Further Structural Study of the Barley and Bamboo Shoot Xyloglucans by Xyloglucanase

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Abstract: To investigate the arrangement of the glucose residues which are not xylosylated at *O*-6 in the β -1,4-D-glucan backbone of xyloglucan from *Gramineae*, a xyloglucan from immature barley plants was hydrolyzed by a purified *Geotrichum* sp. M128 xyloglucan specific endo-1,4- β -D-glucanase (xyloglucanase). The fragment oligosaccharides were then analyzed by matrix-assisted laser-desorption ionization time of the flight mass spectrometry before and after treatment with an oligoxyloglucan-specific glycosidase, oligoxyloglucan reducing end-specific cellobiohydrolase from *Geotrichum* sp. M128, with a mixture of isoprimeverose-producing oligoxyloglucan hydrolase from *Eupenicillium* sp. M9 and β -D-glucosidase from *Trichoderma viride*, or with β -D-galactosidase from *Bacillus circulans*. Tetra-, penta-, hexa-, hepta-, octa- and nona-saccharide fractions were obtained as major oligosaccharide fractions, which might be composed of XX, of XXG, of XXGG, of XXGGG and LXGG, of XLGGG, LXGGG, XXGGGG and GXXGGG, and of XLGGGG, LXGGGG, GXLGGG, GLXGGG and XXGGGGG, respectively. In addition, the oligosaccharide units of bamboo shoot xyloglucan were analyzed. The structure of bamboo shoot xyloglucan was shown to be very similar to that of barley xyloglucan. These results suggest that the *Gramineae* xyloglucan has regions consisting of two, three, four and five contiguous glucose residues which are not xylosylated at *O*-6 in the β -1,4-D-glucan backbone.

Key words: barley, bamboo shoot, xyloglucan oligosaccharides, *Gramineae*, xyloglucanase

Xyloglucan is a major structural polysaccharide of the primary cell-walls of all higher plants.²⁾ A marked characteristic of the xyloglucan is that the polysaccharide consists of a linear backbone chain of 1,4-linked β -D-glucosyl residues and side chains of single α -D-xylosyl residues, α -L-fucosyl-(1→2)- β -D-galactosyl-(1→2)- α -D-xylosyl residues, β -D-galactosyl-(1→2)- α -D-xylosyl residues, and others that are attached to the *O*-6-positions of the backbone chain. Treatment of most xyloglucans from dicotyledonous plants with endo- β -1,4-D-glucanase (cellulase) generates mainly oligosaccharide subunits, XXXG, XLXG, XXLXG, XLLG, XXFG and XLFG.³⁾ Specifically, the xyloglucan of most dicotyledonous plants is built from

XXXG-type subunits.

The xyloglucans from *Gramineae*^{4–6)} and *Solanaceae*^{7–12)} have more unsubstituted Glc residues compared to those from most dicotyledonous plants. We previously investigated the branching pattern and the arrangement of the glucosyl residues which are not xylosylated at *O*-6 in the β -1,4-D-glucan backbone of *Solanaceae* xyloglucans, using a purified *Penicillium* sp. M451 xyloglucan specific endo-1,4- β -D-glucanase (xyloglucanase, Xg-ase), and confirmed that the xyloglucan from most solanaceous vegetables (eggplant, sweet pepper, red pepper and tomato) is built from XXGG-type subunits (XXGG, XLGG, LXGG, XSGG and LSGG).¹³⁾

On the other hand, we previously characterized the structures of xyloglucans obtained from the cell-walls of immature barley and rice plants, and bamboo shoot.^{4–6)} Treatment of *Gramineae* xyloglucans with endo- β -1,4-D-glucanase (cellulase) generated glucose (G) and cellobiose (GG) in addition to oligosaccharides, XXGG, XXG, XX, XG, etc. However, it has not been certain whether or not *Gramineae* xyloglucans are built from oligosaccharide subunits. To clarify this question, we hydrolyzed barley xyloglucan with *Geotrichum* sp. M128 xyloglucan specific endo-1,4- β -D-glucanase (xyloglucanase, Xg-ase) and carried out analysis of the fragment oligosaccharides of barley xyloglucan by digestion with various kinds of enzymes followed by matrix-assisted laser-desorption ionization time of the flight mass (MALDI-TOF-MS). In addition we characterized the structure of fragment oligosaccharides from bamboo shoot xyloglucan. This report deals with the results.

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Abbreviations: Xyl, D-xylose; Glc, D-glucose; Gal, D-galactose; Ara, L-arabinose; IP [α -Xyl-(1→6)-Glc], isoprimeverose; GG, XX, XXG, XXXG, XLXG, XXLXG, XLLG, XXFG, XLFG, XXGG, XLGG, LXGG, XSGG, LSGG, XXGGG, XLGGG, LXGGG, XXGGGG, GXXGGG, XLGGGG, LXGGGG, GXLGGG, GLXGGG and XXGGGGG are the abbreviated names for xyloglucan oligosaccharides according to the nomenclature of Fry *et al.* [Ref. 1] Each (1→4)- β -linked D-glucosyl residue in the backbone is given a one-letter code according to its substituents: G = β -Glc; X = α -Xyl-(1→6)- β -Glc; L = β -Gal-(1→2)- α -Xyl-(1→6)- β -Glc; S = α -Ara-(1→2)- α -Xyl-(1→6)- β -Glc; F = α -Fuc-(1→2)- β -Gal-(1→2)- α -Xyl-(1→6)- β -Glc]. IPase, isoprimeverose-producing oligoxyloglucan hydrolase; Xg-ase, xyloglucanase; OXg-RCBH, oligoxyloglucan reducing end-specific cellobiohydrolase; β -D-galase, β -D-galactosidase; β -D-glcase, β -D-glucosidase; CMCcase, carboxymethylcellulase; HPLC, high-performance liquid chromatography; MALDI-TOF-MS, matrix-assisted laser-desorption ionization time of the flight mass.

MATERIALS AND METHODS

Barley and bamboo shoot xyloglucans. The hemicellulose IIA fraction obtained from cell walls of immature barley plant,⁴⁾ which consists of Glc, Xyl, Gal and Ara in a molar ratio of 57.4 : 30.0 : 9.3 : 3.3, was used as a barley xyloglucan for this study.

Bamboo shoot xyloglucan was prepared as described previously.⁶⁾

Enzymes. A xyloglucan specific endo-1,4- β -D-glucanase (Xg-ase) and an oligoxyloglucan-specific glycosidase, oligoxyloglucan reducing endo-specific cellobiohydrolase (Oxg-RCBH) from *Geotrichum* sp. M128, an isoprimeverose-producing oligoxyloglucan hydrolase (IPase) from *Eupenicillium* sp. M9 and an endo-1,4- β -D-glucanase (carboxymethylcellulase, CMCase) and β -D-glucosidase (β -D-glucase) from *T. viride* which were purified to their respective electrophoretically pure states, were used. M128 Xg-ase can hydrolyze the glucosidic bonds between unbranched Glc residues and O-4 of 4,6-disubstituted Glc residues, but can not hydrolyze Avicel SF, phosphoric acid swollen cellulose, carboxymethyl cellulose, hydroxyethyl cellulose or cellooligosaccharides.¹⁴⁾ M128 Oxg-RCBH has exoglucanase activity, and it recognizes the reducing end of oligoxyloglucan and releases two glucosyl residue segments from the main chain.¹⁵⁾ M9 IPase is highly specific for xyloglucan oligosaccharides and splits off isoprimeverose units from the non-reducing end of the backbone of the substrate; it can not by-pass the glucosyl residues substituted with β -Gal-(1 $\rightarrow$ 2)- α -Xyl, or α -Fuc-(1 $\rightarrow$ 2)- β -Gal-(1 $\rightarrow$ 2)- α -Xyl side chains.¹⁶⁾ β -D-Galactosidase (Biolacta-N5, β -D-galase) from *Bacillus circulans* purchased from Daiwa Kasei K.K., was entirely free from IPase.

General methods. Total carbohydrate was determined by the phenol-sulfuric acid method.¹⁷⁾

Hydrolysis of barley xyloglucan with *Geotrichum* M128 xyloglucanase (Xg-ase). Barley xyloglucan (8.1 mg as Glc equiv. in 180 μ L of 20 mM Na-acetate buffer, pH 5.5) was incubated with *Geotrichum* M128 Xg-ase (0.01 U/20 μ L) at 40°C. After 24 h, the incubation mixture was heated for 10 min in a boiling water-bath, and treated with Amberlite IR-120 (H⁺).

Hydrolysis of barley xyloglucan with *T. viride* CMCase. Barley xyloglucan (8.1 mg as Glc equiv. in 180 μ L of 20 mM Na-acetate buffer, pH 5.5) was incubated with *T. viride* CMCase (0.01 U/20 μ L) at 40°C. After 24 h, the incubation mixture was heated for 10 min in a boiling water-bath, and treated with Amberlite IR-120 (H⁺).

High-performance liquid chromatography (HPLC) of the Xg-ase-hydrolyzate and the CMCase-hydrolyzate.

HPLC was performed on a column (4.6 $\times$ 250 mm) of Lichrosorb NH₂. The hydrolyzates were eluted with CH₃CN: H₂O=70 : 30 at a flow rate of 0.8 mL/min. In the case of the Xg-ase-hydrolyzate, fractions 1 to 18 as shown in Fig. 2 were obtained respectively. Fractions 3, 5, 8, 10, 13 and 17 were rechromatographed on the same column, respectively.

Hydrolysis of xyloglucan oligosaccharide fractions

with a mixture of β -D-glucase and IPase. Fractions of xyloglucan oligosaccharides (50 μ g as Glc equiv. in 50 μ L of 20 mM Na-acetate buffer, pH 5.5) were incubated individually at 40°C with a mixture of β -D-glucase and IPase (0.01 U each/10 μ L). After 48 h, the fractions were heated in a boiling water-bath for 10 min, and deionized with Amberlite IR-120 (H⁺).

Hydrolysis of xyloglucan oligosaccharide fractions with Oxg-RCBH. Fractions of xyloglucan oligosaccharides (50 μ g as Glc equiv. in 50 μ L of 20 mM Na-acetate buffer, pH 5.5) were incubated individually at 40°C with Oxg-RCBH (0.01 U/20 μ L). After 48 h, the fractions were heated in a boiling water-bath for 10 min, and deionized with Amberlite IR-120 (H⁺).

Hydrolysis of xyloglucan oligosaccharide fractions with β -D-galase. Fractions of xyloglucan oligosaccharides (50 μ g as Glc equiv. in 50 μ L of 50 mM Na-acetate buffer, pH 6.0) were incubated individually at 40°C with β -D-galase (0.01 U/10 μ L). After 24 h, the fractions were heated in a boiling water-bath for 10 min, and deionized with Amberlite IR-120 (H⁺).

Matrix-assisted laser-desorption ionization time of the flight mass (MALDI-TOF-MS). An aqueous solution (0.5 μ L, about 50 μ g/mL) of each sample was mixed with an equal volume of 1% 2,5-dihydroxybenzoic acid in 40% acetonitrile aqueous solution. This mixture was applied to the MALDI probe and dried. MALDI-TOF-MS was recorded with a Voyager RP (PerSeptive Biosystems, USA) operating at a 20 kV accelerating voltage.¹⁸⁾

RESULTS AND DISCUSSION

Barley xyloglucan.

1. Hydrolysis of barley xyloglucan with *Geotrichum* Xg-ase and fractionation of the Xg-ase hydrolyzate.

We previously characterized a xyloglucan occurring in the hemicellulose II fraction (HC-IIA, Glc : Xyl : Gal : Ara=57.4 : 30.0 : 9.3 : 3.3) of cell walls of immature barley plants by methylation and fragmentation analyses. The fragmentation of HC-IIA (xyloglucan) was done by hydrolysis with *T. viride* CMCase. The structures of the obtained oligosaccharides were proposed to be XXGG (hexasaccharide), XXG (pentasaccharide), XG (trisaccharide), GG (cellobiose) and G (glucose). In the present study, barley xyloglucan was hydrolyzed with *Geotrichum* sp. M128 xyloglucan specific endo-1,4- β -D-glucanase (xyloglucanase, Xg-ase). HPLC analysis showed that the M128 Xg-ase-hydrolysis products of barley xyloglucan were clearly different from the CMCase-hydrolysis products of barley xyloglucan (Fig. 1). Namely, an unnegligible amount of higher molecular weight oligosaccharides are found in the M128 Xg-ase-hydrolysis products.

The M128 Xg-ase-hydrolysis products were subjected to a preparative HPLC on a column of Lichrosorb NH₂. Fractions 1 to 18 were obtained in a ratio of 5.5 : 0.8 : 4.0 : 2.7 : 11.3 : 2.6 : 2.6 : 17.2 : 4.2 : 25.7 : 2.1 : 1.9 : 9.4 : 3.5 : 1.0 : 0.5 : 4.3 : 0.8 (Fig. 2). Fractions 3, 5, 8, 10, 13 and 17 were purified by the chromatography on the same column. Then, the predominant oligosaccharide fractions were subjected to the respective MALDI-TOF-MS analysis before and after treatment with enzymes.

Fig. 1. HPLC of M128 Xg-ase-hydrolyzate and CMCase-hydrolyzate of barley xyloglucan.

Fig. 2. Separation of the Xg-ase hydrolysis products of barley xyloglucan by HPLC.

Barley xyloglucan (8.1 mg as Glc equiv. in 180 μ L of 20 mM Na-acetate buffer, pH 5.5) was incubated with *Geotrichum* M128 Xg-ase at 40°C. After 24 h, the incubation mixture was heated for 10 min in a boiling water-bath and treated with Amberlite IR-120 (H^+). HPLC was performed on a column (4.6 $\times$ 250 mm) of Lichrosorb NH_2 . The hydrolyzates were eluted with $CH_3CN : H_2O = 70 : 30$ at a flow rate of 0.8 mL/min. Fractions 3, 5, 8, 10, 13 and 17 were rechromatographed on the same column.

2. Analysis of oligosaccharides in fractions 3, 5 and 8.

Fractions 3, 5 and 8 were subjected to MALDI-TOF-MS analysis, respectively. In the mass spectra, the ion peaks at m/z 627.637, 789.016 and 951.162 correspond to the molecular ions as sodium adduct $[M+Na]^+$ of the tetrasaccharide, (hexose)₂(pentose)₂, the pentasaccharide, (hexose)₃(pentose)₂, and the hexasaccharide, (hexose)₄(pentose)₂, were observed (data not shown). In our previous study, tetrasaccharide (Glc : Xyl = 2 : 2), pentasaccharide (Glc : Xyl = 3 : 2) and hexasaccharide (Glc : Xyl = 4 : 2) were isolated from the *T. viride* cellulase hydrolyzate of barley xyloglucan and were characterized to be XX, XXG and XXGG, respectively (Fig. 3). The tetra-, penta- and hexa-saccharides obtained in the present study are considered to be the same oligosaccharides obtained previously. Therefore, further study of these oligosaccharide fractions was not carried out.

Fig. 3. Proposed structures of oligosaccharides in fractions 3, 5 and 8 obtained from barley xyloglucan by *Geotrichum* M128 Xg-ase.

Symbols in figures: Glc, β -Glc-(1 $\rightarrow$ 4)-; Xyl, α -Xyl-(1 $\rightarrow$ 6)-. Symbols in $\square$, see abbreviations.

3. Analysis of oligosaccharides in fraction 10.

Fraction 10 was subjected to MALDI-TOF-MS analysis. In the mass spectra, the ion peaks at m/z 1114.18 and 1130.15, which correspond to the molecular ions as sodium adduct $[M+Na]^+$ and potassium adduct $[M+K]^+$ of the heptasaccharide, (hexose)₅(pentose)₂, were observed (Fig. 4A). Our previous study indicated that barley xyloglucan contained Gal residues as constituent monosaccharide. The (hexose)₅(pentose)₂ may correspond to (Glc)₅(Xyl)₂ and/or (Glc)₄(Xyl)₂(Gal)₁. The branching pattern and the arrangement of the Glc residues in the oligosaccharides, (Glc)₅(Xyl)₂ and/or (Glc)₄(Xyl)₂(Gal)₁ were investigated as follows.

First the heptasaccharide fraction was treated with β -D-galase and the hydrolyzate was subjected to MALDI-TOF-MS analysis. In the mass spectrum (Fig. 4B), peaks at m/z 953.34 and 969.313 corresponding to $[(\text{hexose})_4(\text{pentose})_2 + Na]^+$ and $[(\text{hexose})_4(\text{pentose})_2 + K]^+$, and the peaks at m/z 1115.46 and 1131.43 corresponding to $[(\text{hexose})_5(\text{pentose})_2 + Na]^+$ and $[(\text{hexose})_5(\text{pentose})_2 + K]^+$ were observed (Fig. 4B). The (hexose)₄(pentose)₂ may correspond to a (Glc)₄(Xyl)₂. The (hexose)₅(pentose)₂ is considered to be (Glc)₅(Xyl)₂.

Secondly the heptasaccharide fraction was partially hydrolyzed with M9 IPase and the partial hydrolyzate was subjected to MALDI-TOF-MS analysis. In the MALDI-TOF-MS spectrum of the IPase-partially hydrolyzed heptasaccharide fraction (Fig. 4C), peaks at m/z 528.4 and 544.077 corresponding to $[(\text{hexose})_3 + Na]^+$ and $[(\text{hexose})_3 + K]^+$, peaks at m/z 822.615 and 838.343 corresponding to $[(\text{hexose})_4(\text{pentose})_1 + Na]^+$ and $[(\text{hexose})_4(\text{pentose})_1 + K]^+$ in addition to the original peaks at m/z 1116.78 and 1132.77 corresponding to $[(\text{hexose})_5(\text{pentose})_2 + Na]^+$ and $[(\text{hexose})_5(\text{pentose})_2 + K]^+$, were observed. The (hexose)₄(pentose)₁ and (hexose)₃ are considered to be XGGG [(Glc)₄(Xyl)₁] and GGG [(Glc)₃], respectively. The former and the latter are considered to be produced by splitting off one isoprimeverose (X) unit and two isoprimeverose units from the non-reducing end of the backbone of XXGGG by M9 IPase, respectively. The (hexose)₅(pentose)₂ is considered to be LXGG, because LXGG is not hydrolyzed by IPase.

On the basis of the modes of action of M128 Xg-ase, M9 IPase and β -D-galase, it is reasonable to conclude that heptasaccharide contains XXGGG and LXGG (Fig. 5).

4. Analysis of oligosaccharides in fraction 13.

Fraction 13 was subjected to MALDI-TOF-MS analysis. In the mass spectrum, an ion peak at m/z 1278.1, which corresponds to the molecular ions as sodium adduct

Fig. 4. MALDI-TOF-MS of fraction 10 (A), of β -D-galase treated fraction 10 (B), and of M9 IPase-partially treated fraction 10 (C).

oligosaccharide in fr.10	oligosaccharides obtained by	
	β -D-galase	M9 IPase
$\begin{array}{c} \text{Xyl} \quad \text{Xyl} \\ \quad \\ \text{Glc}-\text{Glc}-\text{Glc}-\text{Glc}-\text{Glc} \\ \boxed{\text{XXGGG}} \end{array}$	$\begin{array}{c} \text{Xyl} \quad \text{Xyl} \\ \quad \\ \text{Glc}-\text{Glc}-\text{Glc}-\text{Glc}-\text{Glc} \\ \boxed{\text{XXGGG}} \end{array}$	$\begin{array}{c} \text{Xyl} \quad \text{Xyl} \\ \quad \\ \text{Glc} \quad \text{Glc} \quad \text{Glc}-\text{Glc}-\text{Glc} \\ \boxed{\text{X}} \quad \boxed{\text{X}} \quad \boxed{\text{GGG}} \end{array}$
$\begin{array}{c} \text{Gal} \\ \\ \text{Xyl} \quad \text{Xyl} \\ \quad \\ \text{Glc}-\text{Glc}-\text{Glc}-\text{Glc} \\ \boxed{\text{LXGG}} \end{array}$	$\begin{array}{c} \text{Gal} \\ \\ \text{Xyl} \quad \text{Xyl} \\ \quad \\ \text{Glc}-\text{Glc}-\text{Glc}-\text{Glc} \\ \boxed{\text{XXGG}} \end{array}$	$\begin{array}{c} \text{Gal} \\ \\ \text{Xyl} \quad \text{Xyl} \\ \quad \\ \text{Glc}-\text{Glc}-\text{Glc}-\text{Glc} \\ \boxed{\text{LXGG}} \end{array}$

Fig. 5. Proposed structures of oligosaccharides in fraction 10, and those of oligosaccharides obtained by hydrolysis of fraction 10 with β -D-galase or M9 IPase.

Symbols in figures: Glc, β -Glc-(1 $\rightarrow$ 4)-; Xyl, α -Xyl-(1 $\rightarrow$ 6)-; Gal, β -Gal-(1 $\rightarrow$ 2)-. Symbols in $\square$, see abbreviations.

$[(\text{hexose})_6(\text{pentose})_2 + \text{Na}]^+$ of the octasaccharide, was observed (data not shown). Fraction 13 was treated with β -D-galase and the hydrolyzate was subjected to MALDI-TOF-MS analysis. In the mass spectrum (Fig. 6A), a peak at m/z 1116.64 corresponding to $[(\text{hexose})_5(\text{pentose})_2 + \text{Na}]^+$ in addition to the peak at m/z 1278.71 corresponding to $[(\text{hexose})_6(\text{pentose})_2 + \text{Na}]^+$ was observed (Fig. 6A). This means fraction 13, $(\text{hexose})_6(\text{pentose})_2$, may correspond to $(\text{Glc})_6(\text{Xyl})_2$ and $(\text{Glc})_5(\text{Xyl})_2(\text{Gal})_1$. On the basis of the modes of action of M128 Xg-ase, the octasaccharides in fraction 13 are supposed to be XLGGG, LXGGG, XXGGGG and/or GXXGGG.

To make clear this supposition, first fraction 13 was

Fig. 6. MALDI-TOF-MS of β -D-galase treated fraction 13 (A), of β -D-galase and M9 IPase treated fraction 13 (B), and of Oxy-RCBH treated fraction 13 (C).

treated with a mixture of β -D-galase and IPase and the product was subjected to MALDI-TOF-MS analysis (Fig. 6B). Peaks at m/z 984.297 corresponding to $(\text{hexose})_5(\text{pentose})_1$ and 1278.44 corresponding to $(\text{hexose})_6(\text{pentose})_2$ were observed. The former is considered to correspond to the oligosaccharide LGGG which was produced from XLGGG by IPase, and the latter is considered to correspond to the oligosaccharide LXGGG which was not hydrolyzed by both β -D-galase and IPase. The oligosaccharides XXGGGG and GXXGGG were hydrolyzed into X (isoprimeverose) and G (glucose) by the β -D-galase and IPase.

Secondly fraction 13 was treated with Oxy-RCBH and the product was subjected to MALDI-TOF-MS analysis (Fig. 6C). The peaks at m/z 954.121 and 659.888 in the mass spectrum correspond to $(\text{hexose})_4(\text{pentose})_2$ and $(\text{hexose})_3(\text{pentose})_1$, respectively. The hexasaccharide, $(\text{hexose})_4(\text{pentose})_2$, and the tetrasaccharide, $(\text{hexose})_3(\text{pentose})_1$, are considered to be LXG and LG, respectively, which were produced from LXGGG and XLGGG. In the mass spectrum the peak at m/z 629.898 corresponding to $(\text{hexose})_2(\text{pentose})_2$ is considered to be XX, which is produced from only XXGGGG by Oxy-RCBH. In addition the peak at m/z 497.93 corresponding to $(\text{hexose})_2(\text{pentose})_1$ is considered to be GX and XG, both of which are produced from only GXXGGG by Oxy-RCBH.

On the basis of the modes of action of M128 Xg-ase, M9 IPase, β -D-galase, β -D-galase and Oxy-RCBH it is reasonable to conclude that octasaccharide contains XLGGG, LXGGG, XXGGGG and GXXGGG (Fig. 7).

oligosaccharide in fr. 13	oligosaccharides obtained by		
	β -D-galase	β -D-glcase + M9 IPase	Oxg-RCBH
XLGGG	XXGGG	LXGGG	LGGG
LXGGG	XXGGG	LXGGG	LXG
XXGGGG	XXGGGG	XXGGGG	XXGGGG
GXXGGG	GXXGGG	GXXGGG	GXXGGG

Fig. 7. Proposed structures of oligosaccharides in fraction 13, and those of oligosaccharides obtained by hydrolysis of fraction 13 with β -D-galase, with β -D-glcase and M9 IPase, or with Oxg-RCBH.

Symbols in figures: Glc, β -Glc-(1 $\rightarrow$ 4)-; Xyl, α -Xyl-(1 $\rightarrow$ 6)-; Gal, β -Gal-(1 $\rightarrow$ 2)-. Symbols in $\square$, see abbreviations.

5. Analysis of oligosaccharides in fraction 17.

Fraction 17 was subjected to MALDI-TOF-MS analysis. In the mass spectrum, an ion peak at m/z 1441.08 which corresponds to the molecular ions as sodium adduct $[(\text{hexose})_7(\text{pentose})_2 + \text{Na}]^+$ of the nonasaccharide was observed (data not shown). Fraction 17 was treated with β -D-galase and the hydrolyzate was subjected to MALDI-TOF-MS analysis. In the mass spectrum, a peak at m/z 1278.95 corresponding to $[(\text{hexose})_6(\text{pentose})_2 + \text{Na}]^+$ in addition to the peak at m/z 1441.08 corresponding to $[(\text{hexose})_7(\text{pentose})_2 + \text{Na}]^+$ was observed (Fig. 8A). This means fraction 17, $(\text{hexose})_7(\text{pentose})_2$ may correspond to $(\text{Glc})_7(\text{Xyl})_2$ and $(\text{Glc})_6(\text{Xyl})_2(\text{Gal})_1$. On the basis of the modes of action of M128 Xg-ase, the nonasaccharides in fraction 17 are supposed to be XLGGGG, GXLGGG, GLXGGG and/or XXGGGGG.

To make clear this supposition, first fraction 17 was treated with a mixture of β -D-glcase and IPase and the product was subjected to MALDI-TOF-MS analysis (Fig. 8B). The peak at m/z 1440.74 corresponding to $(\text{hexose})_7(\text{pentose})_2$ may be derived from LXGGGG, because LXGGGG is not hydrolyzed by β -D-glcase and IPase. The peak at m/z 1278.88 corresponding to $(\text{hexose})_6(\text{pentose})_2$ may be derived from LXGGG, because the non-reducing end Glc residue of GLXGGG is hydrolyzed by β -D-glcase. The peak at m/z 1146.42 corresponding to $(\text{hexose})_6(\text{pentose})_1$ may be derived from LGGGG, because one isoprimeverose (X) molecule is split off from XLGGGG by IPase. The peaks at m/z 984.509 corresponding to $(\text{hexose})_5(\text{pentose})_1$ may be derived from LGGG, because the non-reducing end Glc residue and isoprimeverose (X) residue are split off from GXLGGG by β -D-glcase and IPase. The oligosaccharide XXGGGGG was hydrolyzed completely into X (isoprimeverose) and G (glucose) by β -D-glcase and IPase.

Next fraction 17 was treated with Oxg-RCBH and the product was subjected to MALDI-TOF-MS analysis (Fig. 8C). The peak at m/z 1116.52 corresponding to

Fig. 8. MALDI-TOF-MS of β -D-galase treated fraction 17 (A), of β -D-glcase and M9 IPase treated fraction 17 (B), and of Oxg-RCBH treated fraction 17 (C).

$(\text{hexose})_5(\text{pentose})_2$ may be derived from GLXG, because this GLXG is produced by splitting off one cellobiose (GG) residue from the reducing end of GLXGGG by Oxg-RCBH. The peak at m/z 792.241 corresponding to $(\text{hexose})_3(\text{pentose})_2$ may be derived from both XL and LX, because the former and the latter are produced by splitting off two cellobiose (GG) residues from the reducing end of

oligosaccharide in fr. 17	oligosaccharides obtained by		
	β -D-galase	β -D-glucase + M9 IPase	Oxg-RCBH

Fig. 9. Proposed structures of oligosaccharides in fraction 17, and those of oligosaccharides obtained by hydrolysis of fraction 17 with β -D-galase, with β -D-glucase and M9 IPase, or with Oxg-RCBH.

Symbols in figures: Glc, β -Glc-(1 $\rightarrow$ 4)-; Xyl, α -Xyl-(1 $\rightarrow$ 6)-; Gal, β -Gal-(1 $\rightarrow$ 2)-. Symbols in $\square$, see abbreviations.

XLGGGG and LXGGGG, respectively, by Oxg-RCBH. The peaks at m/z 660.159 and 498.051 corresponding to (hexose)₃(pentose)₁ and (hexose)₂(pentose)₁ may be derived from LG and GX, respectively, because these LG and GX are produced from GXLGGG by Oxg-RCBH.

On the basis of the modes of action of M128 Xg-ase, M9 IPase, β -D-glucase, β -D-galase and Oxg-RCBH, it is reasonable to conclude that nonasaccharide contains XLGGGG, LXGGGG, GXLGGG, GLXGGG and XXGGGGG (Fig. 9).

Analysis of bamboo shoot xyloglucan.

We previously reported that bamboo shoot xyloglucan was built up predominantly of repeating-oligosaccharide units of XXG, XG and GG.⁶⁾ To obtain accurate information on the arrangement of the Glc residues which are not xylosylated at *O*-6 in the β -1,4-D-glucan backbone of bamboo shoot xyloglucan, the fragmentation analysis of bamboo shoot xyloglucan (2.4 mg) was carried out in a manner almost identical with that described for barley xyloglucan. Tetra- (127 μ g), penta- (123 μ g), hexa- (247 μ g), hepta- (347 μ g), octa- (499 μ g) and nona-saccharide (272 μ g) fractions were obtained as major oligosaccharide fractions, which might be composed of XX, of XXG, of XXGG, XLG and LXG, of XXGGG, LXGG, XLGG, LLG and XXXG, of XLGGG, LXGGG, XXGGGG, LLGG and XXLGG, and of XLGGGG, LXGGGG, GXLGGG, GLXGGG and XXGGGGG, respectively. These results suggest that the bamboo shoot xyloglucan also has regions consisting of two, three, four and five contiguous glucose residues which are not xylosylated at *O*-6 in the β -1,4-D-glucan backbone.

General discussion.

Structural analyses of the fragment oligosaccharides re-

vealed regions consisting of two, three, four and five contiguous Glc residues which are not xylosylated at *O*-6 in the β -1,4-D-glucan backbone of barley and bamboo shoot xyloglucans. The previous results¹³⁾ and present results indicate a high degree of regularity in the arrangement of unsubstituted Glc residues along the β -glucan backbone in *Solanaceae* xyloglucans, and no regularity in that in *Gramineae* xyloglucan.

We concluded that the method used for structural study of *Solanaceae* and *Gramineae* xyloglucans involving digestion with xyloglucan-specific enzyme (Xg-ase, IPase and Oxg-RCBH) followed by MALDI-TOF-MS analysis is quite useful for clarifying the branching pattern and the arrangement of the Glc residues which are not xylosylated at *O*-6 in the β -1,4-D-glucan backbone of the xyloglucans.

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キシログルカナナーゼによる大麦 およびタケノコキシログルカンの構造

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大麦幼植物およびタケノコ細胞壁からキシログルカン多糖を分離した。これに、精製キシログルカナナーゼ(Xgase)を作用させ、HPLCにより重合度4-9のキシログルカンオリゴ糖画分を得た。各キシログルカンオリゴ糖画分は、それぞれ、 β -D-ガラクトシダーゼ(β -D-galase), β -D-グルコシダーゼ(β -D-glucase), 精製イソプリメブコース生成酵素(IPase)および精製キシログルカンオリゴ糖分解酵素(Oxg-RCBH)による加水分解を行い、生成物をMALDI-TOF-MS法にて解析した。大麦のキシログルカンを構成している主要オリゴ糖単位として、四糖(XX), 五糖(XXG), 六糖(XXGG), 七糖(XXGGG, LXGG), 八糖(XLGGG, LXGGG, XXGGGG, GXXGGG), および九糖(XLGGGG, LXGGGG, GXLGGG, GLXGGG, XXGGGGG)の存在が確認された。[XX等はFryらによるキシログルカンオリゴ糖の表示法で主鎖の各(1→4)- β 結合のグルコース残基の分岐様式により一文字コードで示される。G= β -D-Glc; X= α -D-Xyl-(1→6)- β -D-Glc; L= β -D-Gal-(1→2)- α -D-Xyl-(1→6)- β -D-Glc]。また、タケノコの場合も大麦と類似した構造単位が確認された。これらの結果より、イネ科植物の大麦およびタケノコのキシログルカンには側鎖をもつ構造単位の間、セロビオース(GG)からセロペンタオース(GGGG)に相当する配列の存在が明らかとなった。