Carbohydrate Research, 218 (1991) 201–209
Elsevier Science Publishers B.V., Amsterdam

Structural studies of amyloglucan and a soluble glucan produced from starch by *Streptococcus sanguis* 1 MC 204

Maeve M. Coogan[1,*], Robert L. Jones[2], Keith L. Manchester[2],
Departments of Oral Pathology[1] and Biochemistry[2], University of the Witwatersrand, Johannesburg, WITS 2050 (South Africa)

and Hendrick W. Viljoen
Institute of Chromatography, University of Pretoria, Pretoria 0001 (South Africa)

(Received July 13th, 1990; accepted for publication January 31st, 1991)

ABSTRACT

Two α-D-glucans, produced from amylopectin by an oral isolate *Streptococcus sanguis* 1 MC 204, were shown to contain both (1→4) and (1→6) linkages. The first α-D-glucan (amyloglucan) was adherent and highly insoluble, and methylation analysis, i.r. spectroscopy, and enzymic analyses showed it to be similar to amylopectin but less branched with longer interior and exterior chains. The second polymer was a non-adherent soluble α-D-glucan that was similar to amyloglucan but with long exterior chains. These α-D-glucans were not synthesised *de novo*, but were the products of the modification of amylopectin.

INTRODUCTION

Coogan and co-workers[1] showed that amylopectin is metabolised by oral streptococci, to give a polymer designated amyloglucan that adheres readily to glass and is insoluble in all the solvents tested. Recently, a second polymer, which is similar to amyloglucan but is soluble and non-adherent, has been isolated. The structures of these polysaccharides are now reported.

Various applications of methylation analysis[2–4], i.r. spectroscopy[5], degradation by enzymes[6], and staining with iodine[7–8] to amylopectins have not yielded a consensus on the fine structure. Nevertheless, the above methods have been applied, because the amyloglucan and the soluble glucan are derived from amylopectin.

RESULTS AND DISCUSSION

Two α-D-glucans were produced from amylopectin by the starch-hydrolysing enzymes. The first dissolved in all of the test solvents and did not adhere to glass, whereas the second (amyloglucan) was adherent and insoluble. In this respect, the latter glucan may be similar to mutan, which accumulates in plaque and enables *S. mutans* to attach to teeth[9]. Furthermore, amyloglucan is less soluble than mutan, which suggests that it would accumulate more readily.

* To whom correspondence should be addressed.

0008-6215/91/$03.50

Amyloglucan may be insoluble for the same reasons that amylose and mutan do not dissolve readily. For example, amylose is a uniform linear molecule that forms strong intermolecular hydrogen bonds, has an extended ribbon-like structure, and contains a few branches[10]. Mutan has similar characteristics, because it is a (1→3)-α-D-glucan that has ribbon-like molecules that are nearly fully extended. The chains pack readily into dense, completely water-insoluble microfilms with extensive intra- and inter-sheet hydrogen bonding[11]. Elongation of the chains in amyloglucan may have enabled the formation of ribbon-like structures with strong intermolecular hydrogen bonding.

The amyloglucan–iodine complex gave a purple stain with an $E1\%$ of 1 (*i.e.*, the theoretical absorbance of a 1% solution of amyloglucan) and had λ_{max} 550 nm, which indicated that amyloglucan is similar to amylopectin but binds iodine poorly. In contrast, the soluble glucan stained blue with iodine (λ_{max} 570 nm, $E1\%$ 17.8), with a value intermediate of those for amylopectin (λ_{max} 540 nm, $E1\%$ 14.5) and amylose (λ_{max} 620 nm, $E1\%$ 99.7), which suggests more branching than in amylose and less than in amylopectin.

The i.r. spectra of amylopectin, amyloglucan, and the soluble glucan were similar, especially in the "fingerprint region" for sugars (1000–650 cm^{-1})[12]. Amyloglucan and the soluble glucan each had a band at 844 cm^{-1} but not at 891 cm^{-1}, consistent with α linkages. The band for (1→4) linkages at 930 cm^{-1} was similar for amylopectin and the soluble glucan, but slightly larger for amyloglucan. The second band for (1→4) linkages at 758 cm^{-1} was more marked for the soluble glucan than for amyloglucan and amylopectin. The bands for (1→6) linkages at 917 and 768 cm^{-1} were present in amyloglucan and the soluble glucan but were difficult to interpret because they were close to those for (1→4) linkages. There were no marked peaks at 793 cm^{-1}, characteristic of (1→3) linkages (Fig. 1).

Methylation analysis of amylopectin, glycogen, amyloglucan, and the soluble glucan (Table I) gave 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol (end groups), 1,4,5-tri-*O*-acetyl-2,3,6-tri-*O*-methyl-D-glucitol [(1→4)-linked units], 1,4,5,6-tetra-*O*-acetyl-2,3-di-*O*-methyl-D-glucitol (branched units), and 1,3,4,5-tetra-*O*-acetyl-2,6-di-*O*-methyl-D-glucitol [(1→3)-linked units] (Fig. 2). More 1,5-di-*O*-acetyl-2,3,4,6-tetra-

TABLE I

Methylation analysis data (see Experimental)

Glucan	*2,3,4,6-Glc*[a]	*2,3,6-Glc*	*2,6-Glc*	*2,3-Glc*
Amylopectin	3.9	82.6	7.7	5.8
Amyloglucan	2.8	89.7	2.6	4.9
Soluble glucan	2.9	84.6	4.8	7.7
T[b]	0.887	0.932	0.957	0.969

[a] 2,3,4,6-Glc = 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol; 2,3,6-Glc = 1,4,5-tri-*O*-acetyl-2,3,6-tri-*O*-methyl-D-glucitol; 2,6-Glc = 1,3,4,5-tetra-*O*-acetyl-2,6-di-*O*-methyl-D-glucitol; 2,3-Glc = 1,4,5,6-tetra-*O*-acetyl-2,3-di-*O*-methyl-D-glucitol. [b] Retention time relative to that of *myo*-inositol hexa-acetate.

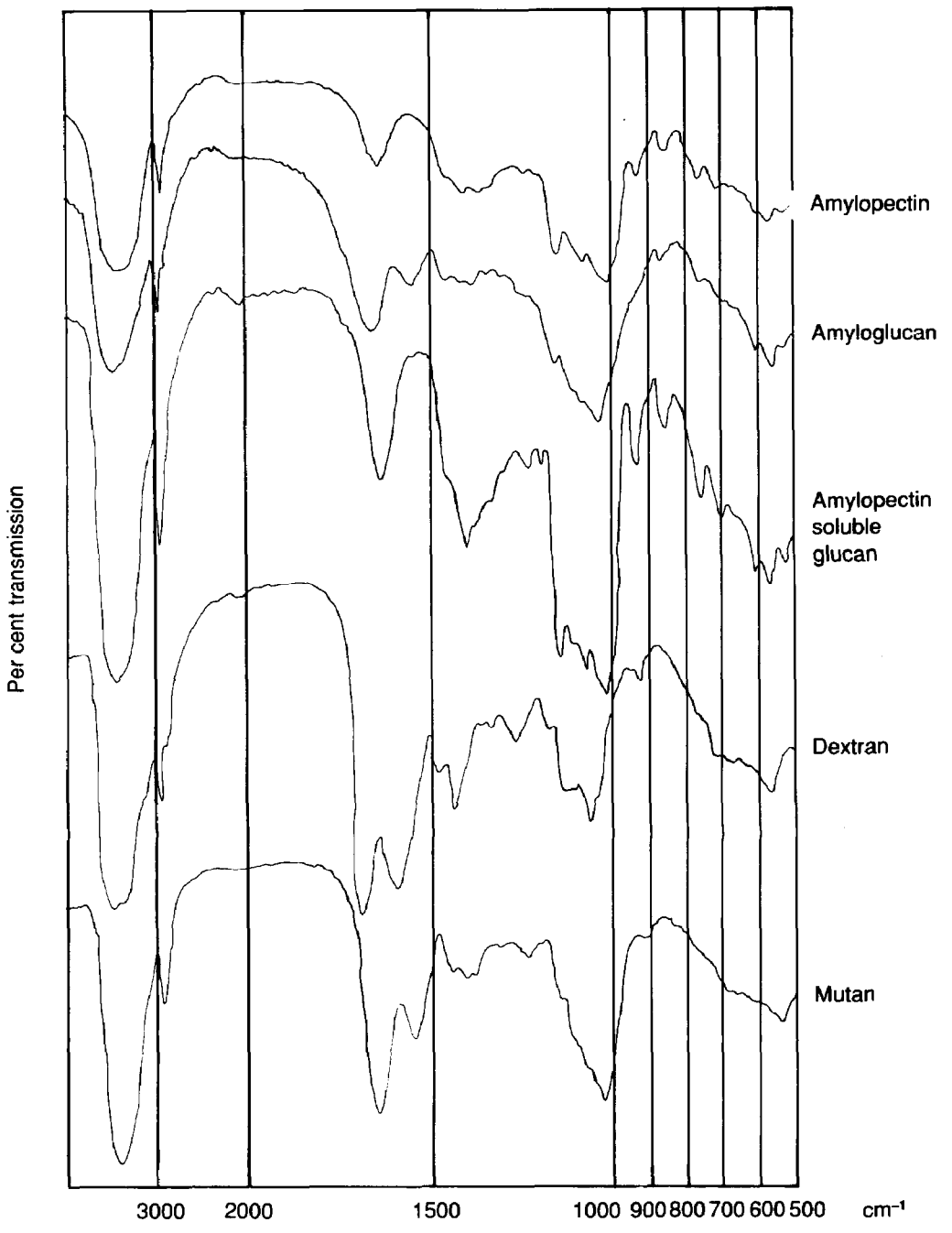

Fig. 1. I.r. spectra of amylopectin, amyloglucan, soluble glucan, the dextran produced from sucrose by *S. sanguis* 1 MC 204, and the mutan produced from sucrose by *S. mutans* ATCC 25175.

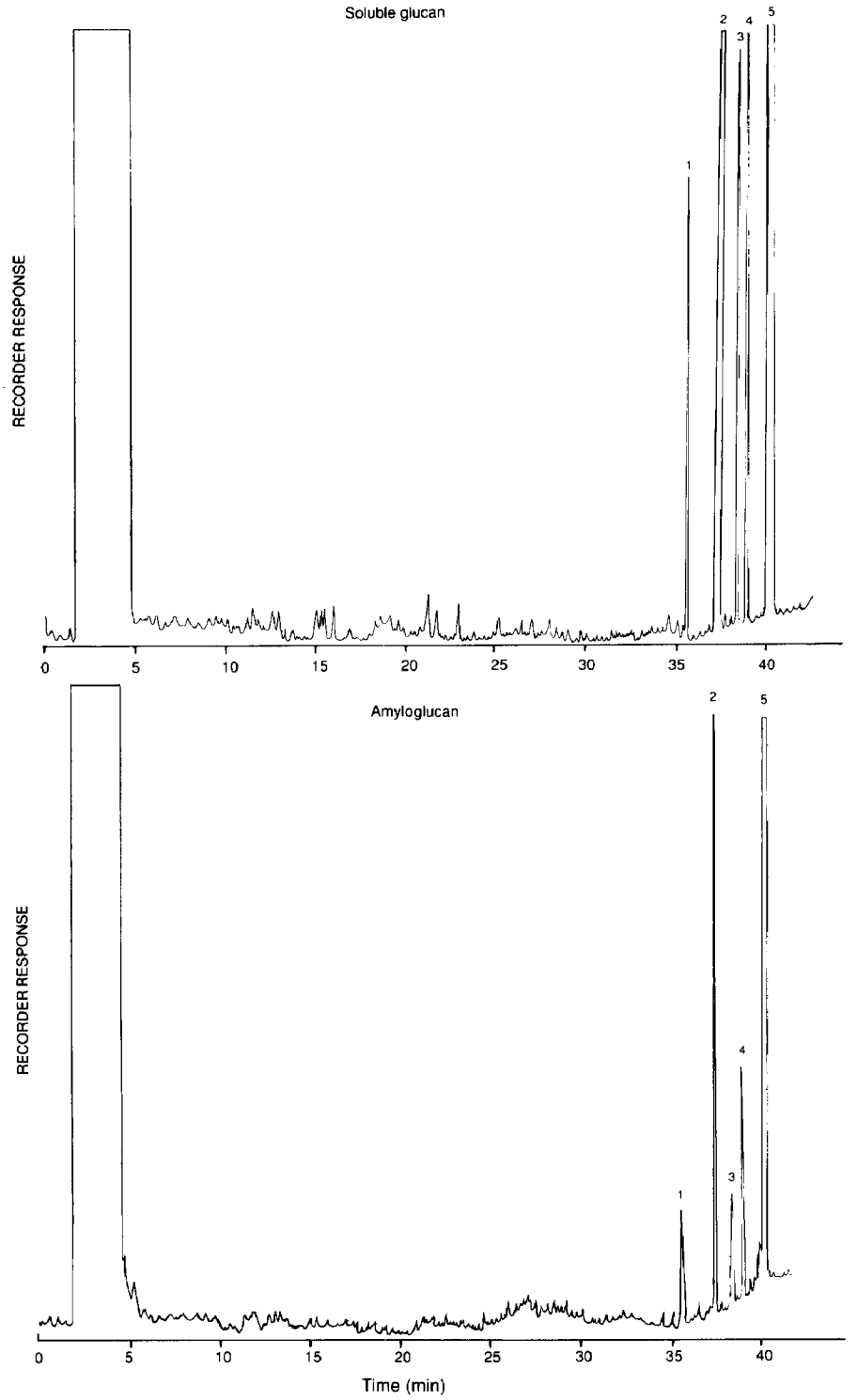

Fig. 2. Gas chromatographs of the alditol acetates formed by the methylation of amyloglucan and the soluble polysaccharide produced from amylopectin: (1) 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol; (2) 1,4,5-tri-*O*-acetyl-2,3,6-tri-*O*-methyl-D-glucitol; (3) 1,3,4,5-tetra-*O*-acetyl-2,6-di-*O*-methyl-D-glucitol; (4) 1,4,5,6-tetra-*O*-acetyl-2,3-di-*O*-methyl-D-glucitol; (5) *myo*-inositol hexa-acetate (internal standard).

TABLE II

Data on amylose, amylopectin[a], the soluble glucan, and amyloglucan produced from amylopectin by *S. sanguis* 1 MC 204

Property	*Amylose*	*Amylopectin*	*Soluble glucan*	*Amyloglucan*
General structure	Essentially unbranched	Branched	Less branched	Less branched
Average chain length	$\sim 10^3$	26(24)	35	36
Exterior chain length		17(15)	23	21
Interior chain length		8(9)	11	14
% Conversion into maltose with beta-amylase	~70	55(56)	59	52

[a] The figures in brackets are from previous studies[14,26].

O-methyl-D-glucitol and 1,4,5-tri-*O*-acetyl-2,3,6-tri-*O*-methyl-D-glucitol were obtained from the amyloglucan and the soluble glucan than from amylopectin. 1,3,4,5-Tetra-*O*-acetyl-2,6-di-*O*-methyl-D-glucitol, usually indicative of (1→3) linkages, was formed in small proportion from each polymer and probably reflected incomplete methylation.

The chain length (26) calculated for amylopectin was intermediate of those reported[8,13,14]. The chain lengths of the soluble glucan and amyloglucan were calculated as 35 and 36, respectively (Table II). The value for amyloglucan may be an underestimate because some of the polysaccharide did not dissolve. The insoluble material may have had long chains with a structure similar to those of mutan[10] and amylose[11].

Three kinds of chains (A–C) are found in amylopectin. A-chains are unbranched, whereas B-chains have one or more A- and B-chains attached to positions 6, and the single C-chain includes the reducing end unit[13,15]. Isoamylase is specific for inter-chain (1→6) linkages and will release all the A and B chains[16].

The chain profile, obtained by gel-permeation chromatography after debranching with isoamylase, showed a bimodal distribution. The two major peaks corresponded to chain lengths of ~20 and ~40. Chains of 20 glucose residues had a partition coefficient (Ka) of 0.75 and presumably consisted of A-chains and short B-chains, whereas the longer chains had a Ka of ~0.3 and represented long B-chains (Fig. 3). The chain profiles for amylopectin, amyloglucan, and the soluble glucan were similar, but the second peak which corresponded to chain lengths of 40 was larger for amyloglucan and the soluble glucan than for amylopectin. This result suggested that the glucans contained longer B-chains than the parent polysaccharide.

The lengths of the exterior (e.c.l.) and interior (i.c.l.) chains were determined from the results obtained from the beta-amylase analysis:

$$\text{e.c.l.} = (\text{c.l.} \times \text{beta-limit}) + 2$$

$$\text{i.c.l.} = \text{c.l.} - \text{e.c.l.} - 1.$$

Both the exterior and interior chains in amyloglucan and the soluble glucan were almost a third longer than those in amylopectin (Table II).

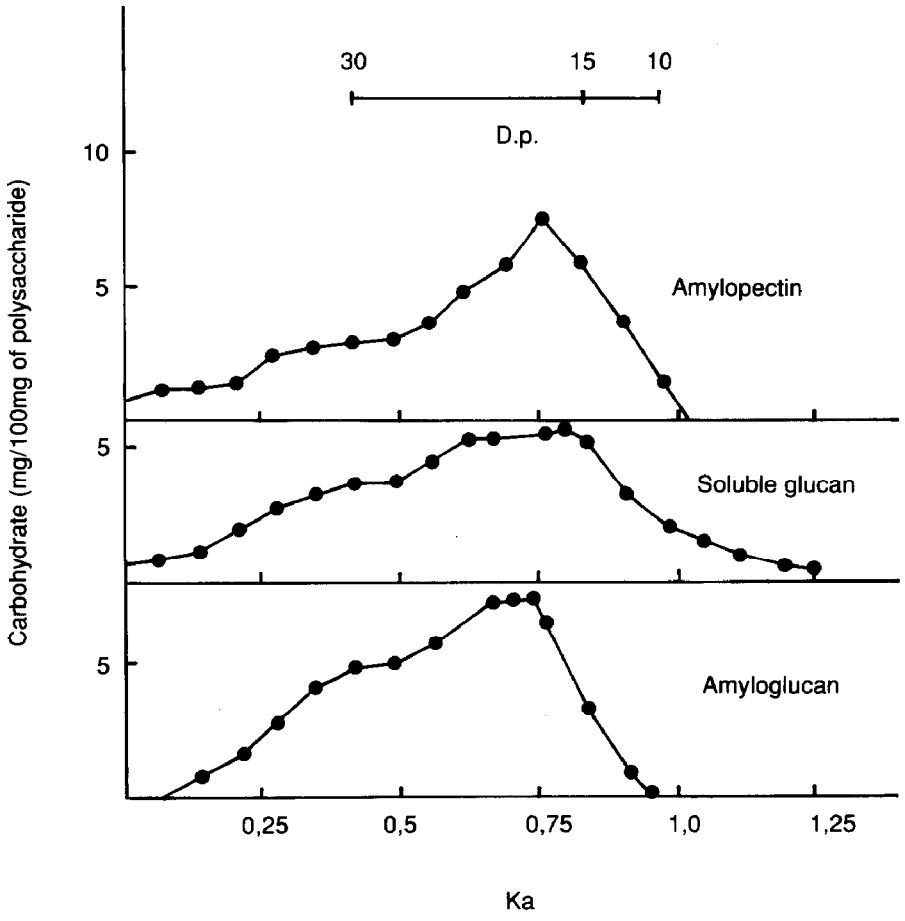

Fig. 3. Fractionation on Sephadex G-50 of the products obtained by the debranching of amylopectin, amyloglucan, and the soluble glucan with isoamylase.

Thus, the amyloglucan is a branched polymer with exterior and interior chains that are longer than those of amylopectin. In contrast, the length of the interior chains in the soluble glucan is similar to those in amylopectin and the exterior chains are similar to those in amyloglucan. If the randomly branched structure suggested by Meyer and Bernfeld[17] is considered, these changes could be caused by the removal of A-chains from amylopectin. The formation of the soluble glucan could be the first stage in this process whereby exterior A-chains are removed, which would increase the length of the remaining chains. Thereafter, further A-chains could be removed from the interior part of the molecule. The formation of oligosaccharides with chain lengths of 10–12 supports this suggestion. Their removal would increase the length of interior and exterior chains and yield the amyloglucan.

The formation of amyloglucan could also be based on the cluster model of amylopectin[18,19], which considers amylopectin to be composed of compact clusters of

chains that are randomly branched. These clusters are linked by long B-chains which extend into two or more clusters. A-chains are unbranched, whereas B-chains carry A- or other B-chains. Hizukuri[14] has shown a tetramodal distribution of chains in potato amylopectin. The first fraction consisted of short A- and B_1-chains which formed a single cluster, the second and third fractions contained B_2- and B_3-chains that extended into two and three clusters, and a further fraction (B_4) contained B-chains that stretched across more than four clusters.

The increased chain length of the soluble glucan (Table II), the presence of short-chain oligosaccharides, and an increase in the number of B_2- and B_3-chains, as shown by debranching and fractionation (Fig. 3), suggest that some of the A-chains and short B_1-chains had been removed to form this polysaccharide. This process would result in a proportionate increase in the number of the longer B_2- and B_3-chains. Further, limited debranching of the soluble glucan followed by inter-chain association of the longer B_3- and B_4-chains could yield the insoluble amyloglucan. This mechanism may be similar to the association process which leads to the formation of amylopectin[20].

The amyloglucan and the soluble glucan could either be synthesised from the degradation products of amylopectin or by modification of amylopectin. There are several reasons to support the latter possibility. Thus, the i.r. spectra for amyloglucan and the soluble glucan were similar to that of amylopectin and not those of the dextran produced from sucrose and the mutan produced by *S. mutans*. Moreover, each polymer contained α-D-glucose residues (Fig. 1) that were variously (1→4)- and (1→4,6)-linked (Table II), and digestion by isoamylase yielded similar chain profiles (Fig. 3).

EXPERIMENTAL

Production of polysaccharide. — *S. sanguis* 1 MC 204 was inoculated into a chemically defined broth[21] (500 mL), which was incubated at 37° for 48 h, then centrifuged at 12,000*g*. The supernatant solution, which contained extracellular enzymes, was passed through a sterile filter (0.2 μm) in order to remove any remaining cells. The suspension was mixed with phosphate-buffered saline (pH 7.2, 500 mL) that contained 1% of potato amylopectin (Sigma, No. A-8515). The mixture was distributed in 100-mL portions in sterile Boston round bottles and incubated at 37° for 5 days. The broth was then decanted into sterile bottles, the material which adhered to the glass was removed, and the suspension was centrifuged at 12,000*g* for 20 min. The polysaccharide pellet (amyloglucan) was washed 4 times with distilled water and dried over phosphorus pentaoxide. This procedure was repeated 6 times on the same litre of broth.

Amylopectin was precipitated by adding 50 mL of ethanol to 100 mL of broth. The broth was centrifuged at 12,000*g* for 20 min and 20 mL of ethanol was added to the supernatant solution. The suspension was centrifuged at 12,000*g* for 20 min, the supernatant solution was discarded, and the precipitate was washed with several changes of aqueous 70% ethanol and dried over phosphorus pentaoxide to give the soluble glucan. The molecular weight of this material may be variable. This polymer was soluble in M acetic acid, M potassium hydroxide, and methyl sulfoxide in which the amyloglucan did not dissolve.

The above supernatant solution (25 mL) was freeze-dried, and a solution of the residue in distilled water (1 mL) was passed through a millipore filter (0.45 μm). The oligosaccharides were then fractionated by h.p.l.c. with a Waters Sugar-pak column, using degassed reagent-quality water as the mobile phase. The sugars were detected with a Waters 401 refractometer.

Characteristics of the polysaccharides. — (*a*) *Iodine staining.* The iodine-staining characteristics of amyloglucan and the soluble glucan were determined and compared with those of amylopectin and amylose, using the method of McCready and Hassid[22].

(*b*) *I.r. spectra.* The i.r. spectra (KBr discs) of dried samples of the amyloglucan, the soluble glucan, the water-insoluble dextran produced from sucrose by *S. sanguis* 1 MC 204, and the mutan produced by *S. mutans* ATCC 25175 (prepared using the method of Nisizawa *et al.*[12]) were recorded with a Jasco A-202 Spectrophotometer.

(*c*) *Methylation analysis.* Samples were dried for 24 h over phosphorus pentaoxide, transferred to McCartney bottles, then methylated, hydrolysed, reduced, and acetylated using the method of Harris *et al.*[4]. The insoluble polysaccharide was ground to a fine powder in an effort to improve the solubility, and the method for the methylation of insoluble samples was used. The preliminary methylation step was repeated 3 times and the sample was heated to 60° for 60 min before proceeding with the methylation; *myo*-inositol was used as the internal standard. The alditol acetates were analysed by g.l.c.–m.s. with a Carlo Erba Fractovap Series 4200 gas chromatograph fitted with an SE 30 column, with hydrogen as the carrier gas, and the temperature program 40° for 3 min, 4°/min to 100°, and 8°/min to 250°. The identity of each peak was confirmed with a Micromas 16F mass spectrometer (ion source at 220°; energy, 70 eV). The mass spectra were identified by comparison with those in the NBS Library Search. The molar percentages of the glucitol acetates were based on the areas of the peaks in g.l.c.

(*d*) *Enzymic analysis.* Each sample (25 mg) was moistened with ethanol (0.3 mL), then stirred with aqueous 10% potassium hydroxide (1 mL). The amyloglucan, which did not dissolve, was ground to a fine powder, suspended in the ethanol–alkali mixture, heated to 60° for 1 h, then stirred at room temperature for 1 week. Some (12%) of the amyloglucan did not dissolve.

The pH of each solution was adjusted to 3.6 with 18M acetic acid and the volume was made up to 5 mL with acetate buffer (pH 3.6). Isoamylase (1.5 U) from *Pseudomonas amyloderamosa* ATCC 21262 (Hayashibara Biochemical Laboratories) was added and each mixture was incubated for 18 h at 37°. The products were eluted from a column (100 × 1.5 cm) of Sephadex G-50 with degassed distilled water. Fractions were analysed for reducing sugars, using the phenol–sulphuric acid method[23].

The pH of suspensions of samples (10 mg) of the polysaccharide in 50mM acetate buffer (pH 4.8) were adjusted to pH 4.8 with 18M acetic acid made up to 3 mL with 50mM acetate buffer (pH 4.8). Beta-amylase (60 U) from *Ipomoea batatas* (sweet potato) (Boehringer Mannheim GmbH) was added and each mixture was incubated at 37° for 18 h. The action of the enzyme was stopped by heating in boiling water for 1 min. The undigested suspensions and the digests were tested for reducing sugars with the Folin

and Wu[24] modification of Fehlings test, as described by Dawson *et al.*[25]. The concentration of copper sulphate in solution A was decreased ten-fold. Absorbance was read at 420 nm. The undigested suspensions were also tested for total carbohydrate, using the phenol–sulphuric acid method[23].

ACKNOWLEDGMENTS

Professor J. F. van Reenen is thanked for his enthusiastic support. This study was supported in part by a grant from the Dental Association of South Africa.

REFERENCES

1 M. M. Coogan, R. L. Jones, D. H. Meyer, and H. W. Viljoen, *J. Dent. Assoc. S. Afr.*, 43 (1988) 515–519.
2 S. Hakomori, *J. Biochem. (Tokyo)*, 55 (1964) 205–208.
3 P. A. Sandford and H. E. Conrad, *Biochemistry*, 5 (1966) 1508–1517.
4 P. J. Harris, R. J. Henry, A. B. Blakeney, and B. A. Stone, *Carbohydr. Res.*, 127 (1984) 59–73.
5 K. Yokobayishi, A. Misaki, and T. Harada, *Agric. Biol. Chem.*, 33 (1969) 625–627.
6 D. J. Manners, in R. D. Hill and L. Munck (Eds.), *Progress in Biotechnology*, Vol. 1, Elsevier, Amsterdam, 1985, pp. 45–54.
7 J. Hollo and J. Szejtli, in J. A. Radley (Ed.), *Starch and its Derivatives*, 4th edn., Chapman and Hall, London, 1968, pp. 203–246.
8 C. T. Greenwood, in W. Pigman and D. Horton (Eds.), *The Carbohydrates: Chemistry and Biochemistry*, 2nd edn., Vol. IIB, Academic Press, New York, 1970, ch. 38.
9 G. Rolla, J. E. Ciardi, K. H. Eggen, W. H. Bowen, and J. Afseth, in R. J. Doyle and J. E. Ciardi (Eds.), *Proceedings Glucosyltransferases, Glucans, Sucrose and Dental Caries*, IRL Press, Washington, DC, 1983, pp. 21–30.
10 R. L. Whistler, *Adv. Chem. Ser.*, 117 (1973) 242–255.
11 R. H. Marchessault and Y. Deslandes, *Carbohydr. Polym.*, 1 (1981) 31–38.
12 T. Nisizawa, S. Imai, H. Akada, M. Hinoide, and S. Aray, *Arch. Oral Biol.*, 21 (1976) 207–213.
13 D. J. Manners, *Cereal Foods World*, 30 (1985) 461–467.
14 S. Hizukuri, *Carbohydr. Res.*, 147 (1986) 342–347.
15 S. Peat, W. J. Whelan, and G. J. Thomas, *J. Chem. Soc.*, (1956) 3025–3030.
16 Z. Gunja-Smith, J. J. Marshall, C. Mercier, E. E. Smith, and W. J. Whelan, *FEBS Lett.*, 12 (1970) 101–104.
17 K. H. Meyer and P. Bernfeld, *Helv. Chim. Acta*, 23 (1940) 875–885.
18 Z. Nikuni, *Chori Kagaku*, 2 (1969) 6–14.
19 D. French, *Denpun Kagaku*, 19 (1972) 8–25.
20 E. Bertoft, *Carbohydr. Res.*, 189 (1989) 195–207.
21 W. M. Janda and H. J. Kuramitsu, *Infect. Immun.*, 14 (1976) 191–202.
22 R. M. McCready and W. Z. Hassid, *J. Am. Chem. Soc.*, 65 (1943) 1154–1157.
23 M. Dubois, K. A. Gilles, J. K. Hamilton, P. A. Rebers, and F. Smith, *Anal. Chem.*, 28 (1956) 350–356.
24 O. Folin and H. Wu, *J. Biol. Chem.*, 41 (1920) 367–374.
25 R. M. C. Dawson, D. Elliott, W. H. Elliott, and K. M. Jones, *Data for Biochemical Research*, Clarendon Press, Oxford, 1969, p. 618.
26 H. Bender, R. Siebert, and A. Stadler-Szoke, *Carbohydr. Res.*, 110 (1982) 245–259.