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Preliminary communication

Location of formate groups in bacterial polysaccharides

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The presence of formate as an integral component of the capsular polysaccharide from *Klebsiella aerogenes* type 54 (strain A3) has been demonstrated by Sutherland[1]. The tetrasaccharide products obtained by depolymerization of the polysaccharide with phage-induced enzymes were shown to contain an appreciable quantity of formate, which was identified as its hydroxamic acid derivative[2] and by its reduction of NAD in the presence of formate dehydrogenase. An estimation of the formate content of the polysaccharide indicated that one molar proportion of formate was present per tetrasaccharide repeating-unit, but the location of these groups has not been determined.

We now report not only the identification but also on the location of formate in the extracellular polysaccharide from *Klebsiella* serotype K63, the primary structure of which has been established by chemical methods[3] and consists of the sugar sequence →3-α-D-Gal*p*-(1→3)-α-D-Gal*p*A-(1→3)-α-L-Fuc*p*-(1→. Bacteriophage depolymerization of this polysaccharide results in the generation of oligomers corresponding to one (trisaccharide), two (hexasaccharide), and three (nonosaccharide) repeating units of the polysaccharide. Fucose was identified as the reducing sugar in each oligosaccharide, and proton magnetic resonance (p.m.r.) spectroscopy indicated the presence of one formic ester group per three sugar residues (signal at δ 5.9). These groups were not detected in the previous study[3], as the polysaccharide was partially depolymerized by hydrolysis with acid prior to analysis by p.m.r. spectroscopy. A red coloration was observed when each of the aforementioned oligomers, as well as the native polysaccharide, was treated with hydroxylamine hydrochloride and ferric chloride (the hydroxamic acid test).

Hydrolysis of the trisaccharide with α-D-galactosidase liberated D-galactose and an aldobiouronic acid, namely, 3-*O*-(α-D-galactosyluronic acid)-L-fucose. The latter gave a positive hydroxamic acid test, and was shown by p.m.r. spectroscopy to contain approximately one molar proportion of formate.

In order to locate the position of the formyl group, the trisaccharide was methylated according to the Hakomori[4] procedure, but was only exposed to the anion for about one minute before the addition of methyl iodide. Hydrolysis of a sample of the methylated product, followed by analysis by gas–liquid chromatography (g.l.c.) of

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the sugars present in the hydrolyzate (as the derived alditol acetates), gave 2,3,4,6-tetra-*O*-methyl-D-galactose and 2,4-di-*O*-methyl-L-fucose. No evidence was obtained, either from the infrared spectrum of the methylated product or from g.l.c., to suggest undermethylation. A further sample was reduced with lithium aluminum hydride in oxolane, and the product hydrolyzed, and the sugars present in the hydrolyzate were analyzed by g.l.c. as the derived alditol acetates. In addition to the two methylated sugars found previously, the hydrolyzate also contained 2,4-di-*O*-methyl-D-galactose and 2-*O*-methyl-D-galactose, resulting from reduction of the D-galactosyluronic residue in the trisaccharide.

Subsequent methylation studies performed on the hexasaccharide, and on the polysaccharide, also indicated varying proportions of 2-*O*-methyl-D-galactose in the hydrolyzates of their methylated derivatives after carboxyl reduction with lithium aluminum hydride. No evidence was obtained to suggest that these residues were present as a result of undermethylation, and, hence, they must have been derived from the D-galactosyluronic residues.

On the basis of the foregoing results it seems reasonable to conclude that, during methylation, a substantial proportion of the formate groups survive treatment with base, and are subsequently removed by reduction, resulting in a free hydroxyl group on C-4 of D-galacturonic acid. This explains the occurrence of 2-*O*-methyl-D-galactose in the hydrolyzates. Studies indicated that the percentage of formyl groups that remain attached during the methylation procedure is dependent on the length of time during which the oligosaccharide is exposed to the base. Behavior of this type was also noted by Sutherland[1], who found that, unlike *O*-acetyl groups, the formic ester is not hydrolyzed by mild alkali, but is, indeed, removed by mild treatment with acid.

This study, performed on the capsular polysaccharide from *Klebsiella* serotype K63, not only substantiates the results of the previous, structural work[3], but also indicates the presence of an *O*-formyl group located at O-4 of each D-galactosyluronic residue in the repeating unit.

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