Carbohydrate Research, 80 (1980) 375–377

Note

Relative molecular masses and structures of some levans elaborated by strains of *Streptococcus salivarius*

KEITH MARSHALL AND HELMUT WEIGEL

The Bourne Laboratory, Royal Holloway College (University of London), Egham, Surrey TW20 OEX (Great Britain)

(Received July 23rd, 1979; accepted for publication, August 15th, 1979)

Strains of *Streptococcus mutans*, *S. salivarius*, and *S. sanguis*, which colonise in the human mouth, produce extracellular polysaccharides when grown on sucrose-containing media[1,2]. They are also thought to elaborate the polysaccharides that make up the bulk of the extracellular material in dental plaque. The glucans elaborated by such organisms have received considerable attention[1] and attempts have been made[1,3] to correlate their structural features with the cariogenicity of the respective bacteria. On the other hand, there are few reports on the fructans elaborated by these organisms. Consequently, their role in the development of dental caries is uncertain, but this, no doubt, depends, *inter alia*, on their relative molecular masses and structures. We have shown[4] that the extracellular, water-soluble polysaccharide elaborated by *S. salivarius* strain 51 is a levan in which β-D-fructofuranosyl residues are linked through C-2 and C-6, as well as C-1, C-2, and C-6. The relative molecular mass of the fructan of *S. salivarius* strain ATCC 13419 has been shown[5] to be 16–23 × 10^6, but no conclusion with regard to the average frequency of branching could be drawn[6] from its ^{13}C-n.m.r. spectrum. We now report on the relative molecular masses of the water-soluble, extracellular polysaccharides of *S. salivarius* strains 51 and NCTC 8606, and on the type and percentages of the glycosidic linkages in those of strains ATCC 13419 and NCTC 8606.

The amount of extracellular polysaccharide present in the culture fluids of *S. salivarius* strains 51, ATCC 13419, and NCTC 8606 reached a maximum at 50, 50, and 100 h, respectively. The subsequent hydrolysis is probably due to induced hydrolases[7]. Large-scale preparations of the polysaccharides were therefore obtained by incubation for periods where ~80% of the maximum amounts of polysaccharides were produced. The levans ATCC 13419 and NCTC 8606 had, respectively, $[\alpha]_D^{20}$ −52.6 and −61.4° (*c* 1.0, water); fructose (determined by the method of Wise *et al.*[8]), 87.6 and 85.4%; ash, 3.5 and 8.3%; N, 0.65 and 0.74%. Properties of the levan of strain 51 have been described earlier[4].

The polysaccharides of *S. salivarius* strains ATCC 13419 and NCTC 8606 were

both shown to contain β-D-fructofuranosyl residues, as indicated by their negative $[\alpha]_D$ values and by the fact that D-fructose was the only saccharide released by acid hydrolysis and by invertase.

On ultracentrifugation at 20°, the fructans of strains 51, ATCC 13419, and NCTC 8606 sedimented rapidly as single peaks with S_c 140 × 10^{-13}, 172 × 10^{-13}, and 121 × 10^{-13}, respectively (c 5 mg/cm^3), indicating that each preparation was homogeneous.

Each fructan was completely excluded from Sepharose 4B (exclusion limit: $M_r = 5 \times 10^6$). However, although the levan of strain ATCC 13419 was also completely excluded from Sepharose 2B (exclusion limit: $M_r = 20 \times 10^6$), those of strains 51 and NCTC 8606 were only partially excluded. These results confirm[5] that the levan of strain ATCC 13419 has a relative molecular mass $M_r > 20 \times 10^6$. Reliable, standard polysaccharides for determination of $M_r > 2 \times 10^6$ by gel filtration are not available, but the results show that the levans of strains 51 and NCTC 8606 have M_r between 5 and 20 million.

The linkage analysis involved essentially the same steps as described before[4], namely, methylation of the polysaccharide by the Hakomori method, hydrolysis, reduction with sodium borodeuteride, acetylation, and g.l.c.–m.s. of the *O*-acetyl-*O*-methylhexitols thus obtained. The conditions used for g.l.c. did not allow separation of the D-glucitol and D-mannitol derivatives. However, three components, *A*, *B*, and *C*, were revealed which had the same retention times as those which had previously been shown by c.i.-m.s. to be tetra-, tri-, and di-*O*-methylhexitol-2-d_1 acetates, respectively. The primary fragments obtained by e.i.-m.s. of components *A*, *B*, and *C* showed that they were 2,5-di-*O*-acetyl-1,3,4,6-tetra-*O*-methyl-, 2,5,6-tri-*O*-acetyl-1,3,4-tri-*O*-methyl-, and 1,2,5,6-tetra-*O*-acetyl-3,4-di-*O*-methyl-hexitol-2-d_1, respectively. We therefore conclude that the fructans of *S. salivarius* strains ATCC 13419 and NCTC 8606 have a branched structure and are, like that of strain 51, composed of β-D-fructofuranose residues linked through C-2, C-2 and C-6, as well as C-1, C-2, and C-6.

G.l.c. does not always separate the tetra-*O*-methylhexitol diacetate *A* sufficiently from materials that arise from the reagents used. However, a second methylation did not affect significantly the molecular proportions of tri-*O*-methyl- (component *B*) to di-*O*-methyl-hexitol (component *C*) derivatives. The methylation was therefore complete, and it was assumed that the molecular ratio of the tetra-*O*-methyl- to di-*O*-methyl-hexitol acetates was unity. It was then calculated that the approximate number of β-D-fructofuranose residues in the average repeating-units of the levans of strains ATCC 13419 and NCTC 8606 is 11 and 8, respectively.

This and our earlier[4] investigations show that the levans elaborated by oral strains of *S. salivarius* have high, relative molecular masses, and that their average repeating-units contain similar numbers of β-D-fructofuranose residues. These features are similar to those of levans elaborated by other micro-organisms[9–11] and are compatible with the suggestion[5] that levans function as substrates for bacterial metabolism within the dental plaque.

We have shown[4], at least for the levan of *S. salivarius* strain 51, that the branches through the β-(2→1) linkage contain up to at least four β-D-fructofuranose residues, and that this polysaccharide possesses[7] chain-types[12] A, B, and C. However, it remains to be demonstrated whether these are general features of bacterial levans.

REFERENCES

1 R. L. SIDEBOTHAM, *Adv. Carbohydr. Chem. Biochem.*, 30 (1974) 371–444.
2 C. F. NIVEN, JR., K. L. SMILEY, AND J. M. SHERMAN, *J. Biol. Chem.*, 140 (1941) 105–109.
3 M. D. HARE, S. SVENSSON, AND G. J. WALKER, *Carbohydr. Res.*, 66 (1978) 245–264.
4 R. A. HANCOCK, K. MARSHALL, AND H. WEIGEL, *Carbohydr. Res.*, 49 (1976) 351–360.
5 E. NEWBRUN AND S. BAKER, *Carbohydr. Res.*, 6 (1968) 165–170.
6 F. R. SEYMOUR, R. D. KNAPP, J. E. ZWEIG, AND S. H. BISHOP, *Carbohydr. Res.*, 72 (1979) 57–69.
7 K. MARSHALL, Ph.D. Thesis, University of London, 1977.
8 C. S. WISE, R. J. DIMLER, H. A. DAVIS, AND C. E. RIST, *Anal. Chem.*, 27 (1955) 33–36.
9 D. J. BELL AND R. DEDONDER, *J. Chem. Soc.*, (1954) 2866–2870.
10 D. S. FEINGOLD AND M. GEHATIA, *J. Polym. Sci.*, 23 (1957) 783–790.
11 R. DEDONDER AND P. SLIZEWICZ, *Bull. Soc. Chim. Biol.*, 40 (1958) 873–886.
12 S. PEAT, W. J. WHELAN, AND G. J. THOMAS, *J. Chem. Soc.*, (1952) 4546–4548.