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STRUCTURAL FEATURES OF TWO AMYLOIDS FROM THE HEMICELLULOSIC FRACTION OF FIELD-BEAN (*Dolichos lablab*) HULLS

PARAMAHANS V. SALIMATH AND RUDRAPATNAM N. THARANATHAN*
Discipline of Biochemistry and Applied Nutrition, Central Food Technological Research Institute, Mysore 570013 (India)
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ABSTRACT

Two amyloid-type fractions were isolated from field-bean (*Dolichos lablab*) hulls by 10% alkali extraction followed by acetylation and solvent fractionation. The major, chloroform-insoluble fraction and a minor, chloroform-soluble fraction were found to be homogeneous in sedimentation analysis and molecular-sieve chromatography. The polysaccharides contained xylose and glucose in various proportions. Methylation analysis, periodate oxidation, Smith degradation, oxidation by chromium trioxide, and oligosaccharide studies indicated a new type of structure for the major fraction (glucose:xylose ratio of 1.9:1) in that it had a backbone of (1→4)-linked β-D-glucose residues interspersed with single or multiple residues of (1→4)-linked β-D-xylose, and to which some single D-xylosyl groups are attached through O-6 of D-glucose. In contrast, the minor fraction (glucose:xylose ratio of 1:3.7) had a backbone of (1→4)-linked β-D-xylose interspersed with (1→4)-β-D-glucose and having a side chain of D-xylose, attached through O-6 of D-glucose. The third fraction was found to be a mixture of linear (1→4)-D-glucan and (1→4)-D-xylan.

INTRODUCTION

In our earlier work[1–3], the isolation and characterization of such starchy and non-starchy polysaccharides as arabinogalactans and pectic polysaccharides from field-bean (*Dolichos lablab*) hulls (FBH) were described. In continuation of our investigations on food carbohydrates of functional and physiological importance, we now report on the main structural features of FBH hemicellulose A[1–3].

EXPERIMENTAL

The general experimental methods have been reported previously[2,3].

Extraction of hemicellulose A. — The FBH residue (189 g) left after successive extraction of water-soluble gums and pectic substances[1,2] was twice extracted with

*To whom correspondence should be addressed.

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TABLE I

COMPOSITION OF HEMICELLULOSE A AND ITS FRACTIONS

Fraction	*Hemi-cellulose A*	*F-1*	*F-2*	*F-3*
Volume (L) of petr. ether per 0.8 L of chloroform solution		Insoluble	0.20	1.40
Yield (%)	(100)	88.75	6.25	0.94
$[\alpha]_D$ (degrees)	[a]	+32° (*c* 1.25 in 0.05M NaOH)	+12° (*c* 0.5 in 0.1M NaOH)	[a]
Constituent sugars				
Xylose (%)	41.60	35.00	75.00	78.00
Glucose (%)	58.40	65.00	25.00	22.00
Periodate-reduced (mol/sugar residue)		1.00	1.06	[a]
Formic acid released (mol/sugar residue)		0.08	0.05	[a]

[a]Not determined.

10% sodium hydroxide (under nitrogen). The alkali-insoluble residue was centrifuged off (146 g). Acidification (to pH 4.5) of the resulting alkaline centrifugate yielded hemicellulose A (18 g) as a cream-coloured product having the composition shown in Table I.

Fractionation of the polysaccharide. — Hemicellulose A (15 g) was acetylated by the method of Carson and Maclay[4]. The fully acetylated polysaccharide (18 g) was shaken with chloroform and the insoluble material (F-1) was filtered off (13.2 g). To the cooled, chloroform solution was added petroleum ether and the precipitated products (F-2 and F-3) were collected (see Table I). Deacetylation (0.2M sodium hydroxide, for 16 h at room temperature) of a part of the fraction, and subsequent acid hydrolysis gave the sugar composition shown in Table I.

Partial hydrolysis. — Fraction F-1 (1 g) was partially hydrolysed with sulphuric acid (0.2M) for 3 h at boiling water-bath temperature. The hydrolysate (left after precipitation of the degraded polysaccharide with alcohol) was made neutral (solid barium carbonate), deionised (Amberlite IR-120, H^+), and concentrated. Oligosaccharides were separated by chromatography on charcoal–Celite and purified by paper chromatography (p.c.) in 7:1:2 (v/v) 1-propanol–ethanol–water. The rate of migration of the oligosaccharides, relative to that of cellobiose (R_{cel}), was determined with precoated cellulose t.l.c. plates (Macherey Nagel Co., Germany) and the aforementioned solvent.

Methylation analyses. — Fraction F-1 (1 g) was methylated with dimethyl sulphate and aq. sodium hydroxide (40%) according to Haworth's method[5]. The addition of reagents was performed on two successive days. The chloroform-soluble (recovered as a fibrous product, 750 mg) and -insoluble (220 mg) products were

recovered. The soluble fraction (200 mg) was dissolved in dry oxolane (tetrahydrofuran) and methylated with dimethyl sulphate and solid, pulverized sodium hydroxide according to the procedure of Falconer and Adams[6]. The partially methylated product (176 mg) was further methylated by the method of Hakomori[7]. The product showed no hydroxyl absorption in its i.r. spectrum. A portion of the methylated polysaccharide (~5 mg) was successively hydrolysed[8], reduced, and *O*-acetylated. Fractions F-2 and F-3 (~5 mg) were directly methylated by the Hakomori procedure[7].

Periodate oxidation. — This was performed as reported earlier[3]. The oxidised polysaccharide was successively reduced (sodium borohydride), depolymerized (0.125M sulphuric acid, 5 h at 100°), and analysed chromatographically.

Oxidation by chromium trioxide[9]. — Fraction F-1 (50 mg) was acetylated with acetic anhydride (2 mL) in the presence of pyridine (2 mL) and formamide (8.9 mL) for 16 h. The product was dissolved in acetic acid (2.5 mL) and chromium trioxide (125 mg) was added to one half of the solution, which was stirred for 12 h, while the remaining half served as a control. The mixture and the control were diluted with water (100 mL) and extracted with chloroform. Analysis of the oxidised sample showed no sugar spots, whereas the control showed both xylose and glucose.

Enzymic method. — The fraction (F-1, 10 mg) was gelatinized by heating it in water (2 mL) and treated with β-D-glucosidase (6 units, E.C. No. 3.2.1.2, Sigma Co., U.S.A.) in phosphate buffer (pH 7.0, 0.05M, 37°). After 12 h of incubation, alcohol (3 vol.) was added and the supernatant solution examined for the release of sugar.

RESULTS AND DISCUSSION

Hemicellulose A, obtained in 9.5% yield from the pectin-free residue[3] of FBH, was a brown-coloured product rich in carbohydrates (82%, see Table I). The fraction was insoluble in water, but was soluble in dilute alkali. Examination of the acid hydrolysate showed xylose and glucose in the proportion of 1:1.4 (Table I). The fraction gave a positive starch-blue colour when treated with iodine–potassium iodide solution, suggesting that hemicellulose A is an amyloid type of polysaccharide, such as have previously been identified in rape-seed[10], tamarind[11], and the like. However, unlike the amyloid of FBH, rape-seed and tamarind amyloids contained, in addition to xylose and glucose, also fucose, rhamnose, and galactose in different proportions. Although the carbazole reaction indicated 7.3% of uronic acid, p.c. revealed no acidic sugars.

Solvent fractionation of hemicellulose A acetate afforded a major amount (~88%) of a chloroform-insoluble fraction (F-1) and two minor, soluble fractions (F-2 and F-3), white and fibrous in nature, in 6.2 and 0.9% yields, respectively. Deacetylation of a part of the fractions and acid hydrolysis showed the insoluble fraction to contain xylose and glucose in 1:1.9 ratio; the soluble fractions had these two sugars in ratios of 3.7:1 and 3.6:1, respectively (Table I).

Fraction F-1 was found to be homogeneous by sedimentation analysis.

TABLE II

METHYLATION ANALYSES OF HEMICELLULOSE A FRACTIONS

Parent sugar	*Peak number*	*Methyl ether*	R_T	*Mode of linkage*	*Molar ratio*
Xyloglucan 1 (F-1):					
Xylose	1	2,3,4-tri-	0.68	D-Xyl*p*-(1→	0.8
	2	2,3-di-	1.52	→4)-D-Xyl*p*-(1→	10.8
Glucose	3	2,3,4,6-tetra-	1.00	D-Glc*p*-(1→	1.0
	4	2,3,6-tri-	2.47	→4)-D-Glc*p*-(1→	16.9
	5	2,3-di-	5.40	→6,4)-D-Glc*p*-(1→	0.6
Xyloglucan 2 (F-2):					
Xylose	1	2,3,4-tri-	0.68	D-Xyl*p*-1(1→	0.7
	2	2,3-di-	1.50	→4)-D-Xyl*p*-(1→	24.5
Glucose	3	2,3,4,6-tetra-	1.00	D-Glc*p*(1→	0.6
	4	2,3,6-tri-	2.45	→4)-D-Glc*p*-1(1→	5.4
	5	2,3-di-	5.40	→6,4)-D-Glc*p*-1(1→	0.5
Xylan and glucan (F-3):					
Xylose	1	2,3,4-tri-	0.68	D-Xyl*p*-(1→	2.1
	2	2,3-di-	1.51	→4)-D-Xyl*p*-(1→	23.1
Glucose	3	2,3,4,6-tetra-	1.00	D-Glc*p*-(1→	1.2
	4	2,3,6-tri-	2.47	→4)-D-Glc*p*-(1→	5.6

Molecular-sieve chromatography on Biogel P-200 of the deacetylated material eluted the fraction in a single, symmetrical peak having $\overline{M}_n$ 60,000. The polysaccharide was positive to the starch–iodine test, and had $[\alpha]_D$ +32°, very much different from the specific rotations of rape-seed (+88°)[10], nasturtium (+83°)[12] and tamarind (+79.6°)[11] amyloids.

Fraction F-1 was methylated first by Haworth's method[5] into chloroform-soluble (75%) and insoluble (20%) fractions. The former was soluble in oxolane, and methylation by the method of Falconer and Adams[6], followed by further methylation by the Hakomori method[7] yielded a fully methylated product (no i.r. absorption at 3500–3200 cm^{-1}). It was then successively hydrolysed by acid, reduced, and *O*-acetylated, and then subjected to g.l.c. and g.l.c.–m.s. analyses (Table II). The preponderance of significant amounts of 2,3,6-tri-*O*-methyl-D-glucose and 2,3-di-*O*-methyl-D-xylose pointed to the existence of (1→4)-glycosidic linkages in the main chain. The identification of 2,3-di-*O*-methyl-D-glucose together with 2,3,4-tri-*O*-methyl-D-xylose, however, suggested a sidechain through O-6 of D-glucose.

Partial acid hydrolysis of F-1 and examination of the neutralised hydrolysate revealed a number of oligosaccharides of various degrees of oligomerization, together with free glucose and xylose. Four of the oligomers were isolated by preparative chromatographic methods and were characterised (Table III). In addition, cellobiose (R_{cel} 1.00), xylobiose (R_{cel} 1.31), and a disaccharide (R_{cel} 1.19) having probably

TABLE III

CHARACTERIZATION OF OLIGOSACCHARIDES FROM F-1

Fraction	R_{cel}	*Sugar composition (mol proportion)*	*Reducing end*	*Methylation data[a] (mole proportion) alditol acetates of*	*Probable structure[b]*
1	0.71	Glc	Glc	2,3,6-Me_3-Glc; 2,3,4,6-Me_4-Glc; 1,2,3,5,6-Me_5-Glc.OH (1:1:1)	Glc(1→4)Glc(1→4)Glc.OH
2	0.59	Glc, xyl (1:1)	Glc	2,3,4,6-Me_4-Glc; 2,3-Me_2-Xyl; 1,2,3,5,6-Me_5-Glc.OH (1:2:1)	Glc(1→4)Xyl(1→4)Xyl(1→4)Glc.OH
3	0.49	Glc, xyl (3:1)	Glc	2,3,4,6-Me_4-Glc; 2,3,6-Me_3-Glc; 2,3-Me_2-Xyl; 1,2,3,5,6-Me_5-Glc.OH (1:1:1:1)	Glc(1→4)Xyl(1→4)Glc(1→4)Glc.OH
4	0.23	Glc, xyl (4:1)	Glc	2,3,4,6-Me_4-Glc; 2,3,6-Me_3-Glc; 2,3-Me_2-Xyl; 1,2,3,5,6-Me_5-Glc.OH (1:2:1:1)	Glc(1→4)Glc(1→4)Xyl(1→4)Glc(1→4)Glc.OH

[a]On reduced saccharides. [b]The sugar sequencing, except at the reducing terminal, is purely arbitrary.

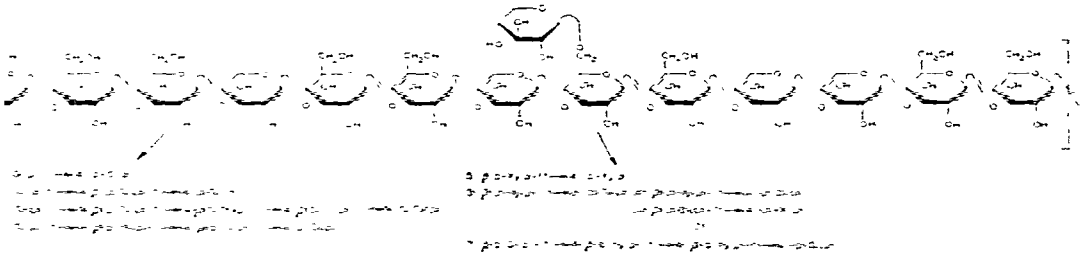

Fig. 1. Possible partial structure of F-1.

either a Glc→Xyl or Xyl→Glc structure were also noted. The liberation of cellobiose and xylobiose suggests the presence of blocks of D-glucose and D-xylose residues in the molecule. Insufficiency of these disaccharides precluded detailed studies on them.

It is evident from the foregoing that F-1 has a structure possessing contiguous residues of glucose and xylose in (1→4) linkage and having one branch point through O-6 of D-glucose; as described in the following partial structure.

The results of periodate-oxidation studies were in good agreement with those expected from a polysaccharide having the repeating unit suggested. The polysaccharide consumed 1.00 mol of periodate and liberated 0.08 mol of formic acid per mol of residues. Theoretically a polysaccharide having the structure shown would consume 1.06 mol of periodate, liberating 0.06 mol of formic acid per mol of residues. Glycerol was the main Smith-degradation product.

Oxidation[9] by chromium trioxide of the peracetylated F-1 resulted in total loss of both glucose and xylose, indicating that the polysaccharide contains mainly β-glycosidic linkages. Support for this conclusion was obtained by the release of glucose after incubation of deacetylated F-1 with β-D-glucosidase.

Fraction F-2 was also homogeneous both by sedimentation and molecular-sieve techniques, and had $\bar{M}_n$ 60,000. In contrast to F-1, F-2 contained a higher proportion of xylose (Table I) and was also starch–iodine positive. The polysaccharide was permethylated and the product hydrolysed and derivatized. The identities of the sugars were established by g.l.c.–m.s. (Table II). Results indicated a backbone of (1→4)-linked D-xylose interspersed with (1→4)-β-D-glucose, together with a side chain of D-xylose attached through O-6 of D-glucose. The molar ratio of xylose and glucose calculated from the ratio of methylated sugars (3.6 : 1) was in good agreement with that found for the native polysaccharide.

Periodate-oxidation and Smith-degradation studies corroborated well with the proposed structure. By analogy with F-1, it is assumed that the glycosidic links in F-2 are also of the β-D type. Because of the insufficiency of material, sequential analysis on F-2 could not be carried out.

Although a homogeneity test on F-3 could not be performed (because of its extreme insolubility), permethylation analyses revealed the presence in F-3 of two polymers, a linear, (1→4)-linked D-glucan and D-xylan. Interestingly, this fraction also gave a positive starch–iodine test.

Thus far xyloglucans, present in the endospermic tissues of various plant materials[13], have been regarded as reserve as well as structural polysaccharides. The latter function, of particular value during seed dormancy, is supported by germination studies[14]. Except in rape seed[15], there are no reports about their occurrence in hulls. The fucose-containing xyloglucan from rape-seed hulls forms a distinct subgroup and possesses a structure similar to that of jojoba-seed xyloglucan[16]. The structure of FBH xyloglucan(s) is quite unusual and significantly different from the classical structures. Unlike other xyloglucans, which invariably contain side-chain residues of fucose and galactose, FBH xyloglucan has none. However, as with any other amyloid, this xyloglucan also gave a positive starch–iodine test, and the colour yield was significant and comparable. It is hard to speculate at present on the biological role of this unusual polysaccharide from the hulls.

It has been observed in the literature that certain linear polysaccharides having a sequence of at least three β-(1→4)-linked D-glucose, 2-amino-2-deoxy-D-glucose, D-xylose, or D-mannose residues shows a positive starch–iodine colour only in concentrated, aqueous salt solutions[17]. The blue colour of the latter, clearly distinct from that of the amylose–iodine complex, is due to the ability of the polysaccharide, in the divalent salt solution, to trap a linear array of iodine atoms. Such entrapment may result in the formation of a tubular structure capable of containing strings of iodine atoms. The weak chemical stability of these amyloid complexes is consistent with such an assumption.

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