Carbohydrate Research, 161 (1987) 161–166
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands

Note

A mucilaginous acidic polysaccharide from black gram (*Phaseolus mungo*): structure–function characteristics

U. RAMADAS BHAT, PARAMAHANS V. SALIMATH, AND RUDRAPATNAM N. THARANATHAN*

Department of Food Chemistry, Biochemistry Section, Central Food Technological Research Institute, Mysore–570 013 (India)

(Received April 10th, 1986; accepted for publication, September 22nd, 1986)

Mucilaginous polysaccharides with various functional attributes are widely known in the plant kingdom[1], and that from black gram *(Phaseolus mungo)* is reported[2,3] to stabilise against thermal disruption the foam formed by its surface-active proteins. The carbohydrate composition of black gram has been investigated[4] and we now report on the structure of a functionally important polysaccharide, and on the relation of structure to function.

Extraction of black-gram endosperm with aqueous 10% trichloroacetic acid furnished a mucilaginous polysaccharide (~6%) containing little (0.65%) protein. Carbohydrates extracted with trichloroacetic acid are usually[5] protein-free and, also, any inadvertent enzyme activity is arrested. Any hydrolysis of the polysaccharide by trichloroacetic acid was ruled out by the observation[6] that similar treatments (aqueous 10% acid, 4°, 6 h) of commercial larchwood arabinogalactan and citrus pectic material did not release any mono- or oligo-saccharides.

The native polysaccharide was only sparingly soluble in water and contained L-rhamnose, L-arabinose, and D-galactose in the molar ratios 1:9:3. Also, there were traces of xylose and 18.5% of galacturonic acid.

Carboxyl-reduction[7] of the native polysaccharide gave a neutral polysaccharide (CRP) that was completely soluble in water, had $[\alpha]_D$ −29° (*c* 0.25), and gave a solution having low viscosity. CRP was homogeneous by gel filtration, ultracentrifugation, and electrophoresis on cellulose acetate. Its molecular weight, determined on Sephacryl S-400, was 118,000. G.l.c. analysis revealed it to be composed of rhamnose, arabinose, and galactose in the molar ratios 1:9:5.

Due to its poor solubility in methyl sulfoxide, the native polysaccharide was methylated sequentially by the methods of Haworth[8], Falconer and Adams[8], and Hakomori[9]; the CRP was methylated directly by the Hakomori method[9]. Each methylated polymer was hydrolysed with acid and the products were converted[9] into [1-^{2}H]alditol acetates[10]. The results in Table I show that, in CRP, essentially

*To whom correspondence should be addressed.

0008-6215/87/$ 03.50

TABLE I

ALDITOL ACETATES DERIVED FROM METHYLATION ANALYSIS OF CRP-3

Alditol acetate of	*Molar ratio*	*Mode of linkage*
2,3,5-Me_3-Ara[a]	8.0	Ara-(1→
3,4-Me_2-Rha	0.4	→2)-Rha-(1→
2,3,4,6-Me_4-Gal	0.3	Gal-(1→
2,3-Me_2-Ara	4.1	→5)-Ara-(1→
5-Me-Ara	Tr	→2,3)-Ara-(1→
3-Me-Rha	2.2	→2,4)-Rha-(1→
2,4,6-Me_3-Gal	2.2	→3)-Gal-(1→
2-Me-Ara	1.1	→3,5)-Ara-(1→
2,3,6-Me_3-Gal	5.9	→4)-Gal-(1→
3-Me-Ara	3.5	→2,5)-Ara-(1→
Ara	2.6	→2,3,5)-Ara-(1→
2,4-Me_2-Gal	0.3	→3,6)-Gal-(1→

[a]2,3,5-Me_3-Ara = 1,4-Di-*O*-acetyl-2,3,5-tri-*O*-methylarabinitol, *etc.*

all of the rhamnose residues are (1→2)-linked and a significant portion is also substituted at O-4 with (1→3)- and (1→4)-linked galactose residues and/or highly branched arabinose side-chains. The formation of significant amounts of 2,3,5-tri-*O*-methylarabinose, which is in good molar agreement with the 3-*O*- and 5-*O*-methyl plus non-methylated arabinose derivatives, lends support for high branching. A small proportion of galactose was also found at non-reducing terminals. The higher proportion of 2,3,6-tri-*O*-methylgalactose formed from CRP indicated all the galacturonic acid to be (1→4)-linked.

CRP consumed 0.77 mol of periodate and released 0.06 mol of formic acid per mol of "anhydro sugar". Smith degradation of the reduced oxopolysaccharide gave glycerol, threitol, arabinose, galactose, and rhamnose. The presence of unoxidised sugar residues was consonant with high branching in the polysaccharide.

Oxidation of acetylated CRP with chromium trioxide[11] destroyed the galactose but not the rhamnose, suggesting the former to be β and the latter α.

Partial hydrolysis of the native polysaccharides with H_2SO_4 and CF_3COOH furnished three neutral, and two neutral and three acidic oligosaccharides, respectively, with considerable amounts of free arabinose and some galactose, indicating the arabinose to be furanosidic. Some of the oligosaccharides were characterised (Table II) and the results showed blocks of rhamnose and galactose residues, the former from the backbone and the latter probably from the side-chains. Apart from the tetrasaccharide consisting of galacturonic acid and rhamnose (1:3), no other oligomer of the former could be characterised.

The structure of the polysaccharide from black gram is comparable with that of pectic polysaccharides in possessing a rhamnogalacturonan core with blocks of (1→2)-linked rhamnosyl and probably (1→4)-linked galactosyluronic acid residues[12]. The presence in the main chain of contiguous rhamnosyl residues was

TABLE II

OLIGOSACCHARIDES RELEASED ON PARTIAL ACID HYDROLYSIS OF THE NATIVE POLYSACCHARIDE

	R_{MT}[a]	R_{GalA}[b]	*Neutral sugars (molar proportions)*	*GalA content (%)*	*Reducing end*	*O-Methyl ethers*[c] *(mol proportion)*	*Possible structure*
Hydrolysis with H_2SO_4							
1	1.53	—	Ara,Gal 1:1	—	Gal	N.c.[d]	—
2	1.16	—	Ara,Gal 1:2	—	Gal	N.c.	—
3	0.65	—	Rha	—	Rha	1,3,4,5-Me_4-Rha; 2,3,4-Me_3-Rha; 3,4-Me_2-Rha (1:1:2)	Rha-(1→2)-Rha-(1→2)-Rha-(1→2)-Rha-OH
Hydrolysis with CF_3COOH							
4	1.57	—	Gal	—	Gal	1,2,3,5,6-Me_5-Gal; 2,3,4,6-Me_4-Gal (1:1)	Gal-(1→4)-Gal.OH
5	1.01	—	Gal	—	Gal	1,2,3,5,6-Me_5-Gal; 2,3,4,6-Me_4-Gal; 2,3,6-Me_3-Gal (1:1:1)	Gal-(1→4)-Gal-(1→4)-Gal.OH
6	0.42	0.62	Rha	25.6	Rha	1,3,4,5-Me_4-Rha; 2,3,4,6-Me_4-Gal; 3,4-Me_2-Rha (1:1:2)	GalA-(1→2)-Rha-(1→2)-Rha-(1→2)-Rha.OH
7	0.22	0.33	Rha,Gal	45.4	Rha	N.c.	—
8	0.07	0.11	Rha,Gal	55.0	Rha	N.c.	—

[a]Mobility with respect to that of maltotriose in 1-butanol–pyridine–water (6:4:3). [b]Mobility with respect to that of galacturonic acid in ethyl acetate–pyridine–acetic acid–water (5:5:1:3). [c]Derived from borohydride-reduced oligosaccharides. [d]Not characterised.

TABLE III

VISCOSITY AND FOAM STABILITY OF THE NATIVE AND THE CARBOXYL-REDUCED POLYSACCHARIDES

	Ratio of polysaccharide–CMC–2M $NaBH_4$	*GalA*	*Viscosity (mPa.s)*	*Foam stability*[a]
Native	—	19.5	—	+++
CRP-1	1:2:4	8.5	17.6	++
CRP-2	1:5:10	3.2	11.7	+
CRP-3	1:10:20	1.0	4.3	—

[a]Key: +, low; ++, average; +++, high.

indicated by the release of a tetrasaccharide of rhamnose and an acidic oligosaccharide having three rhamnose residues attached to a single non-reducing galacturonic acid. A similar backbone structure has been reported for the acidic polysaccharide complexes derived from soybean cotyledon meal[13] and white-willow bark[14], whereas the rhamnogalacturonan I of suspension-cultured sycamore cells has a backbone consisting of →4)-GalA-(1→2)-Rha-(1→ repeating-units[15]. The rhamnose residues are α as in the mucilages of roots[16] and cambium[17]. The arabinose side-chains are highly branched and attached to position 4 of rhamnose, as reported for some acidic polysaccharides[18–21]. Some rhamnosyl residues are also substituted at position 4 by (1→3)- and (1→4)-linked galactose. Single or multiple residues of side-chain-linked galactose have been reported in a few polysaccharides[13,22].

Black-gram mucilage stabilises against thermal disruption the foam formed by the surface-active proteins[2]. The viscogenic nature of the polymer is partly responsible for this property, as a few other polysaccharides, such as guar gum, with dissimilar structures but having high viscosities also exhibit a foam-stabilising property. When the mucilage was reduced to different extents and the effects on foam stabilisation were examined (Table III), it was found that, as the content of uronic acid diminished (18.5%→~1% in CRP-3), the solubility was enhanced (CRP-1 <CRP-2 <CRP-3), the viscosity decreased, and the foam-stabilising ability decreased. CRP-3, which was highly soluble in water, did not stabilise foam. It is likely that the uronic acid carboxyl groups in the native polysaccharide are, at least partly, responsible for its viscosity and hence are of functional significance in preparations of leavened food from black gram.

EXPERIMENTAL

In addition to the various analytical and structural methods reported earlier[23,24], h.p.l.c. was performed with a Waters Associates liquid chromatograph fitted with a refractive index detector and a carbohydrate column, using acetonitrile–water (85:15).

Extraction of the mucilage. — The mucilaginous polysaccharide was extracted from the defatted/depigmented powdered endosperm (100 g) with aqueous 10% trichloroacetic acid (800 mL, 4°, 6 h) followed by precipitation with acetone (3 vol.). A solution of the precipitate in water was dialysed and lyophilised (5.9 g).

Partial hydrolysis with acid. — The native polysaccharide was partially hydrolysed either with conc. H_2SO_4 (0°, 30 min) or CF_3COOH (0.25M, 96°, 6 h). The excess of acid was removed and the released oligosaccharides were separated by preparative p.c. and purified by h.p.l.c.

Viscosity measurements. — Measurements were made at room temperature, using a Haake Rotovisco Viscometer with a cone and plate sensor system PK IV (cone angle, 1°).

Determination of foam stabilisation. — A steipel-type foam meter was used as described earlier[2].

Preparation of CRP-1, CRP-2, and CRP-3. — The native polysaccharide was carboxyl-reduced to different extents, according to the method of Taylor and Conrad[7], by varying the contents of 1-cyclohexyl-2-(4-methylmorpholino)ethyl-carbodi-imide *p*-toluenesulfonate (CMC) and sodium borohydride (2M solution), as detailed in Table III. The reduced products were dialysed and lyophilised.

ACKNOWLEDGMENTS

The authors thank Dr. H. Mayer (Max Planck Institut für Immunobiologie, Freiburg, W. Germany) and Dr. M. McNeill (Department of Chemistry, Colorado, U.S.A.) for the g.l.c.–m.s. analyses. One of us (U.R.B.) thanks the C.S.I.R. (New Delhi) for the award of a senior research fellowship.

REFERENCES

1 M. Glicksman (Ed.), *Gum Technology for the Food Industry,* Academic Press, New York, 1973.
2 N. S. Susheelamma and M. V. L. Rao, *J. Sci. Food Agric.*, 25 (1974) 665–673.
3 N. S. Susheelamma and M. V. L. Rao, *J. Agric. Food Chem.*, 26 (1978) 1434–1437.
4 U. Ramadas Bhat and R. N. Tharanathan, *Cereal Chem.*, 63 (1986) 376–377.
5 A. H. Tran and P. Nordin, *Staerke*, 29 (1977) 153–156.
6 R. N. Tharanathan and U. Ramadas Bhat, unpublished results.
7 R. L. Taylor and H. E. Conrad, *Biochemistry,* 11 (1972) 1383–1388.
8 E. L. Hirst and E. Percival, *Methods Carbohydr. Chem.*, 5 (1965) 287–296.
9 S. I. Hakomori, *J. Biochem. (Tokyo)*, 55 (1964) 205–208.
10 P.-E. Jansson, L. Kenne, H. Liedgren, B. Lindberg, and J. Lonngren, *Chem. Commun. Univ. Stockholm*, 8 (1976) 1–20.
11 J. Hoffman, B. Lindberg, and S. Svensson, *Acta Chem. Scand.*, 26 (1972) 661–663.
12 A. M. Stephen, in G. O. Aspinall (Ed.), *The Polysaccharides*, Vol. 2, Academic Press, New York, 1983, pp. 154–161, and references therein.
13 G. O. Aspinall, I. W. Cottrell, S. V. Egan, I. M. Morrison, and J. C. N. Whyte, *J. Chem. Soc., C,* (1967) 1071–1080.
14 R. Toman, S. Karacsonyi, and M. Kubackova, *Carbohydr. Res.*, 43 (1975) 111–116.
15 J. M. Lau, M. McNeil, A. G. Darvill, and P. Albersheim, *Carbohydr. Res.*, 137 (1985) 111–125.
16 M. Tomoda, M. Arai, Y. Suzuki, M. Ohmura, and H. Takayama, *Chem. Pharm. Bull.*, 28 (1980) 1506–1511.

17 B. W. SIMSON AND T. E. TIMELL, *Cellul. Chem. Technol.*, 12 (1978) 79–84.
18 R. W. STODDART, A. J. BARRETT, AND D. H. NORTHCOTE, *Biochem. J.*, 102 (1967) 194–204.
19 K. W. TALMADGE, K. KEEGSTRA, W. D. ZAUER, AND P. ALBERSHEIM, *Plant Physiol.*, 51 (1973) 158–173.
20 I. R. SIDDIQUI AND P. J. WOOD, *Carbohydr. Res.*, 50 (1976) 97–107.
21 D. A. REES AND N. J. WIGHT, *Biochem. J.*, 115 (1969) 431–439.
22 G. O. ASPINALL, K. HUNT, AND I. M. MORRISON, *J. Chem. Soc. C*, (1967) 1080–1086.
23 P. V. SALIMATH AND R. N. THARANATHAN, *Carbohydr. Res.*, 106 (1982) 251–257.
24 U. RAMADAS BHAT AND R. N. THARANATHAN, *Carbohydr. Res.*, 148 (1986) 143–147.