Poly(Glucosylglycerol Phosphate) Teichoic Acid in the Walls of *Bacillus stearothermophilus* B65

By ALISTAIR J. ANDERSON and A. RONALD ARCHIBALD

Microbiological Chemistry Research Laboratory, University of Newcastle upon Tyne,
Newcastle upon Tyne NE1 7RU, U.K.

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1. Walls of *Bacillus stearothermophilus* B65 contain a glycerol teichoic acid in which repeating structures consisting of 1-*O*- α -D-glucopyranosylglycerol phosphate are held together by phosphodiester linkage between the glycerol and glucose moieties of adjacent units. 2. The walls are not agglutinated on incubation with concanavalin A, nor does the isolated teichoic acid form a precipitate with this lectin. 3. No evidence was obtained of the presence of the glucosylated (1 $\rightarrow$ 2)-poly(glycerol phosphate) teichoic acid which has previously been reported to occur in walls of this bacterium.

Most Gram-positive bacteria contain (1 $\rightarrow$ 3)-poly(glycerol phosphate) teichoic acids which are associated with the cytoplasmic membrane; similar teichoic acids are also present in the cell walls of certain species (Archibald, 1974; Baddiley, 1972). These polymers generally contain D-alanyl and glycosyl substituents. Walls of *Bacillus licheniformis* A.T.C.C. 9945 and *Lactobacillus plantarum* C106 contain structurally different glycerol teichoic acids in which the glycosyl substituents form part of the main polymer chain, so that repeating structures are held together by phosphodiester linkages between the glycerol and glycosyl moieties of neighbouring units. Alkali hydrolysis of these teichoic acids gives glycosylglycerol phosphates (Burger & Glaser, 1966; Archibald & Coapes, 1971*b*). Similar products would also be formed on alkali hydrolysis of glycosylated (2 $\rightarrow$ 3)-poly(glycerol phosphate) teichoic acids and such teichoic acids have been reported to be present in walls of *Actinomyces antibioticus* 39 (Zaretskaya *et al.*, 1971) and *Bacillus stearothermophilus* B65 (Wicken, 1966). The major repeating structure in the teichoic acid from walls of *B. stearothermophilus* was shown to be 1-*O*- α -D-glucopyranosylglycerol phosphate. Since α -glucosyl moieties in a 2 $\rightarrow$ 3-linked poly(glycerol phosphate) teichoic acid are not involved in interunit linkages they should be available for interaction with concanavalin A (Archibald & Coapes, 1971*a*; Reeder & Ekstedt, 1971). In the course of further studies on the interaction of this lectin with teichoic acids and bacterial cell walls we have found that concanavalin does not agglutinate walls of *B. stearothermophilus* B65 nor does it form a precipitate with the isolated wall teichoic acid of this bacterium. We have therefore further examined the structure of this teichoic acid and have confirmed the earlier report (Wicken, 1966) that it contains 1-*O*- α -D-glucopyranosylglycerol units; however, the

glucosyl residues form part of the main polymer chain in which units are held together by phosphodiester linkages between glycerol and the 6 position of the glucosyl residue of the adjacent unit.

Materials and Methods

Materials

Enzymes, chemicals and reagents were purchased from the suppliers previously described (Archibald & Coapes, 1971*b*). Concanavalin A was that prepared previously (Archibald & Coapes, 1971*a*).

Methods

Analytical and chromatographic methods were those described previously (Archibald & Coapes, 1971*b*). The solvents used for paper chromatography were: A, propan-1-ol-aq. NH₃ (sp.gr. 0.88)-water (6:3:1, by vol.); B, ethyl acetate-pyridine-acetic acid-water (5:5:1:3, by vol.). Products were detected by using procedures previously described (Archibald & Coapes, 1971*b*). Electrophoresis was carried out on Whatman no. 1 paper by using pyridine-acetic acid-water (10:4:186, by vol.; pH 5.3) and a voltage gradient of 50 V/cm for 2 h.

For determination of radioactivity, samples were adjusted to 1.5 ml with water and mixed with scintillant (10 ml), composed of Triton X-100 (1 litre), toluene (2 litres), 2,5-diphenyloxazole (8 g) and 1,4-bis-(4-methyl-5-phenyloxazol-2-yl)benzene (0.2 g). Samples were counted for radioactivity in a Beckman LS-150 liquid-scintillation counter to a counting error of less than 2%. The counting efficiency, determined by using a [³H]lysine standard, was 25%.

Cultures of *B. stearothermophilus* B65 were obtained from Dr. K. C. Oo, Department of Biochemistry, University of Malaya, Kuala Lumpur, Malaya,

and Professor A. J. Wicken, School of Microbiology, University of New South Wales, Australia.

Experimental and Results

B. stearotheophilus B65, obtained as a spore suspension from Dr. Oo, was grown in batch culture at 55°C in a medium similar to that of Forrester & Wicken (1966) and consisting of 1% (w/v) peptone (Oxoid Ltd., London E.C.4, U.K.), 0.5% (w/v) yeast extract (Difco Laboratories, West Molesey, Surrey, U.K.), 0.25% (w/v) K_2HPO_4 , 0.2% (w/v) glucose and 0.1% (v/v) salts solution [containing 0.5% (w/v) $FeCl_3$, 0.5% (w/v) $CaCl_2$ and 0.5% (w/v) $MgSO_4 \cdot 7H_2O$].

Batches (10 litres), inoculated with an 18 h culture (500 ml) were aerated (10 litres of air/min) and stirred (300 rev./min) in a New Brunswick Fermentor. Towards the end of exponential growth, the culture was pumped into ice and then harvested by continuous centrifugation in an Alfa-Laval Lab. 102B-24 separator at a flow rate of 500 ml/min; the cell paste so obtained was freeze-dried.

Cells were disrupted by shaking a suspension (30% dry wt./vol. in cold 0.9% NaCl) with an equal volume of no. 11 Ballotini beads for 2.5 min in a Braun M.S.K. homogenizer with cooling. Walls were separated from intact cells by centrifugation, suspended in boiling water for 10 min to inactivate autolytic enzymes and then digested with trypsin as described by Archibald & Stafford (1972). The walls were recovered by centrifugation and washed with 0.9% NaCl (four times), followed by water (four times) and then freeze-dried. These walls contained 5.3% P.

Interaction with concanavalin A

Agglutination studies were performed as previously described (Archibald & Coapes, 1971a) but in a buffer of 0.05 M-Tris-HCl, pH 7.3, instead of sodium acetate. Suspensions of *B. stearotheophilus* walls (3 mg/ml; 0.2 ml) were mixed with a solution (5 mg/ml; 0.2 ml) of concanavalin A and the mixture was examined at intervals for agglutination, by using a control without concanavalin for comparison. No agglutination was observed after incubation for several hours at room temperature (20°C). Cell walls of *Lactobacillus plantarum* C106, *Bacillus subtilis* 3610 and *B. subtilis* W23 were also examined as controls. Walls of *B. subtilis* W23 were not agglutinated, but those of *L. plantarum* and *B. subtilis* 3610 rapidly agglutinated and settled and this reaction was shown to be specific by the absence of agglutination when concanavalin was equilibrated with α -methyl mannoside (10 mg/ml) before addition of the wall suspensions.

Samples of teichoic acid were prepared by extraction of walls with dilute alkali (Archibald *et al.*, 1969)

and by dissolution of walls by incubation with lysozyme (Doyle & Birdsall, 1972). The interactions of these samples with concanavalin A (all at concentrations of 5–10 mg/ml) were examined in agarose gels in 0.05 M-Tris-HCl buffer, pH 7.3 (Duckworth *et al.*, 1972). No precipitin lines were observed with teichoic acid preparations from *B. stearotheophilus* B65, whereas distinct lines were formed with teichoic acids isolated from walls of *B. subtilis* 3610 and *L. plantarum* C106.

Extraction of teichoic acid

Cell walls (1 g) were stirred with 0.5 M-NaOH (40 ml) for 1 h at 25°C. The suspension was then centrifuged for 20 min at 17000g and the supernatant solution was passed through a column (20 mm $\times$ 300 mm) of Dowex 50 (NH_4^+ form) resin. The column eluate was rotary evaporated and the crude teichoic acid was freeze-dried. This procedure was found to extract about 75% of the wall P and gave 250 mg of crude teichoic acid. This alkali-extracted teichoic acid (300 mg) was dissolved in water (100 ml) and applied to a column (30 mm $\times$ 500 mm) of DE 32 DEAE-cellulose (HCO_3^- form). After washing with water to remove neutral material, phosphates were eluted with a linear gradient of 0–0.5 M- $(NH_4)_2CO_3$ (700 ml total volume). A single phosphate-containing peak was eluted in 0.35–0.44 M- $(NH_4)_2CO_3$; fractions containing this material were combined, rotary-evaporated to remove $(NH_4)_2CO_3$ and then freeze-dried.

Purified teichoic acid contained 9.25% P, and about 2.2% of this P was present as monoester (determined by incubation with phosphatase). The molar proportions of P, glycerol, reducing sugar and D-glucose present in a sample which had been hydrolysed in 1 M-HCl for 3 h at 100°C were 1:0.98:0.72:0.68.

Alkali hydrolysis

Teichoic acid (40 mg) was hydrolysed at 100°C for 3 h in 1 M-NaOH (3 ml). The hydrolysate as passed through a column (15 mm $\times$ 90 mm) of Dowex 50 (NH_4^+ form) resin and the eluate was rotary-evaporated to dryness. The residue was dissolved in water (50 ml) and applied to a column (15 mm $\times$ 170 mm) of DE 52 DEAE-cellulose (HCO_3^- form). After being washed with water (200 ml) to remove neutral products, the column was eluted with a linear gradient of 0–0.5 M- $(NH_4)_2CO_3$ (total volume 500 ml). Three phosphate peaks, which were eluted at 0.1, 0.18 and 0.21 M- $(NH_4)_2CO_3$, contained respectively 89.6, 8.7 and 1.7% of the total phosphate eluted from the column. Fractions constituting each peak were combined and rotary-evaporated to remove $(NH_4)_2CO_3$. The components present in each fraction were identified as follows.

Fraction 1: monophosphates. 1-O- α -D-Glucopyranosylglycerol monophosphate and glycerol

monophosphate. Chromatography in solvent A revealed two phosphates (R_{glycerol} 0.25 and 0.33). These gave, respectively, a blue colour slowly and a purple colour rapidly with the periodate-Schiff reagent, and the latter phosphate co-chromatographed with glycerol monophosphate. Dephosphorylation gave glucosylglycerol, which was subsequently characterized, and a little glycerol. Electrophoresis of fraction (1) showed phosphates with the mobility of glycerol monophosphate ($m_{\text{glycerol diphosphate}}$ 0.74) and a slower-moving component ($m_{\text{glycerol diphosphate}}$ 0.48).

Fraction 2: diphosphates. Glycerol diphosphates and 1-*O*- α -D-glucopyranosylglycerol diphosphates. These components were not separated by chromatography in solvent A, but electrophoresis gave two phosphates ($m_{\text{glycerol diphosphate}}$ 1.0 and 0.75). No colour was produced with periodate-Schiff reagent, and dephosphorylation gave glycerol and the same glucosylglycerol which was obtained on dephosphorylation of fraction 1. No organic phosphate was detected after dephosphorylation. The components of fraction 2 were separated by preparative electrophoresis on acid-washed (2M-acetic acid) Whatman no. 1 paper, the compounds being eluted from the paper with water. The compound with the mobility of glycerol diphosphate gave glycerol and P_i on enzymic dephosphorylation and was unaffected by acid hydrolysis. The compound with the lower mobility was hydrolysed in 1M-HCl at 100°C for 2h and gave products which had the electrophoretic mobilities of glycerol monophosphate and glucose 6-phosphate; no glycerol diphosphates were detected.

Fraction 3: diglycerol triphosphate. This component could not be examined extensively owing to the small quantity of material available. Chromatography in solvent A revealed a phosphate which gave no colour with periodate-Schiff reagents. Enzymic dephosphorylation gave a single product which had the same chromatographic (solvent A) and electrophoretic mobility as diglycerol phosphate and which gave a colour reaction with the periodate-Schiff spray characteristic of compounds which produce formaldehyde on oxidation.

Isolation of 1-O- α -D-glucopyranosylglycerol

Teichoic acid (60mg) was hydrolysed in 1M-NaOH (5ml) for 3h at 100°C. The hydrolysate was passed through a column of Dowex 50 (NH_4^+ form) resin, and the eluate was rotary evaporated to dryness. The residue was dissolved in 0.05M- $(\text{NH}_4)_2\text{CO}_3$ (50ml) containing calf intestinal phosphatase (2.5mg) and incubated for 24h at 37°C. After removal of $(\text{NH}_4)_2\text{CO}_3$ by repeated rotary evaporation, the residue was dissolved in CO_2 -free water (2.5ml) and applied to a column (15mm $\times$ 400mm) of Dowex 1 (OH^- form) resin, prepared as described by Austin *et al.* (1963). The column was eluted with

CO_2 -free water (400ml), and fractions (5ml) were collected. Glycerol (fractions 15–21, 25 μmol) and glucosylglycerol (fractions 34–41, 130 μmol , calculated as hexose) were detected. The glucosylglycerol had similar chromatographic properties to an authentic sample of 1-*O*- α -D-glucopyranosylglycerol and on acid hydrolysis gave equimolar proportions of glucose and glycerol. The glucosylglycerol had $[\alpha]_D^{25} + 112^\circ$, and on oxidation with sodium periodate 3.2 molar proportions of periodate were reduced and 0.96 molar proportion of formaldehyde was formed. These values are closely similar to those previously obtained with the 1-*O*- α -D-glucopyranosylglycerol isolated from the wall teichoic acid of *L. plantarum* C106 (Adams *et al.*, 1969; Archibald & Coapes, 1971b).

Location of the phosphodiester residue

(a) **Periodate oxidation.** Teichoic acid (10mg) was dissolved in 25mM-sodium periodate (4ml), and the solution was incubated at room temperature (20°C) in the dark. The reaction was followed as previously described (Archibald & Coapes, 1971b). Oxidation was complete after 72h, when 1.9mol of periodate was reduced per mol of P and 0.033 molar proportion of formaldehyde was formed. Excess of periodate was destroyed by addition of ethylene glycol (0.1mmol) and the solution was incubated for a further 3h at room temperature and then freeze-dried.

(b) **Action of base on oxidized teichoic acid.** Periodate-oxidized teichoic acid (8mg) and unoxidized teichoic acid (8mg) were separately dissolved in 0.25M-glycine-NaOH buffer, pH 10.5 (8ml), and incubated at room temperature for 5h (Archibald & Coapes, 1971b), when 77% of the P in the oxidized teichoic acid was present as monoester, whereas only 3% of the P in the unoxidized teichoic acid was monoester.

(c) **Reduction with KB^3H_4 .** Oxidized teichoic acid (10mg) was desalted by passage through a column (15mm $\times$ 400mm) of Sephadex G-25. Elution with water gave a single peak of phosphate-containing material which was eluted in the void volume. This material was adjusted to 4ml, mixed with KB^3H_4 (0.5M; 1ml: 1.5mCi) and left at room temperature for 20min. The solution was then dialysed against water for 5h and the non-diffusible material was freeze-dried and desalted, as before, on Sephadex G-25. All of the radioactivity was eluted in the void volume and this material was hydrolysed in 1M-HCl as previously described (Archibald & Coapes, 1971b). After removal of acid *in vacuo* over KOH pellets, the hydrolysate was applied to a column (8mm $\times$ 50mm) of DE 52 DEAE-cellulose (acetate form) and neutral products were eluted with water (20ml). Phosphates were eluted with a linear gradient (total volume 200ml) of 0–0.5M-pyridinium acetate, pH 5.3. A single peak of phosphate containing

almost all of the radioactivity was eluted in 0.05–0.07M-pyridinium acetate and fractions containing this material were combined and rotary-evaporated to remove buffer. On chromatography in solvents A and B the major radioactive component had the same chromatographic mobility as glycerol monophosphate. The material gave a purple spot rapidly with the periodate–Schiff reagent and on enzymic dephosphorylation yielded a radioactive compound with the chromatographic properties of glycerol. To determine the radioactivity of the KB^3H_4 used in the reduction, a sample of glucose 6-phosphate (20mg) was reduced with this KB^3H_4 solution (1 ml) at the same time. The reaction was allowed to proceed at room temperature for 20 min and then the excess of KB^3H_4 was destroyed by addition of 1 M-acetic acid. After freeze-drying, the residue was applied to a column (10mm $\times$ 250mm) of DE 52 DEAE-cellulose (acetate form). Neutral products were eluted with water and phosphates were eluted with a linear gradient (total volume 300ml) of 0–0.5M-pyridinium acetate, pH 5.3. Fractions representing the single peak of radioactivity were combined and the buffer was removed by repeated rotary evaporation. The specific radioactivities of the various materials were as follows: glucitol 6-phosphate, 9.13×10^4 d.p.s./ μmol of P; oxidized and reduced teichoic acid, 1.42×10^5 d.p.s./ μmol of P; glycerol monophosphates, 4.95×10^4 d.p.s./ μmol of P.

In a further experiment teichoic acid (10mg) was oxidized and reduced with KB^3H_4 as before. Desalted material (2mg) was dissolved in 1 M-HCl (0.2ml) and the solution was incubated at 30°C for 1 h (Kennedy, 1974). After neutralization of acid by the addition of 1 M-NaOH (0.2ml) the hydrolysate was applied to a column (15 mm $\times$ 300mm) of Sephadex G-25 and the column was eluted with water. Only a small amount of polymer remained undegraded and the radioactive label was present in approximately equal amounts in the phosphate-containing and phosphate-free peaks (Fig. 1). Tubes containing radioactive phosphate included by the gel were combined and the material was examined by paper chromatography in solvents A and B. In each case a single spot was detected with periodate–Schiff reagent and this coincided with a standard of diglycerol phosphate. The specific radioactivities of the various materials were as follows: glucitol 6-phosphate, 5.16×10^4 d.p.s./ μmol of P; oxidized and reduced teichoic acid, 8.27×10^4 d.p.s./ μmol of P; diglycerol phosphate, 4.9×10^4 d.p.s./ μmol of P.

Examination of the wall teichoic acid present in bacteria grown from a second culture of B. stearotherophilus B65

Since the above results showed that the structure of the teichoic acid differed from that reported earlier (Wicken, 1966), a second culture of *B. stearothermo-*

Fig. 1. Chromatographic fractionation of the products of gentle acid hydrolysis of oxidized and reduced teichoic acid

For details see the text. ○, P; △, radioactivity.

philus was obtained. This was a freeze-dried culture, kindly given by Professor Wicken, which gave a pure culture of bacilli which had cellular and colonial morphology indistinguishable from that of the culture first examined. The bacteria were grown at 55°C in an orbital incubator shaking at 300 rev./min. Flasks (2 litres) containing the medium (500ml) described by Forrester & Wicken (1966) were inoculated with a culture (25ml) which had been grown under similar conditions. Bacteria were harvested at the end of exponential growth and walls were prepared as described above. The walls did not agglutinate with concanavalin A and the isolated teichoic acid (which contained P and glucose in the molar ratio 1:0.72) did not form a precipitate with the lectin. Hydrolysis with alkali and subsequent enzymic dephosphorylation gave glycerol and glucosylglycerol. The oxidized teichoic acid readily underwent β -elimination to give phosphomonoesters. After reduction with KB^3H_4 acid hydrolysis gave ^3H -labelled glycerol phosphates, as before. The results obtained were closely similar to those described above and show that both wall preparations contain poly(glucosylglycerol phosphate) teichoic acids in which units are held together by phosphate linkages between glycerol and the 6-hydroxyl group of the glucose residue of the adjacent unit.

Discussion

Concanavalin A has been shown to form precipitates with teichoic acids which contain α -D-glucopyranosyl or α -D-2-acetamido-2-deoxyglucopyranosyl substituents in which the hydroxyl groups at positions 3, 4 and 6 of the sugar are unsubstituted (Archibald & Coapes, 1971a; Reeder & Ekstedt, 1971; Doyle & Birdsell, 1972). Walls of *B.*

stearothermophilus B65 have been reported to contain a 2→3-linked poly(glycerol phosphate) teichoic acid in which α -D-glucopyranosyl substituents are attached to the 1 position of most of the glycerol residues (Wicken, 1966). This teichoic acid would therefore be expected to react specifically with concanavalin A. However, we have found that neither walls nor whole cells of *B. stearothermophilus* B65 are agglutinated with concanavalin A and that the isolated wall teichoic acid does not form a precipitate with this lectin.

Doyle & Birdsell (1972) have reported that the interaction between concanavalin A and the teichoic acid from walls of *B. subtilis* 168 is inhibited by the presence of NaCl, an effect which these authors suggest may be due to alterations in the conformation of the teichoic acid induced by the presence of salt. It therefore seemed possible that the failure of the teichoic acid from *B. stearothermophilus* B65 to interact with concanavalin A, in either the presence or the absence of NaCl, might be due to the adoption by 1→2-linked glycerol teichoic acids of a conformation which precluded interaction with the lectin. An alternative explanation, now shown to be correct, was that appropriate α -glucopyranosyl substituents were not present in the wall teichoic acid.

B. stearothermophilus B65 was obtained from Dr. K. C. Oo, who had also supplied the culture used by Dr. Kennedy in his study of the biosynthesis of the wall teichoic acids of this organism (Kennedy, 1974). The culture was obtained as a spore suspension, which on germination gave a pure culture of a thermophilic bacillus. The mean generation times of the bacteria grown at 37°C and 55°C as described by Forrester & Wicken (1966) were approx. 26 and 20 min, values similar to those of 32 and 18 min previously reported. Walls isolated from bacteria which had been grown at 55°C under conditions similar to those described by Wicken (1966) were similar in overall composition to those studied by that author. They contained the same major amino acids and a similar amount of P; the isolated teichoic acid had a similar ratio of glucose to P and the major products of alkali hydrolysis were the 1-O- α -D-glucopyranosylglycerol phosphates previously described. Alkali hydrolysis of isolated teichoic acid gave small amounts of glycerol and glucosylglycerol, together with a mixture of organic phosphates which was fractionated by ion-exchange chromatography. The major phosphate was 1-O- α -D-glucopyranosylglycerol monophosphate, but a small amount of a related diphosphate was also isolated. On acid hydrolysis this gave no glycerol diphosphate, but compounds having the chromatographic properties of glucose phosphate and glycerol monophosphate were produced. This diphosphate is thus similar to that previously described (Wicken, 1966) and consists of glucosylglycerol which carries phosphate groups

on both glucose and glycerol moieties. This compound could not arise by hydrolysis of glucosylated 2→3-poly(glycerol phosphate), but its formation, in small quantities, from poly(glucosylglycerol phosphate) may be explained either by hydrolysis through a possible cyclic intermediate involving the 4-hydroxyl group of glucose (see Archibald & Stafford, 1972) or by elimination of phosphate groups from glycerol through the intermediate formation of epoxides (Brown & Usher, 1963). Such processes might also explain the small amount of glucosylglycerol formed on alkali hydrolysis, though the presence in the chain of adjacent glycerol and glucosylglycerol units cannot be excluded. The other products of alkali hydrolysis were glycerol, its mono- and di-phosphates and a small amount of material which had the chromatographic properties of diglycerol phosphate (Kelemen & Baddiley, 1961). The formation of these products indicates that glycerol residues which are not substituted by glucose are present in chains of 1→3-linked glycerol phosphate residues, possibly present as a separate polymer. In the earlier study (Wicken, 1966) the main evidence that the teichoic acid was a glucosylated (2→3)-poly(glycerol phosphate) was the isolation from alkali-hydrolysed teichoic acid of a small amount of material which was identified as glucosyldiglycerol triphosphate. Such material was not detected in the present work.

Oxidation of the teichoic acid with sodium periodate resulted in the reduction of 1.9 mol of periodate/mol of phosphate. The oxidized teichoic acid was extensively depolymerized on incubation at room temperature at pH 10.5, whereas unoxidized teichoic acid was stable under these conditions. As previously discussed (Archibald & Coapes, 1971b) this behaviour is readily explained by β -elimination of phosphate from the 6 position of oxidized glucose residues in a structure of the type shown in Fig. 2. Confirmation of this structure was obtained by reduction of a sample of oxidized teichoic acid with KB^3H_4 . This resulted in the reduction of approximately two aldehydic groups per oxidized glucose residue. Acid hydrolysis of this material gave radioactive glycerol phosphate; this can arise only by reduction at C-4 of an oxidized glucosyl residue which carries a phosphate group at C-6, and so its formation confirms the presence of glucose as an integral component of the chain as shown in Fig. 2. After gentle acid hydrolysis most of the P was present as diglycerol phosphate, the specific radioactivity of which showed that it contained equal amounts of glycerol derived from that originally present and derived from C-4, C-5 and C-6 of glucose. The small amount of P not converted into diglycerol phosphate presumably includes the 1→3-linked chains of glycerol phosphate which are also present in the walls.

It is clear from these results that the major teichoic acid component of the wall is a poly(glucosylglycerol

Fig. 2. Location of the phosphodiester linkage between repeating structures in *B. stearothermophilus* wall teichoic acid

phosphate). The chemical data do not exclude the presence of very small amounts of α -glucosylated (2 $\rightarrow$ 3)-poly(glycerol phosphate), although the absence of agglutination with concanavalin A suggests that the walls do not contain any appreciable amount of such teichoic acid.

These findings thus differ from those of Wicken (1966) and are also in contrast with results obtained in a biosynthetic study (Kennedy, 1974) which showed that incubation of a particulate enzyme preparation from *B. stearothermophilus* B65 with CDP-glycerol and UDP-glucose led to incorporation of 1-*O*- α -D-glucopyranosylglycerol phosphate into a high-molecular-weight polymer. It was shown that the incorporated glucose was not linked directly to phosphate and that it could be removed by selective degradation without depolymerizing the synthesized material. The results obtained did not show that the incorporated glucosylglycerol was doubly linked through phosphate and so did not strictly show that (2 $\rightarrow$ 3)-poly(glycerol phosphate) was formed. However, it is difficult to envisage what else the synthesized material could have been, and so this study reinforces the earlier work (Wicken, 1966) in indicating that *B. stearothermophilus* B65 can synthesize glucosylated (1 $\rightarrow$ 2)-poly(glycerol phosphate). Such teichoic acid was not present in walls of this bacterium grown from either of the cultures that we examined. The bacteria were grown under conditions similar to those used by Wicken (1966) and it seems unlikely that any

slight difference in these conditions could result in such a major alteration in the structural organization of the wall teichoic acid. It is clear that the walls are closely similar in overall composition to those studied earlier (Wicken, 1966). The reason for the apparent major difference in the structural organization of the teichoic acid is obscure, though it may be a reflexion of some unknown alteration in the culture since the earlier work was carried out.

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References

- Adams, J. B., Archibald, A. R., Baddiley, J., Coapes, H. E. & Davison, A. L. (1969) *Biochem. J.* **113**, 191–193
- Archibald, A. R. (1974) *Adv. Microb. Physiol.* **11**, 53–95
- Archibald, A. R. & Coapes, H. E. (1971a) *Biochem. J.* **123**, 665–667
- Archibald, A. R. & Coapes, H. E. (1971b) *Biochem. J.* **124**, 449–460
- Archibald, A. R. & Stafford, G. H. (1972) *Biochem. J.* **130**, 681–690
- Archibald, A. R., Coapes, H. E. & Stafford, G. H. (1969) *Biochem. J.* **113**, 899–900
- Austin, P. W., Hardy, F. E., Buchanan, J. G. & Baddiley, J. (1963) *J. Chem. Soc. London* 5350–5353
- Baddiley, J. (1972) *Essays Biochem.* **8**, 35–77
- Brown, D. M. & Usher, D. A. (1963) *Proc. Chem. Soc. London* 309–310
- Burger, M. M. & Glaser, L. (1966) *J. Biol. Chem.* **241**, 494–506
- Doyle, R. J. & Birdsell, D. C. (1972) *J. Bacteriol.* **109**, 652–658
- Duckworth, M., Archibald, A. R. & Baddiley, J. (1972) *Biochem. J.* **130**, 691–696
- Forrester, I. T. & Wicken, A. J. (1966) *J. Gen. Microbiol.* **42**, 147–154
- Kelemen, M. V. & Baddiley, J. (1961) *Biochem. J.* **80**, 246–254
- Kennedy, L. D. (1974) *Biochem. J.* **138**, 525–535
- Reeder, W. J. & Ekstedt, R. D. (1971) *J. Immunol.* **106**, 334–340
- Wicken, A. J. (1966) *Biochem. J.* **99**, 108–116
- Zaretskaya, M. Z., Naumova, I. B. & Shabarova, Z. A. (1971) *Biokhimiya* **36**, 97–107