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Application of Malononitrile Derivatization Method for Structural Glycomics Study in Matrix-assisted Laser Desorption/Ionization Time-of-flight Mass Spectrometry

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Structural analyses of oligosaccharide-malononitrile derivatives were conducted by matrix-assisted laser desorption/ionization post-source decay (MALDI-PSD) analysis in positive ion mode. The malononitrile derivatives of oligosaccharides, which were developed for highly sensitive detection of multi-component oligosaccharides by negative ion electrospray ionization mass spectrometry (ESI MS), were detected by positive-ion MALDI with the detection limit of 2 pmol level from the crude derivatization sample. The used matrix affected drastically the analytical results of oligosaccharide-malononitrile derivative by matrix-assisted laser desorption/ionization mass spectrometry (MALDI MS). The malononitrile derivatization of oligosaccharide also affect the patterns of MALDI-PSD spectra and give much more structural information than the free oligosaccharide.

key words: MALDI-TOF, Malononitrile derivatization, Oligosaccharide, Glycomics, Mass spectrometer

INTRODUCTION

Since it is recognized that carbohydrates, as free oligosaccharides and constituents of glycoconjugates, play important roles in many biological functions of glycoproteins, a variety of analytical techniques have been developed for highly sensitive detection and analysis of complex oligosaccharides obtained from a variety of biological media [1]. Presently, among many analytical techniques, mass spectrometry has been recognized to be a highly powerful technique for the determination of molecular weights and for structural analysis for a variety of carbohydrates. Because of the low proton affinity and ionizability of free oligosaccharides, especially neutral oligosaccharides, the mass sensitivity and the detectable mass range of free oligosaccharides are generally poor in comparison with those of proteins and nucleic acids in positive ion mode. In order to solve these problems, a number of derivatization methods such as permethylation, peresterification, reductive amination and malononitrile addition have been developed [2-6]. Furthermore in the structural analyses of oligosaccharides by CID experiments [7, 8] with ESI mass spectrometry or by PSD experiments [9, 10] in MALDI-TOF mass spectrometry, these derivatization techniques have additional advantages. Firstly the mass shift of oligosaccharide derivatives, corresponding to the mass of an attached labeling group, facilitates the interpretation of the resulting peak patterns of fragment ions. Secondly the occurrence of a variety of fragmentation patterns, due to the direction of ion fragmentation in the oligosaccharide derivative, can give more information on the structure of the oligosaccharide.

Among several derivatization techniques the malononitrile addition method has been developed recently by our group, and applied effectively for the separation and high-sensitivity detection of multi-component oligosaccharide mixture using on-line capillary liquid chromatography (LC)/ESI-MS in negative ion mode [11]. The malononitrile derivatization of oligosaccharide is accomplished easily under mildly basic aqueous conditions without any reagent except for malononitrile, which is a volatile compound with a small molecular weight, so that excess reagent is easily removed and/or does not interfere with the spectra of interest. Thus we could accomplish directly the high-sensitivity analyses of unpurified oligosaccharide derivatives using negative-ion ESI-MS without any sample pre-treatment procedure.

In the present study we introduce the application of malononitrile derivatization method for structural analyses of oligosaccharides in matrix-assisted laser desorption/ionization time-of-flight mass spectrometry.

EXPERIMENTAL MALDI-TOF MS

Positive-ion MALDI-TOF MS was performed using a Voyager-DE STR time-of-flight mass spectrometer (PerSeptive Biosystems, Framingham, MA) equipped with a delayed-extraction system, with flight paths of 1.3 m for the linear mode and 2.0 m for the reflectron mode. The spectrometer was equipped with a nitrogen laser with 3 ns pulse width at 337 nm for evaporation and ionization of sample. The sam-

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ple was accelerated at 20 kV for linear and reflectron mode in the ion source, with a delay ranging from 50 to 200 ns. For PSD experiments, after ions of interest were separated from undesired ones by the timed ion selector, the ions could be focused on the detector by adjusting the potential of the reflectron in a stepwise manner. The obtained segments of the fragment ion spectra were stitched together by software to create a complete PSD spectrum.

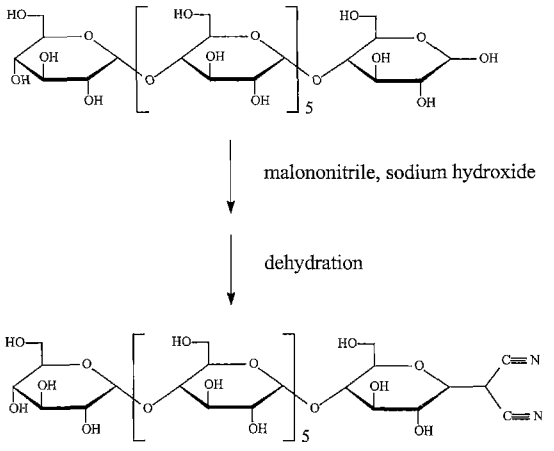
Derivatization of Oligosaccharides and Analyses

In an Eppendorf tube malononitrile (0.12 mg, 1.8 μmol) dissolved in methanol (7.5 μL) was added to the aqueous solution (2.5 μL) of maltoheptaose (0.12 mg, 0.1 μmol). Sodium hydroxide (0.02 mg, 0.5 μmol) dissolved in water (0.5 μL) was added to the mixture. The reaction tube was shaken for several seconds, and incubated at ambient temperature for about 4 hr.

Matrix solutions of 2,5-dihydroxybenzoic acid (DHBA) and (α-cyano-4-hydroxycinnamic acid (CHCA) were prepared each to a concentration of 10 mg/mL in 50% aqueous ethanol. For the sample preparation, a portion of the reaction mixture (1 μL) was taken up and diluted with distilled water (100 μL). 0.2 μL of the aqueous solution was taken up and mixed with the matrix solution (0.2 μL) prepared separately. The analyte matrix mixture was loaded on a sample probe and dried.

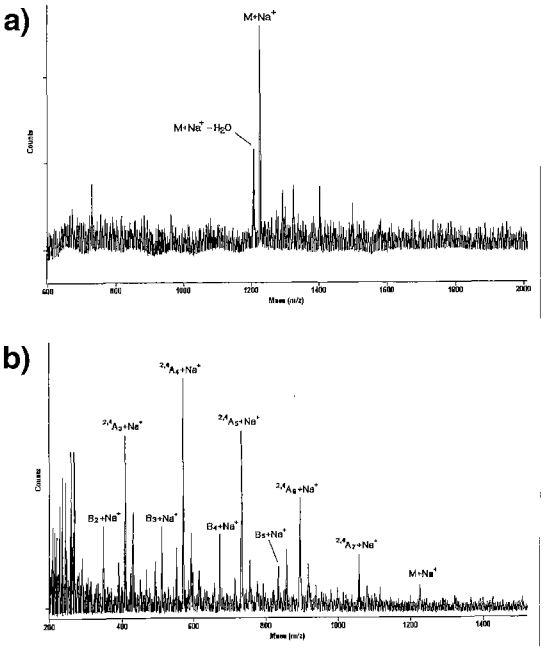
RESULTS AND DISCUSSION

All signals of molecular and fragment ions obtained in the study were recorded as sodium ion adducts. DHBA and CHCA as matrix compound were used for the analyses of free oligosaccharide and oligosaccharide-malononitrile derivatives. When the reactions were carried out for 4 hours, the malononitrile derivatives of oligosaccharides were detected with the best sensitivity (Scheme 1). The MALDI spectra



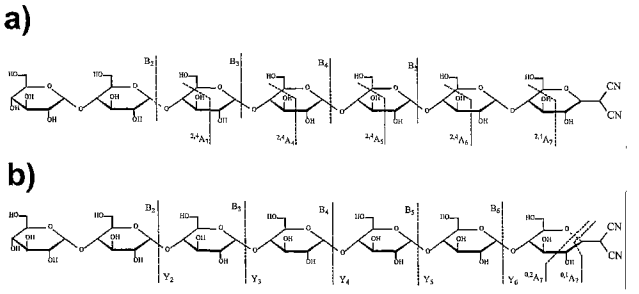
Scheme 1. Derivatization of oligosaccharide with malononitrile.

obtained from the crude maltoheptaose-malnonitrile derivative using the matrix DHBA are shown in Figures 1a. Although the malononitrile derivatization method for oligosaccharides was originally designed and developed for high-sensitivity analyses of oligosaccharides by negative ion ESI-MS, the maltoheptaose-malononitrile derivative was detected as a sodium ion adduct (m/z 1223.4) with a good signal-to-noise (S/N) ratio in positive ion mode, even for crude samples loaded without any pre-treatment. The sodium ion adduct of the fragment due to dehydration of maltoheptaose-malononitrile derivative was also detected as a minor peak at m/z 1205.4. Although several peaks due to minor impurities were also detected, we could analyze the oligosaccharide derivative even without any purification procedure for the derivatization mixture.



Figures 1.Positive-ion MALDI-TOF MS spectra obtained from 2 pmol of the unpurified maltoheptaose-malononitrile derivative using the matrix DHBA (a) and using the matrix CHCA (b).

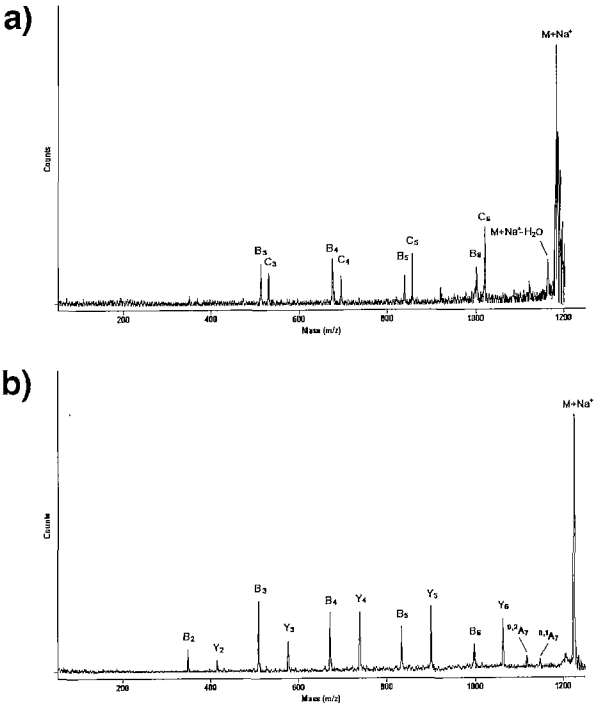
We also examined the effect of matrix on the MALDI analysis of oligosaccharide-malononitrile derivatives. The MALDI-TOF-MS spectrum of crude maltoheptaose-malononitrile derivative, obtained in linear mode using CHCA as a matrix, gave a very interesting result shown in Figure 1b. In addition to the expected molecular ion $[M+Na]^+$, a series of peaks with a mass interval of 162 Da was observed, and assigned to the sodium ion adducts of the [2,4]A fragment series produced from ring cleavages of the oligosaccharide derivative (Scheme 2a). Another series, corresponding the sodium ion adducts of the B fragment series produced from glycosidic bond cleavages of the oligosaccharide derivative was also observed. It is assumed tentatively that this result is due to the effective energy transfer from the hot matrix, CHCA, to the maltoheptaose-malononitrile derivative intrinsically labile.



Scheme 2. Fragmentation patterns of maltoheptaose-malononitrile derivative by positive-ion MALDI-TOF analysis using the matrix CHCA (a) and by positive-ion MALDI-PSD analysis using the matrix DHBA (b).

While DHBA can be classified as a cool matrix suitable for the detection of intact molecular ions with high resolution, CHCA can be classified as a hot matrix able to transfer energy more effectively from matrix to sample molecules [12, 13]. Thus the use of CHCA as a matrix in MALDI-TOF mass analysis for oligosaccharide-malononitrile derivatives, afford some resemble results such as obtaining from the MALDI-PSD analysis. It can provide additional information in structural analyses of oligosaccharides.

Next we conducted structural analyses of the malononitrile derivative of oligosaccharide by PSD experiments using the MALDI-TOF mass spectrometer and results were compared with the results obtained from free oligosaccharide. DHBA was used as matrix. Figures 2a and 2b show the MALDI-PSD fragment spectra obtained from free maltoheptaose and from the maltoheptaose-malononitrile derivative, respectively. The spectrum obtained from free maltoheptaose shows a considerably different peak pattern in comparison with that obtained from the maltoheptaose-malononitrile derivative. The peaks due to the sodium ion adducts of the B and C-series fragments of maltoheptaose appear together with the peak due to the sodium ion adduct of the dehydrated oligosaccharide, at m/z 1158.3, as well as the precursor ion $[M+Na]^+$ at m/z 1176.3. The spectrum obtained from the maltoheptaose-malononitrile derivative, as shown in the MALDI-PSD fragment spectrum (Figure 2b), shows the peaks due to the sodium ion adducts of the A and Y-series fragments of maltoheptaose- malononitrile derivatives besides the peaks due to the B-series (Scheme 2b). The series due to B and Y-type fragmentations originates from the cleavage of glycosidic bonds. However, it is noticeable here that Y-type fragmentations, containing the reducing end of the oligosaccharide derivative, were observed with high peak intensity. The Y-series was not observed from the analysis of free oligosaccharide. We think tentatively this result was due to that the sequential degradation starting from the reducing end in gas phase was blocked by the presence of malononitrile moiety. Besides the fragmentations mentioned above, A-type fragmentations derived from ring cleavage of



Figures 2. Positive-ion MALDI-PSD fragment spectra obtained from 1 nmol of free maltoheptaose (a) and of the crude maltoheptaose-malononitrile derivative (b), using the matrix DHBA.

the hexose unit bearing the labeling group were observed with significant peak intensity and unique feature obtainable from malononitrile derivative. These results could be additional merits of the malononitrile derivatization method in structural analysis of oligosaccharides.

CONCLUSIONS

The malononitrile derivatization method developed originally for high-sensitivity analysis of oligosaccharides can also be a powerful tool for structural analyses of oligosaccharides. The MALDI analyses of malononitrile derivative of oligosaccharide were affected drastically by the choice of the matrix used (DHBA and CHCA). Furthermore the MALDI-PSD analyses of the derivative gave some different results and much more information than the corresponding analyses of the free oligosaccharides.

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