THE PRESENCE OF *N*-ACETYLGLUCOSAMINE 1-PHOSPHATE IN THE LINKAGE UNIT THAT CONNECTS TEICHOIC ACID TO PEPTIDOGLYCAN IN *STAPHYLOCOCCUS AUREUS*

John COLEY, A. Ronald ARCHIBALD and James BADDILEY

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

Received 19 May 1977

1. Introduction

We have shown [1,2] that the ribitol teichoic acid in *Staphylococcus aureus* H is held in the wall by attachment to an oligomer of three glycerol phosphate residues which is, in turn, covalently linked to peptidoglycan. Similar 'linkage units' are thought to occur in walls of other bacteria containing teichoic acid. The presence of such linkage units in *S. aureus* has been confirmed in biosynthetic studies [3,4] showing that the glycerol phosphate oligomer is synthesized from CDP–glycerol. However, synthesis of the linkage unit also requires the presence of UDP–*N*-acetylglucosamine and is inhibited by tunicamycin [5,6]. Together with the finding that the phosphate moiety of the muramic acid phosphate residues in *Bacillus licheniformis* is derived from UDP–*N*-acetylglucosamine [7] and not from CDP–glycerol, these observations suggest that the glycerol phosphate linkage unit might be attached to muramic acid through one or more *N*-acetylglucosamine 1-phosphate residues rather than directly through a phosphodiester linkage between glycerol and muramic acid. This possibility is supported by the demonstration of ordered sequence of incorporation of *N*-acetylglucosamine and glycerol phosphate from the appropriate nucleotides into wall [7] and lipid precursor of linkage unit [3,4]. We now report chemical evidence demonstrating the involvement of *N*-acetylglucosamine in the linkage between teichoic acid and peptidoglycan in a mutant of *S. aureus* H.

2. Methods

S. aureus H gol^{-1} ϕ^{R} 71, a mutant lacking the *N*-acetylglucosaminyl substituents that occur on the ribitol residues of the wall teichoic acid in the parent strain, was grown and walls were prepared as described previously [1]. Conditions for oxidation of the walls with sodium periodate, reduction with potassium borohydride and for fractionation and analysis of hydrolysis products were as described previously [1,2].

3. Results and discussion

Isolated walls contained 3.7% (w/w) phosphorus, most of which was present as the poly(ribitol phosphate) that has previously been shown to have a chain length of about 40 units [1]. After a procedure of oxidation and reduction which destroys the poly(ribitol phosphate), about 12% of the original phosphate remained associated with the wall residue; this corresponds to about four or five residual phosphates for each teichoic acid chain.

Treatment of the wall residue with 0.1 M HCl at 100°C for 7 min gave 73% of the residual phosphate as a soluble fraction which, when examined by paper electrophoresis at pH 5.3, migrated as a single component with $R_{\text{glycerol 1-phosphate}}$ 0.95. A maximum release of 80% of the phosphate was obtained after hydrolysis for 15 min, but the extracted material had undergone partial degradation, as shown by the presence of small amounts of glycerol mono- and

diphosphates on paper electrophoresis. The material with $R_{\text{glycerol 1-phosphate}}$ 0.95 was therefore isolated by hydrolysis of walls for 7 min followed by chromatography of the extract on DEAE-cellulose to remove possible traces of glycan that might have been solubilized. Hydrolysis of the isolated material in 2 M HCl 100°C for 3 h gave glycerol and its mono- and diphosphates, ethylene glycol phosphate and glucosamine. The identity of the glucosamine was confirmed by gas–liquid chromatographic analysis of its alditol acetate on a column of ECNSS-M. Enzymic dephosphorylation of the acid hydrolysate gave inorganic phosphate, glycerol, and glucosamine in the molar proportions 1 : 0.74 : 0.26. Examination of the products of reduction of the phosphate with potassium borohydride, followed by hydrolysis in 2 M HCl at 100°C for 3 h, showed that all of the glucosamine had been reduced; the presence of glucosaminitol phosphate showed that glucosamine was attached to glycerol through a phosphodiester bond. The chromogen formed on treatment of the intact phosphate with the Elson-Morgan reagents [8] had an absorption maximum at 530 nm, indicating [9] that the *N*-acetylglucosamine was substituted at its C-4 position. These results support the structure shown in fig.1. The ethylene glycol phosphate residue is derived from oxidation and reduction of the phosphate-terminal ribitol of the teichoic acid and is attached to a chain of three glycerol phosphate residues which is joined to the C-4 position of an *N*-acetylglucosamine residue. Since this product was isolated from walls that had been reduced with borohydride, it follows that the reducing group of the *N*-acetylglucosamine was liberated during the controlled acid hydrolysis; such lability suggests that the *N*-acetylglucosamine was present in the linkage unit as its 1-phosphate.

Treatment of the oxidized and reduced wall residue with 0.5 M NaOH at 22°C for 4 h released 75% of the phosphate as a soluble fraction which, on paper electrophoresis at pH 5.3, migrated as a single component with $R_{\text{glycerol 1-phosphate}}$ 1.35. This product had been isolated in our previous study [1] and was shown to be an oligomer of three glycerol phosphate residues attached to an ethylene glycol phosphate residue. We had earlier supposed that this oligomer was directly attached to muramic acid through a phosphodiester linkage, although we reported [1] that the amount of muramic acid phosphate formed on acid hydrolysis of walls was greater than would be expected for such a structure because hydrolysis would be likely to proceed preferentially by cyclization of the phosphate on to the glycerol moiety. Interposition of *N*-acetylglucosamine 1-phosphate between the glycerol phosphate oligomer and muramic acid, as shown in fig.2, does however give a structure from which high yields of muramic acid phosphate would be expected in view of the ready hydrolysis of the sugar 1-phosphate bond in acid. Phosphodiester linkages between glycerol and the C-4 position of *N*-acetylglucosamine are known to be particularly labile in alkali [10] so that the structure shown in fig.2 adequately accounts for the known lability of the linkage to both alkali and acid.

In the present work muramic acid phosphate was formed by vigorous acid hydrolysis (4 M HCl/100°C) of the wall residue after either alkali or acid treatment.

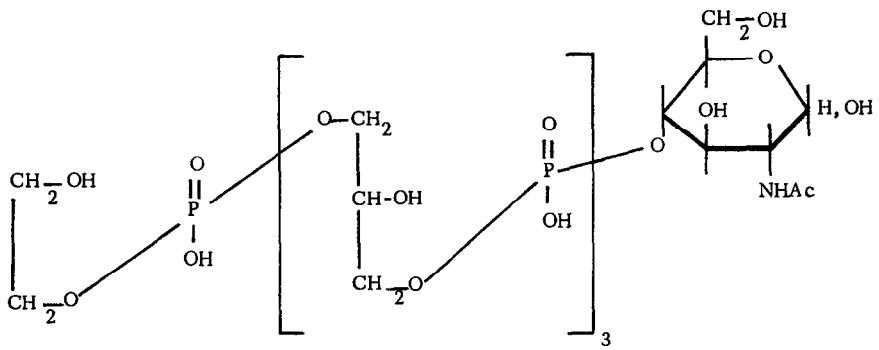

Fig.1. Product isolated after mild acid extraction of oxidized and reduced cell walls from *S. aureus* (mutant).

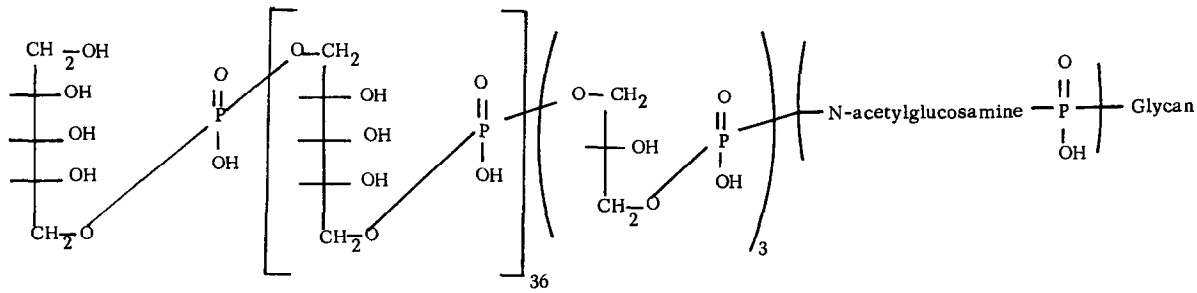

Fig.2. Scheme for the linkage of ribitol teichoic acid to peptidoglycan in a mutant of *S. aureus* H.

This contrasts with the earlier study in which all of the phosphate remaining in the peptidoglycan fraction after alkali extraction was apparently associated with the extracted glycerol phosphate oligomer. The earlier study was carried out on a solubilized phosphate–glycan complex, so that the residual glycan was less readily separated from the glycerol phosphate oligomer; this probably explains why the residual phosphate was not detected.

It has long been known that the linkage between teichoic acid and peptidoglycan is labile towards both acid and alkali. The present study shows that this lability involves two different linkages, both of which are involved in a rather complex linkage unit. Thus, acid extraction of teichoic acids requires fission of the *N*-acetylglucosamine 1-phosphate to give a teichoic acid terminating in a reducing group of the amino sugar, whereas alkali causes fission at the point of attachment of glycerol phosphate to the 4-position on *N*-acetylglucosamine, thereby leaving the amino sugar attached to the peptidoglycan.

Acknowledgement

We thank the Science Research Council for financial support.

References

[1] Heckels, J. E., Archibald, A. R. and Baddiley, J. (1975) Biochem. J. 149, 637–647.
[2] Coley, J., Archibald, A. R. and Baddiley, J. (1976) FEBS Lett. 61, 240–242.
[3] Hancock, I. C. and Baddiley, J. (1976) J. Bacteriol. 125, 880–886.
[4] Bracha, R. and Glaser, L. (1976) J. Bacteriol. 125, 872–879.
[5] Hancock, I. C., Wiseman, G. and Baddiley, J. (1976) FEBS Lett. 69, 75–80.
[6] Bracha, R. and Glaser, L. (1976) Biochem. Biophys. Res. Commun. 72, 1091–1098.
[7] Wyke, A. W. and Ward, J. B. (1977) FEBS Lett. 73, 159–163.
[8] Randle, C. J. M. and Morgan, W. T. J. (1955) Biochem. J. 61, 586.
[9] Janczara, E., Perkins, H. R. and Rogers, H. J. (1911) Biochem. J. 80, 82–92.
[10] Archibald, A. R., Baddiley, J., Heckels, J. E. and Heptinstall, S. (1971) Biochem. J. 125, 353–359.