Structure of Succinoglucan, an Exocellular Acidic Polysaccharide of *Alcaligenes faecalis* var. *myxogenes**

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ABSTRACT: Succinoglucan, a succinylated polysaccharide ($[\alpha]_D^{25}$ $-15°$, water) produced by *Alcaligenes faecalis* var. *myxogenes* 10C3, consists of D-glucose, D-galactose, and succinic acid, in the approximate molar ratio of 7:1:1.5. The polysaccharide moiety was shown by methylation and periodate oxidation to consist of β-1→3-, 1→4-, and 1→6-linked glucose residues, and a small proportion of β-1→3-linked galactose residues. Removal of succinyl residues by alkali treatment furnished a desuccinylated polysaccharide ($[\alpha]_D^{25}$ $-26°$, water), which was oxidized by periodate at essentially the same rate as the native polysaccharide, and consumed 0.65 mole of periodate/sugar residue with production of 0.16 mole of formic acid. On acid hydrolysis the methyl derivative derived from the desuccinylated polysaccharide yielded 2,3,4,6-tetra-, 2,3,4-tri-, 2,3,6-tri-, 2,4,6-tri-, and 2,3-di-*O*-methyl-D-glucose and 2,4,6-tri-*O*-methyl-D-galactose (1:1.5:4:5:1:2 molar ratio). Periodate oxidation of the polysaccharide, followed by borohydride reduction and mild hydrolysis with acid yielded *O*-β-D-galactosylerythritol, *O*-β-D-glucosylerythritol, *O*-β-laminaribiosylerythritol, and *O*-β-laminaribiosylglycerol, together with small amounts of erythritol and glycerol.

The significance of these findings in relation to the structure of the polysaccharide is discussed.

Previous papers (Harada *et al.*, 1964, 1965) showed that *Alcaligenes faecalis* var. *myxogenes* strain 10C3, produces a water-soluble acidic polysaccharide when grown in synthetic media containing ethylene glycol and various sugars, such as glucose, galactose, or sucrose. This polysaccharide, designated as succinoglucan, was shown to be composed of D-glucose (70–80%) and succinic acid (10%) which appeared to be linked through an ester linkage, together with small amounts of galactose and mannose (Harada, 1965). Its unusual viscosity property in the presence of salts seemed to be due to the succinic acid (Harada and Yoshimura, 1965).

A naturally occurring mutant of this organism, which does not synthesize succinoglucan, produces a water-insoluble, gel-forming glucan, designated as curdlan, containing exclusively β-1→3-D-glucosidic linkages (Harada *et al.*, 1966a,b; Maeda *et al.*, 1967; Harada *et al.*, 1968b; Saito *et al.*, 1968). Thus, it is interesting to establish the structure of succinoglucan, to compare the structural and biosynthetic relationships between the two polysaccharides. This paper reports studies on the glycosidic linkages in succinoglucan, as revealed chiefly by methylation and Smith degradation.

Materials and Methods

Preparation and Purification of Succinoglucan. Alcaligenes faecalis var. *myxogenes*, strain 10C3 was grown in a medium containing 4% glucose, 0.5% yeast extract, and 1% $CaCO_3$, at 30° for 5 days (Harada *et al.*, 1965). After removal of the cells and insoluble materials by centrifugation, the crude succinoglucan (Ca form) was precipitated from the clarified broth by addition of 2 volumes of acetone (Harada, 1965). This precipitation procedure was repeated three times, and the fibrous polysaccharide was dehydrated with acetone, and dried *in vacuo*. The yield to the initial glucose was 31.5%. For purification, the above polysaccharide preparation (4.5 g) was dissolved in water, passed through Amberlite IR-120 (H^+ form), and 10% aqueous solution of cetylpyridinium chloride was added to precipitate the acidic polysaccharide as an insoluble complex. The cetylpyridinium chloride complex was then dissolved in 10% NaCl solution, centrifuged to remove a trace of impurity, and the polysaccharide (Na form) was precipitated with ethanol. It was then dissolved in water, dialyzed against water, and precipitated again with acetone. After washing with acetone, ether, and petroleum ether (bp 30–60°), the purified succinoglucan was dried *in vacuo* (recovery, 3.2 g).

Analytical Methods. Paper chromatography was usually performed on Toyo-roshi No. 51A paper by the descending method with the following solvent systems (v/v); (A) butanol–ethanol–water (4:1:5), (B) butanol–acetic acid–water (4:1:5), (C) butanol–pyridine–water (6:4:3), and (D) butanone–water azeotrope. Preparative paper chromatography was done on Whatman No. 3MM paper. Reducing sugars were detected by spraying the chromatograms with *p*-anisidine hydrochloride, or alkaline silver nitrate, and sugar alcohols and nonreducing sugars were detected with alkaline silver nitrate.

Gas–liquid partition chromatography was carried out with a Hitachi K53 Chromatograph, fitted with a flame ionization detector, using a stainless steel column (0.4-cm i.d.). Sugars and sugar alcohols were converted into the corresponding trimethylsilyl derivatives (Sweeley *et al.*, 1963), and separated on a column (2 m length) of 5% Ucon LB 550X on Gas-Chrom (Nishio Ind. Co., Tokyo). Methyl sugars were converted into the methyl glycosides and separated on a column (1 m length) of 15% butanediol succinate polyester on Neo-

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sorb NC (Nishio Ind. Co.). Nitrogen was used as a carrier gas, at a flow rate of 60 ml/min.

Determination of carbohydrates was carried out generally with phenol–sulfuric acid (Dubois *et al.*, 1956). A glucostat reagent (Worthington Biochemical Co.) was also used for assay of glucose. Formaldehyde was determined with chromotropic acid, and sugar alcohols were also determined with the same reagent by the procedure of Hanahan and Olley (1958). In periodate oxidation studies, the periodate consumption was determined by the usual arsenite method (Fleury and Lange, 1933), and the formic acid production by titration with 0.01 N NaOH.

Electrophoresis of polysaccharide was carried out on Whatman GF 81 glass-fiber paper, in 1 N NaOH, at 110 V for 10 hr in a cold room (5°), and the spot was detected by spraying with 0.5% $KMnO_4$ in 1 N NaOH (Lewis and Smith, 1958).

Infrared spectra were measured on KBr disks (polysaccharide) or in chloroform solution (methylated product), with a Hitachi Infrared Spectrophotometer, Model EP 1-2.

Sedimentation analysis of the polysaccharide was carried out in a Spinco Model E analytical ultracentrifuge. For determination of molecular weight by the Archbald (1947) method, the value of specific volume, 0.619 cm^3/g was adopted.

Desuccinylation and Resuccinylation. Succinoglucan (3.2 g) was heated with 0.1 N NaOH (400 ml) at 70° for 2.5 hr, in a nitrogen atmosphere. After neutralization with 1 N HCl, the neutral solution was dialyzed, and the resulting neutral polysaccharide was precipitated by addition of acetone (recovery, 2.66 g). Chemical succinylation of the neutral polysaccharide was carried out by the method of McIntire *et al.* (1967) with sodium succinate and succinic anhydride.

Methylation Analysis. The desuccinylated polysaccharide (2.3 g) was acetylated in formamide with pyridine and acetic anhydride in the usual way. The acetylated polysaccharide (1.7 g) was simultaneously deacetylated and methylated four times with methyl sulfate and 30% NaOH by the Haworth (Haworth and Percival, 1932) method. The methylated product (1.2 g) still showed a weak OH band (3400–3600 cm^{-1}) in the infrared spectrum. To complete the methylation the product was further methylated by the Hakomori (1964) method with methylsulfinyl carbanion, prepared according to Sandford and Conrad (1966), and methyl iodide, and then by the usual Purdie (Purdie and Rose, 1906) method. The fully methylated polysaccharide thus obtained was precipitated from the chloroform solution by addition of petroleum ether (yield, 880 mg).

The methylated polysaccharide (480 mg) was subjected to methanolysis by heating with 3% methanolic HCl for 24 hr. After neutralization with Ag_2CO_3, the methanolysate was concentrated in an air current. A portion of the methanolysate was used for gas–liquid partition chromatography, and the remainder was hydrolyzed (1 N H_2SO_4, 100°, 24 hr). After neutralization with $BaCO_3$ and concentration to a syrup, a mixture of the methyl sugars (270 mg) was fractionated by cellulose–hydrocellulose column using solvent D (Misaki *et al.*, 1962), and then by thick paper chromatography (solvent A or D). Each of the methyl sugars was examined by gas–liquid partition chromatography as the methyl glycoside, and finally identified as a crystalline compound, usually as an aniline derivative.

Smith Degradation. The polysaccharide (200 mg) was oxidized with 0.05 M periodic acid (200 ml) at 5° for 22 days, and the oxidized polysaccharide was reduced with borohydride in the usual way. The resulting polyalcohol was heated with 1 N H_2SO_4, at 100° for 5 hr. The hydrolysate was neutralized with $BaCO_3$, passed through Amberlite IR-120, and evaporated to dryness, the boric acid in the residue being removed by repeated evaporation with methanol at room temperature. The sugar and sugar alcohols in the hydrolysate were separated by paper chromatography (solvent C). Each component was extracted with water and determined colorimetrically. For gas–liquid partition chromatography, the hydrolysate was reduced with sodium borohydride, and the borate in the reduced product was removed completely before trimethylsilylation by treatment with 1% methanolic HCl and subsequent deionization with Amberlite IR-120 (H^+ form) and Duolite A-4 (OH^- form).

In the controlled Smith degradation, the desuccinylated polysaccharide (1.2 g), which was oxidized and reduced as described previously, was hydrolyzed with 0.1 N H_2SO_4, at 25° for 18 hr (Goldstein *et al.*, 1965). After neutralization and removal of borate, the hydrolysate (460 mg) was fractionated on a column (1.7 × 20 cm) of Dowex 1-X4 (200–400 mesh) according to Austin *et al.* (1963), and the compounds eluted with water were further fractionated by thick paper chromatography (solvent B or C).

Results and Discussion

The previous study (Harada, 1965) showed that the constitution of the acidic polysaccharide prepared from cultures of *Alcaligenes faecalis* var. *myxogenes*, strain 10C3, grown on different carbon sources were not significantly different. Therefore, in the present study, succinoglucan was prepared from a culture grown in glucose medium. This strain also produces a small amount of a neutral β-1,3-glucan, identical with curdlan (Harada *et al.*, 1968a), but it precipitates during fermentation and is readily removed by centrifugation. The water-soluble succinoglucan was precipitated from the supernatant fluid with 50% acetone. In order to remove the contaminating neutral polymers, an aqueous solution of the crude polysaccharide was treated with cetylpyridinium chloride to precipitate succinoglucan as an insoluble complex. The cetylpyridinium chloride complex dissolved in NaCl solution was dialyzed and the purified polysaccharide was precipitated with acetone.

The purified succinoglucan thus obtained was free from nitrogen, and gave a single spot on a glass-fiber paper electrophoresis (Lewis and Smith, 1958). The homogeneity of the polysaccharide was further assessed by ultracentrifugal analysis, as shown in Figure 1. It gave a single peak ($s_{20,w}$ = 2.46 S), and had a molecular weight of 3×10^5, as calculated by the Archbald (1947) method.

The polysaccharide had $[\alpha]_D^{25}$ −15° (*c* 1, water) and an acid equivalent weight of 1100 (free form). Acid hydrolysis (1 N H_2SO_4, 100°, 8 hr) and quantitative determinations of the constituents indicated that the polysaccharide is composed of residues of D-glucose, D-galactose, and succinic acid, in the approximate molar ratio of 7.0:1.0:1.5. A small proportion (1.9%) of mannose present in the previous preparation (Harada, 1965) could not be detected, suggesting that mannose is not an essential constituent.

Treatment of succinoglucan with dilute alkali at 70° re-

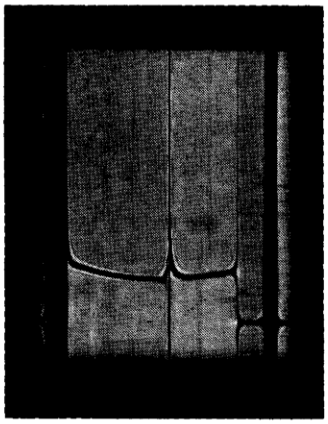


FIGURE 1: Sedimentation diagram of purified polysaccharide of *A. faecalis* var. *myxogenes* 10C3. Measurement was made at a concentration of 5 mg/ml of polysaccharide (sodium form) in 0.1 M NaCl. The photograph was taken at 130 min after attaining top speed (59,800 rpm) at 20°, with a Spinco Model E. ultracentrifuge (sedimented from right to left).

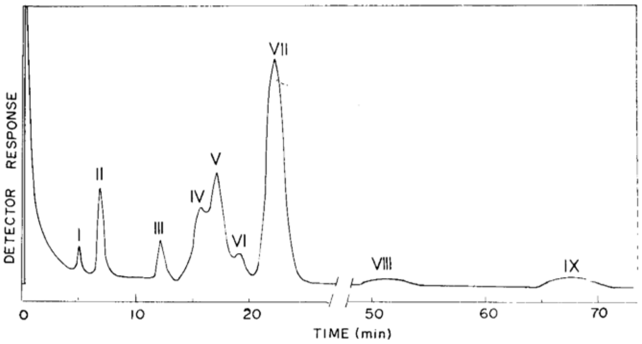


FIGURE 2: Gas–liquid partition chromatogram of the methanolysate of methylated polysaccharide of *A. faecalis* var. *myxogenes* 10C3. Conditions: 15% butanediol succinate polyester on Neosorb N.C., 175°, 60 cc of nitrogen/min. (I and II) methyl 2,3,4,6-tetra-*O*-methyl-D-glucoside (β, α), (III) methyl 2,3,4-tri-*O*-methyl-D-glucoside (β), (IV) methyl 2,4,6-tri-*O*-methyl-D-glucoside (β), (V) mixture of methyl 2,3,4-(α)-, and 2,3,6-tri-*O*-methyl-D-glucoside (β), (VI) methyl 2,4,6-tri-*O*-methyl-D-galactoside (β), (VII) mixture of methyl 2,3,6-, and 2,4,6-tri-*O*-methyl-D-glucosides (α), and 2,4,6-tri-*O*-methyl-D-galactoside (α), and (VIII and XI), methyl 2,3-di-*O*-methyl-D-glucoside (β,α).

leased all the succinic acid to afford a neutral polymer having $[\alpha]_D^{25}$ −26.6° (*c* 0.5, water). The infrared spectrum of the polysaccharide after removal of succinic acid (desuccinylated polysaccharide) showed no absorption peak at 1740–1760 cm^{-1}, due to an ester linkage. When the desuccinylated polymer was succinylated chemically, the product containing 9.3% succinic acid had a spectrum almost identical with that of the native succinoglucan. This confirms the previous results (Harada, 1965) that the succinic residues are joined by ester linkages to some of the sugar residues, probably through one of the carboxyl groups of the succinic acid.

The desuccinylated polysaccharide was methylated by successive applications of the Haworth (Haworth and Percival, 1932), Hakomori (1964), and Prudie (Prudie and Rose, 1906) methods. The fully methylated polysaccharide, which had $[\alpha]_D^{25}$ −10.9° (*c* 2.2, chloroform), was hydrolyzed with acid and the methyl sugar fragments were separated by column and paper chromatographies, giving 2,3,4,6-tetra-, a mixture of 2,3,4-, 2,3,6-, and 2,4,6-tri-*O*-methyl-D-glucose, 2,4,6,-tri-*O*-methyl-D-galactose, and 2,3-di-*O*-methyl-D-glucose (1:11.2:2:1.3 molar ratio). These sugars were identified as the crystalline compounds. In addition, a trace of 2,4-di-*O*-methyl-D-glucose was detected, which for the present is regarded as an artifact probably formed by incomplete methylation or demethylation during acid hydrolysis. The methyl sugars were also analyzed as their methyl glycosides by gas–liquid partition chromatography, which gave the separation shown in Figure 2. The tri-*O*-methyl sugars were not completely resolved from each other by this separation, but the result indicated the over-all proportions of tetra-, tri-, and di-*O*-methylhexoses as 1.0:12.8:1.0.

In another experiment, a mixture of tri-*O*-methylhexoses, including 2,3,4-, 2,3,6-, and 2,4,6-tri-*O*-methyl-D-glucoses, and 2,4,6-tri-*O*-methyl-D-galactose was separated from the tetra- and di-*O*-methyl-D-glucoses on a thick paper (Whatman No. 3MM) chromatogram, and these were reduced to the corresponding alditols with sodium borohydride. On periodate oxidation the reduced product consumed 0.46 mole of periodate/mole of tri-*O*-methylalditol with liberation of 0.12 mole of formaldehyde/mole. This indicates that the approximate ratio of 2,3,4-:2,3,6-:2,4,6-tri-*O*-methylalditols was 1.0:2.8:4.6, assuming that 2,3,4-trimethylalditol consumes 1 mole of periodate with liberation of 1 mole of formaldehyde/mole, the 2,3,6-trimethylalditol consumes 1 mole of periodate without liberation of formaldehyde, while the 2,4,6-trimethylalditol is resistant to periodate oxidation. Table I summarizes the identities and relative proportions of the cleavage fragments of the methylated polysaccharide. With regard to the gross structure of the polysaccharide, Table I shows that the polysaccharide possesses a low degree of branching, having repeating units with an average of 14–15 sugar residues, where one D-glucose residue is doubly substituted at the C-4 and C-6 positions and the side chain is terminated with D-glucose residue; the remaining nonterminal residues consist of one or two 1→6-linked D-glucoses, four 1→4-linked D-glucoses, five 1→3-linked D-glucoses, and two 1→3-linked D-galactoses.

The negative specific optical rotation (−26.6°) of the desuccinylated polysaccharide and also its absorption at about 890 cm^{-1} in the infrared spectrum indicate that most of the

TABLE I: Hyrolysis Products of Methylated Polysaccharide of *A. faecalis* var. *myxogenes* 10C3.

Compound	Molar Ratio	Structural Feature Indicated
2,3,4,6-Tetra-*O*-methyl-D-glucose	1	Glc-(1→
2,3,4-Tri-*O*-methyl-D-glucose	1.5	→6)-Glc-(1→
2,3,6-Tri-*O*-methyl-D-glucose	4	→4)-Glc-(1→
2,4,6-Tri-*O*-methyl-D-glucose	5	→3)-Glc-(1→
2,4,6-Tri-*O*-methyl-D-galactose	2	→3)-Gal-(1→
		↓
		6
		|
2,3-Di-*O*-methyl-D-glucose	1	→4)-Glc-(1→

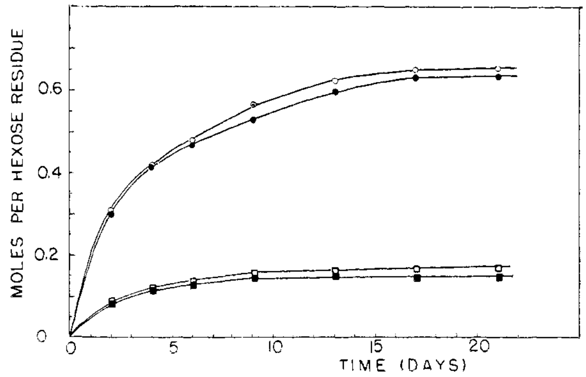


FIGURE 3: Rates of periodate consumption and formic acid production during oxidation. Consumption of periodate: (-●-) native polysaccharide; (-○-) desuccinylated material. Production of formic acid: (-■-) native polysaccharide; (-□-) desuccinylated material. Polysaccharides (150 mg) were oxidized with 0.04 M $NaIO_4$ (100 ml) at 5°.

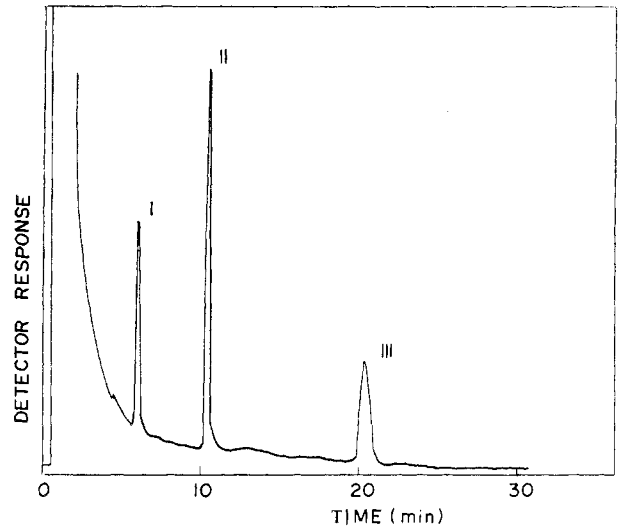


FIGURE 4: Gas–liquid partition chromatogram of trimethylsilyl derivatives of reduced products after Smith degradation of desuccinylated polysaccharide. Conditions: 5% Ucon oil LB 550X on Gas-Chrom, 105–195° (programed at a rate of 7°/min, 60 ml of nitrogen/min). (I) Glycerol, (II) erythritol, and (III) mixture of glucitol and galactitol.

glycosidic bonds joining these sugar residues are of the β type. This was confirmed by characterization of the products of the controlled Smith degradation, as described later.

These structural features were supported by the results of periodate oxidation. When the native and desuccinylated polysaccharides were subjected to periodate oxidation with 0.04 M sodium periodate at 5°, they were oxidized essentially to the same extent, as shown in Figure 3. The desuccinylated polysaccharide consumed 0.65 mole of periodate with production of 0.16 mole of formic acid/sugar residue, in good agreement with the values 0.68 and 0.17 mole, respectively, expected from the results obtained by the methylation analysis (Table I).

The desuccinylated polysaccharide that was oxidized at 5° for 22 days was reduced with borohydride to the corresponding polyalcohol, and then completely hydrolyzed with acid, according to the procedure of Abdel-Akher *et al.* (1952). Paper chromatography revealed the presence of glycerol, erythritol, glucose, and galactose in the hydrolysate. The molar ratio of these components was estimated by colorimetric determination after paper chromatographic separation, indicating the ratio of glycerol:erythritol:monosaccharide (glucose and galactose) as 1.0:1.9:2.7. The presence of these sugars and sugar alcohols was confirmed by gas–liquid partition chromatography, in which the hydrolysis mixture was treated with sodium borohydride before trimethylsilylation, to eliminate multiple peaks due to anomeric isomers of reducing sugars (Cayle *et al.*, 1968). Gas–liquid partition chromatographic separation of the trimethylsilyl derivatives gave peaks corresponding to glycerol, erythritol, and a mixture of glucitol and galactitol, as shown in Figure 4. Although the latter two alditols were not separated under these conditions, comparison of peak areas of the hydrolysis products provided quantitative information on the different linkages involved in the polysaccharide. It is clear in inspection of Table I that the glycerol must arise from the terminal ends and also the 1→6-linked D-glucose units, the erythritol from the 1→4-linked D-glucose units and the branch points joined through the C-1, C-4, and C-6 positions of the glucose units, and the glucose and galactose surviving periodate cleavage should arise from the 1→3-linked sugar units. Thus, the values for the molar ratio of glycerol, erythritol, and a mixture of glucose and galactose were consistent with the results deduced both from the methylation and periodate oxidation studies.

The native polysaccharide containing succinyl groups consumed 0.63 mole of periodate with production of 0.14 mole of formic acid/sugar residue, and no detectable succinic acid was released during oxidation. On complete acid hydrolysis the polyalcohol derived from the oxidized polysaccharide by borohydride reduction yielded glycerol, erythritol, and a mixture of glucose and galactose in the molar ratio of 1.0:2.1:2.9, in addition to succinic acid. These values are very close to those obtained for the desuccinylated polysaccharide. The exact location of the succinic acid residues cannot be deduced from the present results, but the above data suggest that these residues are linked to the sugar residues in such a manner that the substitution does not block periodate cleavage of the sugar residues. For instance, this might be by substitution at the C-6 position of the 1→4-linked D-glucose unit, though the possibility that the substitution occurs either at the C-2, C-4, or C-6 position of the 1→3-linked sugar unit cannot be excluded.

As indicated by the methylation and periodate oxidation studies, the polysaccharide appears to have a complicated structure. However, since it contains relatively large proportions of 1→3-glycosidic linkages, information on the sequential arrangements of some of the sugar residues, particularly the linkages between 1→3-linked residues and their neighboring sugar residues should be obtained by the controlled Smith degradation (Goldstein *et al.*, 1965). This procedure, which involves mild acid hydrolysis of the polyalcohol derived from a polysaccharide by periodate oxidation and borohydride reduction, has been successfully applied in structural studies on 1→3-linked D-glucans, *e.g.*, oat glucan (Goldstein *et al.*, 1959), isolichenin (Fleming and Manners, 1966), and also yeast glucan (Misaki *et al.*, 1968). If a 1→3-linked sugar residue is joined by a 1→6 bond to the neighboring sugar residue in the succinoglucan molecule, mild hydrolysis of the polyalcohol gives a glyceryl glycoside, whereas if it is joined by a 1→4 bond an erythrityl glycoside is formed, because of the marked instability of true acetal toward dilute acid.

The polyalcohol derived from the desuccinylated polysaccharide was hydrolyzed with 0.1 N acid at 25° for 18 hr, and the hydrolysate was fractionated on a column of Dowex 1 resin (OH^- form), the compounds being eluted from the column with water (Austin *et al.*, 1963). The fractions containing two or three compounds were further fractionated by thick paper (Whatman No. 3MM) chromatography, affording the following compounds: glycerol (7% by weight), erythritol (10%), *O*-β-D-galactosylerythritol (20%), *O*-β-D-glucosylerythritol (23%), a mixture of *O*-β-laminaribiosylglycerol, and *O*-β-laminaribiosylerythritol (36%), and a higher oligosaccharide (4%) presumed to be *O*-β-laminaritriosylerythritol.

The first glycoside eluted from the column was crystallized (mp 192–193°; $[\alpha]_D^{25}$ +3.4°, *c* 2.9, water). It was characterized as 2-*O*-β-D-galactosyl-D-erythritol by the fact that (a) acid hydrolysis gave equal proportions of galactose and erythritol, (b) on periodate oxidation it releases 1 mole of formaldehyde/mole, and (c) it had a low optical rotation, compared with a known α-linked D-galactosylerythritol ($[\alpha]_D^{25}$ +134°, reported for 4-*O*-α-D-galactosyl-D-erythritol; Charlson and Perlin, 1956). The identity of this compound clearly indicates that the 1→3-linked D-galactose residues, which would be recognized as 2,4,6-tri-*O*-methyl-D-galactose in the methylation analysis, are present as single 1→3-linked units joined by β-1→4 bonds to the neighboring D-glucose units. Since partial acid hydrolysis of succinoglucan yielded a trisaccharide, tentatively characterized as 3-*O*-β-cellobiosyl-D-galactose (unpublished result), all D-galactose units in the polysaccharide chain must be located as single β-1→3-linked units flanked by β-1→4-linked D-glucose units, as shown in Scheme I (sequence I).

The second glycoside (mp 189°; $[\alpha]_D^{25}$ −17°, *c* 2, water) eluted from the column was similarly characterized as 2-*O*-β-D-glucosyl-D-erythritol (Goldstein *et al.*, 1965). This *O*-β-glucosylerythritol must have arisen from 1→3-linked D-glucose units flanked by 1→4-linked D-glucose units, as shown in sequence II or in the sequence: →6)-Glc-(1→3)-Glc-(1→4)-Glc-(1→.

The sequence of two contiguous 1→3-linked D-glucose units was shown by isolation of 1-*O*-β-laminaribiosylglycerol ($[\alpha]_D^{25}$ −22°; *c* 2, water) and 2-*O*-β-laminaribiosylerythritol ($[\alpha]_D^{25}$ −16°; *c* 1.5, water), which were partially purified and characterized in the same manner described previously. These glycosides of laminaribiose must have arisen from two contiguous β-1→3-linked D-glucose units joined by a β-1→6 and 1→4 bond, respectively, to the neighboring glucose units, as shown in sequences III and IV. In addition, a small amount (4%) of a higher oligosaccharide behaving like laminaritriosylerythritol was present, suggesting that a few of the three contiguous β-1→3-linked D-glucose units are contained in the polysaccharide molecule.

These cleavage fragments which arose from different sections of the polysaccharide chain all have the β configuration, confirming that the glycosidic linkages of the sugar units are largely of the β type.

The small amount of glycerol found in the polyalcohol after mild hydrolysis is believed to originate from the terminal nonreducing glucose residues, as seen in Scheme I since the polysaccharide contains a few 1→6-linked D-glucose residues and most of these residues must appear as *O*-β-2-laminaribiosylglycerol. The presence of free erythritol in the hydrolysate indicates that some of the 1→4-linked D-glucose residues are located either as contiguous 1→4-linked units or in the sequence: →6)-Glc-(1→4)-Glc-(1→, but the absence of cellotriose in the partial acid-hydrolysate of the polysaccharide is in favour of the latter system (unpublished result). The isolation of 2,3-di-*O*-methyl-D-glucose from the methylated polysaccharide showed that the polysaccharide has a small degree of branching, one per 14–15 sugar units. The side chains are attached to the main chain at the C-6 position of the 1→4-linked D-glucose residues, as shown in sequence V, or at the alternative position. However, the present results do not indicate whether the side chains consist of single D-glucose units or whether 1→4- and/or 1→3-linked sugar units are interposed between the main chain and the terminal ends of the side chains.

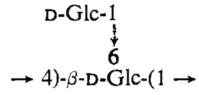
SCHEME I: Partial Structural Features of the Polysaccharide of *A. faecalis var. myxogenes* 10C3.

→4)-β-D-Glc-(1→3)-β-D-Gal-(1→4)-β-D-Glc-(1→
I

→4)-β-D-Glc-(1→3)-β-D-Glc-(1→4)-β-D-Glc-(1→
II

→3)-β-D-Glc-(1→3)-β-D-(1→6)-β-D-Glc-(1→
III

→3)-β-D-Glc-(1→3)-β-D-Glc-(1→4)-β-D-Glc-(1→
IV



$$\begin{array}{c} \text{D-Glc-1} \\ \downarrow \\ 6 \\ \rightarrow 4)\text{-}\beta\text{-D-Glc-(1} \rightarrow \end{array}$$

V

The exact proportions of the cleavage fragments obtained by the controlled Smith degradation could not be deduced because recovery from the Dowex 1 column was relatively low (70%). However, the foregoing evidence indicates that the D-glucose residues which are the main carbohydrate constituents of succinoglucan are located as alternate 1→3 and 1→4 linkages, and as sequences of two or three 1→3-linked units joined by 1→4 or 1→6 bonds to the neighboring glucose units; the remainder would be present either as contiguous 1→4 linkages or as alternate 1→4 and 1→6 linkages. In addition, a small proportion of D-galactose residues (12% of the total carbohydrate) are present in the same polysaccharide molecule as the sequence: →3)-Gal-(1→4)-Glc-(1→.

A naturally occurring mutant, mutant K, of *Alcaligenes faecalis* var. *myxogenes* 10C3 is known to produce a water-insoluble polysaccharide, curdlan, instead of the water-soluble succinoglucan (Harada *et al.*, 1966a,b; Maeda *et al.*, 1967). Recent studies established that curdlan is a linear β-1,3 D-glucan though it contains a very few 1→6 linkages (Harada *et al.*, 1968b; Saito *et al.*, 1968). The more recent work (Harada *et al.*, 1968a) showed that different mutants derived from the parent strain by treatment with a chemical mutagen produce both succinoglucan and curdlan; the ratio of the two polysaccharides differed in the individual strain. From these findings it was expected that the basic structural units would be analogous to those of curdlan, *e.g.*, relatively long "blocks" of β-1→3-linked glucose units present in the succinoglucan molecule. However, the present results show that the structure of succinoglucan is very different. Sugar residues with different types of linkages seem to be randomly distributed along the polysaccharide chain. Since it does not contain an ordered "repeating oligosaccharide unit," the sequential additions of monosaccharide units, but not transfer of oligosaccharide units, would be involved in elongation of the polysaccharide chain. Investigations on the mechanism of biosynthesis of the present polysaccharide are in progress.

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