ELSEVIER

www.elsevier.nl/locate/carres

Carbohydrate Research 330 (2001) 285–288

CARBOHYDRATE RESEARCH

Note

Isolation and NMR characterisation of a (4-*O*-methyl-D-glucurono)-D-xylan from sugar beet pulp

Elisabeth Dinand, Michel R. Vignon*

Centre de Recherches sur les Macromolécules Végétales, C.N.R.S., and Université Joseph Fourier, BP 53, 38041 Grenoble cedex 9, France

Received 6 April 2000; received in revised form 28 September 2000; accepted 18 October 2000

Abstract

Stable aqueous suspensions of purified and homogenised sugar beet pulp (SBP) cellulose were subjected to various TFA treatments which induced a flocculation of the suspension and the release of a number of polysaccharides. Among these, a 4-*O*-methyl glucuronoxylan was isolated and characterized by ^{1}H and ^{13}C NMR spectroscopy. In this polysaccharide the molar proportions of D-Xyl and 4-*O*-Me-D-GlcA were found to be 7:1. The presence of a glucuronoxylan at the surface of the cellulose microfibrils is very likely involved in the stability of the suspensions. To our knowledge, the presence of a 4-*O*-methyl-glucuronoxylan in the sugar beet cells has not been described previously.

Keywords: Sugar beet pulp; Parenchymal cellulose; 4-*O*-Methyl-glucuronoxylan; ^{1}H and ^{13}C NMR spectroscopy

Cellulose isolated from sugar beet pulp (SBP) consists of slender microfibrils that possess interesting rheological properties in aqueous suspensions. These cellulose microfibrils can find a large number of applications.[1–3] The neutral sugar analysis of SBP, in addition to Glc shows the presence of Ara, Gal, Xyl and Rha and minute amounts of Man and Fuc.[2,3]

The structures of galacturonans, rhamnogalacturonans and arabinans from SBP have been studied in detail,[4–8] but there is little information about the structure of other polysaccharides isolated from SBP. In general, xylan and xyloglucans are often found strongly associated with cellulose. Xylan is a generic name for polymers consisting of a backbone of (1→4)-linked β-D-xylopyranose. The xylopyranosyl residues can be substituted in C-2 and/or C-3 position by short and flexible side chains. These chains are constituted mainly of units of α-D-glucuronic acid or 4-*O*-methyl-α-D-glucuronic acid and some units of α-L-arabinofuranose, α-D-xylopyranose or α-D-galactopyranose.[9–14] In addition, acetyl groups located at O-2 and/or O-3 are often found on the backbone xylopyranosyl residues.[15] These hemicelluloses are abundant in cell walls of mono- and dicotyledones of angiosperms, in the cellulosic mucilage and in smaller amount, in cell walls of gymnosperms. In this note we describe a 4-*O*-methyl-glucuronoxylan isolated from SBP and its charac-

* Corresponding author. Tel.: +33-4-76037614; fax: +33-4-76547203.

E-mail address: michel.vignon@cermav.cnrs.fr (M.R. Vignon).

0008-6215/01/$ - see front matter
PII: S0008-6215(00)00273-1

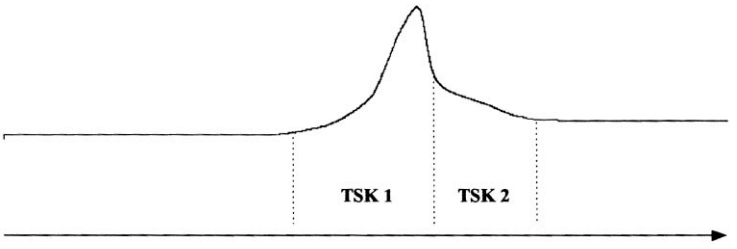

Fig. 1. Separation of TFA hydrolysate on the TSK gel column.

terisation by proton and ^{13}C NMR spectroscopy.

The initial composition of SBP, as presented already,[1–3] was 22% cellulose, 32% hemicelluloses, 27% pectin and 9% minerals. After removal of pectic and hemicellulosic polysaccharides under alkaline conditions,[1–3] SBP contained 1% of ashes and 2% of uronic acids.[1] Among the neutral sugars present in the sample, there were Glc 87.5, Xyl 5.7, Man 2.9, Ara 1.7, Gal 1.4, Fuc 0.4 and Rha 0.3 (mol%). These data indicated that the sample was mainly cellulosic, but that some pectins and hemicelluloses were still bound to the cellulose microfibrils. Transmission electron microscopy (TEM) observation of the sample showed disentangled microfibrils, individual or in bundles that contained a limited number of parallel microfibrils. The individual microfibrils were a few nanometers in width with an average length of several micrometers.[3] These microfibrils gave stable suspensions in water.

It is known that TFA preferentially hydrolyses pectins and hemicelluloses without hydrolysis of the crystalline cellulose.[16] After a treatment with TFA for different durations, the microfibrils were separated by filtration and extensively washed until neutrality of the filtrate. The microfibrillar residues were redispersed in the same amount of water. The suspensions were not stable any more, and a phenomenon of decantation was observed. The level of the microfibrils suspension phase under a clear water phase was inversely related to the time of reaction. This decantation phenomenon induced by TFA treatment indicated that the stabilisation properties of microfibrils suspensions were due to non-cellulosic constituents that can be removed by TFA treatments. After a TFA treatment for 30 min, the yield of dry matter in the filtrate was 4% (w/w). The TFA filtrate gave 0.2% of ashes and contained 7.4% acidic sugars and 85.2% neutral sugars. Among the neutral sugars present in the sample, there were Xyl 61.2, Glc 16.2, Gal 12.1, Ara 9.6 and Rha 0.9 (mol %). The TFA filtrate was fractionated by size-exclusion chromatography on a TSK gel column (Fig. 1) and finally on a polyacrylamide Biogel P4 column (Fig. 2).

Two fractions were obtained, and the fraction P4-1 was characterised by sugar analysis and NMR spectroscopy. An hydrolysate of P4-1 fraction revealed that it contained 78.9% neutral sugars. Xyl and Ara in the proportion 97:3 were the only sugars detected. The uronic acid content determined by colourimetric method, was estimated to be 14%. The ^{13}C NMR spectrum of P4-1 shows five main signals at δ 102.43 (C-1), 73.47 (C-2), 74.45 (C-3), 77.14 (C-4) and 63.76 (C-5) corresponding to (1→4)-linked β-D-Xyl residues. Among the other signals observed at δ 177.57, 98.32, 83.17 and 60.63 ppm, respectively, were characteristic signals of C-6, C-1, C-4 and

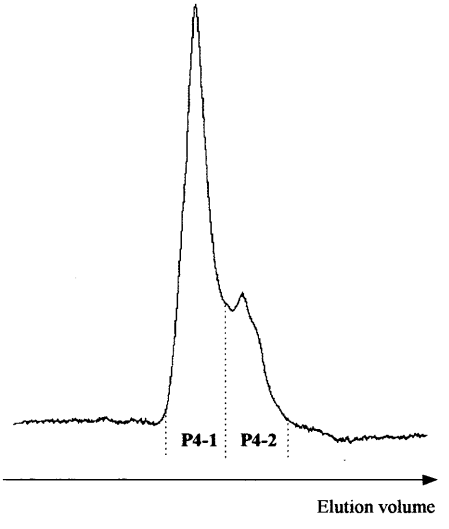

Fig. 2. Separation of the TSK-1 fraction on the Biogel P4 column

Table 1
^{13}C NMR data of P4-1 fraction at 303 K

Glycosyl residues	Chemical shift [a]						
	C-1	C-2	C-3	C-4	C-5	C-6	OCH_3
(1→4)-α-D-Xyl*p*	102.43	73.47	74.45	77.14	63.76		
(1→4)-α-D-Xyl*p*-2-*O*-Glc*p*A	102.13	77.35	73.02	76.77	63.66		
4-*O*-Me-α-D-Glc*p*A	98.32	72.04	77.61	83.17	73.16	177.57	60.63

[a] In ppm relative to the signal of internal acetone in deuterium oxide, at 2.1 ppm (^1H) or at 31.5 ppm (^{13}C)

methoxyl group of a 4-*O*-methyl-α-D-glucuronic acid residue. All the data reported in Table 1 are in good agreement with the structures of (4-*O*-methyl-α-D-glucurono)-β-D-xylan already described in other plants.[15,17–19]

In the ^1H spectrum of P4-1 all the signals characteristic of a (4-*O*-methyl-α-D-glucurono)-β-D-xylan were present.[15,17,20] The proton spectrum showed three doublets in the anomeric region at 4.36 [(1→4)-β-D-Xyl*p*], 4.51 [(1→4)-β-D-Xyl*p*-2-*O*-Glc*p*A] and 5.12 (4-*O*-methyl-α-D-Glc*p*A) with an intensity ratio of 6:1:1.

The X-ray diffraction pattern of the microfibrils obtained after the TFA treatment is that of native cellulose I of the primary wall type,[1] indicating that no conformational change occurred. Moreover the neutral carbohydrate analysis showed a great enrichment in Glc, with the presence of only Glc and Xyl in the ratio of 98:2. The uronic acid content measured by colourimetry was estimated to be less than 1%.

Thus it can be concluded that the cellulose microfibrils obtained from sugar beet pulps are associated with a (4-*O*-methyl-D-glucurono)-D-xylan of a structural type already observed in several plants. It is likely that this acidic material at the surface of the microfibrils, which is removed during the TFA treatment, is responsible for the suspension stability of dispersed microfibrils in water as it enables the electrostatic repulsion of charged entities.

1. Experimental

Material.—The fresh SBP, a by-product of the sugar industry, was supplied by Saint-Louis Sucre, Nassandres, France.

General methods.—The uronic acid content was determined according to Blumenkrantz and Asboe Hansen.[21] Neutral sugars were released by Seaman hydrolysis and analysed by GLC as their corresponding alditol acetates[22] using a Packard and Becker 417 instrument coupled to a Hewlett–Packard 3380 A integrator. Glass column (3 mm × 2 m) packed with 3% SP 2340 on Chromosorb W-AW DMCS (100–120 mesh) was used.

Purification.—The initial SBPs were treated according to Dinand et al.;[1–3] they were enriched in cellulose after two NaOH extractions (NaOH 2%, 2 h, 80 °C) and two $NaClO_2$ treatments ($NaClO_2$, pH 4.9, 1h, 70 °C). The purified SBP was disrupted in a Waring Blender operated at 18,000 rpm for 15 min at a concentration ranging from 1 to 2%. The samples, which had reached a temperature of 60 °C were immediately treated with a laboratory scale Manton–Gaulin homogeniser 15MR-8TBA, from APV France, Evreux. Fifteen passes in the homogeniser at 500 bars led to non-flocculating microfibrillar suspension.

The purified microfibrillar sample was extracted in 0.1 M TFA at 100 °C (liquid/solid = 170), for several periods of time from 30 min to 5 h, cooled in ice water, filtered on a glass funnel and washed with water. TFA was removed by evaporation from the filtrate which was then freeze-dried, solubilised in a minimum of distilled water and fractionated by gel filtration on a column of TSK-gel HW (20 × 500 mm, CH265230, Interchim) eluted with water. The flow rate (108 mL h^{-1} was controlled by a peristaltic pump (Milton–Roy, Touzart et Matignon, Courtaboeuf, France), and the refractive index was measured with a differential refractometer (R 403, Waters). The pseudo polysaccharidic fraction (TSK-1,

Fig. 1) was fractionated again on a Biogel P4 column (50 mm × 1 m, 200–400 mesh, Bio-Rad) eluted with water (flow rate and refractive index as mentioned above). Two fractions were obtained, named P4-1 and P4-2 (Fig. 2).

NMR spectroscopy.—^{1}H and ^{13}C NMR experiments were recorded on an AC 300 Bruker spectrometer (^{1}H frequency of 300 MHz and ^{13}C frequency of 75.468 MHz). Sample was studied as solution in D_2O at 303 K in 5 mm o.d. tube (internal acetone ^{1}H (CH_3) at 2.1 ppm relative to Me_4Si and ^{13}C (CH_3) at 31.5 ppm relative to Me_4Si).

Acknowledgements

The authors acknowledge the financial help of Saint-Louis Sucre. They thank also Miss C. Fraschini and Dr A. Heyraud for help in purification and gel filtration of samples.

References

1. Dinand, E.; Chanzy, H.; Vignon, M. R. *Food Hydrocolloids* **1999**, *13*, 275–283.
2. E. Dinand, A. Maureaux, H. Chanzy, I. Vincent, M.R. Vignon, US Patent 5964983, 1999.
3. Dinand, E.; Chanzy, H.; Vignon, M. R. *Cellulose* **1996**, *3*, 183–188.
4. Thibault, J. F.; Guillon, F.; Rombouts, F. M. In *The Chemistry and Technology of Pectins*; Walter, R., Ed.; Academic Press: San Diego, 1991; pp. 119–133.
5. Thibault, J. F.; Renard, C. M. G. C.; Guillon, F. In *Modern Methods of Plant Analysis, Vegetables and Vegetables Products*; Linskens, H. F.; Jackson, J. F., Eds.; Springer: Berlin, 1994; Vol. 16, pp. 23–55.
6. Guillon, F.; Thibault, J. F. *Carbohydr. Res.* **1991**, *190*, 85–96.
7. Guillon, F.; Thibault, J. F.; Rombouts, F. M.; Voragen, A. G. J.; Pilnik, W. *Carbohydr. Res.* **1991**, *190*, 97–108.
8. Oosterveld, A.; Beldman, G.; Schols, H. A.; Voragen, A. G. J. *Carbohydr. Res.* **1996**, *288*, 143–153.
9. Timell, T. E. *Wood Sci. Technol.* **1967**, *1*, 45–70.
10. Garregg, P. J.; Han, M. *Svensk Papperstidn* **1968**, *71*, 331–334.
11. Wilkie, K. C. *Adv. Carbohydr. Chem.* **1979**, *36*, 215–264.
12. Carpita, N. C.; Whittern, D. *Carbohydr. Res.* **1986**, *146*, 129–140.
13. Darvill, J. E.; MacNeil, M.; Darvill, A. G.; Albersheim, P. *Plant Physiol.* **1980**, *66*, 1135–1139.
14. Waldron, K. M.; Brett, C. T. In *Topics in Plant Physiology II*; Black, M.; Chapman, J., Eds.; Hyman: London, 1990.
15. Lindberg, B.; Mosihuzzaman, M.; Nahar, N.; Abeysekera, R. M.; Brown, R. G.; Willison, J. H. M. *Carbohydr. Res.* **1990**, *207*, 307–310.
16. Chambat, G.; Barnoud, F.; Joseleau, J. P. *Plant Physiol.* **1984**, *74*, 687–693.
17. Vignon, M. R.; Gey, C. *Carbohydr. Res.* **1998**, *307*, 107–111.
18. Kardosova, A.; Matulova, M.; Malovikova, A. *Carbohydr. Res.* **1998**, *308*, 99–105.
19. Bazus, A.; Rigal, L.; Gaset, A.; Fontaine, T.; Wieruszeski, J. M.; Fournet, B. *Carbohydr. Res.* **1993**, *243*, 323–332.
20. Andersson, R.; Hoffman, J.; Nahar, N.; Scholander, E. *Carbohydr. Res.* **1990**, *206*, 340–346.
21. Blumenkrantz, P. N.; Asboe-Hansen, G. *Anal. Biochem.* **1973**, *54*, 484–489.
22. Selvendran, R. R.; March, J. F.; Ring, S. G. *Anal. Biochem.* **1979**, *96*, 282–292.