Post-source Decay Fragment Analysis of a Highly Branched Nonasaccharide of Xyloglucan Using MALDI-TOF Mass Spectrometry

Tohru Yamagaki,[†,††] Yasushi Mitsuishi,[†] and Hiroshi Nakanishi*[†]
[†]*National Institute of Bioscience and Human-Technology, AIST, 1-1 Higashi, Tsukuba, Ibaraki 305*
[††]*Department of Biochemistry, Saitama University, Urawa, Saitama 338*

(Received October 8, 1997; CL-970779)

A highly branched nonasaccharide of xyloglucan was analyzed by MALDI-TOFMS post-source decay fragment analysis. The fragmentation occurred only at glycosidic bonds at the non-reducing terminal side of the glycosidic oxygen. In the MALDI-PSD fragment spectrum, almost all fragment ions, possibly produced by a three-site cleavage, were observed. The large amount of fragment mass information allowed us to determine the sequentially fine structure of the highly branched oligosaccharide.

Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOFMS) has been applied to biomolecules such as oligosaccharides.[1-3] Recently, reflectron-TOF-MS has enabled the detection of metastable fragment ions as post-source decay (PSD) fragment ions. The MALDI-PSD fragment analysis gave the sequential information of oligosaccahrides.[4-7] We have already reported the MALDI-PSD fragment analysis of xyloglucan heptaose.[8] In this study we report a more complex and highly branched xyloglucan nonasaccharide (see **1**), in which a cellulose backbone bonded D-glucopyranose together by β1-4 glycosidic linkages, D-xylopyranose and D-glucopyranose residues were bonded by α1-6 linkage at the branched points, and D-galactopyranose and D-xylopyranose residues were bonded by β1-2 linkage to lengthen the branched chains (see **1**). The reducing-end glucose was reduced in **1** in order to distinguish the reducing-end glucose from the galactose residues at the non-reducing end.

All MALDI-TOF mass spectra were acquired on a KOMPACT MALDI IV (Shimadzu Corporation, Japan). Operation conditions were as follows; nitrogen laser (337 nm; about 40 μJ in PSD fragment measurements), acceleration energy of 20 kV, reflectron mode, positive-ion detected. 2,5-Dihydroxybenzonic acid (DHBA) was used as matrix.

In the MALDI-PSD fragment spectrum of **1**, nineteen fragment ions (A through S) constituting a sodium adduct and the precursor ion $[M+Na]^+$ at m/z 1412 were observed (Figure. 1). Since ion intervals are 132, 162, and 182 amu, corresponding to the loss of anhydroxylose, anhydroglucose or anhydrogalactose, and glucitol residues, only glycosidic linkages were cleaved at the non-reducing terminal side of the glycosidic oxygen; sugar ring fragmentations were not observed in the MALDI-PSD fragmentation. The chemical species corresponding to ions A through K had glucitol at the reducing end, but those of ions L through S did not have the glucitol residue, indicating that these nineteen PSD fragment ions can be classified into two series (i.e., with or without glucitol residues), one being from ions A through K as the reducing-end ions (summarized in Table 1) and the other from ions L through S as the non-reducing-end ions (summarized in Table 2). Thus, the MALDI-PSD fragment spectrum using only the positive polarity mode provided mass information from both the reducing and non-reducing ends with one measurement.

Comparing the FAB-MS data[9] and MALDI-PSD fragment spectrum of **1**, only four fragment ions have been detected in the negative FAB-MS spectrum[9] among the reducing-end ions of the MALDI-PSD fragment spectrum (Table 1). In the positive FAB-MS spectrum,[9] only two ions have been observed among the non-reducing-end ions in the MALDI-PSD fragment spectrum (Table 2). The MALDI-PSD fragment analysis thus provides clearer and more detailed structural information on sugar compounds from the large number of fragment ions by only the positive measurement. Our previous study of the xyloglucan heptasaccharide confirmed these results.[8] These characteristics give MALDI-TOFMS an advantage over FAB-MS for saccharides analyses.

In the highly branched oligosaccharides such as **1**, using other analytical methods, it was difficult to detect all possible fragment ions because they required multi-site cleavage at their

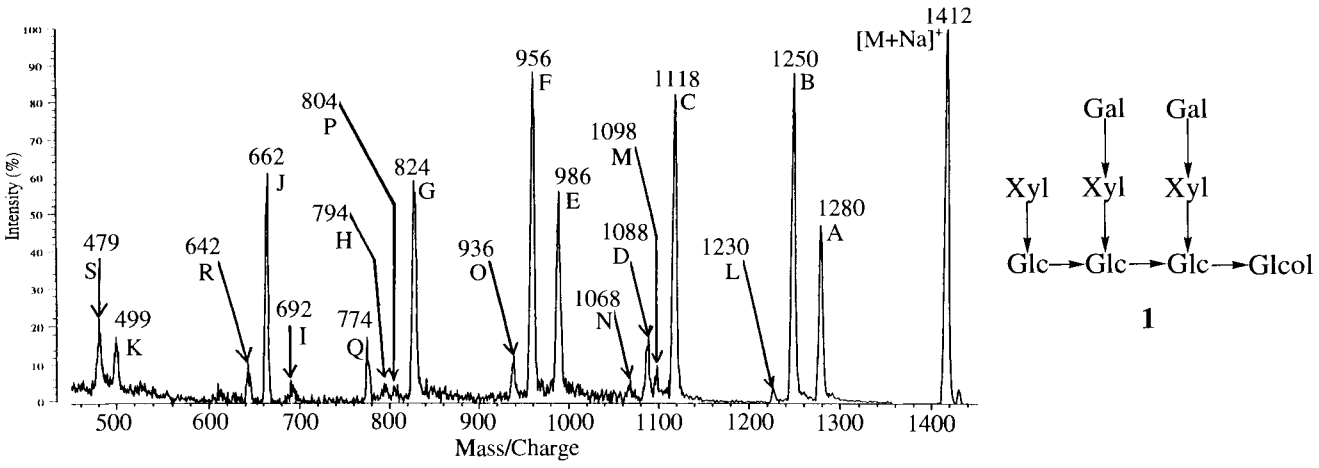

Figure 1. The MALDI-PSD fragment spectrum of **1**.

Table 1. Chemical species of the reducing-end ions in the MALDI-PSD fragment spectrum of **1**

Mass number	Ion[a] type	Lost residues[b] -X	-Gal	-G	Ion composition
1412					$[Gal_2Xyl_3Glc_3Glcol + Na]^+$
1280 (A)[c]	Y	1	0	0	$[Gal_2Xyl_2Glc_3Glcol + Na]^+$
1250 (B)[c]	Y	0	1	0	$[Gal_1Xyl_3Glc_3Glcol + Na]^+$
1118 (C)[c]	Y	1	1	0	$[Gal_1Xyl_2Glc_3Glcol + Na]^+$
1118[c]	Y	1	0	1	$[Gal_2Xyl_2Glc_2Glcol + Na]^+$
1088 (D)	Y	0	2	0	$[Xyl_3Glc_3Glcol + Na]^+$
986 (E)	Y	2	1	0	$[Gal_1Xyl_1Glc_3Glcol + Na]^+$
956 (F)	Y	1	2	0	$[Xyl_2Glc_3Glcol + Na]^+$
956	Y	1	1	1	$[Gal_1Xyl_2Glc_2Glcol + Na]^+$
824 (G)	Y	2	2	0	$[Xyl_1Glc_3Glcol + Na]^+$
824	Y	2	1	1	$[Gal_1Xyl_1Glc_2Glcol + Na]^+$
794 (H)	Y	1	2	1	$[Xyl_2Glc_2Glcol + Na]^+$
692 (I)	Y	3	2	0	$[Glc_3Glcol + Na]^+$
662 (J)	Y	2	2	1	$[Xyl_1Glc_2Glcol + Na]^+$
662[c]	Y	2	1	2	$[Gal_1Xyl_1Glc_1Glcol + Na]^+$
499 (K)	Y	2	2	2	$[Xyl_1Glc_1Glcol + Na]^+$

a: fragmentation of *Y*-type initially proposed by Domon and Costello in reference 10.
b: -X means the loss of anhydroxylose from the molecule.; -Gal, the loss of anhydrogalactose; -G, the loss of anhydroglucose.
c: ions corresponding to the negative ions in the FAB-MS spectrum from reference 9 with the mass increments in the mass region higher than about m/z 500.

branched points. When we simulated all of the possible chemical species of sample **1** exceeding trisaccharides, all of the corresponding ions in the reducing-ends could be observed in the MALDI-PSD fragment spectrum (Table 1), as well as almost all ions of the non-reducing-ends (Table 2). The clear detection of ion I, for example, strongly indicates that a three-site cleavage of glycosidic linkages occurred at least in the MALDI-PSD fragmentation.

The possible structures assumed from the obtained MALDI-PSD fragment ions were focused on the structure of **1** (the glucose and galactose residues were not distinguished from each other). Ions A, B, and D indicate that one xylose and two galactose residues are present at the non-reducing end in the sample. Ions C and D showed that two xylose residues are not released simultaneously without the release of galactose residue, and indicate the presence of Gal→Xyl→ units at the branch. Ions E and F suggest that three hexose (Gal and Glc) residues are not released simultaneously without the release of the xylose residue. Three xylose residues were released with two galactose residues (ion I), indicating the branched species of Xyl→ and two Gal→Xyl→ on the cello-tetraose backbone structure. Ion L indicates that the glucitol at the reducing end is not substituted by these branched units except for one glycosidic bond to the rest of the molecule, because the presence of ion L was not expected at this mass number if the glucitol residue was substituted. The

Table 2. Chemical species of the non-reducing-end ions in the MALDI-PSD fragment spectrum of **1**

Mass number	Ion[a] type	Lost residues[b] -X	-Gal	-G	-Gol	Ion composition
1230 (L)[c]	B	0	0	0	1	$[Gal_2Xyl_3Glc_3 + Na]^+$
1098 (M)	(B, Y)[a]	1	0	0	1	$[Gal_2Xyl_2Glc_3 + Na]^+$
1068 (N)	(B, Y)	0	1	0	1	$[Gal_1Xyl_3Glc_3 + Na]^+$
936 (O)	(B, Y)	1	1	0	1	$[Gal_1Xyl_2Glc_3 + Na]^+$
936	(B, Y)	1	0	1	1	$[Gal_2Xyl_2Glc_2 + Na]^+$
804 (P)	(B, Y)	2	1	0	1	$[Gal_1Xyl_1Glc_3 + Na]^+$
774 (Q)	(B, Y)	1	2	0	1	$[Xyl_2Glc_3 + Na]^+$
774[c]	B or (B, Y)	1	1	1	1	$[Gal_1Xyl_2Glc_2 + Na]^+$
642 (R)	(B, Y)	2	2	0	1	$[Xyl_1Glc_3 + Na]^+$
642	(B, Y)	2	1	1	1	$[Gal_1Xyl_1Glc_2 + Na]^+$
479 (S)	(B, Y)	2	2	1	1	$[Xyl_1Glc_2 + Na]^+$
479	(B, Y)	2	1	2	1	$[Gal_1Xyl_1Glc_1 + Na]^+$

a: fragmentation of *B*- and *Y*-type initially proposed by Domon and Costello in reference 10; (B, Y) mean that two types fragmentaion occurred simultaneously when the ions were produced.
b: -Gol means the loss of anhydroglucitol at the reducing end from the molecule.; -X, the loss of anhydroxylose; -Gal, the loss of anhydrogalactose; -G, the loss of anhydroglucose.
c: the same ions as described in reference 9.

eight ions from L through S also confirmed the structure of **1** noted earlier. These results indicate that the sequentially fine structure of **1** could be determined from the PSD fragment spectral analyses. Thus, the elegant structure analysis using the MALDI-PSD fragment method can be applied to the fine structure analysis of more complicated highly branched saccharides in the future.

We thank to Dr. Shin-ichirou Kawabata (Shimadzu Co., Kyoto, Japan) for his technical advice and helpful discussions.

References

1 T. Yamagaki, M. Maeda, K. Kanazawa, Y. Ishizuka, and H. Nakanishi, *Biosci. Biotech. Biochem.*, **60**, 1222 (1996).
2 T. J. P. Naven and D. J. Harvey, *Rapid Commun. Mass Spectrom.*, **10**, 829 (1996).
3 Y. Kimura, S. Kajiyama, J. Kanaeda, T. Izukawa, and M. Yonekura, *Biosci. Biotech. Biochem.*, **60**, 2099 (1996).
4 T. Yamagaki, Y. Ishizuka, S. Kawabata, and H. Nakanishi, *Rapid Commun. Mass Spectrom.*, **10**, 1887 (1996).
5 T. Yamagaki, Y. Ishizuka, S. Kawabata, and H. Nakanishi, *Rapid Commun. Mass Spectrom.*, **11**, 527 (1997).
6 B. Kuster, T. J. P. Naven, and D. J. Havrvey, *Rapid Commun. Mass Spectrom.*, **10**, 1645 (1996).
7 G. Talbo and M. Mann, *Rapid Commun. Mass Spectrom.*, **10**, 100 (1996).
8 T. Yamagaki, Y. Mitsuishi, and H. Nakanishi, *Biosci. Biotech. Biochem.*, **61**, 1411 (1997).
9 W. S. York, H. Halbeek, A. G. Darvill, and P. Albersheim, *Carbohydr. Res.*, **200**, 9 (1990).
10 B. Domon andC. E. Costello, *Glycoconjugate J.*, **5**, 397 (1988).