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Determination of Structural Isomers of Xyloglucan Octasaccharides using Post-source Decay Fragment Analysis in MALDI-TOF Mass Spectrometry

Tohru Yamagaki,[a,b] Yasushi Mitsuishi,[a] and Hiroshi Nakanishi,[a*]

[a]*National Institute of Bioscience and Human-Technology, AIST, 1-1, Higashi, Tsukuba, Ibaraki 305-8566, Japan*
[b]*Department of Biochemistry, Saitama University, Urawa, Saitama 338-8570, Japan*

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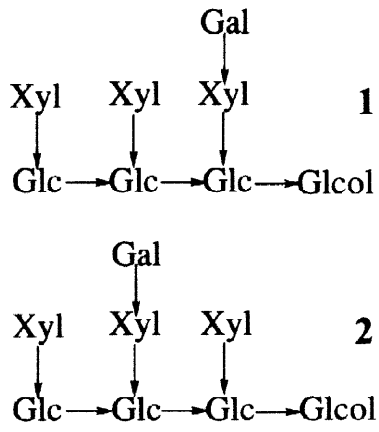
Abstract
Post-source decay (PSD) fragment analyses by MALDI-TOFMS were applied to the characterization of two analogous structure isomers of xyloglucan octasaccharides (XXLGol and XLXGol) from Tamarind seed. Almost all possible fragment ions were detected by a multi-site cleavage at the glycosidic linkages. The same fragment ions were observed in their spectra, but the relative intensities of some of the ions differed greatly. The detailed investigation of the relative intensities of the fragment ions enabled definitely to distinguish the analogous structure isomers of the highly branched octasaccharides.

Keywords: Carbohydrate, Isomer, Plants, Mass spectrometry.

In the study[1,2] of enzyme degradation of xyloglucan from Tamarind seed, xyloglucan octasaccarides were obtained. They consist of four β-D-glucopyranose residues (Glc) bonded by β1-4 glycosidic linkages, three α-D-xylopyranose residues (Xyl) substituted to each Glc by an α1-6 linkage, and one β-D-galactopyranose residue (Gal) substituted to Xyl by a β1-2 linkage. There were two analogous structural isomers (XXLG and XLXG[3]) in which the substituted Gal positions differed (see **1** and **2**). Until now, it was very difficult to distinguish them, even using NMR spectroscopy and FAB-MS methods. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOFMS) was used for oligosaccharide analysis.[4-6] The sequential analysis was performed by using a reflectron-TOFMS instrument, which enables the detection of metastable fragment ions as post-source decay (PSD) fragment ions. MALDI-PSD fragment analysis revealed the sequential information of oligosaccharides.[7-14] We previously reported the sequential analysis of xyloglucan oligosaccharides.[13,14] In this paper, we distinguish the analogous structural isomers of the highly branched xyloglucan octasaccharides of **1** and **2** using MALDI-PSD fragment methods. These analyses were performed by a detailed examination of the relative intensities of the fragment ions.



1

2

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The reducing-end glucose in the xyloglucan octasaccharide samples was reduced in order to distinguish the reducing-end glucose from the galactose residues at the non-reducing end for the TOF-MS analysis. The MALDI-TOF mass spectra were acquired on a KOMPACT MALDI IV (a nitrogen laser 337 nm; about 40 μJ in PSD fragment measurements; Shimadzu Corporation, Japan). We used 2,5-Dihydroxy-benzonic acid (DHBA) as a matrix. The MALDI-PSD fragment spectra were measured as the average of one hundred shots at different spots and were smoothed by the average function. The precursor ion and all fragment ions were generated and detected under the same conditions, since the instrument utilizes a curved field reflectron[15,16] as a reflector. The reproducibility of the fragment spectral patterns was satisfactorily high. These measurements enable us to discuss the relative intensities of the fragment ions.[7-9]

In the MALDI-PSD fragment spectrum of **1**, seventeen fragment ions (A through Q) and a molecular ion were observed as a sodium adduct (Fig. 1). The ion intervals were 132, 162, and 182 amu, corresponding to anhydroxylose, anhydroglucose (or anhydrogalactose), and glucitol residues. The intervals indicated that MALDI-PSD fragmentation occurred at only glycosidic linkages. These fragment ions were classified into two series produced from the reducing end (ions A through I) and from the non-reducing end (ions J through Q) with or without the glucitol residue at the reducing end in their chemical species. The former ions were produced by Y-type fragmentation, the latter ions were produced by B-type fragmentation and double cleavage of Y- and B-type fragmentation.[17] In the ion series produced from the reducing end (ions A through I), the ion type was unified.

Simulating the cleavage of glycosidic linkages in **1**, almost all possible fragment ions were observed in species greater than the trisaccharides species. For example, the detection of ion G at m/z 691 indicated that a three-site cleavage of the glycosidic linkage occurred at least in the MALDI-PSD fragmentation, because the chemical species corresponding to ion G was produced by the loss of three anhydroxylose and anhydrogalactose residues from the molecule (Fig. 2). These multi-site cleavages yielded seventeen fragment ions both from the reducing and non-reducing end sides in the positive measurement of the MALDI-PSD fragment analysis, although

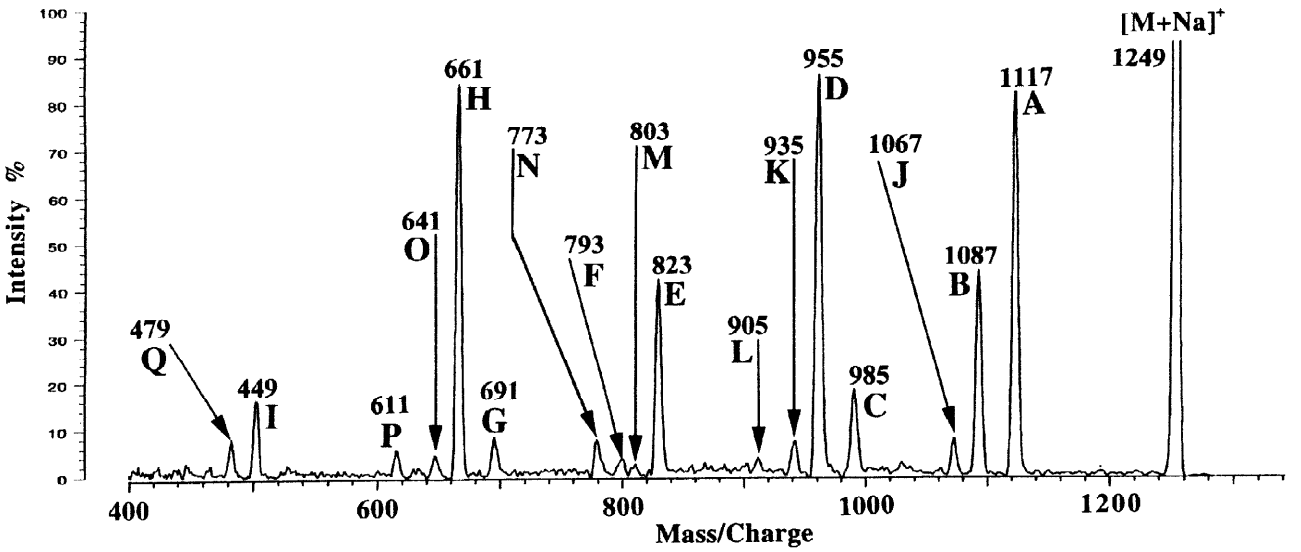

Figure 1. The MALDI-PSD fragment spectrum of **1**.

only eight fragment ions were observed in the total measurements of the positive and negative-ion modes by FAB-MS analyses.[2]

In the MALDI-PSD fragment spectra of **2**, the same fragment ions were observed as in the case of **1**. Therefore, these analogous saccharide isomers **1** and **2** could not be distinguished by only the mass numbers of the fragment ions. Their structural differences were analyzed in detail by comparing the relative intensities of the fragment ions between **1** and **2**. In the first stage of the fragement analysis, we focused our attention on the cleavage of the same glycosidic linkages of α1-6 between Glc and Xyl as shown in Fig. 2.

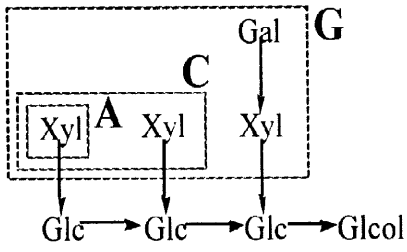

Figure 2. The chemical species of ions A, C, and G. The square denote the lost saccharide residues.

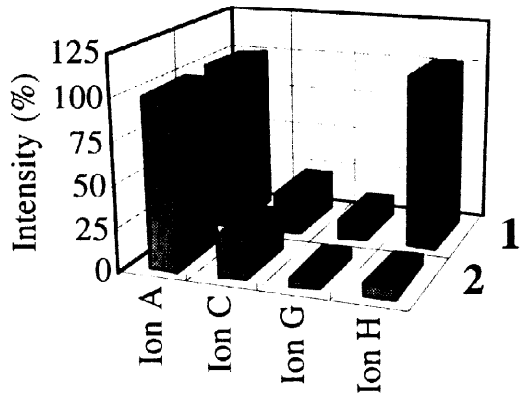

Figure 3. The relative intensities of ions A, C, G, and H.

The relative intensities of ions A, C, and G for **1** and **2** are shown in Fig. 3. They were calculated from the peak area of each ion and normalized to that of ion A at m/z 1117 as 100%. When ions A, C, and G were produced, the glycosidic linkages between Xyl and Glc cleaved at one, two, and three sites, respectively. Thus, ion A at m/z 1117 was produced by the loss of anhydroxylose residue (Fig. 2). Ion C at m/z 985 was produced by the loss of two anhydroxylose residues (Fig. 2). Ion G at m/z 691 was produced by the loss of three anhydroxylose and anhydrogalactose residues (Fig. 2). The relative intensity of ion A, produced by a one-site cleavage, was much higher than that of ion C, produced by a two-site cleavage, which was higher than that of ion G, produced by a three-site cleavage (Fig. 3). These indicate that a one-site cleavage occurred with higher probability than a two-site cleavage, which occurred with higher probability than a three-site cleavage in MALDI-PSD fragmentation. Since ion A was originated from two possible species, the relative intensity of ion A was much higher than those of ions C and G with only one possible species. These trends in the relative intensities of PSD fragment ions did not differ between

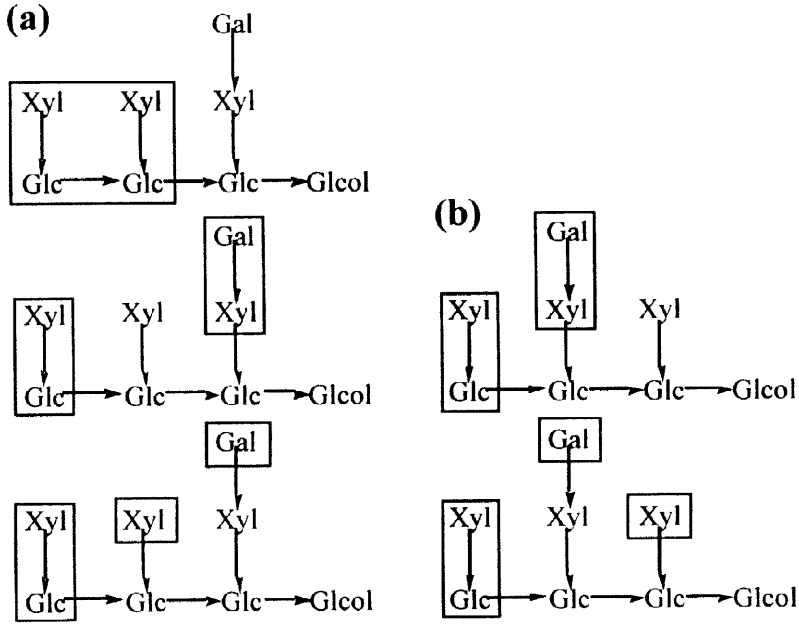

Figure 4. The chemical species of ion H of **1** (a), and **2** (b). The dark square denote the lost saccharide residues.

1 and **2** (Fig. 3). No large differences were observed in the other fragment ion intensities, except for that of ion H. As shown in Fig. 3, the relative intensities of ion H for **1** and **2** differed greatly. The possible chemical species of ion H are listed in Fig. 4. Ion H of **1** originated from a one-site cleavage species as well as a two- and three-site ones, as shown in Fig. 4 (a). In contrast, the possible species of **2** for ion H were not produced by a one-site cleavage, but they were produced by only two- and three-site cleavage (Fig. 4 (b)). Therefore, the relative intensity of ion H for **1** with three possible species, produced mainly by a one-site cleavage, was much higher than that for **2** with two possible species, originated from only the two- and three-site cleavage species. These characterization method enables us to definitively distinguish the analogous structural isomers of xyloglucan octasaccharides **1** and **2**. If we should have other substitution isomer **3** as a degradation product, we would be able to recognize the structure very easily by the ion intensity analyses of the MALDI-PSD fragment method of **3**. These results strongly suggest the potential possibility that MALDI-PSD fragment analysis is a powerful tool for detailed sequential structure analysis of highly branched oligosaccharides. The detailed investigation of the relative intensities of the fragment ions gives very important information about the analogous structure isomers of complexed oligosaccharides.

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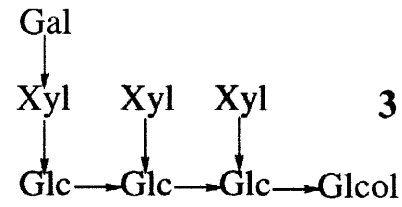

References

[1] W. S. York, L. K.Harvey, R. Guillen, P. Albersheim, and A. G. Darvill, *Carbohydr. Res.*, **248**, 285-301 (1993).
[2] W. S. Herman, H. Halbeek, A. G. Darvill, and P. Albersheim, *Carbohydr. Res.*, **200**, 9-31 (1990).
[3] S. .C. Fry, W. S. York, P. Albersheim, A. Darvil, T. Hayashi, J. P. Joseleau, Y. Kato, E. P. Lorences, G. A. Maclachlan, M. McNeil, A. Mort, J. S. G. Reid, H. U. Seitz, R. R. Selverdran, A. G. J. Voragen, and A. W. White, *Physiol. Plant.*, **89**, 1-3 (1993).
[4] T. Yamagaki, M. Maeda, K. Kanazawa, Y. Ishizuka, and H. Nakanishi, *Biosci. Biotech. Biochem.*, **60**, 1222-1228 (1996).
[5] T. J. P. Naven and D. J. Harvey, *Rapid Commun. Mass Spectrom.*, **10**, 829-834 (1996).
[6] Y. Kimura, S. Kajiyama, J. Kanaeda, T. Izukawa, and M. Yonekura, *Biosci. Biotech. Biochem.*, **60**, 2099-2102 (1996).
[7] T. Yamagaki, Y. Ishizuka, S. Kawabata, and H. Nakanishi, *Rapid Commun. Mass Spectrom.*, **10**, 1887-1890 (1996).
[8] T. Yamagaki, Y. Ishizuka, S. Kawabata, and H. Nakanishi, *Rapid Commun. Mass Spectrom.*, **11**, 527-531 (1997).
[9] T. Yamagaki, Y. Mitsuishi, and H. Nakanishi, *Rapid Commun. Mass Spectrom.*, **12**, 1-5 (1998).
[10] R. Kaufmann, *J. Biotechnol.*, **41**, 155-175 (1995).
[11] B. Kuster, T. J. Naven, and D. J. Harvey, *Rapid Commun. Mass Spectrom.*, **10**, 1645-1651 (1996).
[12] G. Talbo and M. Mann, *Rapid Commun. Mass Spectrom.*, **10**, 100-103 (1996).
[13] T. Yamagaki, Y. Mitsuishi, and H. Nakanishi, *Biosci. Biotech. Biochem.*, **61**, 1411-1414 (1997).
[14] T. Yamagaki, Y. Mitsuishi, and H. Nakanishi, *Chem. Lett.*, **1998**, 57-58.
[15] T. J. Cornish and R. J. Cotter, *Rapid Commun. Mass Spectrom.*, **8**, 781-785 (1994).
[16] T. J. Cornish and R. J. Cotter, *Rapid Commun. Mass Spectrom.*, **7**, 1037-1040 (1993).
[17] B. Domon and C. E. Costello, *Glycoconjugate J.*, **5**, 397 (1988).