A NOVEL TEICHOIC ACID-LIKE POLYMER FROM THE CELL WALLS OF *BACILLUS COAGULANS* NRS T2007

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We recently reported (Forrester and Wicken, 1966) that a glycerol teichoic acid-like polymer, containing glucose and galactose but lacking amino acid substituents, could be extracted with cold trichloracetic acid from the cell walls of *Bacillus coagulans* NRS T2007. We have now established the structure of this polymer (Figure 1).

The polymer had $[\alpha]_D^{30} = +99.8^\circ$ (c=1.023) and appeared homogeneous by its elution as a single symmetrical peak from a column of Sephadex G200. Potentiometric titration showed a ratio of phosphodiester to phosphomonoester groups of 38:1. Analysis[1] of acid hydrolysates of the polymer showed mole ratios of D-galactose:D-glucose:phosphorus:glycerol of 1.54:0.77:0.95:1.00. The polymer was resistant to alkaline hydrolysis and the only organic phosphates detected after hydrolysis in 2N HCl for 3 hr. at 100° were glyceromonophosphates; glycerodiphosphates were entirely absent.

The polymer was hydrolysed in 60% HF for 6 hr. at 0° (Glaser and Burger, 1964) to a mixture of glycerolglycosides, phosphate containing material and a trace of free galactose. The mixture of glycerolglycosides was resolved chromatographically into four components, designated 1a, 2a, 2b, and 3.

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[1] The following analytical methods were used: Glucose and galactose by their respective oxidases, essentially after Huggett and Nixon (1957). Total sugar by Dubois et al (1956). Glycerol by the chromotropic acid method of Roberts, Buchanan and Baddiley (1963) and phosphorus by Chen, Toribara and Warner (1956). Periodate was estimated by a standard micro arsenite procedure and formic acid by the method of Manners and Archibald (1957).

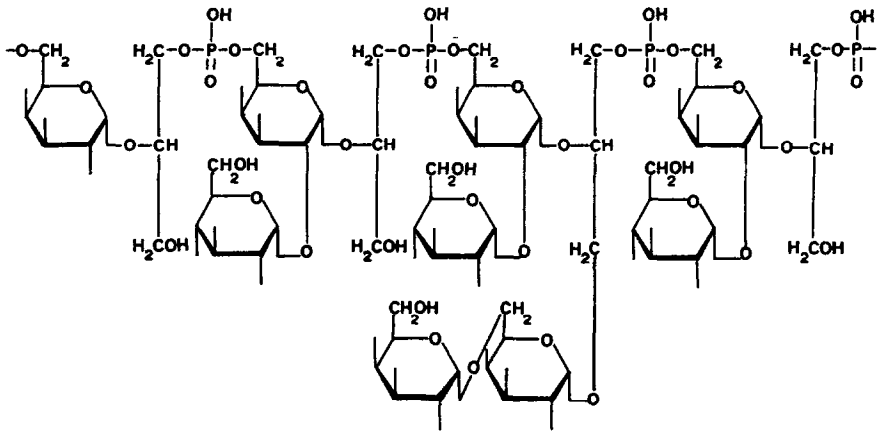

Figure 1. Structure of a statistical repeating unit of the teichoic acid-like polymer from the cell walls of B. coagulans NRS T2007.

Hydrolysis of the polymer in 66% formic acid for 30 min. at 100° gave glucose, galactose, glycosides 1a, 2a and 3 and a new glycoside, 1b.

Glycoside 1a: 2-O-α-D-Galactopyranosylglycerol. The glycoside gave a blue colour slowly with the periodate-Schiff's reagents (Baddiley et al, 1956). Acid hydrolysis gave only galactose and glycerol in the mole ratio 1.00: 1.07. Incubation of the glycoside with α-galactosidase[2] gave glycerol and galactose. β-Galactosidase (Sigma Chemical Co.) was without effect.

Glycoside 1b: 1-O-α-D-Galactopyranosylglycerol. The glycoside gave a purple colour rapidly with the periodate-Schiff's reagents characteristic of formaldehyde which would be produced from a 1-substituted glycerol (Roberts, Buchanan and Baddiley, 1963). Acid hydrolysis gave galactose and glycerol in the mole ratio 1.00:1.10 and, like the 2-isomer, the glycoside was hydrolysed with α-galactosidase but not β-galactosidase. Periodate oxidation during 30 min. at 2° followed by reduction with sodium

[2] α-Galactosidase was prepared from an acetone powder of Epidinium ecaudatum kindly provided by Professor B.H. Howard. The preparation contains an α-glucosidase as well as α-galactosidase but is inactive against β-glucosides and β-galactosides (Bailey and Howard, 1963 a and b).

borohydride and acid hyrolysis did not produce arabinose which would be expected from a galactofuranose residue. A parallel oxidation of whole polymer gave similar results.

Glycoside 2a: O-α-D-Glucopyranosyl-(1→2)-O-α-D-galactopyranosyl-(1→2)-glycerol. The glycoside gave a yellow colour with the periodate-Schiff's reagents characteristic of 1→2 linked sugars (Wicken and Baddiley, 1963). It had a mole ratio of glucose:galactose of 1.00:1.05 and total sugar: glycerol of 1.70:1.00 and was hydrolysed with α-galactosidase to a mixture of glycoside 1a, glucose, galactose and glycerol.

Glycoside 2b: O-α-D-Galactopyranosyl-(1→6)-O-α-D-galactopyranosyl-(1→1)-glycerol. The glycoside gave a purple colour rapidly with the periodate-Schiff's reagents and had a mole ratio of galactose:glycerol of 1.70:1.00. It was completely hydrolysed by α-galactosidase to galactose and glycerol.

Glycoside 3: O-α-D-Glucopyranosyl-(1→2)-O-α-D-galactopyranosyl-(1→2)-O-[-α-D-galactopyranosyl-(1→6)-O-α-D-galactopyranosyl-(1→1)]-glycerol. The glycoside gave a yellow colour with the periodate-Schiff's reagents. It had mole ratios of glucose:galactose of 1.00:2.81 and total sugar:glycerol of 3.60:1.00. Treatment with α-galactosidase gave a mixture of glycosides 2a and 1a, galactose, glucose and glycerol. Mild acid hydrolysis (0.015N HCl for 5 hr. at 100°) gave the four glycosides, 1a, 1b, 2a, 2b, as well as free sugars and glycerol. The glycoside consumed 6.9 molar proportions of periodate with the production of 2.6 molar proportions of formic acid. Galactose oxidase (plus catalase) oxidised 69% of the galactose residues in the glycoside. These results are only consistent with a 1→6 linkage between the two galactose residues attached to C1 of glycerol and is considered presumptive evidence for a similar linkage for glycoside 2b.

Phosphodiester linkages: It is clear, from the analytical data and hydrolysis products of the polymer, that phosphate must be esterified to a glycerol and a sugar hydroxyl group. Glycoside 3 cannot be the sole repeating unit of the polymer from the observed whole polymer glucose:

galactose ratio of 1:2. A repeating structure which is in agreement with the analytical data is shown in Figure 1. A glucose residue is omitted from 1 in every 4 glycerol units as the glucose:glycerol ratio for the polymer proved consistently to be 0.77:1.00. Such a structure would be expected to be alkali stable. The alternative structure involving phosphodiester linkage through a galactose residue on C1 of each glycerol is not compatible with analysis and would be alkali labile unless C2 of each glycerol moiety bore a glycosyl substituent.

Assignment of a phosphate ester bond to C6 of the galactose residue on C2 of each glycerol residue is based on periodate oxidation of the polymer under conditions where phosphate bonds remain intact (pH 6.0). Oxidation during 72 hr. followed by reduction with sodium borohydride and acid hydrolysis gave only glycerol. The absence of resistant sugars and erythritol precludes positions C3 and C4 of either sugar residue for phosphate esterification. The polymer consumed 16.2 moles periodate per 4 moles phosphorus with the production of 5.96 moles formic acid. Phosphate esterification to C2 of glucose would result in the production of only 2 moles formic acid per 4 moles phosphorus. The two remaining C6 positions were distinguished by the action of galactose oxidase on the polymer. In the proposed structure only 16.7% of the total galactose residues have their C6 positions free whereas the alternative phosphate esterification to C6 of glucose would result in 83.5% of the galactose having free C6 positions. Treatment of the polymer with galactose oxidase resulted in the oxidation of 6% of the total galactose. The enzyme preparation used (Miles Laboratories, Elkhart, USA) was shown to possess α-glucosidase activity and released free glucose from the polymer during incubation for 48 hr. These results are consistent only with the structure shown in Figure 1.

The repeating unit illustrated is to be regarded as a statistical one as the actual sequence of partially and fully glycosylated glycerol moieties in the polymer is not known. Like the teichoic acid from B. stearothermo-

philus cell walls (Wicken, 1966), this polymer appears to be bound firmly to glycosaminopeptide, probably through a phosphodiester linkage to hexosamine, and has a back-bone structure involving C2 and C3 of glycerol rather than the more usual C1 and C3. It is debatable whether the B. coagulans polymer can be regarded as a teichoic acid. The absence of amino-acyl substituents and linkage of polyol through sugar phosphate is structurally closer to some of the pneumococcal capsular polysaccharides of the type $\left[(\text{hexose})_x - \text{polyol} - P\right]_y$ (c.f. Roberts, Buchanan and Baddiley, 1963) and the polygalactosylglycerophosphate recently found in the cell walls of B. licheniformis ATCC 9945 (Burger and Glaser, 1966). A similar polymer of glycerophosphate and N-acetyl-D-glucosamine from the cell walls of Staphylococcus lactis has also been described (Archibald, Baddiley and Button, 1965). In each of these latter cases, however, C1 and C3 of the glycerol residues have been involved in the linkage of the polymer back-bone.

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