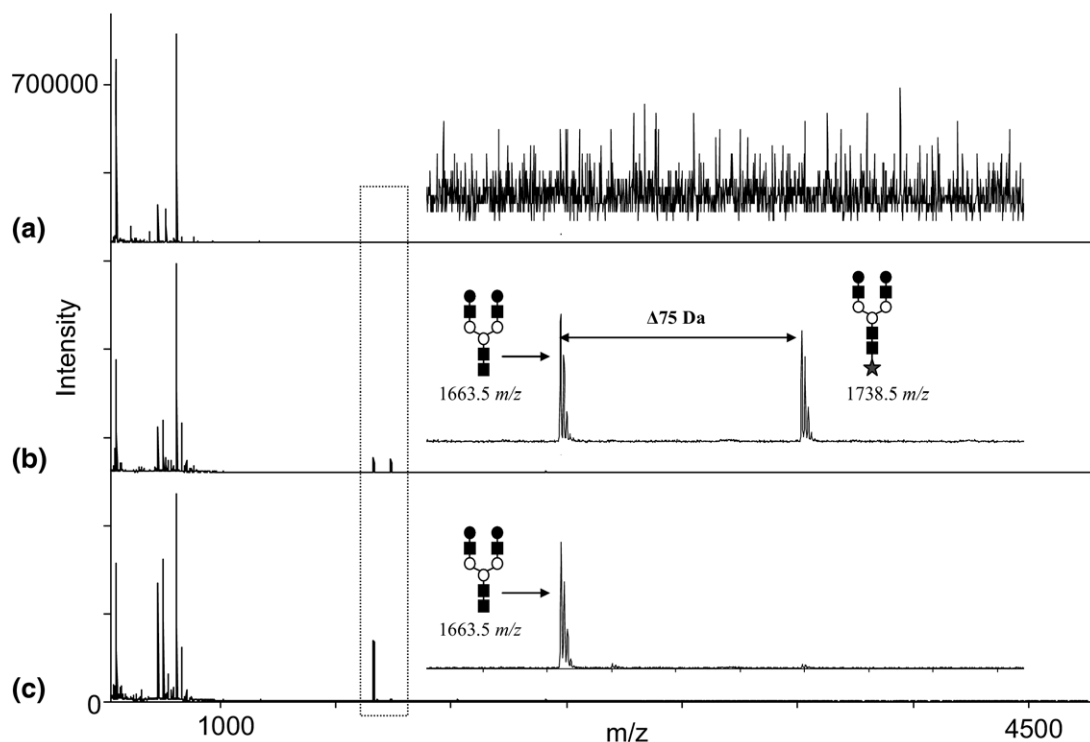


Figure 3. Reflector mode MALDI-TOF mass spectra of a peptide mixture containing glycopeptides treated with PNGase F acquired in DHB (a), DHB/An (b), and DHB/DMA (c). Expansions are shown in the insets. $[M + Na]^+$ ions of selected native glycan and its Schiff base derivative are labeled therein.

cated by an extensive number of peptide sodium adducts, as observed in previous reports on the use of analogous matrix systems [18, 19, 23]. The two mass spectra clearly differ in the region displaying the $[M + Na]^+$ native glycan peak at 1663.5 m/z and the $[M + Na]^+$ aniline Schiff base peak at 1738.5 m/z , as seen in Figure 3 (II). The algorithm identified only one peak as corresponding to a glycan, at 1663.5 m/z .

The second sample tested was composed of a tryptic digest of human α_1 -acid glycoprotein spiked with maltoheptaose. The mass spectra of this sample acquired in DHB, DHB/An, and DHB/DMA are given in Figure 4. The only difference between DHB/An and DHB/DMA spectra is attributed to the Schiff base derivative of maltoheptaose formed in DHB/An. Despite the complexity of the mass spectra, maltoheptaose was identified by the algorithm as the only glycan present in the mixture (Figure 4b).

Quantitative Analysis of Oligosaccharides: General Observations

Previous work has shown that DHB/DMA matrix may be used for quantitative analysis of neutral oligosaccharides using MALDI-TOF MS [18, 19]. This matrix system allowed for sensitive detection and accurate relative quantitation of glycans in a sample. Moreover, by using an internal standard, it was possible to estimate the absolute amounts of the analyte oligosaccharides deposited within a sample spot.

The main challenge in analyzing sialylated oligosaccharides samples by MALDI MS is associated with the labile and negatively charged nature of sialic acid residues. In-source and post-source losses of sialic acid often lead to inaccurate representation of the degree of sialylation of glycans [9]. Thus, sialylated glycans may be under-represented, whereas the relative amounts of the asialo species may be exaggerated in the mass spectra. Sialylated oligosaccharides give rise to $[M + (n + 1)Na - nH]^+$ peaks in the positive-ion mode and $(n + 1)$ peaks are typically observed, where n is the number of sialic acid residues in a given glycan. These peak patterns may serve as signatures for the presence of sialylated species. The occurrence of *N*-glycolylneuraminic acid (NeuGc) in glycans further complicates the mass spectra [20]. Chemical modification of sialic acid residues is often used to improve their stability for subsequent mass spectrometric analysis and to reduce the degree of sodiation, thus simplifying interpretation of the mass spectra [24]. Negative-ion extraction mode MALDI analysis has also proved to be very useful in the analysis of sialylated oligosaccharides [25].

Analysis of Sialylated Oligosaccharides

The ionization patterns of sialylated N-linked glycans released from human α_1 -acid glycoprotein were investigated in the positive-ion mode, using DHB/DMA matrix in both linear and reflector TOF modes, without chemical derivatization. Samples at three different lev-

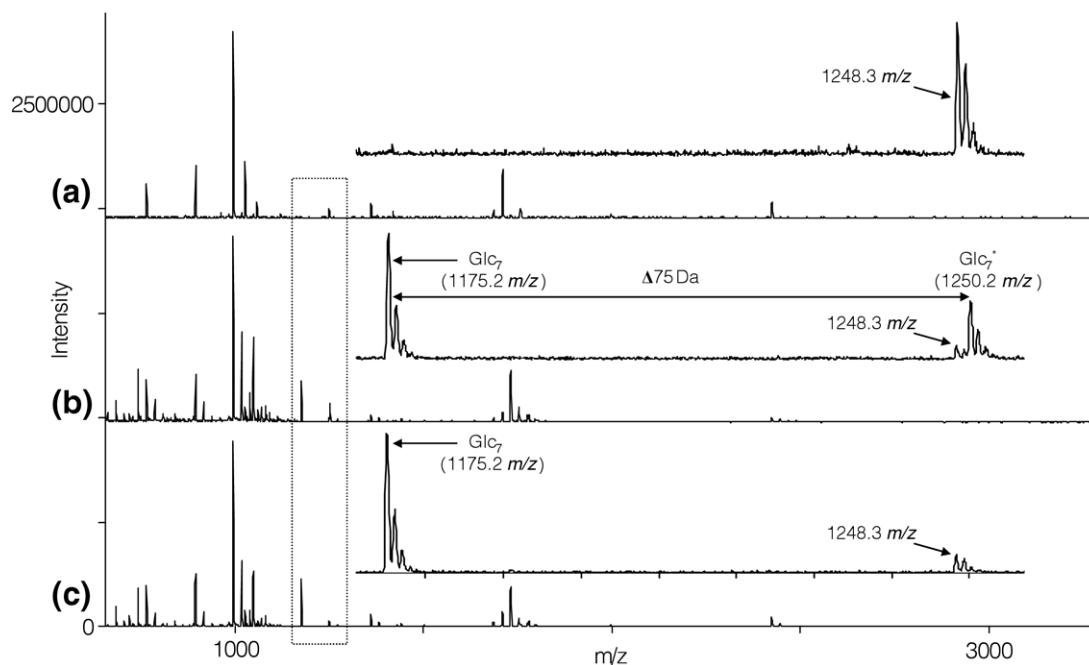


Figure 4. Reflector MALDI-TOF mass spectra of a human α_1 -acid glycoprotein tryptic digest mixture spiked with approximately 4.6 ng (4 pmol) of maltoheptaose acquired in DHB (a), DHB/An (b), and DHB/DMA (c). Expansion are shown in the insets. Note that the peak at 1248.3 m/z corresponds to a $[M + H]^+$ peptide ion present in the mixture. Maltoheptaose and its Schiff base derivative are observed as $[M + Na]^+$ ions.

els of sialylation (untreated, partially desialylated, and fully desialylated) were prepared according to the procedure outlined in the Sample Preparation section.

The degree of sialylation of the glycans was initially assessed in the linear mode using the untreated and partially desialylated samples (Figure 5). This was done

primarily to evaluate the partial desialylation procedure used in the proposed method. It was important to have a sample with an intermediate degree of sialylation with respect to the untreated (fully sialylated) and fully desialylated samples. Mass spectra were acquired at the laser power setting yielding the highest abun-

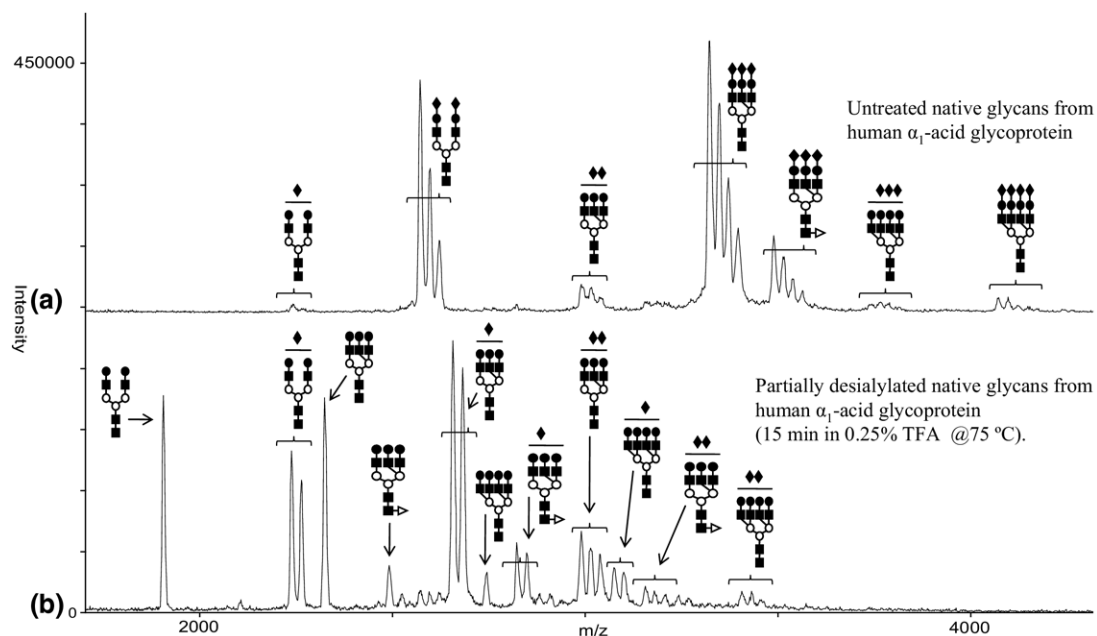


Figure 5. Linear mode MALDI-TOF mass spectra of the untreated sample (a) and partially desialylated sample (b) deposited in DHB/DMA matrix. The peaks corresponding to the $[M + Na]^+$ ions of some of the glycan structures observed are labeled in the mass spectra.

dance ratio of fully sialylated species to species one sialic acid short of full occupancy for the untreated and partially desialylated samples. This was done to minimize in-source desialylation. This setting (20% laser power) also resulted in the complete absence of fully desialylated glycan ions in the mass spectrum of the untreated sample (Figure 5a), suggesting that all glycans in the sample have at least one sialic acid residue. Acquisition at a lower laser power resulted in a significant overall decrease of signal intensity, suggesting that the initial 20% setting was just above threshold for ionization of the glycans in the positive-ion mode. Oligosaccharides with different degrees of sialylation were observed for both samples and various glycan structures were easily assigned to the peaks, as illustrated in Figure 5.

The same two samples, untreated and partially desialylated, were then analyzed in the positive-ion mode using the reflector, at the same 20% laser power setting as used earlier (spectra not shown). Surprisingly, no peaks corresponding to sialylated species were observed. Signal intensities of the asialo oligosaccharide ions remained relatively unchanged for the partially desialylated sample (within a 10% margin) with respect to those seen in the linear mode mass spectra. Also, the reflector mode mass spectrum of the untreated sample lacked any peaks in the region of interest (i.e., above m/z 1500), in sharp contrast with features observed in the linear mode mass spectrum (Figure 5a). These observations imply that none of the sialylated glycan ions reached the second detector, likely arising from post-

source fragmentation. It is also clear that the laser power used was above threshold for the formation of oligosaccharide ions, given that asialo glycan ions were abundant in the mass spectrum of the partially desialylated sample. Characteristically broad metastable peaks, often observed as a result of post-source fragmentation of analyte ions, were completely absent from the mass spectra of both samples.

To further investigate these observations and to attempt quantitative analysis, all three samples were spiked with maltoheptaose as an internal standard. The untreated sample contained maltoheptaose at approximately 17 ng/ μ L (\sim 15 pmol/ μ L), whereas the other two contained maltoheptaose at about 34 ng/ μ L (\sim 30 pmol/ μ L). The following volumes of spiked samples were deposited onto a MALDI target, as three spots: 2 μ L of untreated sample and 1 μ L of each of the partially desialylated and fully desialylated samples. Thus, twice the glycan material was deposited in the case of the fully sialylated sample relative to both of the others, whereas all three spots contained 34 ng (30 pmol) of maltoheptaose internal standard. The purpose of this experiment was to improve the detection of low-abundance ions in the reflector mode mass spectrum of the fully sialylated sample.

Mass spectra of these three samples, acquired in the reflector mode, are presented in Figure 6. Amounts of the main four fully desialylated glycans in the three samples were determined relative to the maltoheptaose internal standard (by peak integration) and were all within 10% deviation for three successive acquisitions

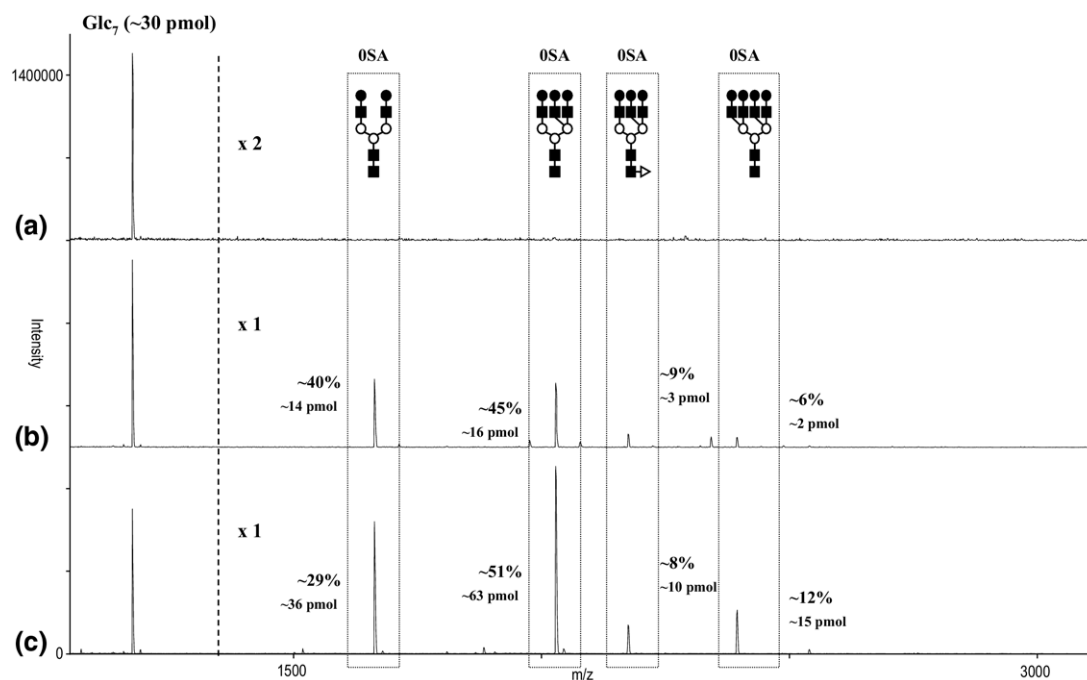


Figure 6. Reflector mode MALDI-TOF mass spectra of the three glycan samples spiked with maltoheptaose in DHB/DMA matrix. (a) Untreated sample, (b) partially desialylated glycans, (c) fully desialylated glycans. The laser power used to acquire these mass spectra was identical to the one used in the acquisition of the mass spectra presented in Figure 5. All peaks correspond to $[M + Na]^+$ ions.

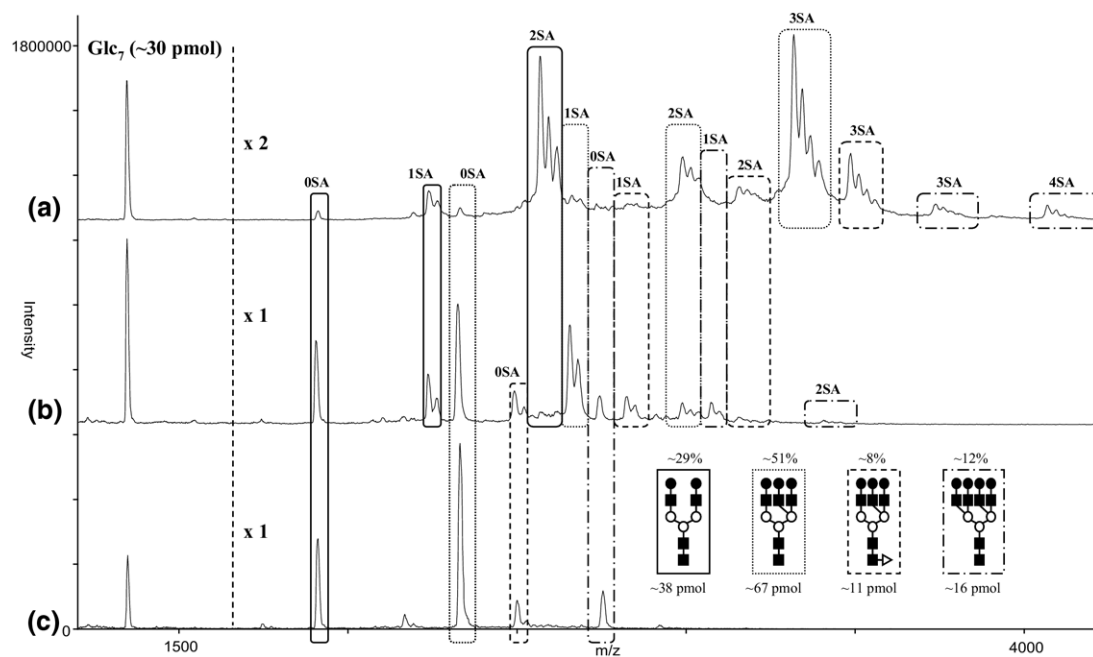


Figure 7. Linear mode MALDI-TOF mass spectra of oligosaccharides detached from α_1 -acid glycoprotein in DHB/DMA matrix acquired at a slightly higher laser power. (a) Untreated sample, (b) partially desialylated glycans, (c) fully desialylated glycans. Peaks corresponding to $[M + Na]^+$ ions of the four dominant glycan structures with varying degrees of sialylation are labeled. Note the spectral differences relative to the mass spectra shown in Figure 5.

(see Figure 6). Sample composition, in terms of stable glycan structures without labile residues or groups and in terms of glycan antennarity, was thus obtained.

The same sample spots were then analyzed in linear mode. The mass spectrum of the fully desialylated sample contained only the $[M + Na]^+$ peaks corresponding to fully desialylated glycan structures. The relative abundances and calculated amounts of fully desialylated species in all three samples were identical to those calculated using the reflector mode mass spectra.

When the laser power was increased from 20 to 23%, the relative intensities of the glycan signals in the mass spectra of the untreated and partially desialylated samples did decrease, as shown in Figure 7. Peaks corresponding to oligosaccharides with lower sialylation states increased in area, whereas those of glycans with a higher number of sialic acid residues decreased with respect to areas observed in Figure 5. In the reflector mode (not shown), $[M + Na]^+$ signal intensities of asialo glycans appeared to increase with respect to those observed in Figure 6 [using the optimal (20%) laser power]. Interestingly, peaks corresponding to metastable ions and to sialylated species together with peaks corresponding to sialylated species began to emerge at this laser power (23%), although only at low signal intensity.

On the basis of these observations on the analysis of sialylated oligosaccharides in the linear and reflector modes, it is possible to propose a method for relative

and absolute quantitation of oligosaccharides by positive-ion MALDI-MS.

In the first step, the laser power and sample size are optimized to produce a strong signal in the linear mode without any noticeable in-source loss of sialic acid, using an untreated sample. At this optimal laser setting, there should be no signal observed for sialylated glycans in the reflector mode. Asialo glycans are then quantified using an appropriate internal standard, preferably in the reflector mode [18, 19]. There would be no interference from sialylated glycan peaks.

In the second step, a portion of the fully desialylated sample is analyzed under the same conditions as cited earlier, in both linear and reflector modes, and asialo glycans are again quantified. For each glycan type, the difference between the calculated amount of the asialo glycans in untreated and fully desialylated samples corresponds to the total amount of sialylated species. Thus, (1) determining the abundance ratios of glycans of a particular type at different levels of sialylation in the linear mode in the untreated sample and (2) applying these ratios to the difference calculated above, make it possible to indirectly determine the amount of each sialylated species for each glycan type present in the untreated sample.

This method does not involve the use of sialic acid-containing oligosaccharide standards. Also, neutral glycans may be used as internal standards for the analysis of acidic glycans [18, 19]. This method rests on the assumptions that no in-source losses of sialic acid

occur under the chosen conditions and that the ionization efficiency of sialylated glycans does not depend on the number of sialic acid residues. These assumptions hold for the purpose of estimating—with reasonable precision and accuracy—the quantities of sialylated oligosaccharides contained in a sample.

Conclusions

This study demonstrates applications of DHB/An and DHB/DMA matrices for MALDI-based mass spectrometric analyses of oligosaccharides from glycoproteins. The use of both matrices allows for simple automated identification of glycans because of their partial derivatization with aniline and subsequent application of an algorithm-based mass difference discrimination procedure. Thus, the occurrence of both the aniline Schiff base and native glycan signals may be exploited as a key variable in data processing for automated detection and identification of glycans. The use of DHB/DMA matrix for quantitative analysis of native neutral oligosaccharides presents advantages over other MALDI-based quantitative analysis methods in terms of simplicity and sensitivity. It was shown how sialylated glycans may be analyzed quantitatively using DHB/DMA MALDI matrix. The algorithm discussed herein may also be used for automated identification of sialylated glycans, although additional adjustments to the peak identification procedure would have to be made to account for the multiple peaks representing all sialylated species in a sample.

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