Teichoic Acids Possessing Phosphate-Sugar Linkages in Strains of *Lactobacillus plantarum*

By J. B. ADAMS, A. R. ARCHIBALD, J. BADDILEY, HILARY E. COAPES AND A. L. DAVISON

Microbiological Chemistry Research Laboratory, Department of Organic Chemistry,
University of Newcastle upon Tyne, NE1 7RU

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Cell walls of strains of *Lactobacillus plantarum* lacking the group D precipitinogen (a glucosylribitol teichoic acid) contain glucosylglycerol teichoic acid in which the glycosidic substituents are attached to the primary hydroxyl group of glycerol. Three distinct repeating units have been isolated from the teichoic acid preparation of strain C106, indicating either that the polymer is complex or that the wall contains a mixture of teichoic acids. Walls of streptobacteria differ from those of *L. plantarum* and contain neither teichoic acid nor diaminopimelic acid.

The taxonomy of the lactobacilli is well developed (Sharpe, 1962). The cell walls of many species contain teichoic acids (Ikawa & Snell, 1960; Archibald, Baddiley & Buchanan, 1961; Baddiley & Davison, 1961) and the group A, D and E precipitinogens have been identified as teichoic acids (Sharpe, Davison & Baddiley, 1964). Strains of *Lactobacillus plantarum* lacking the group D precipitinogen normally found in this species, and streptobacteria differing from both *L. casei* and *L. plantarum*, have been encountered (Naylor & Sharpe, 1958*a,b*; Sharpe, 1957). The present paper compares their cell-wall composition with that of strains of *L. plantarum* previously studied and describes the properties and general nature of the wall teichoic acid of *L. plantarum* C106.

METHODS

Organisms. *Lactobacillus plantarum* N.I.R.D. A164, AB41, C106 and unclassified streptobacteria N.I.R.D. A41, A101 and D45 were obtained from Dr M. E. Sharpe. Intestinal phosphomonoesterase was purchased from Sigma Chemical Co. (St Louis, Mo., U.S.A.) and α -glucosidase from Koch-Light Laboratories Ltd. (Colnbrook, Bucks.). Methods for the determination of total hexose, D-glucose, D-alanine, total and inorganic phosphate, glycerol, reduction of periodate, production of formaldehyde, enzymic hydrolysis of phosphomonoesters, potentiometric titration and paper chromatography are as previously described (Archibald, Baddiley & Button, 1968*a*; Archibald, Baddiley & Shaukat, 1968*b*).

Lactobacilli were grown at 30° for 16 hr. in a liquid medium (181.) with the following composition: tryptone (Oxoid), 20 g.; glucose (AnalaR), 20 g.; sodium acetate (AnalaR) 10 g.; yeast extract (Difco), 5 g.; K₂HPO₄ (AnalaR), 5 g.; inorganic salts B (Barton-Wright, 1946), 5 ml.; demineralized water, 1 l. Cell walls were prepared as previously described (Davison & Baddiley, 1963) and

teichoic acids were isolated by extraction with cold trichloroacetic acid. Samples of teichoic acid were hydrolysed in M-HCl or M-NaOH at 100° for 3 hr. and products were examined by paper chromatography in solvent A.

Paper chromatography. Whatman no. 1 paper, previously washed with 2M-acetic acid solution and then with water, was used for descending chromatography. The following solvent systems were employed: A, butan-1-ol-ethanol-aq.NH₃ (sp.gr. 0.88)-water (40:10:1:49, by vol.); B, butan-1-ol-pyridine-water (6:4:3, by vol.).

Products were detected by spray reagents previously described (Archibald *et al.* 1968*b*).

RESULTS

Streptobacteria strains A41, A101 and D45. Walls of these strains contained no teichoic acid or diaminopimelic acid and thus differ in these respects from those of *L. plantarum*.

L. plantarum A164. Walls of this group D strain possessed a glucosylribitol teichoic acid indistinguishable from that of *L. plantarum* 17-5 (Archibald *et al.* 1961). It contained ribitol, monoglucosylribitol and diglucosylribitol [R_{ribitol} 1.0, 0.5 and 0.25 in solvent A]; unlike the walls of strain 17-5, however, no rhamnose-containing polysaccharide (Ikawa & Snell, 1960; Archibald *et al.* 1961) was present in the walls of this strain.

L. plantarum AB41. Walls of this strain contained a glucosylglycerol teichoic acid. On hydrolysis in acid or alkali, followed by enzymic dephosphorylation, the teichoic acid gave products chromatographically identical with those obtained by similar hydrolysis of the teichoic acid of walls of strain C106.

L. plantarum C106. Walls of this strain contained 2.5% (w/w) of phosphorus corresponding to

approximately 45% (w/w) of teichoic acid, the molecular proportions phosphate: muramic acid: glucosamine: glutamic acid: diaminopimelic acid: alanine (total): alanine (ester-linked) in the wall being 1.8:1.2:1.1:1.1:3.0:7. The ester-linked alanine had the D-configuration and was attached to the teichoic acid. Samples of teichoic acid, $[\alpha]_D + 104^\circ$ (c 1.2% in water), isolated by extraction with cold trichloroacetic acid solution contained 5.6% of phosphorus, the molecular proportions phosphate: D-glucose: D-alanine: glycerol being 1.1:78.0:6.0:95. Treatment with phosphomonoesterase and potentiometric titration showed that 95% of the phosphate represents phosphodiester linkages, the remainder representing terminal phosphomonoester groups. Acid hydrolysis gave alanine, glucose and glycerol monophosphates, together with a smaller amount of a reducing phosphate that gave glucose on enzymic dephosphorylation.

The products obtained by hydrolysis of the teichoic acid in alkali, followed by enzymic dephosphorylation, also differed from those previously obtained from teichoic acids of *Lactobacilli*. Three products were isolated by chromatography on Dowex 1 (OH⁻ form) resin (Austin, Hardy, Buchanan & Baddiley, 1963). Compounds I, II and III had R_{glucose} 0.93, 0.48 and 0.74 (solvent B) respectively and gave the fast-developing purple colour with the periodate-Schiff spray reagents, characteristic of compounds that give formaldehyde on oxidation with periodate. All three were hydrolysed to glucose and glycerol by acid and by α -glucosidase.

Glucoside I, $[\alpha]_D + 111^\circ$ (c 0.81% in water), (molecular ratio D-glucose:glycerol, 1:1) was chromatographically identical with synthetic α -D-glucopyranosyl-(1 $\rightarrow$ 1)-glycerol (Hardy, 1961; kindly supplied by Dr P. W. Austin), and on oxidation with sodium metaperiodate it reduced 3 mol.prop. of oxidant and gave 1 mol.prop. of formaldehyde. Reduction of the product with sodium borohydride, and subsequent acid hydrolysis, gave glycerol and ethylene glycol thus confirming its structure.

Glucoside II, $[\alpha]_D + 145^\circ$ (c 0.89% in water), (molecular ratio D-glucose:glycerol, 1.95:1) reduced 3.94 mol.prop. of periodate and gave 0.98 mol.prop. of formaldehyde; the oxidation product gave glycerol and ethylene glycol after subsequent reduction and acid hydrolysis. Glucoside II is therefore α -D-glucopyranosyl-(1 $\rightarrow$ 2)- α -D-glucopyranosyl-(1 $\rightarrow$ 1)-glycerol.

The isomeric diglucoside III, $[\alpha]_D + 141^\circ$ (c 0.74% in water) (molecular ratio D-glucose:glycerol, 1.95:1) reduced 2.9 mol.prop. of periodate and gave 0.9 mol.prop. of formaldehyde; the reduction product gave glycerol, ethylene glycol and glucose on subsequent reduction and acid hydrolysis. This

glucoside is therefore α -D-glucopyranosyl-(1 $\rightarrow$ 3)- α -D-glucopyranosyl-(1 $\rightarrow$ 1)-glycerol.

The diglucosides were formed in approximately equal proportions and the monoglucoside was produced in smaller amounts.

DISCUSSION

The group-specific precipitinogen of the species *Lactobacillus plantarum* is a glucosylribitol teichoic acid in the wall (Sharpe *et al.*, 1964; Archibald *et al.*, 1961). Walls of *L. plantarum* A164 (group D) have now been found to contain a ribitol teichoic acid similar to or identical with those of strains 17-5 and N.C.T.C. 7220 (Archibald *et al.* 1961; Sharpe *et al.* 1964), but do not contain the rhamnose polysaccharide that occurs in addition to teichoic acid in the walls of strain 17-5 (Ikawa & Snell, 1960; Archibald *et al.* 1961).

Walls of two strains of *L. plantarum* lacking the group D precipitinogen contain glycerol teichoic acids differing in structure from teichoic acids previously found in lactobacilli. Although alanine, glucose and glycerol monophosphates were formed on acid hydrolysis of these teichoic acids, no glycerol or its diphosphates (normally found in similar proportions to the monophosphates) were detected. Moreover, the quantitative formation of a mixture of isomeric glucosylglycerol phosphates confirms that the teichoic acid cannot have a (1 $\rightarrow$ 3)-linked poly(glycerol phosphate) chain (cf. Archibald & Baddiley, 1966). The enzymic dephosphorylation of these phosphates gave a mixture of a monoglucosylglycerol and two isomeric diglucosylglycerols in which it was shown that glucose is attached to the primary hydroxyl group of glycerol. It is not yet established whether these three components occur in separate polymer chains or in a mixed polymer.

These observations indicate that the teichoic acid is either a glucosylated (1 $\rightarrow$ 2)-linked poly(glycerol phosphate) (Wicken, 1966; Naumova & Zaretskaya, 1964) or is a poly(glycosylglycerol phosphate) of the type found by Burger & Glaser (1966) in walls of a strain of *Bacillus subtilis*. Both types of structure would hydrolyse in alkali to products of the kind

Fig. 1. Suggested structure of the wall teichoic acid (alanine ester omitted) in *Lactobacillus plantarum* C106.

formed from *L. plantarum* C106. However, in the present case the absence of glycerol diphosphates and the presence of a glucose phosphate in acid hydrolysates indicates that the phosphodiester linkages connect glycerol to glucose. A partial structure of the polymer is given in Fig. 1.

The teichoic acid from strain C106 thus differs fundamentally in structure from those of strains of *L. plantarum* containing the group D precipitinogen, and might be expected to possess an immunochemical specificity quite distinct from those previously studied (Sharpe *et al.* 1964; Oeding, Myklestad & Davison, 1967).

Although it is now apparent that different strains of *L. plantarum* may possess widely different wall composition, as is shown by the structure of their teichoic acids, it is noteworthy that all strains examined possess diaminopimelic acid, presumably as a component of their peptidoglycan (Cummins & Harris, 1956; Ikawa & Snell, 1960); the strains C106, A164 and AB41 are no exceptions to this rule. On the other hand, walls of streptobacteria that differ biochemically from *L. plantarum* contain neither teichoic acid nor diaminopimelic acid. Clearly, the species *L. plantarum* as presently constituted is not entirely homogeneous and contains some immunochemically distinct strains with unusual glycerol teichoic acids.

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