Pergamon

0031-9422(95)00979-1

Phytochemistry, Vol. 39, No. 4, pp. 821-823, 1995

0031-9422/95 $9.50 + 0.00

ANALYSIS OF STRUCTURE OF SUGAR-BEET PECTIN BY ENZYMATIC METHODS

TATSUJI SAKAMOTO and TAKUO SAKAI

Department of Applied Biological Chemistry, College of Agriculture, University of Osaka Prefecture, 1-1 Gakuen-cho, Sakai City, Osaka 593, Japan

(*Received in revised form* 7 *November* 1994)

Key Word Index—*Beta vulgaris*; sugar-beet; pectin; rhamnogalcturonan; arabinan; arabinogalactan.

Abstract—The structure of alkali-soluble pectin (ASP) prepared from sugar-beet pulp was determined. ASP was sequentially degraded by pure galactanase, rhamnogalacturonase and arabinosidase and the sugar compositions and NMR analysis of the products used to assign the following tentative structure to ASP: a backbone based on units of →4)-α-D-Gal*p*A-(1 → 2)-α-L-Rha*p*-(1 →, and side chains both of arabinan and of arabinogalactan.

INTRODUCTION

Pectins are one of the main components of the primary cell walls of dicotyledons. They are heteropolysaccharides composed for the most part of galacturonic acid, arabinose, rhamnose and galactose, and small amounts of xylose, glucose, 2-*O*-metylxylose, 2-*O*-metylfucose, and apiose [1]. Pectins in plant cell walls contained homogalacturonan (smooth) regions that are degraded by endopolygalacturonase and endopectin lyase, and resistant (hairy) regions that are rich in neutral side chains [2]. The chemical structure of the hairy fragments along the rhamnogalacturonan backbone of sugar-beet pectins has been studied by means of methylation analysis and characterization of the products obtained after acid hydrolysis [3] or enzymatic degradation [4].

In our previous studies, the chemical structures of two fragments released from ASP by enzymatic degradation were determined. We elucidated the structure of these oligomers by sequential digestion with α-L-arabinofuranosidase, *endo*-1,5-α-L-arabinase, *endo*-1,4-β-D-galactanase, and protopectinase-T (PPase-T), which cleaves galactopyranosyluronic–rhamnopyranosyl linkages in rhamnogalacturonan, by NMR analysis. These oligomers had a tetramer backbone based on units of →4)-α-D-Gal*p*A-(1 → 2)-α-L-Rha*p*-(1 → with rhamnose at the non-reducing end, indicating that the chemical structure of ASP contains a backbone of alternating rhamnose and galacturonic acid [5]. However, one part of the hairy fragments split from ASP by treatment with PPase-T was identified as an L-arabinan with a main structure consisting of α-1,5-L-arabinan to which α-L-arabinofuranosyl units were attached to hydroxy groups of the 3-positions of the arabinan to form one-unit side chains [6]. In this paper, we deal with the determination of the structure of sugar-beet pectin by an enzymatic method.

RESULTS AND DISCUSSION

The so-called hairy regions are rich in arabinose, rhamnose, and galactose and this study is concerned with the structural relationship between these regions and the rhamnogalacturonan and arabinan already described. ASP was prepared from sugar-beet pulp by extraction under hot, alkaline conditions as described in the Experimental section. ASP was assumed to contain the hairy regions of pectin as main components, because the methyl-esterified homogalacturonan regions are unstable to alkaline treatment, being depolymerized into oligogalacturonides by transeliminative degradation. First, ASP was partially hydrolysed with *endo*-1,4-β-D-galactanase in 20 mM acetate buffer, pH 5 at 40°. After denaturation of the enzyme by boiling for 5 min, the reaction products were loaded on a DEAE-Toyopearl 650 M (2 × 16 cm; Tosoh Corp., Tokyo) column equilibrated with 50 mM acetate buffer, pH 5.0, and the bound saccharides were eluted by a linear gradient of acetate buffer (200 ml, 50–1000 mM, pH 5). In this step, two (unbound and bound) fractions with different sugar compositions (Table 1) were collected. The unbound and bound fractions were called ASPG-1 and -2, respectively. ASPG-1 seemed to be the hairy regions split by the galactanase from the ASP bound on the column. No galacturonic acid was found in ASPG-1. The material released by the galactanase contained not only galactose residues (42%) but also arabinose residues (58%). The arabinose content in ASPG-1 was increased up to 95% when this fraction was dialysed against water, which indicated that the arabinose residues were in oligomeric chains of higher d.p. than those containing the galactose residues that were produced by the action of the galactanase. Arabinan chains obtained in this step could be split by protopectinase-C, an *endo*-1,5-α-L-arabinase isol-

Table 1. Sugar composition (mol%) of sugar-beet pectin fractions treated with β-1,4-galactanase

Pectin fraction	Galacturonic acid	Rhamnose	Galactose	Arabinose
ASP	8	5	15	72
ASPG-1	0	0	42	58
ASPG-2	10	7	16	67

Detailed descriptions of the different pectin fractions are given in the text.

Sugars present in trace amounts are not reported.

ated from the culture filtrate of *Bacillus subtilis* [7]. The facts that when ASP is digested with an α-L-arabinofuranosidase and an *endo*-1,5-α-L-arabinase, the arabinose residues are completely separated from ASP while the galactose residues are retained in rhamnogalacturonan [5] may indicate that galactan chains are directly linked to the rhamnogalacturonan main chain. These results also suggest that some arabinan chains existed as arabinogalactan, which are linked to rhamnogalacturonan through an interposed β-1,4-D-galactan chain.

Albersheim [8] has reported that rhamnogalacturonan in sycamore cells is attached at the reducing end of the arabinogalactan molecule, and also that arabinogalactan seems to consist of a chain of arabinose coupled to another chain of galactose. Our data indicated that the structure of the hairy regions in sugar-beet protopectin was substantially the same as in sycamore cells, with rhamnogalacturonan attached to other cell wall constituents by interposed chains of arabinogalactan.

Next, the hairy regions that were not released by the galactanase were studied. ASPG-2 was treated with PPase-T, which would release the material in the hairy regions connecting with a rhamnose reisdue in the rhamnogalacturonan of sugar-beet pectin, in 20 mM acetate buffer, pH 5, at 45°. The reaction products were chromatographed using DEAE-Toyopearl column and a linear gradient of acetate buffer (200 ml, 50–1000 mM, pH 5). Three fractions were obtained in this step and they were termed ASPGT-1, -2, and -3, respectively (Fig. 1). ASPGT-1 was from the hairy region split from ASPG-2 by treatment with PPase-T and was composed almost completely of arabinose. This indicated that some arabinan chains were attached directly to rhamnogalacturonan. The arabinan obtained was partially digested with α-L-arabinofuranosidase [9], which catalysed the hydrolysis of single L-arabinofuranosyl residues attached to the main chain in L-arabinan and thus supported the proposed structure. Undegraded polysaccharide was purified by the method of Tagawa and Kaji [10]. It was identified as α-1,5-L-arabinan according to the ^{13}C NMR data [6]. ASPGT-3 might be material derived from the rhamnogalacturonan region.

From these results, it was confirmed that the ASP contains a backbone based on residues of →4)-α-D-Gal*p*A-(1→2)-α-L-Rha*p*-(1→ and side chains of both arabinan and arabinogalactan. There is an indication of a

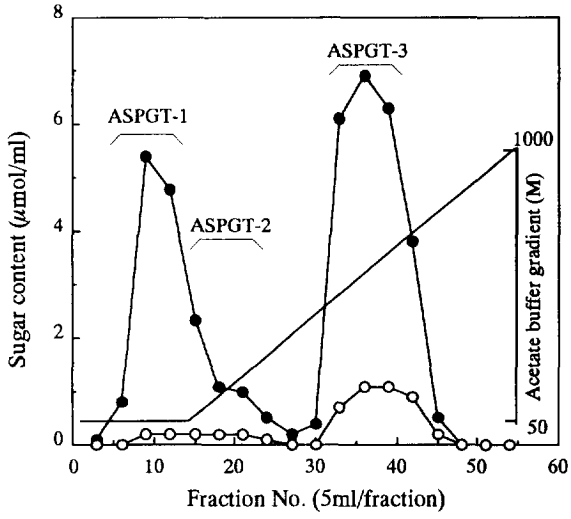

Fig. 1. Chromatography on DEAE-Toyopearl of the reaction products of ASPG-2 treated with PPase-T. ● Neutral sugar; ○ galacturonic acid.

single unit of galactose being attached to the 4-position of a rhamnose residue in the rhamnogalacturonan backbone [5]. Furthermore, we found that a very small quantity of galactose was released in company with a lot of arabinose from ASP treated with arabinase (data not shown). This fact indicated that a little of the galactose was present in side chains on the arabinan. Figure 2 shows one possible structure for the sugar-beet pectin that can be envisaged from the results. However, arabinan chains in the hairy fragments of sugar-beet pectin are tentatively proposed to be attached only directly to rhamnose residues of rhamnogalacturonan [4]. We can not explain these different results based on the structure of the sugar-beet pectin hairy region.

In the determination of the chemical structure of pectins, polygalacturonase, pectin lyase, arabinase and galactanase have been used for degradation of specific sites in pectins. PPase-T, which is a rhamnogalacturonase, is also seen to be useful in studies of the structures of complex pectic polysaccharides. Another rhamnogalacturonase has been isolated by Schols *et al.* [11].

EXPERIMENTAL

Preparation of alkali-soluble pectin (ASP). The remaining dry material left after sugar extraction from sugar-beet pulp (*Beta vulgaris* L. Var. *rapa*; Tienen Sugar Co., Tienen, Belgium) was used for pectin extraction. The pulp was boiled in 0.1 N NaOH for 1 hr and the extract loaded on an anion-exchange column. The bound fraction was eluted with 1 M acetate buffer, pH 5.0, dialysed against H_2O, and finally lyophilized, giving ASP [5].

Enzyme preparations. α-L-Arabinofuranosidase [9] was prepared from culture filtrate of *Aspergillus niger* K1 strain (kindly supplied by Prof. A. Kaji, Kagawa University, Japan). Pure *endo*-1,4-β-D- galactanase [12] was kindly provided by Dr. Y. Tominaga (Osaka Municipal

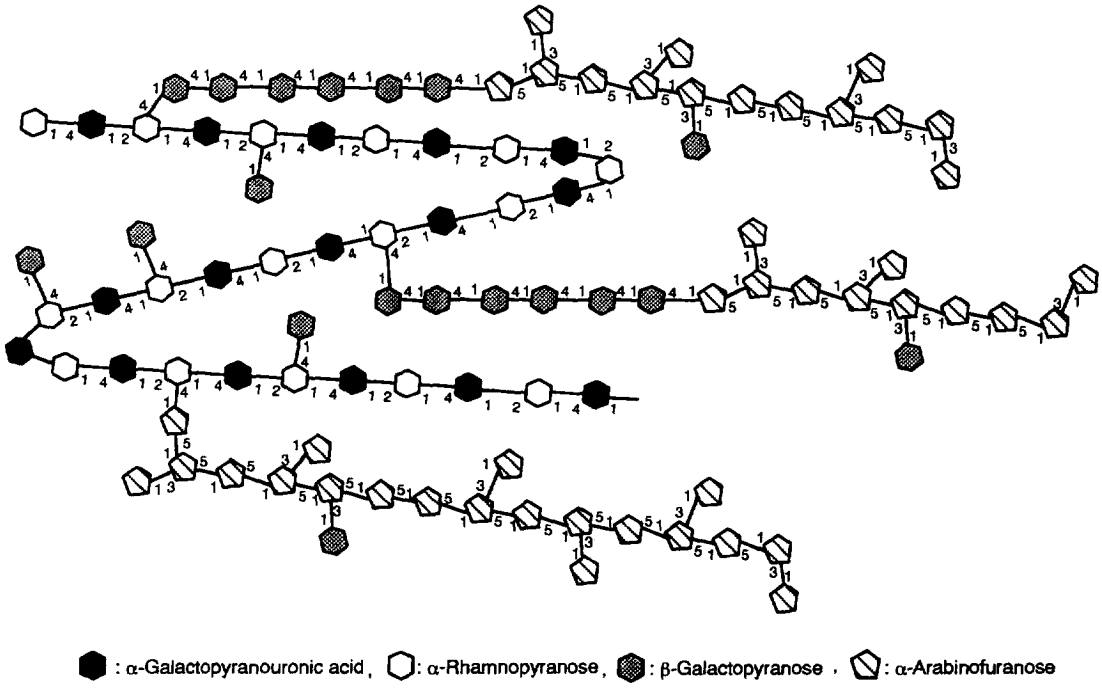

Fig. 2. Postulated structure of sugar-beet pectin.

Technical Research Institute, Japan). Protopectinase-T (PPase-T) [13] was purified from the culture filtrate of *Trametes sanguinea*.

Analytical methods. Galacturonic acid was determined by the method of ref. [14]. Neutral sugars were determined by the phenol H_2SO_4 method [15], using D-galacturonic acid and D-galactose as the standards, as the difference between the total absorbance (which corresponds to both neutral and acidic sugars) and the one corresponding to the galacturonic acid previously quantified. The composition of neutral sugars was analysed by HPLC after hydrolysis with 1 N HCl at 110° for 2 hr.

REFERENCES

1. Lau, J. M., McNeil, M., Darvill, A. G. and Albersheim, P. (1985) *Carbohydr. Res.* **137**, 111.
2. Saulnier, L. and Thibault, J.-F. (1987) *Carbohydr. Polym.* **7**, 345.
3. Guillon, F. and Thibault, J.-F. (1989) *Carbohydr. Res.* **190**, 85.
4. Guillon, F., Thibault, J.-F., Rombouts, F. M., Voragen, A. G. J. and Pilnik, W. (1989) *Carbohydr. Res.* **190**, 97.
5. Sakamoto, T. and Sakai, T. (1994) *Carbohydr. Res.* **259**, 77.
6. Sakamoto, T., Yoshinaga, J., Shogaki, T. and Sakai, T. (1993) *Biosci. Biotech. Biochem.* **57**, 1832.
7. Sakai, T. and Sakamoto, T. (1990) *Agric. Biol. Chem.* **54**, 879.
8. Albersheim, P. (1975) *Sci. Am.* **6**, 81.
9. Tagawa, K. and Kaji, A. (1988) *Methods Enzymol.* **160**, 707.
10. Tagawa, K. and Kaji, A. (1969) *Carbohydr. Res.* **11**, 293.
11. Schols, H. A, Geraeds, C. C. J. M., Searle-van Leeuwen, M. F., Kormelink, F. J. M. and Voragen, A. G. J. (1990) *Carbohydr. Res.* **206**, 105.
12. Nakano, H., Takenishi, S. and Watanabe, Y. (1986) *Agric. Biol. Chem.* **50**, 3005.
13. Sakamoto, M., Shirane, Y., Naribayashi, I., Kimura, K., Morishita, N., Sakamoto, T. and Sakai, T. (1994) *Eur. J. Biochem.* **226**, 285.
14. Blumenkrantz, N. and Asboe-Hansen, G. (1973) *Anal. Biochem.* **54**, 484.
15. Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A. and Smith, F. (1956) *Anal. Chem.* **28**, 350.