Carbohydrate Research, 83 (1980) 406–408
Elsevier Scientific Publishing Company, Amsterdam – Printed in The Netherlands

Note

The sugar sequence of a streptococcal, immunogenic tetraheteroglycan: a revision

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(Received August 6th, 1979, accepted for publication in revised form, October 10th, 1979)

It was reported recently that an immunogenic tetraheteroglycan from the cell wall of *Streptococcus bovis*, strain C_3, consists of a main chain of →3)-6-deoxy-L-talose-(1→3)-L-rhamnose-(1→3)-D-galactose-(1→2)-L-rhamnose-(1→ and side chains of single D-glucuronic acid groups at O-4 of the 3-substituted rhamnose residues[1] The immunogenicity of a cell-wall glycan is attributable to the nature of the monosaccharide constituents, the sugar sequence, and the glycosidic bond-types[2]. We have now found that the sequence proposed for the main chain of this glycan needs to be revised to the following →3)-6-deoxy-L-talose-(1→3)-D-galactose-(1→3)-L-rhamnose-(1→2)-L-rhamnose-(1→ with side chains of single D-glucuronic acid groups at O-4 of the 3-substituted L-rhamnose residues This revision involves a reversal of the order of the D-galactose residue and the 3-substituted L-rhamnose residue It is based on new data from experiments on the chemical degradation of the tetraheteroglycan by alkaline β-elimination, oxidation with ruthenium tetraoxide, and degradation by base and acid followed by reduction and methylation analysis of the resulting oligosaccharide

Fig 1, frame B, shows a g l c pattern for the methylated alditol acetates from the reduced oligosaccharide These derivatives were identified as 3-*O*-acetyl-1,2,4,5,6-penta-*O*-methylgalactitol, 1,5-di-*O*-acetyl-2,3,4-tri-*O*-methylrhamnitol, and 1,3,5-tri-*O*-acetyl-6-deoxy-2,4-di-*O*-methyltalitol The three derivatives possessed retention times identical to those of reference derivatives (frames A and C of Fig 1), and the derivatives of L-rhamnose and 6-deoxy-L-talose yielded mass-spectral data identical with the data of the reference compounds The foregoing results establish that the sequence of residues in the oligosaccharide derived from the degraded glycan is L-rhamnose-(1→3)-6-deoxy-L-talose-(1→3)-D-galactose In the native glycan, the D-galactosyl residue is attached to a second L-rhamnosyl residue by a 1→3 linkage The second rhamnosyl residue is, in turn, attached by a 1→2 linkage to the terminal L-rhamnose group of another oligosaccharide and is substituted at O-4 by a D-glucosyluronic acid group Repetition of this structure several times yields the glycan molecule[1]

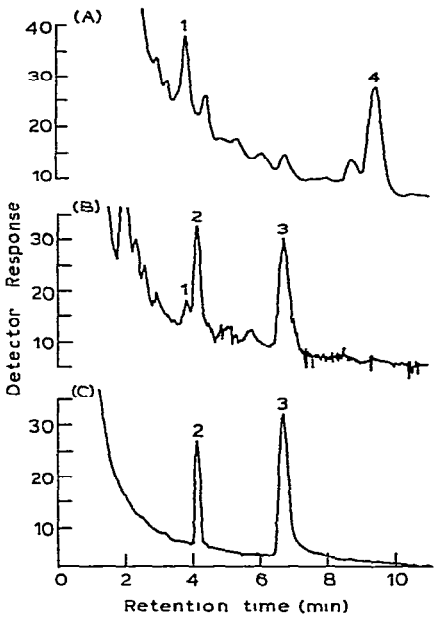

Fig 1 A photograph of the g l c charts for (A) the methylated alditol acetates from reduced *O*-D-galactosyl-(1→3)-D-galactose, (B) the oligosaccharide from the tetraheteroglycan oxidized by ruthenium tetraoxide and then degraded by base and acid, and (C) reference derivatives of L-rhamnose and 6-deoxy-L-talose 1 = 3-*O*-acetyl-1,2,4,5,6-penta-*O*-methylgalactitol, 2 = 1,5-di-*O*-acetyl-2,3,4-tri-*O*-methylrhamnitol, 3 = 1,3,5-tri-*O*-acetyl-6-deoxy-2,4-di-*O*-methyltalitol, and 4 = 1,5-di-*O*-acetyl-2,3,4 6-tetra-*O*-methylgalactitol

The earlier data[1] from methylation analysis, periodate oxidation, and β-elimination experiments are in harmony with the revised structure for the tetraheteroglycan The revised structure for the repeating unit of the glycan is thus

→3)-6-deoxy-L-Tal*p*-(1→3)-D-Gal*p*-(1→3)-L-Rha*p*-(1→2)-L-Rha*p*-(1→
4
↑
1
D-Glc*p*A

EXPERIMENTAL

Selective oxidation of the tetraheteroglycan with ruthenium tetraoxide — The tetraheteroglycan[3] from *Streptococcus bovis*, strain C_3 (25 mg), was methylated by the Hakomori procedure[4] The methylated glycan was purified by chromatography on Sephadex LH-20 and the purified product subjected to β-elimination to remove the D-glucosyluronic acid groups[5] The product was rechromatographed on Sephadex LH-20, dried in a stream of nitrogen, and oxidized with ruthenium tetraoxide[6] A syrupy, methylated glycan devoid of D-glucuronic acid groups was obtained

Degradation of the methylated and oxidized tetraheteroglycan — The methylated and oxidized glycan was degraded to an oligosaccharide derivative by treatment first

with base and then with acid, essentially as described in a published procedure[6]. However, in order to effect complete hydrolysis of the product, treatment of the sample with sodium methoxide was extended to 4 h As revealed in later analyses, some degradation of the reducing-end residue of the oligosaccharide occurred As a result, methylation analysis gave a lower yield of 3-*O*-acetyl-1,2,4,5,6-penta-*O*-methylgalactitol than of the other derivatives (frame B of Fig 1)

Structural analysis of the methylated oligosaccharide — The methylated oligosaccharide, obtained as just described, was reduced to the corresponding alditol with sodium borohydride The product was dried overnight and then remethylated[4] The product was successively hydrolyzed, reduced, and acetylated, and the derivatives were identified by g l c –mass spectrometry[3,7] The results of the g l c analysis are shown in Fig 1, frame B

Reference derivatives and analytical methods — *O*-β-D-Galactopyranosyl-(1→3)-D-galactose[8] (2 mg) was reduced with sodium borohydride, and the product subjected to methylation analysis The g l c pattern for the derivatives from the reduced oligosaccharide is shown in frame A of Fig 1 L-Rhamnose (5 mg) and the native tetraheteroglycan (3 mg) were methylated and subjected to the conventional series of reactions to obtain methylated alditol acetates[3,7] The 2,4-dimethyl ether of 6-deoxy-L-talose possessed the shortest retention-time of the derivatives from the tetraheteroglycan The g l c pattern for this derivative and for the trimethyl ether of L-rhamnose is shown in frame C of Fig 1 The g l c pattern for other derivatives from the tetraheteroglycan is not shown in Fig 1

G l c analysis was performed in a 1 83 m × 3 2 mm stainless-steel column packed with 3% OV-225 on 80–100 Supelcoport A Varian Aerograph 1400 gas chromatograph was used at a temperature of 190° Mass spectrometry was performed with a Dupont 21–490 mass spectrometer The source temperature was 240°, the electron potential 70 eV, and the ionization current 125 μA Mass-spectral data were obtained for all derivatives obtained from the oligosaccharide from the glycan except for 3-*O*-acetyl-1,2,4,5,6-penta-*O*-methylgalactitol, which was present in low concentration The mass-spectral data for the derivatives of L-rhamnose and 6-deoxy-L-talose have been recorded previously[3,7] Data for the reference compound 3-*O*-acetyl-1,2,4,5,6-penta-*O*-methylgalactitol were as follows (relative abundances in parentheses) *m/e* 45 (100), 89 (80), 101 (90), 133 (20), 205 (15), and 248 (10)

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