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Note

Fractionation of okra mucilage and structural investigation of an acidic polysaccharide

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Numerous varieties of okra *(Hibiscus esculentus)* having various contents of mucilage are available. Due to these differences, variation in the extraction procedure, or the uncertain homogeneity of the resulting preparations, the composition of okra mucilage has been the subject of controversy[1–3]. As part of our study of the structure–function relationship of food carbohydrates, the structure of okra mucilage has been investigated.

The mucilage, extracted in ~1% yield, was rich in carbohydrate and contained little protein (1.6%). Such a protein-free preparation was not reported earlier[1–3]. Hydrolysis of the mucilage gave rhamnose, galactose, and glucose as the major neutral sugars (Table I). Galacturonic acid was the only uronic acid present (20.5%).

The mucilage was insoluble in water but was soluble (up to 80%) in aqueous 1% sodium borohydride, a useful solvent which does not cause any degradation/decomposition of water-insoluble gums[4,5]. The composition of borohydride-soluble and -insoluble fractions given in Table I reveals that the non-starch glucan impurity is largely removed in the latter. The glucan gave a negative reaction with I_2–KI.

Cetavlon fractionation of the borohydride-soluble fraction yielded a precipitate (80%) and a soluble (18%) fraction. The latter still contained ~11% of uronic acid which could not be removed in spite of repeated fractionations. The sugar compositions of these fractions are given in Table I. The precipitate had higher amounts of rhamnose, galactose, and galacturonic acid (25.2%), and was further fractionated on DEAE-cellulose (CO_3^{2-} form). Three fractions, eluted with 0.1 (→AP-1, 11%) and 0.2M ammonium carbonate (→AP-2, 36%) and 0.4M sodium hydroxide (→AP-3, 50%) were obtained. AP-1 and AP-2 contained rhamnose, galactose, and galacturonic acid in various proportions (Table I), and AP-2 also contained glucose. AP-3 was heterogeneous and contained rhamnose, xylose, mannose, galactose, glucose, and galacturonic acid. Since AP-2 and AP-3 were heterogeneous in gel filtration, they were not studied further.

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TABLE I

CHEMICAL COMPOSITION (%) OF NATIVE OKRA MUCILAGE AND ITS DERIVED FRACTIONS

	Native	*Borohydride-soluble*	*Borohydride-insoluble*	*Borohydride-soluble fraction*		*Cetavlon-precipitable fraction*		
				Cetavlon-precipitable	*Cetavlon-non-precipitable*	*AP-1*	*AP-2*	*AP-3*
Yield	1.0	80.0	20.0	80.0	18.0	11.5	36.0	50.0
Total sugar	89.5	86.0	82.0	90.0	74.5	93.2	90.8	91.1
Uronic acid	20.5	18.2	19.3	25.2	11.5	13.7	25.0	28.7
Sugars detected								
Rhamnose	18.6	18.4	3.4	19.9	38.7	19.6	20.8	3.1
Arabinose	2.2	1.8	1.1	Tr[a]	17.1	Tr	0.9	Tr
Xylose	0.8	0.7	1.9	Tr	—	—	—	19.3
Mannose	1.1	Tr	2.5	1.7	—	—	—	15.7
Galactose	29.3	43.3	12.1	41.9	3.2	59.9	42.5	1.3
Glucose	17.4	3.6	40.9	Tr	4.0	—	1.8	22.7

[a]Tr = trace.

AP-1 had $[\alpha]_D$ +68° (*c* 0.5, water) and contained L-rhamnose and D-galactose in the molar ratio 1:3 together with D-galacturonic acid (13.7%). Sedimentation analysis of AP-1 gave a sharp, single peak, and molecular-sieve chromatography on Sepharose-4B resulted in quantitative elution as a single peak within the bed volume and having an average molecular weight ($\bar{M}_n$) of ~10^6. Microzone electrophoresis performed in various buffer systems also indicated homogeneity.

Carboxyl-reduction[6] (performed twice) of AP-1 gave a product containing rhamnose and galactose in the molar ratio 1:4. Only traces of galacturonic acid could be detected by p.c. of a hydrolysate. Methylation of native and carboxyl-reduced AP-1 and conversion of the products conventionally into alditol acetate derivatives gave (Table II) derivatives of 3-*O*-methylrhamnose, 2,3,4,6-tetra-*O*-methylgalactose, and 2,3,6-tri-*O*-methylgalactose in the molar ratios 1:2.1:1.1 and 1.0:0.9:2.1, respectively. Small proportions of the derivatives of 2,3,4-tri-*O*-methylrhamnose and 2,3,4-tri-*O*-methylgalactose were also formed. Discrepancies in the molar ratios (Table II) of methylated sugars could be attributed to β-elimination caused by the strong base used for Hakomori methylation. Such a phenomenon has been reported in the methylation of acidic polysaccharides[7].

The methylation data revealed that a small amount of rhamnose together with ~60% of galactosyl residues existed as non-reducing terminal units. The bulk of the rhamnose constituted (1→2)-linked chain units with branches composed of (1→4)-linked galactose. The higher proportion of 2,3,6-tri-*O*-methylgalactose formed from carboxyl-reduced AP-1 indicated that all the galactopyranuronic acid residues were (1→4)-linked. The galactose in the side chains could be present as singly [as (1→4)-linked] or doubly [as (1→6)-linked] substituted residues. That all the galactose originated from the side chains was evident from the partial hydrolysis studies. Thus, galactose (~90%) was the only sugar to be released under mild conditions of hydrolysis (CF_3COOH or H_2SO_4, 0.1–1.0M, 3–8 h, ~100°). Hydrolysis of

TABLE II

G.L.C.–M.S. OF THE ALDITOL ACETATES DERIVED FROM PERMETHYLATED NATIVE AND CARBOXYL-REDUCED AP-1

Methyl ether	*Molar ratio*		*Diagnostic mass fragments* (m/z)	*Mode of linkage*
	Native	*Carboxyl-reduced*		
2,3,4,6-Me_4-Gal	3.4	1.9	45, 118, 162, 205	Gal*p*-(1→
3-Me-Rha	1.6	2.1	130, 143, 190, 203	→2,4)-Rha*p*-(1→
2,3,6-Me_3-Gal	1.8	4.5	45, 102, 118, 162, 173, 233	→4)-Gal*p*-(1→
2,3,4-Me_3-Rha	0.16	Tr	89, 118, 131, 143, 175, 206	Rha*p*-(1→
2,3,4-Me_3-Gal	0.21	Tr	102, 118, 129, 146, 162, 189, 206, 233	→6)-Gal*p*-(1→

the precipitated, degraded polysaccharide gave only rhamnose and galacturonic acid. The occurrence of any galactofuranosyl residues in AP-1 was excluded on the basis of the methylation data.

Fraction AP-1 consumed 0.97 mol of periodate per "anhydrosugar" unit and 0.35 mol of formic acid was released. These results accord with the theoretical values expected of a polysaccharide having the structural features discussed above. Smith degradation of the oxopolysaccharide gave rhamnose and threitol, the former indicating unattacked branch-points and the latter (1→4)-linked galactosyl residues. Hence, it is concluded that AP-1 has a backbone of (1→4)-linked D-galactopyranosyluronic acid residues interspersed with (1→2)-linked L-rhamnosyl residues. Essentially all the rhamnosyl residues carry, as side chains, (1→4)- and/or (1→6)-linked single or double residues of D-galactose.

Oxidation of acetylated AP-1 with chromium trioxide did not destroy the rhamnose and galactose residues, indicating these sugars to be α.

The foregoing results for AP-1 of okra mucilage are consistent with the structural features reported for pectic rhamnogalacturonans[8]. Pectic fractions of rapeseed[9] and mustard seed[10] carry highly branched chains of arabinose, whereas, in tragacanthic acid[11] and pollen fraction[12], single residues of xylose are attached to the rhamnogalacturonan main-chain.

EXPERIMENTAL

Immature okra (variety, *Tusa sawani*) pods from a single harvest were obtained from a local field. In addition to the various general methods reported previously[13,14], gel filtration on Sepharose-4B was performed with 0.1M sodium chloride as eluent.

Extraction of the mucilage. — The de-seeded pods were cut into thin slices, and stirred with 0.1M hydrochloric acid at 4° for 6–8 h. The slurry was squeezed through a nylon cloth and the extraction was repeated thrice. Addition of acetone (3 vol.) to the combined extracts gave a white fibrous material. The native mucilage was solubilised in aqueous 1% sodium borohydride[4], the solution was centrifuged, and the supernatant solution was dialysed thoroughly and then lyophilised.

Fractionation of the mucilage. — The borohydride-soluble polysaccharide was fractionated by dropwise addition of aqueous 3% Cetavlon until precipitation was complete. The precipitate and the soluble fractions were recovered after centrifugation in yields of 80 and 18%, respectively. The Cetavlon-precipitable polysaccharide was further resolved by chromatography on DEAE-cellulose (CO_3^{2-} form) by sequential elution with water, ammonium carbonate (0.1–0.5M, pH 9.3), and sodium hydroxide (0.1–0.4M).

Methylation analysis[15]. — The native and the carboxyl-reduced[6] polysaccharides were methylated by the Hakomori method. The methylated product was hydrolysed, and the products were converted into the alditol acetates using sodium borodeuteride and then analysed by g.l.c.–m.s.

Periodate oxidation and Smith degradation. — The polysaccharide was oxidised with sodium metaperiodate (10mM) at 4°, and the consumption of IO_4^- was monitored[16] and the formic acid liberated[17] was determined by established methods. The oxidised polysaccharide was reduced with borohydride and then hydrolysed with acid.

Chromium trioxide oxidation[18]. — The fully acetylated polysaccharide was treated with chromium trioxide for 2 h at 60° and the oxidised product was analysed for sugars after hydrolysis.

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