The Distribution of the Glucosyl Substituents along the Chain of the Teichoic Acid in Walls of *Lactobacillus buchneri* N.C.I.B. 8007

By A. R. ARCHIBALD, J. BADDILEY and S. HEPTINSTALL
Microbiological Chemistry Research Laboratory, Department of Organic Chemistry,
University of Newcastle upon Tyne, NE1 7RU

(Received 17 October 1968)

Studies on the structures of teichoic acids that had been extracted with cold trichloroacetic acid from walls and membranes of Gram-positive bacteria revealed considerable differences in the nature and extent of the glycosidic substitution of the polymers (cf. Archibald & Baddiley, 1966). Since, under careful conditions of extraction, teichoic acids have been isolated in which every alditol unit bears a glycosidic substituent (Armstrong, Baddiley & Buchanan, 1960; Wicken & Baddiley, 1963), it was believed that the lesser extent of substitution found in several other teichoic acids (Ellwood, Kelemen & Baddiley, 1963; Critchley, Archibald & Baddiley, 1962; Shaw & Baddiley, 1964) is not a consequence of loss of sugar during extraction. Degradative studies on the latter teichoic acid preparations showed that they consist of chains of poly(glycerol phosphate) in which both glycerol residues and glycosylglycerol residues occur together in the same chain. In contrast with this finding, studies on other teichoic acid preparations by immunological techniques and biosynthetic arguments have shown them to consist of mixtures of fully glucosylated and glucose-free poly(alditol phosphate) (Glaser & Burger, 1964; Hughes, 1965; Chin, Burger & Glaser, 1966; Burger & Glaser, 1966). Further, the α - and β -N-acetylglucosaminyl substituents of the wall teichoic acid of *Staphylococcus aureus* (Baddiley, Buchanan, RajBhandary & Sanderson, 1962) are present on separate chains (Torii, Kabat & Beyer, 1964).

Fully glycosylated (1 $\rightarrow$ 3)-linked glycerol teichoic acids, such as those present in the membrane of group D streptococci (Wicken & Baddiley, 1963) and the wall of *Bacillus subtilis* N.C.T.C. 3610 (Glaser & Burger, 1964), are stable towards alkali since they possess no free hydroxyl groups adjacent to phosphate. Hydrolysis of phosphodiester in alkali requires the presence of such hydroxyl groups and normally proceeds through the formation of intermediate cyclic phosphates. The action of alkali on a mixture of fully glycosylated and glycosyl-free chains of (1 $\rightarrow$ 3)-poly(glycerol phosphate) would therefore cause degradation of the unsubstituted teichoic acid chains (cf. Kelemen & Baddiley, 1961) but would not affect the sub-

stituted chains (cf. Wicken & Baddiley, 1963). Thus the membrane teichoic acid of *B. subtilis* N.C.T.C. 3610 is a mixture of glucosylated and glucose-free (1 $\rightarrow$ 3)-poly(glycerol phosphate) and the former is stable towards alkali. The formation of glycosylglycerol by the action of alkali on such teichoic acids can occur only when the glycosylglycerol unit is flanked by unsubstituted glycerol phosphate residues. The production of substantial proportions of glycosylglycerol on alkali hydrolysis of the wall teichoic acid of *Staphylococcus albus* N.T.C.C. 7944 (Ellwood, Kelemen & Baddiley, 1963) and the membrane teichoic acids of *Lactobacillus arabinosus* 17-5 (Critchley, Archibald & Baddiley, 1962) and *Staphylococcus aureus* H (RajBhandary & Baddiley, 1963), showed that these teichoic acid preparations contain poly-(glycerol phosphate) chains in which only a few of the glycerol residues are glycosylated.

We were fortunate in obtaining a small sample of walls of *Lactobacillus buchneri* (N.C.I.B. 8007) from the batch used for earlier structural studies (Shaw & Baddiley, 1964). The teichoic acid (molecular ratio phosphorus:glucose 1:0.26) had previously been isolated by extraction with trichloroacetic acid. Substantial proportions of 2-O- α -D-glucopyranosylglycerol had been obtained by hydrolysis of the material with alkali, thus indicating the occurrence of adjacent substituted and unsubstituted glycerol phosphate residues in the polymer chain. Further evidence supporting this was obtained by isolation from the hydrolysate of a phosphodiester containing two glycerol residues, only one of which possessed a glycosyl substituent. Although these observations established that the material examined was a partially glucosylated poly(glycerol phosphate), it remained possible that this had arisen from a fully substituted teichoic acid by loss of glucose during the extraction with trichloroacetic acid. In the present study teichoic acid was released from the walls by heating in 0.1 N-NaOH at 100° for 1 hr., after which 99% of the total phosphate was in solution. After removal of the remaining walls by centrifugation, and further hydrolysis by N-NaOH at 100° for 3 hr. the molecular ratio phosphate:hexose in the extract was 1:0.26; this

is the same as the value found earlier for the material that had been extracted with trichloroacetic acid. It is noteworthy that teichoic acids prepared by acid extraction from *B. subtilis* W23 and *Bacillus licheniformis* N.C.T.C. 6346 possess much higher molecular glucose:ribitol ratios than do the walls themselves. In these two organisms the walls contain mixtures of fully glucosylated and glucose-free ribitol teichoic acid. In the present case, however, no enrichment of glucose was found in teichoic acid prepared by extraction with trichloroacetic acid; this would be consistent with the conclusion from the chemical evidence discussed below that the teichoic acid is partly glucosylated.

The neutral products and phosphates formed by the action of alkali were separated by passing the hydrolysate through a column of Dowex 50 (NH_4^+ form) resin, evaporation to small volume and chromatography on a column of Dowex 2 (CO_3^{2-} form) resin, when 50% of the total hexose compounds were eluted with water. Adsorbed phosphates and glucose derivatives were recovered by elution with 2M- $(\text{NH}_4)_2\text{CO}_3$; this removed all of the phosphates and the rest of the hexose derivatives as a complex mixture that gave on paper chromatography in propan-1-ol-aq. NH_3 (sp.gr. 0.88)-water (6:3:1, by vol.) several spots with mobilities consistent with the phosphates previously characterized (Shaw & Baddiley, 1964). Chromatographic examination in butan-1-ol-ethanol-water-aq. NH_3 (sp.gr. 0.88) (40:10:49:1, by vol.; upper phase) of the aqueous extract showed it to contain glycerol and a glucosylglycerol. The latter had the chromatographic mobility ($R_{\text{glucose}} 1.1$) of the previously characterized 2-O- α -D-glucopyranosylglycerol and gave the same colour with the periodate-Schiff spray reagents. A sample of the glucoside was isolated by preparative paper chromatography and then hydrolysed with 2N-HCl for 3 hr. at 100°, when glucose and glycerol (identified by paper chromatography with the periodate-Schiff and AgNO_3 spray reagents) were the only products. The amount of glycerol formed (visual estimation from colour on paper chromatograms) was much less than the amount of free glycerol present on the preparative chromatogram, as would be expected if glycerol and glucosylglycerol were present in the approximate molecular ratio 4:1.

These results show that formation of glucosylglycerol on alkali hydrolysis of the *L. buchneri*

teichoic acid does not depend upon prior treatment with acid. The amount of glucosylglycerol formed (corresponding to 50% of the total hexose in the extract) is about that expected from hydrolysis of a randomly substituted polymer containing glucose and phosphate in the molecular ratio 1:4, so that it is clear that the teichoic acid present in isolated walls of this organism consists of partially glucosylated chains of poly(glycerol phosphate). It seems likely also that the partially substituted teichoic acids previously examined in this Laboratory have this type of structure.

It is not clear whether partially glucosylated chains are the direct product of biosynthesis or whether fully glucosylated chains are first synthesized, followed by loss of glucose during cell metabolism. It is most unlikely that enzymic loss of glucose residues occurred during the isolation of subcellular fragments, as the isolated teichoic acid contains a substantial proportion of D-alanyl ester residues attached to those glycerol residues not possessing a glucosyl substituent; it is improbable that loss of sugar during fractionation of the cell would be followed by introduction of alanine. In the wall teichoic acid from *Staphylococcus albus*, apparently all of the glycerol residues that do not possess N-acetylgalactosaminyl substituents possess alanyl residues, so that in this case the above reasoning is even more conclusive.

This work was carried out during the tenure of a Science Research Council Studentship by S.H.

- Archibald, A. R. & Baddiley, J. (1966). *Advanc. Carbohydr. Chem.* **21**, 323.
- Armstrong, J. J., Baddiley, J. & Buchanan, J. G. (1960). *Biochem. J.* **76**, 610.
- Baddiley, J., Buchanan, J. G., RajBhandary, U. L. & Sanderson, A. R. (1962). *Biochem. J.* **82**, 439.
- Burger, M. M. & Glaser, L. (1966). *J. biol. Chem.* **241**, 494.
- Chin, T., Burger, M. M. & Glaser, L. (1966). *Arch. Biochem. Biophys.* **116**, 358.
- Critchley, P., Archibald, A. R. & Baddiley, J. (1962). *Biochem. J.* **85**, 420.
- Ellwood, D. C., Kelemen, M. V. & Baddiley, J. (1963). *Biochem. J.* **86**, 213.
- Glaser, L. & Burger, M. M. (1964). *J. biol. Chem.* **239**, 3187.
- Hughes, R. C. (1965). *Biochem. J.* **96**, 700.
- Kelemen, M. V. & Baddiley, J. (1961). *Biochem. J.* **80**, 246.
- RajBhandary, U. L. & Baddiley, J. (1963). *Biochem. J.* **87**, 429.
- Shaw, N. & Baddiley, J. (1964). *Biochem. J.* **93**, 317.
- Torii, M., Kabat, E. A. & Bezer, A. E. (1964). *J. exp. Med.* **120**, 13.
- Wicken, A. J. & Baddiley, J. (1963). *Biochem. J.* **87**, 54.