Review Letter

THE LINKAGE BETWEEN TEICHOIC ACID AND PEPTIDOGLYCAN IN BACTERIAL CELL WALLS

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1. Introduction

The major polymers in the walls of most Gram-positive bacteria are peptidoglycan and teichoic acid [1–3]. Whereas peptidoglycan is insoluble because of extensive cross-linkage of glycan chains through oligopeptide bridges, teichoic acids can be extracted from walls as highly soluble polymers by the action of dilute acid or alkali under gentle conditions [4–6]. The simple teichoic acids comprising chains of glycerol phosphate or ribitol phosphate units, with D-alanyl ester substituents and in some cases sugar residues, can often be removed from their association with peptidoglycan with little or no degradation of the polymer chain. Those teichoic acids and related polymers possessing sugar 1-phosphate linkages in the chain are readily degraded by acid; however, even in these cases most of the polymer can be removed from the wall by acid with only partial chain degradation if conditions are chosen carefully [7]. In all cases, however, extraction is relatively slow and the association with peptidoglycan can not be explained exclusively as electrostatic attraction between acidic and basic centres in the two polymers. It seems then that attachment involves covalent linkages that are generally more labile towards acids and bases than are the inter-unit linkages in the teichoic acid chain.

Much effort has been directed towards establishing the details of the chemistry of the linkage between these wall polymers, despite the considerable difficulties associated with the characterization of small amounts of the components of the linkage region when accompanied by much larger amounts of chemically similar products of degradation of the main chain of both polymers. The effort has been made because a knowledge of the molecular architecture of linkage is fundamental to the discussion of the process of wall assembly in relation to cell division and growth, as well as for the understanding of the details of teichoic acid biosynthesis and of general control mechanisms in cell wall synthesis. This account summarizes the results of recently published papers on studies with several different organisms and also describes new work in an attempt to draw conclusions on the similarities and differences of linkage in widely different bacterial species.

Earlier work on teichoic acids showed that, after prolonged extraction from walls with cold trichloroacetic acid, the chain is terminated by a phosphomonoester residue [8–10]. When, however, cell walls of *Staphylococcus aureus* were dissolved by enzymic hydrolysis of the peptidoglycan, a teichoic acid preparation was obtained to which fragments of peptidoglycan were still covalently attached. This preparation contained no phosphomonoester groups, and it was concluded that attachment to the peptidoglycan involved a phosphodiester linkage representing the P-terminal phosphate of the teichoic acid [11,12]. It was also shown that acid hydrolysis of many cell walls containing teichoic acids gave muramic acid phosphate [13–15] and it seemed possible that this muramic acid phosphate represented the point of attachment between the two polymers. The conclusion that the linkage is a simple phosphodiester between the P-terminus of the teichoic acid and a muramic acid residue in the peptidoglycan was unjustified, however, because it is unlikely that such a structure would be more labile than other phosphodiester groupings in the molecule towards both acids and bases. Several

alternative structures were considered in an attempt to explain the chemical behaviour of the linkage [16–18] but the lack of suitable models and inadequate knowledge of the precise nature of the bonds undergoing hydrolysis prevented further progress.

2. The nature of linkage units

Wall preparations from Gram-positive bacteria usually contain variable amounts of adherent membrane, and the detection of glycerol phosphates in hydrolysates is therefore not surprising. Nevertheless, it was observed in these laboratories on a number of occasions that hydrolysates of relatively clean walls prepared from organisms that possess only a ribitol teichoic acid or a sugar 1-phosphate polymer contained small amounts of glycerol phosphates [19–21]. The possibility that glycerol phosphate was covalently attached to the wall, and that it might form a part of the linkage between teichoic acid and peptidoglycan, was therefore examined [21,22] in wall preparations that had been rigorously purified by treatment with trypsin and 40% aqueous phenol, procedures that remove proteins and phospholipids. Walls prepared in this way from a mutant (*gol*$^-$$\phi^R$71) of *S. aureus* H, in which the teichoic acid is a simple poly(ribitol phosphate), gave small amounts of glycerol mono- and diphosphates on hydrolysis. The ribitol phosphate polymer was destroyed by oxidation with periodate, either in the wall itself or as a soluble teichoic acid–peptidoglycan complex obtained by the action of an amidase. After reduction of the product with borohydride and gentle treatment with alkali, a compound was obtained in which an ethylene glycol phosphate residue is attached to a linear tri(glycerol phosphate) as shown in fig.1. Quantitative considerations relating the chain-length of the ribitol phosphate polymer, the number of end groups detected and the proportion of

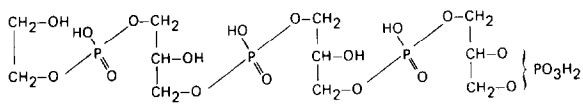

Fig.1. Product containing ethylene glycol phosphate and three glycerol phosphate residues obtained from *Staphylococcus aureus gol*$^-$$\phi^R$71 walls by oxidation with periodate, reduction with borohydride and treatment with alkali. The ethylene glycol phosphate residue arises from the P-terminal ribitol phosphate of the teichoic acid.

the total wall phosphorus accounted for as the structure shown in fig.1, indicated that the ethylene glycol phosphate residue could correspond to the phosphate together with two carbon atoms of the P-terminal ribitol phosphate residue in the teichoic acid. It would follow that the main poly(ribitol phosphate) chain of the teichoic acid is attached at its P-terminus to the tri(glycerol phosphate) in a linkage unit that is itself attached to the peptidoglycan.

In the parent organism, *S. aureus* H, each D-ribitol 5-phosphate residue in the teichoic acid chain bears an *N*-acetylglucosaminyl substituent at its D-4 position; so in a similar degradation to that described for the mutant [23] the presence of a sugar residue on the terminal ribitol phosphate would modify the course of the oxidation. As expected, the product of oxidation and subsequent reduction contained no ethylene glycol substituent, but in its place was a glycerol residue, corresponding to three carbon atoms of the P-terminal ribitol, and attached to this glycerol were the fragments of the oxidized and reduced *N*-acetylglucosaminyl residue. The two degradative studies thus establish conclusively the attachment of the poly(ribitol phosphate) chain to the tri(glycerol phosphate) moiety in the linkage unit.

Studies with cell-free membrane preparations on the mechanism of biosynthesis of the linkage unit, and on the attachment of the polymer chain to the unit, showed that CDP-glycerol contributed glycerol phosphate but that UDP-*N*-acetylglucosamine was also required [24,25]. Moreover, the glycerol phosphate residue from CDP-glycerol is transferred to a polyisoprenyl phosphate carrier before the main polymer chain becomes attached, and it was found that the nucleotides were required in the sequence UDP-*N*-acetylglucosamine followed by CDP-glycerol [26,27]. It was also found that, in *Bacillus licheniformis*, attachment of the teichoic acid to the wall in a wall-membrane preparation required a similar sequence of nucleotide additions, and the phosphate residue on muramic acid in the peptidoglycan originated from UDP-*N*-acetylglucosamine rather than from CDP-glycerol [28]. It seemed possible, then, that the linkage unit contained an *N*-acetylglucosamine residue that had escaped detection in the chemical degradative studies. This was supported by analyses of fractions obtained by enzymic hydrolysis of walls of *S. aureus*, where an excess of glucosamine was

observed in the teichoic acid fractions [27].

The presence of an *N*-acetylglucosamine residue attached to the tri(glycerol phosphate) moiety in the linkage unit would not have been revealed readily in the degradative sequence described above for *S. aureus*. Depending upon the position of the tri(glycerol phosphate) substituent, the amino sugar would either have been oxidized, and so not easily recognized, or it would have remained with the peptidoglycan after the alkali treatment and thus eluded detection. Consequently, a different degradative sequence was adopted [29] in an effort to establish whether the linkage unit in the wall did indeed include an *N*-acetylglucosamine 1-phosphate residue. The oxidized and reduced walls of the mutant were treated with 0.1 M HCl at 100°C for 7 min, conditions suitable for the hydrolysis of sugar 1-phosphate. The product contained an ethylene glycol phosphate residue, three glycerol phosphate residues and a reducing *N*-acetylglucosamine; in the Morgan-Elson reaction for reducing amino sugars it gave a chromogen with an $A_{530\ nm}$ max, characteristic of a 4-substituted *N*-acetylglucosamine. It follows that this product has the structure shown in fig.2.

The *N*-acetylglucosamine residue in the product shown in fig.2 thus forms a part of the linkage unit. It had been unaffected by the periodate oxidation, consistent with the presence of a substituent at its 4-position; and since it had been unaffected by borohydride whilst attached to the peptidoglycan, it follows that the attachment must have involved the 1-position. The sensitivity of this linkage towards acid hydrolysis is in agreement with a sugar 1-phosphate and thus is consistent with the biosynthetic evidence. A sugar 1-phosphate linkage would be expected to be more labile towards acid hydrolysis than the other phosphodiester linkages in both the linkage unit and in the main polymer chain; this accounts for the ease of removal of relatively undegraded teichoic acid from a wall under acidic conditions. Moreover, the presence of the tri(glycerol phosphate) substituent at the 4-position on an *N*-acetylglucosamine residue explains the ease of removal of the teichoic acid from the wall with alkali; it is known that a phosphodiester linkage joining glycerol to the 4-position on *N*-acetylglucosamine 1-phosphate, a structure encountered [30] in the repeating unit of the main chain of the teichoic acid in the walls of *Micrococcus* sp. I3, is readily hydrolysed through cyclization onto glycerol under controlled alkaline conditions that do not cause the hydrolysis of other glycerol phosphodiester linkages.

After the removal of the main polymer chain and the linkage unit from the peptidoglycan with acid, one phosphate residue remained attached to the peptidoglycan. It was shown that this is on a muramic acid, since vigorous acid hydrolysis of this peptidoglycan gave muramic acid phosphate. The complete structure of the teichoic acid–peptidoglycan complex is given in fig.3, and this also shows the acid- and alkali-labile linkages that are hydrolysed during extraction of the teichoic acid from the wall. The proposal that the *N*-acetylglucosamine in the linkage unit is attached directly to the phosphate on muramic acid is based upon the expected lability of such a linkage towards acid.

3. The attachment of sugar 1-phosphate polymers

It was of interest to know whether similar linkage units occur in the walls of different bacteria, and to establish the extent of structural variation in such cases. Of particular interest was the possible occur-

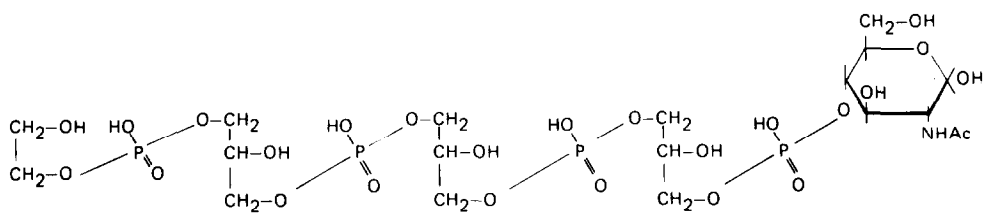

Fig.2. Product containing *N*-acetylglucosamine from the *Staphylococcus aureus* mutant walls. Prepared by oxidation with periodate, reduction with borohydride and hydrolysis in 0.1 M HCl.

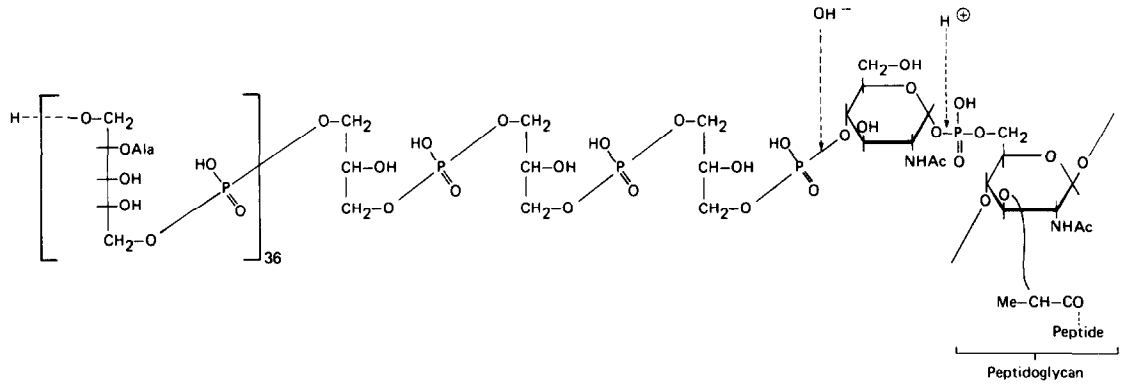

Fig.3. Structure of the teichoic acid–linkage unit–peptidoglycan complex comprising the wall of the *Staphylococcus aureus* mutant. The removal of teichoic acid from the wall by dilute acid or alkali occurs through hydrolysis at the points indicated by arrows.

rence of a linkage unit in those organisms where the anionic wall polymer is a poly(sugar 1-phosphate). Like teichoic acids, such polymers can be extracted readily by treatment of walls with dilute alkali [5,6]. An example is *Micrococcus* sp. 2102, where the wall polymer is poly(*N*-acetylglucosamine 1-phosphate) [21] in which the phosphodiester linkages are between the 1-position and the 6-position of the sugar in the neighbouring repeating units. A sequence of degradative studies analogous to those developed for *S. aureus* showed that a similar or identical linkage unit occurs in the micrococcus [31]. The sugar units in the polymer chain were readily oxidized with periodate and the removal of the oxidized residues from the wall was achieved by β-elimination at pH 10.5. Further alkali treatment (0.5 M NaOH at room temperature) yielded a tri(glycerol phosphate) to which was attached an additional phosphate originating from the P-terminal sugar phosphate unit of the main polymer chain. Confirmation of the nature of the linkage between the sugar phosphate polymer and the tri(glycerol phosphate) was obtained by vigorous alkaline hydrolysis of walls; a minor product was a triphosphate in which glucosamine 6-phosphate is attached at its 1-position through a phosphodiester linkage to the 1-position on glycerol 2-phosphate (fig.4). Under alkaline conditions phosphodiesters of glycerol are hydrolysed through 1,2-cyclic phosphate intermediates, and it was therefore expected that such hydrolysis of the tri(glycerol phosphate) component of the linkage unit would give a mixture of

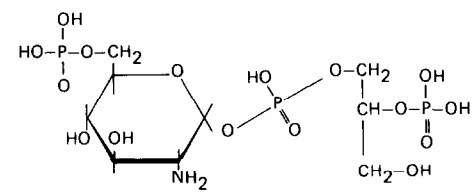

Fig.4. Minor product of vigorous alkaline hydrolysis of walls of *Micrococcus* sp. 2102. The main sugar 1-phosphate polymer chain is hydrolysed, leaving a phosphomonoester at the 6-position on glucosamine. The phosphomonoester at the 2-position on the glycerol residue, arising during hydrolysis of the glycerol phosphate trimer, stabilizes the phosphodiester in this product against further attack by alkali.

isomeric phosphomonoesters, including a small amount of the one shown in fig.4, where further hydrolysis of the single phosphodiester linkage had been prevented by the presence of a phosphate at the 2-position in the glycerol residue. Thus in this product the glucosamine 6-phosphate originates from the P-terminal sugar residue in the main chain, and the phosphodiester residue represents the point of attachment of the main chain to the linkage unit.

The presence of an *N*-acetylglucosamine 1-phosphate residue in the linkage unit in the micrococcus was established in a manner similar to that described for *S. aureus* H; however, in this case, because of the acid lability of the sugar 1-phosphate linkages in the main polymer chain, direct acid hydrolysis of the wall itself conveniently yielded a compound with the

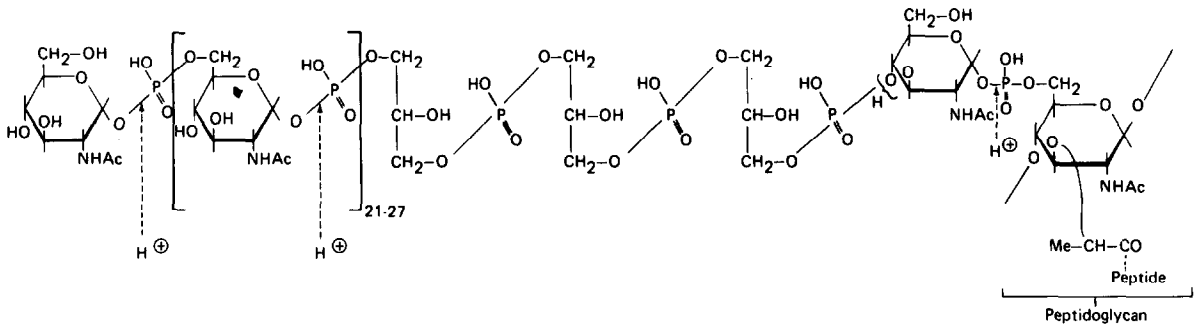

Fig.5. *N*-Acetylglucosamine (1→6) phosphodiester polymer–linkage unit–peptidoglycan complex comprising part of the wall of *Micrococcus* sp. 2102. The arrows indicate points of hydrolysis by acid under controlled conditions.

structure corresponding to that shown in fig.2, but in which the ethylene glycol residue was absent. The quantitative data on the amount of phosphate remaining in the peptidoglycan after acid hydrolysis, and the acid lability at the point of attachment of linkage unit to peptidoglycan indicate a direct phosphodiester linkage between the 1-position of *N*-acetylglucosamine in the linkage unit and a muramic acid in the peptidoglycan. The structure of the sugar 1-phosphate polymer–linkage unit–peptidoglycan complex comprising the wall of *Micrococcus* sp. 2102 is shown in fig.5.

4. Linkage in *Bacillus subtilis* W23

To extend the studies on the linkage of teichoic acid to peptidoglycan the nature of this region in a bacillus was investigated; the results are now described. A mutant of *Bacillus subtilis*, strain W23 (ϕR M12) was chosen, since its wall teichoic acid consists of poly(ribitol 5-phosphate) (R. S. Green and A. R. Archibald, unpublished observation) and is thus similar to that of the mutant of *S. aureus* H referred to earlier. A sequence of degradations analogous to those already described for *S. aureus*, viz. sequential oxidation with periodate, reduction with borohydride, solubilization with an amidase and controlled hydrolysis with alkali were carried out on clean wall preparations from the bacillus; the experimental procedures were similar to those described in the earlier work. This sequence of steps resulted in the formation of the same glycerol phosphate trimer, terminating in an ethylene glycol phosphate residue (fig.1), as had been obtained in the study of the *S. aureus* H mutant. However, the rate of release of this fragment from the glycan was much lower in the present case, suggesting that the nature of the attachment of the glycerol phosphate trimer to the peptidoglycan was different. Nevertheless, the involvement of an *N*-acetylglucosamine 1-phosphate residue was implicated, since the teichoic acid could be extracted readily from the walls of the bacillus by gentle hydrolysis with acid (0.1 M HCl, 100°C, 15 min) giving a polymer that contained about one reducible glucosamine residue for each teichoic acid chain. The chain contained 30–35 ribitol phosphate units, calculated from the amount of formaldehyde produced from chain ends after oxidation with periodate.

The oxidized and reduced walls were treated with acid (0.1 M HCl, 100°C, 10 min) and, although this released a homogeneous phosphorus-containing product, it was different in electrophoretic mobility from that obtained in the parallel study carried out on the walls of the *S. aureus* mutant. Analysis of the compound showed that it did not contain glucosamine, but was apparently composed of a glycerol phosphate tetramer together with an ethylene glycol phosphate residue. It contained equimolar amounts of phosphate and glycerol, and one of the glycerol residues was oxidizable by periodate giving 0.2 molecular proportions of formaldehyde per phosphate.

It appeared then that in the bacillus the *N*-acetylglucosamine in the linkage unit was readily oxidized with periodate and is therefore unsubstituted at its 3- and 4-positions. Thus the glycerol phosphate trimer

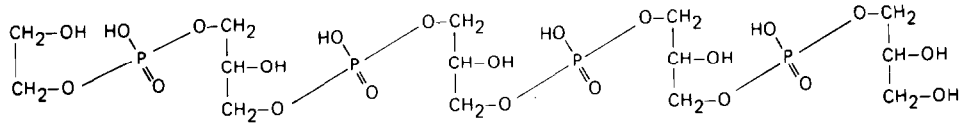

Fig.6. Glycerol phosphate tetramer with an ethylene glycol substituent from the mutant *Bacillus subtilis* W23 obtained by oxidation of walls with periodate, reduction with borohydride and acid treatment.

must be attached to the 6-position on glucosamine, and periodate oxidation of the wall had not only destroyed the ribitol phosphate chain, but had also caused fission of the bond between the vicinal free hydroxyl groups at positions 3- and 4- in the *N*-acetylglucosamine 1-phosphate residue. Subsequent reduction and treatment with acid would cause hydrolysis of the acyclic acetal giving the observed glycerol phosphate tetramer (fig.6). In a test of the validity of these conclusions, walls were treated with borohydride in order to reduce glycan chain ends and then treated with alkali (1 M NaOH, 100°C, 3 h) in order to effect complete hydrolysis of the alkali-labile bonds. It was expected that those phosphodiesters that possessed a hydroxyl group on an adjacent carbon atom would undergo fission, and consequently most of those linkages in the wall would be destroyed. However, hydrolysis of phosphodiester linkages in the glycerol phosphate oligomer, proceeding through 5-membered cyclic phosphate intermediates, would occasionally give rise to a phosphate substituent at the 2-position on the glycerol phosphate attached to the *N*-acetylglucosamine to give a structure (fig.7) in which further hydrolysis through 5-membered cyclic phosphate intermediates would not be possible. The product

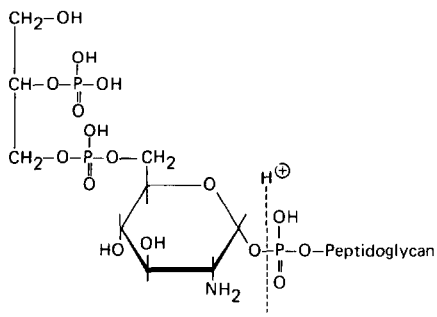

Fig.7. Alkali-stable minor product obtained from reduced walls of the mutant *Bacillus subtilis* W23 by vigorous hydrolysis with alkali. Treatment with acid causes hydrolysis at the dotted line.

would consequently be stable towards alkali; the formation of this product is analogous to the formation of the stable phosphodiester shown in fig.4 arising from the action of alkali on the walls of *Micrococcus* sp. 2102. After hydrolysis of the walls with alkali and subsequent re-*N*-acetylation, the polymeric material remaining was treated with acid (0.1 M HCl, 100°C, 10 min) and the phosphorus-containing product that was released from the polymer was purified by filtration through a dialysis membrane, gel filtration and paper chromatography; it contained about 2% of the phosphorus originally present in the wall. Analysis of this material showed that half of the phosphorus was present as phosphomonoester. Acid hydrolysis gave glycerol diphosphate and glucosamine as major products, and by treatment of the hydrolysate with alkaline phosphatase the molecular proportions of P:glycerol:glucosamine were shown to be 1.0 : 0.53 : 0.47. When the material was subjected to sequential oxidation with periodate and reduction with borohydride the glucosamine residue was destroyed and equimolar amounts of phosphate and glycerol were found after hydrolysis of the product with acid and phosphatase.

Additional evidence in support of the structure of the linkage unit in the bacillus was obtained from the Morgan-Elson reaction for reducing amino sugars. A chromogen with an $A_{535\ nm}$ max was observed, in agreement with 6-substitution by the phosphodiester on the amino sugar. Finally, the fragment was reduced with borohydride and the resulting substituted alditol was oxidized with periodate and reduced with borohydride. Acid hydrolysis (2 M HCl, 100°C, 3 h) gave inter al. ethylene glycol phosphate, which can only arise from substitution of the phosphodiester at the 6-position on the *N*-acetylglucosamine.

It would therefore appear that the alkali-resistant phosphate is derived from the terminal glycerol phosphate moiety that is joined through a phosphodiester to the 6-position of *N*-acetylglucosamine 1-phosphate

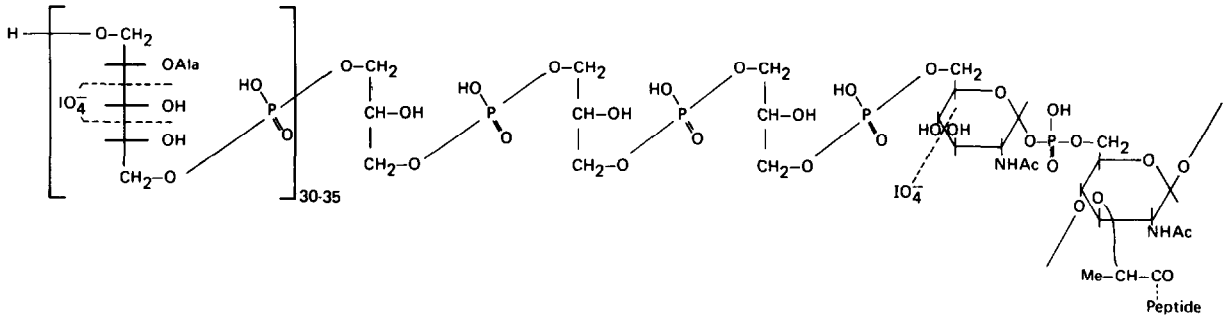

Fig.8. Structure of the teichoic acid–linkage unit–peptidoglycan complex comprising the wall of the mutant *Bacillus subtilis* W23. Periodate oxidation occurs at the points indicated by dotted lines.

in the linkage unit (fig.8). The formation of the alkali-stable material shown in fig.7 thus confirms that in the bacillus the position of attachment of glycerol phosphate is to the 6-position in the *N*-acetylglucosamine 1-phosphate residue. Although not directly established in the present work, it seems likely that the linkage between the *N*-acetylglucosamine 1-phosphate and muramic acid is as shown in fig.8. The ordered incorporation of residues from appropriate nucleotides into wall-membrane preparations from *B. subtilis* W23 is consistent with this structure [35].

5. Linkage in glycerol teichoic acids

The possible occurrence of linkage units, similar to those found with ribitol teichoic acids and sugar 1-phosphate polymers, has been examined in organisms where the wall contains a glycerol teichoic acid of the poly(glycerol phosphate) type. This is of considerable interest because a glycerol phosphate trimer, comprising a part of such a linkage unit, would be chemically indistinguishable from the glycerol phosphate units in the main polymer chain. The chemical demonstration of such a unit would therefore have to be confined to the detection of an *N*-acetylglucosamine 1-phosphate residue attached to the P-terminus of the poly(glycerol phosphate) chain.

In *Bacillus subtilis* NCIB 3610 the wall teichoic acid is a glucosylated poly(glycerol phosphate) [32] that, like many other teichoic acids, can be extracted into alkali under gentle conditions. Treatment of teichoic acid extracted in this way with potassium [^{3}H]borohydride did not result in the incorporation of radioactivity, whereas isotope was incorporated on similar reduction of a sample of teichoic acid that had been extracted by treatment of walls with 0.1 M HCl at 100°C for 10 min. Subsequent hydrolysis of the reduced teichoic acid gave [^{3}H]glucosaminitol, indicating that the acid-extracted teichoic acid terminated in a reducing glucosamine, or more probably *N*-acetylglucosamine, residue that could have arisen by hydrolysis of an *N*-acetylglucosamine 1-phosphate. Some of the glycerol phosphate residues in the teichoic acid do not possess glycosyl substituents and it is possible that these represent glycerol phosphate residues in a linkage unit similar to those in walls of the other bacteria described earlier. This conclusion is strengthened by the consideration that it is unlikely that the teichoic acid would be extracted readily in dilute alkali were such an alkali-labile linkage unit not present.

6. Conclusions

It seems clear that the ease with which teichoic acids and related sugar 1-phosphate polymers are extracted by treatment of walls with dilute acid or alkali, often with little or no marked hydrolysis of inter-unit linkages in the main polymer chain, can be adequately explained by the presence, in a specialized linkage unit, of an acid-labile *N*-acetylglucosamine 1-phosphate linkage and an alkali-labile phosphodiester linkage. The location of these latter linkages, and consequently their ease of hydrolysis in alkali, may vary. Thus the glycerol phosphate trimer in *B. subtilis* appears to be attached to the 6-position of the *N*-acetylglucosamine residue, whereas in the staphylo-

coccus and micrococcus the 4-position of the *N*-acetylglucosamine is attached to glycerol phosphate. Although this latter linkage is more labile to alkali than are the inter-unit linkages in many teichoic acids, its lability is comparable to that of the similar linkage that connects the repeating structures in the wall teichoic acid of *Micrococcus* sp. I3 [30]. There is indeed, a striking relationship between linkage unit and the *Micrococcus* sp. I3 teichoic acid which can be regarded as a polymer of a modified linkage unit containing only one glycerol phosphate residue. The phosphorylated polysaccharide of *Micrococcus* sp. 2102 could, at least chemically, be regarded as being related to the *Micrococcus* sp. I3 teichoic acid but lacking the glycerol phosphate moiety.

The presence of apparently similar or closely related linkage units in walls of widely differing bacterial species containing structurally distinct teichoic acids might reflect a common biosynthetic mechanism essential for the linking together of teichoic acids or phosphorylated polysaccharides to peptidoglycan. Considerable similarities have already been observed in the biosynthesis of teichoic acid–linkage unit complexes in several bacteria [24–28,33,35]. These complexes are precursors of the teichoic acid–linkage unit–peptidoglycan representing the major part of the wall, and in the examples studied all are built up by the same general route. The main polymer chain is assembled by the transfer of appropriate groupings from nucleotide precursors (e.g., CDP-ribitol, UDP-*N*-acetylglucosamine) to an incompletely characterized lipid known as the lipoteichoic acid carrier (LTC). The linkage unit is assembled by a similar transfer to polyprenyl phosphate of, firstly, *N*-acetylglucosamine (from UDP-*N*-acetylglucosamine) and then of, successively, three glycerol phosphate residues (from CDP-glycerol). The resulting prenyl-pyrophosphate-linkage unit then accepts the main polymer chain by transfer from its LTC compound.

It is of interest that a specialized linkage unit, also terminating in *N*-acetylglucosamine 1-phosphate but lacking glycerol phosphate, has been observed in recent chemical [36] and biosynthetic [34,37] studies on the teichuronic acid in *Micrococcus lysodiekticus*. That this linkage unit, like that of the teichoic acids, is assembled on an undecaprenyl phosphate carrier [34] similar to that involved in peptidoglycan biosynthesis suggests that the formation of such linkage units could be an important point of coordination and regulation of wall polymer assembly.

The unexpectedly complicated mechanism of attachment of teichoic acids to peptidoglycan is perhaps related to the vectorial features of the process of assembly. Nucleotide precursors of both teichoic acid and peptidoglycan are synthesized intracellularly, whereas the final polymeric products are outside the membrane. The point in the biosyntheses where translocation through the membrane occurs, and the mechanism of this translocation are unknown, but it is possible that translocation imposes requirements which dictate the structure of the linkage unit and its mode of integration into the final product.

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