MECHANISM AND CONTROL OF CELL WALL SYNTHESIS IN BACTERIA

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ABSTRACT

The structure of the principal components of the walls of Gram-positive bacteria is discussed. The relationship between teichoic acids in walls and membranes and their location within the outer layers of bacteria is described, as is their role in cation balance in the vicinity of the membrane. An account is given of the steps in the biosynthesis of peptidoglycan and teichoic acid, including the participation of polyprenol phosphate intermediates, and mechanisms of control of synthesis are suggested. It is shown how, during studies on teichoic acid biosynthesis, the presence of a phosphorylated glycoprotein was established in the bacterial membrane.

The outer layers of bacteria comprise two main structures; the wall and the membrane. The membrane is a typical phospholipid bilayer containing protein and is similar to membranes found in other cells. The wall, however, does not occur in all cells; as found in bacteria, it possesses considerable mechanical strength and consequently protects the delicate underlying membrane against external mechanical and osmotic forces and against attack by aggressors. It is important for a number of reasons. First it comprises an appreciable proportion (10–20 per cent) of the dry weight of the cell and consequently is a major metabolic product; it allows the free passage of metabolites and (see later) participates in ion-exchange and cation balance. In the wall are located macromolecular substances that are in many cases important bacterial antigens. Moreover, a number of antibiotics exert their characteristic inhibitory effect on bacteria by specific inhibition of steps in the biosynthesis of the cell wall.

There has been a considerable amount of work done on the biosynthesis of bacterial wall components and this lecture represents no more than a somewhat superficial review of aspects of the subject. Although the components of the wall are characteristically bacterial, the mechanism of their synthesis shows features common to the synthesis of macromolecular components of many cells, in particular the polysaccharides. It is necessary before considering the details of the formation of bacterial walls to outline briefly the structure of the major wall components. In order to simplify this discussion, attention will be confined to Gram-positive bacteria: the reason for this is that their walls are simpler than those of Gram-negative bacteria and consequently they are better understood.

An important component of all bacterial cell walls is a material known as peptidoglycan or mucopeptide (for a review of peptidoglycan structure see ref. 1). This comprises oligosaccharide chains crosslinked with peptides (*Figure 1* illustrates the structure in *Staphylococcus aureus*). In the glycan chains the sugar components are *N*-acetylglucosamine and *N*-acetylmuramic acid; these are arranged in regular alternation, and peptide residues are attached to the carboxyl groups of muramic acid residues. There is some variation in different bacteria in the number and nature of the amino acid residues in the crosslinkages but in all bacteria both D and L amino acids occur in these peptide residues. The peptides are crosslinked between chains to form an open meshwork. This crosslinkage is frequently incomplete and consequently both amino and carboxyl groups occur in many walls.

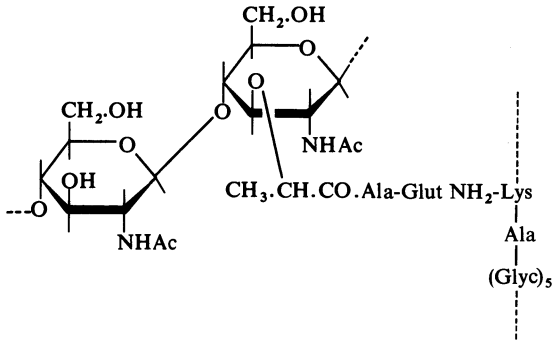
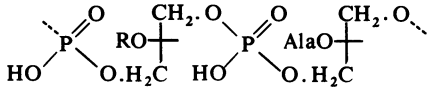


Figure 1. Peptidoglycan from *Staphylococcus aureus*



R = glycosyl

Figure 2. Glycerol teichoic acid

Peptidoglycan usually accounts for 50 per cent or more of the dry weight of the wall. The rest is made up of either acidic polysaccharide containing uronic acid residues, sometimes referred to as teichuronic acids, or in many cases phosphorylated polymers called teichoic acids[2]. In some organisms the teichoic acid can be replaced by teichuronic acid when growth has occurred under phosphate-limitation[3]. The teichoic acids are polymers containing glycerol phosphate (*Figure 2*) or ribitol phosphate residues and sugar residues together with D-alanine in ester linkage. In some cases the sugars are appendages to the main polyol phosphate chain, whereas in others the sugars form a part of the chain itself (*Figure 3*). In fact, we now know of many structurally different teichoic acids[4], but all contain glycerol phosphate or ribitol phosphate and D-alanine, and most contain sugar residues of one kind or another.

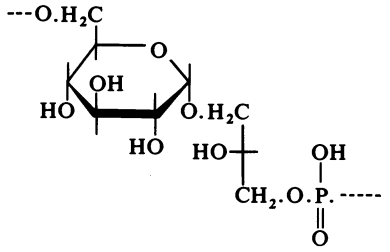


Figure 3. Wall teichoic acid from *Bacillus licheniformis*

The teichoic acids are covalently attached to peptidoglycan through their terminal phosphate residues. The details of the nature of the chemical linkage between teichoic acid and peptidoglycan are still the subject of study, but it is known that the linkage involves a phosphodiester linking the teichoic acid to a muramic acid residue in the glycan chain of peptidoglycan. A study[5] of the nature of the arrangement of the two polymers in *Staphylococcus lactis* I 3 has shown that each teichoic acid is attached to its individual glycan chain in the peptidoglycan; no glycan chain has attached to it more than one teichoic acid. Since the number of teichoic acid chains in this case is less than the total number of glycan chains, there will be some glycan chains not possessing a teichoic acid. From a consideration of the quantitative aspects of this study, and particularly from the fact that no glycan has attached to it more than one teichoic acid, we are able to eliminate certain possibilities about the arrangement of these polymers within the cell wall itself. *Figure 4* illustrates two extreme situations in the possible arrangement of these polymers. In one case the glycan chains lie perpendicular to the surface of the cell, and in this case the teichoic acid chains could form a largely external layer. In the other case the glycan chains lie in the plane of the cell surface, and consequently the teichoic acid chains must permeate deeply into the wall and no discrete layered structure would occur. There are of course intermediate stages between these two extremes.

One of the most readily demonstrated general functions of teichoic acids is their role in cation balance. It has been shown that wall teichoic acids bind cations[6,7] and this binding is important in maintaining an appropriate cation concentration in the region of the membrane. The membrane contains many enzymes, a number of which are known to require rather high (15–50 mM) divalent cation concentrations for optimal activity. Teichoic acids have a particular affinity for divalent cations and assist in making them available to the membrane-bound enzymes[8]. In this connection it is important to consider briefly those teichoic acids which are in fact associated with the membrane rather than the wall. These membrane teichoic acids belong to the class of lipoteichoic acids. They comprise in all cases polymers of glycerol phosphate with or without sugar residues but always with D-alanine ester residues[4]; the terminal phosphate of the polymer chain is attached to the 6-position of a sugar residue in a glycolipid (*Figure 5*). The lipid part of the molecule is intercalated with the lipids of the membrane bilayer. Lipoteichoic acids have been found in very nearly all Gram-positive bacteria, including those that lack wall teichoic acid[9–11]. Like the wall polymers, they also play

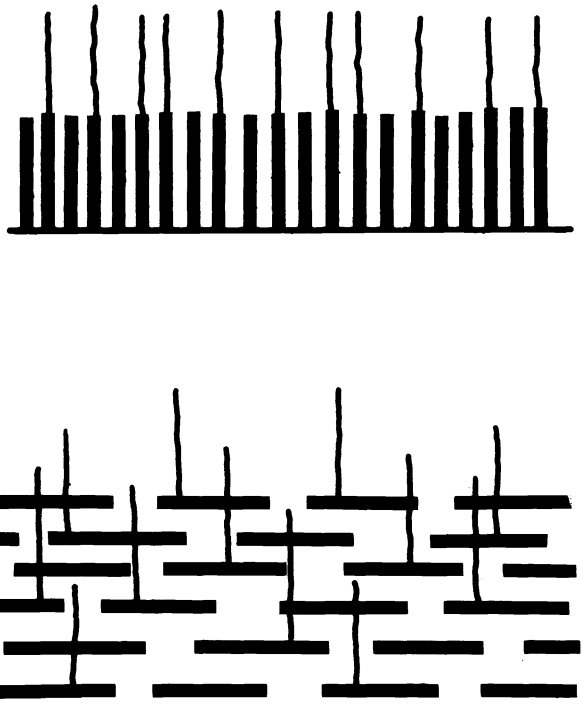


Figure 4. Arrangement of glycan and teichoic acid chains in wall of *Staphylococcus lactis* I 3

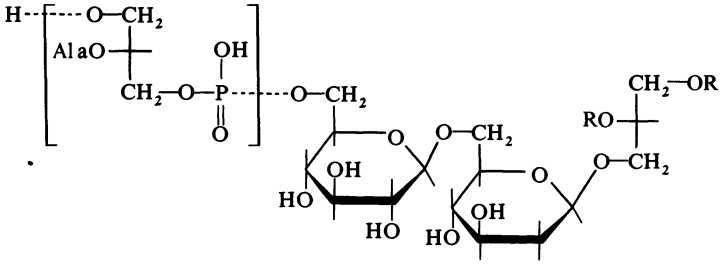


Figure 5. Lipoteichoic acid from membrane of *Staphylococcus aureus*

an important part in cation balance, and it is not surprising that there are indications that the poly(glycerol phosphate) chains of these molecules extend from the surface of the membrane deeply into the wall structure and in some cases[12] through the wall to the outer surface of the cell (*Figure 6*).

It is found[13] that *Micrococcus lysodeikticus* lacks both wall and membrane teichoic acids. This organism, however, possesses in its membrane a new type of compound, a lipomannan, in which some of the sugar hydroxyl groups are esterified by long-chain acy residues and others are substituted with nega-

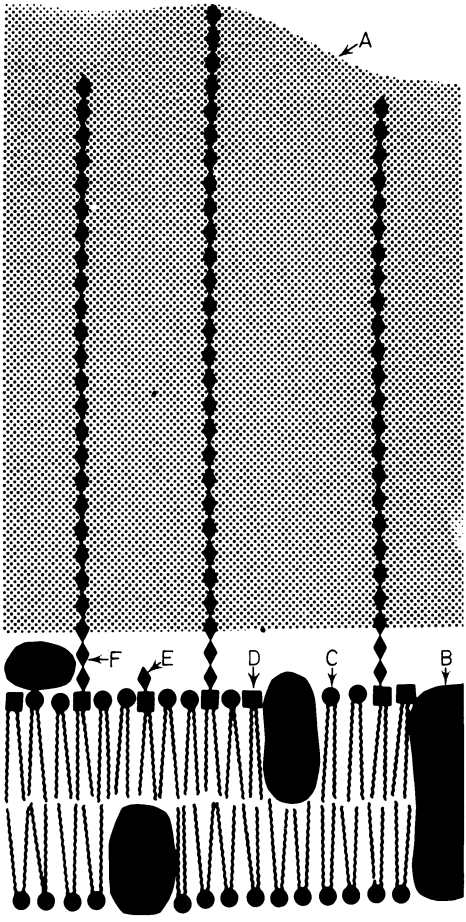


Figure 6. Penetration of membrane lipoteichoic acid chains through the wall

tively charged succinic ester residues. This compound has properties similar to those of the lipoteichoic acids and strongly binds divalent cations.

The biosynthesis of peptidoglycan follows the general pattern of polysaccharide biosynthesis, in which sugar residues are transferred from nucleoside diphosphate sugars to the growing polymer chain[14]. In the case of peptidoglycan one of the nucleotide precursors is uridine diphosphate *N*-acetylmuramic acid to which is attached a peptide. In *Figure 7* the nucleotide precursor for the biosynthesis of peptidoglycan in *Staphylococcus aureus* is illustrated. The pentapeptide chain, which is built up on UDP-*N*-acetylmuramic acid, in this case closely resembles the peptides in the peptidoglycan itself; the principal difference is in the possession of an additional D-alanine at its carboxyl end and the absence of five glycine residues.

Another important feature of peptidoglycan synthesis is the participation of the isoprenoid lipid undecaprenol as its phosphate[15]. This lipid phosphate

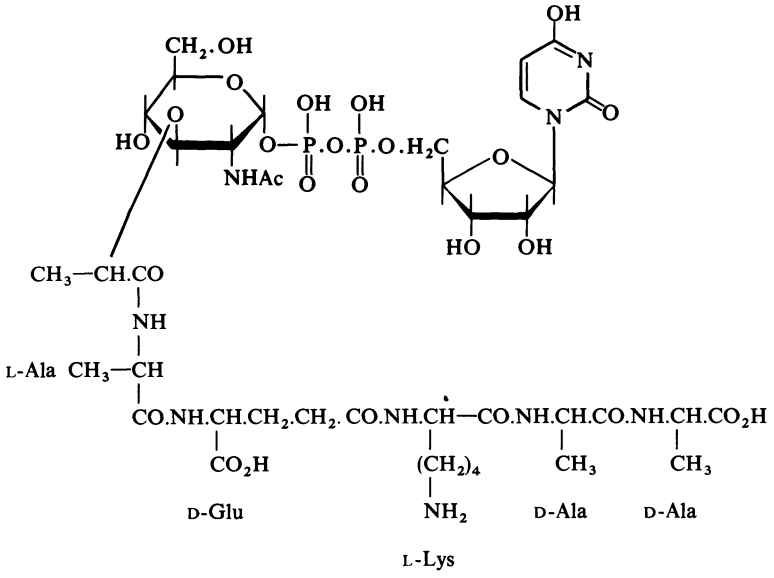
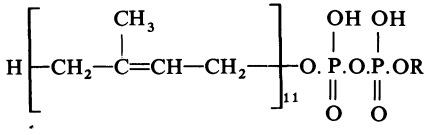


Figure 7. Uridine diphosphate *N*-acetylmuramyl pentapeptide

accepts *N*-acetylmuramyl pentapeptide together with a phosphate from the nucleotide precursor to form a lipid intermediate (*Figure 8*), in which undecaprenol is joined through a pyrophosphate to the muramyl pentapeptide. Further steps in the synthesis are shown in *Figure 9*. The lipid intermediate



(12)

Figure 8. Undecaprenol pyrophosphate sugar intermediate for peptidoglycan synthesis

accepts *N*-acetylglucosamine from the other nucleotide precursor UDP-*N*-acetylglucosamine. Modification then occurs to the peptide part of the molecule, i.e. the attachment of a pentaglycine unit by transfer from glycyl RNA and amidation of the carboxyl of the glutamyl residue. At this point the peptidoglycan unit is transferred from the lipid pyrophosphate to the growing glycan chain, the other product of this reaction being undecaprenol pyrophosphate. The cycle is completed by the monodephosphorylation of the pyrophosphate to undecaprenol monophosphate, which is then able to accept further building units. It is of interest that the dephosphorylation of undecaprenol pyrophosphate to its monophosphate is specifically inhibited by bacitracin, and this is believed to be the mechanism of the action of this antibiotic. Moreover, vancomycin and ristocetin specifically inhibit the

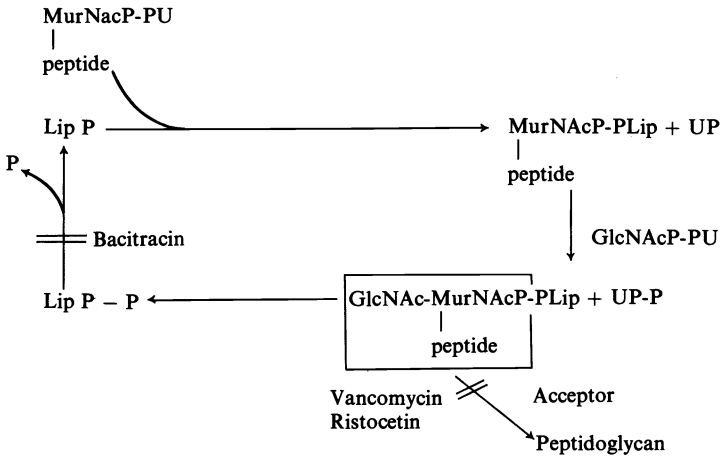


Figure 9. Synthetic cycle for peptidoglycan

transfer of the glycan unit from the undecaprenol pyrophosphate intermediate to the growing peptidoglycan chain. Although the detailed mechanism of chain extension is not yet clear, it has been shown that, as in certain other cases of polysaccharide biosynthesis, glycan chain extension occurs from the reducing end of the chain.

The final stage of peptidoglycan synthesis is the crosslinking of peptide chains; this is illustrated in *Figure 10*, where it is seen that the additional D-alanine residue is displaced from the carboxyl terminus of a peptide chain by a glycine from a neighbouring chain. The result is the linking together of adjacent peptide chains with the elimination of D-alanine. It is, of course, this transpeptidation reaction that is specifically inhibited by penicillin, and

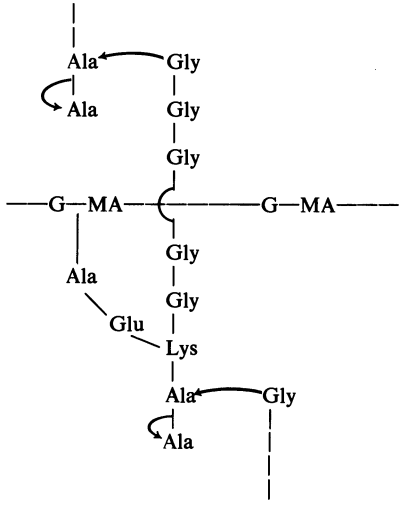


Figure 10. Crosslinkage reaction in peptidoglycan synthesis

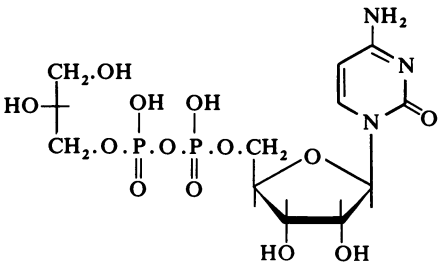


Figure 11. Cytidine diphosphate glycerol

undoubtedly an important feature of the mechanism of the inhibitory action of penicillin on bacterial growth is inhibition of the transpeptidase[16].

Teichoic acids are synthesized from cytidine diphosphate glycerol (*Figure 11*) and cytidine diphosphate ribitol[17,18]. Chain extension occurs through the transfer of polyol phosphate by transphosphorylation. Where sugar residues occur as appendages on the main chain, these can be transferred from the appropriate nucleoside diphosphate sugar after the chain has been formed, whereas when the sugar residues form a part of the chain, then the transfer occurs alternately to the transfer of polyol phosphate. In those teichoic acids where sugar 1-phosphate residues occur in the main chain, these are transferred as intact units from the appropriate nucleoside diphosphate sugar. Chain extension in teichoic acids occurs in the opposite direction from that in the glycan chains of peptidoglycan, i.e. polyol phosphate residues are added to the polyol rather than the phosphate end of the growing chain[19].

It has not been possible to demonstrate the participation of undecaprenol phosphate in the synthesis of teichoic acids comprising poly(ribitol phosphate) chains, and there is also some doubt about this participation in the synthesis of simple poly(glycerol phosphate). However, in those teichoic acids where sugar residues form a part of the chain, it has been shown that undecaprenol phosphate participates in a manner analogous to that for peptidoglycan[20]. This is well illustrated for the synthesis of the teichoic acid in the wall of *Bacillus licheniformis* (*Figure 12*). In this case undecaprenol phosphate accepts a glucose residue from UDP-glucose to form undecaprenol-monophosphate-glucose in which the glycosidic linkage is β (in UDP glucose the linkage is α). A glycerol phosphate is then transferred from CDP-glycerol to the 6-position on the glucose residue in the lipid intermediate to form an undecaprenol phosphate derivative containing the complete repeating unit of the teichoic acid (but lacking alanine). This residue is then transferred to the growing teichoic acid chain and the other product of the reaction is undecaprenol monophosphate which is now available for repetition of the cycle[21]. The alanine ester residues are believed to be introduced after the chain has been formed and two enzymes are thought to be involved in their incorporation[22].

The very small amounts of lipid intermediates for teichoic acids which are normally present in bacterial membranes have so far precluded the direct

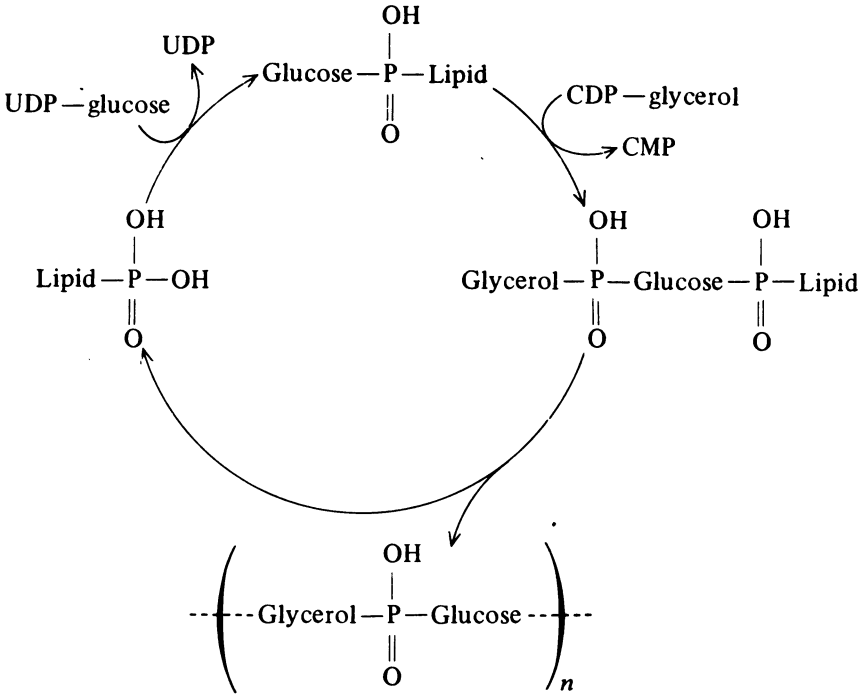


Figure 12. Synthesis of wall teichoic acid in *Bacillus licheniformis*

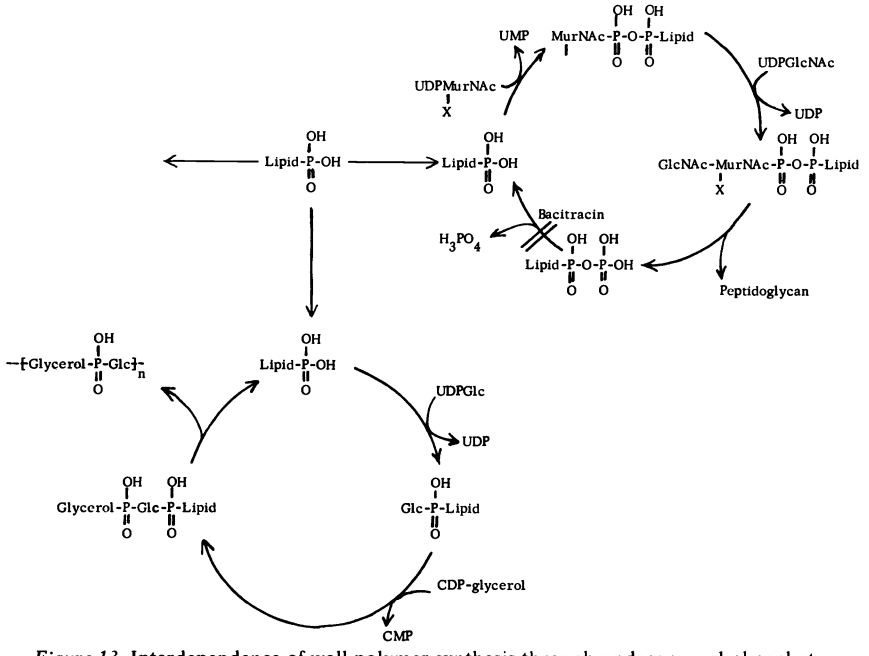


Figure 13. Interdependence of wall polymer synthesis through undecaprenol phosphate

demonstration that the lipid is indeed undecaprenol. However, careful examination of the total prenols from hydrolysed extracts of *Bacillus licheniformis* has failed to reveal the presence of any example other than undecaprenol. An indirect demonstration that the lipid is in fact undecaprenol has been achieved[23]. If it is assumed that undecaprenol phosphate is an intermediate in the synthesis of both polymers and also that there is a common pool of this lipid phosphate in the preparation, then interference with the synthesis of one polymer should be reflected in the rate of synthesis of the other. A particulate membrane preparation which was capable of synthesizing both peptidoglycan and teichoic acid was obtained; in this system peptidoglycan synthesis occurs by the usual route (*Figure 13*) and the teichoic acid synthesis is that already described. The synthesis of the peptidoglycan is inhibited by bacitracin, whereas that of the teichoic acid is unaffected, because undecaprenol pyrophosphate is not an intermediate in that synthesis; consequently the rate of synthesis of the teichoic acid is unaffected by the presence of bacitracin. However, if the system also contains nucleotide precursors for peptidoglycan synthesis, then undecaprenol phosphate is diverted away from the teichoic acid cycle into the peptidoglycan cycle, where it accumulates because the bacitracin prevents its return to the common pool. The result is a diminution in the rate of teichoic acid synthesis by at least 50 per cent. The rate of formation of either polymer is reduced when synthesis of the other polymer is occurring simultaneously owing to direct competition for undecaprenol phosphate in the pool; the inhibitory effect of bacitracin is additional to this.

It follows from these studies that undecaprenol phosphate is common to both synthetic pathways. It also follows that the amount of undecaprenol phosphate in the cell-free system is rate-limiting. The latter conclusion is interesting because it suggests that control of wall synthesis might be achieved by regulating the amount of undecaprenol phosphate available in a common pool, thereby controlling the over-all rate of the several synthetic routes. This supports the previous proposal that undecaprenol kinase and undecaprenol phosphate phosphatase could together regulate the amount of undecaprenol phosphate and thereby control the rate of wall synthesis[24].

Evidence for the participation of undecaprenol phosphate in the synthesis of polymers of glycerol phosphate and ribitol phosphate is inconclusive. Despite considerable effort, no prenol phosphate intermediate containing ribitol has been found. Nevertheless evidence for the participation of a different kind of lipid intermediate in these cases has been presented[25]. It is suggested that the polymer chain grows by transfer of residues from the appropriate nucleotide precursor to a lipid which has some resemblance to lipoteichoic acid. The details of this route require further clarification and it is possible that this might be assisted by the discovery that, at least with some organisms, the wall teichoic acid synthesizing complex can be released from the membrane by a simple process of multiple freezing and thawing[26]. The soluble enzyme preparation is free from most of the important membrane-bound enzymes (*Table 1*). Further purification by Sephadex and ion-exchange chromatography gives a highly active preparation. It contains protein and phospholipids, the latter comprising about 40 per cent of the weight of the preparation. Amongst these phospholipids is undecaprenol

Table 1. Release of enzymes from membranes by freezing and thawing

Enzyme	MR	CS
Alkaline phosphatase	80*	8
Acid phosphatase	89	1.5
Succinate dehydrogenase	97.5	0
NADH dehydrogenase	81.5	17.5
poly GP synthetase	55	409
poly GPGlc synthetase	80	196

* Relative specific activities; activity in original membrane = 100.
MR = membrane residue CS = soluble fraction

phosphate, and it can be shown that this accepts and transfers residues from nucleotide precursors to the growing polymer.

The soluble enzyme system should be helpful in studying the possibility that control mechanisms operate at the lipid intermediate level. Such experiments might be rather difficult but control of synthesis at the nucleotide precursor level has been easier to study. Using relatively unpurified enzyme preparations, it has been shown[27] that a number of control steps occur in the synthesis of nucleotide precursors for both polymers (*Figure 14*). The synthesis of UDP-muramyl pentapeptide is inhibited by CDP-glycerol at an early stage, i.e. at the formation of UDP-*N*-acetylglucosamine from *N*-acetylglucosamine 1-phosphate. The pentapeptide itself inhibits this synthesis at the same point and is also inhibitory towards the subsequent formation of the enolpyruvate derivative. Similarly the synthesis of CDP-glycerol from glycerol 1-phosphate is inhibited by UDP-*N*-acetylmuramyl pentapeptide. Both syntheses are inhibited by ADP, and clearly the energy charge of the cell is a controlling factor. These regulatory effects have not been examined quantitatively but this should be possible using more carefully purified enzyme preparations.

One of the exciting features of scientific research is the hope that from time to time investigations will yield bonuses in the form of discoveries in other fields. This has happened in our recent work on the biosynthesis of wall polymers when it was found (J. Baddiley, J. P. Burnett, I. C. Hancock and J. Heptinstall, unpublished work) that membrane preparations from *Bacillus licheniformis*, when incubated with UDP-*N*-acetylglucosamine, gave rise to membrane-bound material containing *N*-acetylglucosamine. Apart from peptidoglycan itself, the wall of this organism contains no other component derived from *N*-acetylglucosamine. Nor were there any lipids known in the membrane containing *N*-acetylglucosamine. The material containing the amino sugar residues, which is in fact a relatively minor membrane component, could be extracted with water at 60°C, and after purification by several chromatographic procedures it had the properties of a glycoprotein. It is an interesting fact that although there has been extensive work on glycoproteins in membranes of cells of higher organisms, not much is known about such compounds in bacterial membranes. There have been one or two reports of their presence in bacteria but relatively little chemical study has been carried out. The compound isolated from *Bacillus licheniformis* is strongly acidic, and although structural investigations are

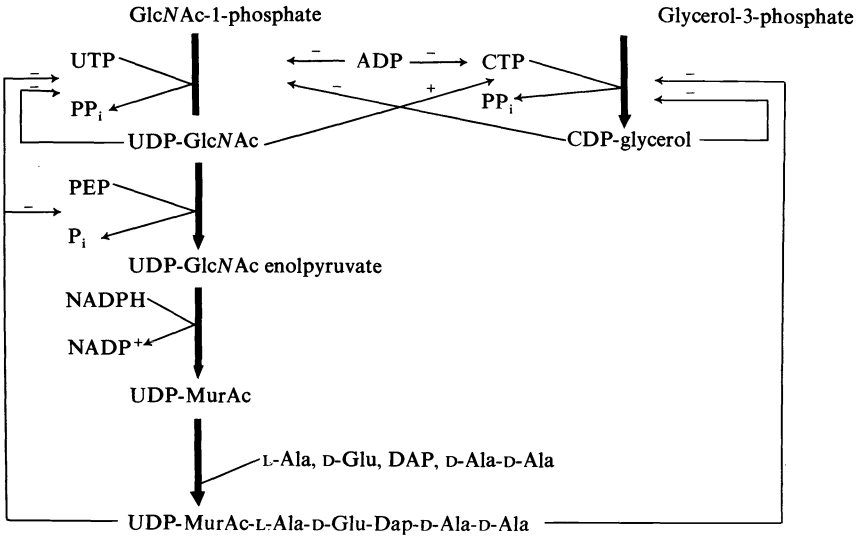


Figure 14. Control of biosynthesis of nucleotide precursors for peptidoglycan and teichoic acid

still in progress, it is already clear that this glycoprotein is unusual. Degradation has yielded strongly acidic peptides containing *N*-acetylglucosamine and glucose, and hydrolysis has given phosphates of both of these sugars. It seems, then, that sugar phosphate linkages are present in the molecule. Such phosphoglycoproteins have been described previously in one or two cases, namely in *Hansenula holstii*[28], *Cladosporium*[29] and *Penicillium charlesii*[30].

Clearly glycoproteins of this kind are of great interest. Although their function is at present unknown, it is worth noting that membrane proteins are likely to be associated with several different kinds of biological activity. They may be enzymes, e.g. those concerned in electron transport, oxidative phosphorylation or the synthesis of wall polymers. Moreover, some organisms including *Bacillus licheniformis* excrete extracellular enzymes into the surrounding medium. Another possible function is in the permease systems, i.e. the proteins involved in selective transport of metabolites across the membrane. It is hoped that further studies will help to clarify the function of these phosphoglycoproteins, which have now been detected in several other species of bacteria.

REFERENCES

[1] H. J. Rogers and H. R. Perkins, *Cell Walls and Membranes*. Spon: London (1968).
[2] J. Baddiley, *Essays in Biochemistry*, **8**, 35–77 (1972).
[3] D. W. Tempest, J. W. Dicks and D. C. Ellwood, *Biochem. J.* **106**, 237–243 (1968).
[4] A. R. Archibald, J. Baddiley and N. L. Blumsom, *Advanc. Enzymol. Relat. Areas Mol. Biol.* **30**, 223–253 (1968).
[5] A. R. Archibald, J. Baddiley and J. E. Heckels, *Nature New Biol.* **241**, 29–31 (1973).
[6] J. L. Meers and D. W. Tempest, *J. Gen. Microbiol.* **63**, 325–331 (1970).
[7] S. Heptinstall, A. R. Archibald and J. Baddiley, *Nature (Lond.)*, **225**, 519–521 (1970).

[8] A. H. Hughes, I. C. Hancock and J. Baddiley, *Biochem. J.* **132**, 82–93 (1973).
[9] K. W. Knox and A. J. Wicken, *J. Gen. Microbiol.* **63**, 237–248 (1971).
[10] P. Toon, P. E. Brown and J. Baddiley, *Biochem. J.* **127**, 399–409 (1972).
[11] J. Coley, M. Duckworth and J. Baddiley, *J. Gen. Microbiol.* **73**, 587–591 (1972).
[12] A. J. Wicken and K. W. Knox, *J. Ultrastruct. Res.* **43**, 483–497 (1973).
[13] D. A. Powell, M. Duckworth and J. Baddiley, *FEBS Letters*, **41**, 259–263 (1974).
[14] P. M. Meadow, J. S. Anderson and J. L. Strominger, *Biochem. Biophys. Res. Commun.* **14**, 382–390 (1964).
[15] Y. Higashi, J. L. Strominger and C. C. Sweeley, *J. Biol. Chem.* **245**, 3697–3702 (1970).
[16] K. Izaki, M. Matsuhashi and J. L. Strominger, *Proc. Nat. Acad. Sci. USA*, **55**, 656–663 (1966).
[17] J. J. Armstrong, J. Baddiley, J. G. Buchanan, B. Carss and G. R. Greenberg, *J. Chem. Soc.* 4344–4354 (1958).
[18] M. M. Burger and L. Glaser, *J. Biol. Chem.* **239**, 3168–3177 (1964).
[19] L. D. Kennedy and D. R. D. Shaw, *Biochem. Biophys. Res. Commun.* **32**, 861–865 (1968).
[20] H. Hussey and J. Baddiley, *Biochem. J.* **127**, 39–50 (1972).
[21] I. C. Hancock and J. Baddiley, *Biochem. J.* **127**, 27–37 (1972).
[22] V. M. Reusch and F. C. Neuhaus, *J. Biol. Chem.* **246**, 6136–6143 (1971).
[23] R. G. Anderson, H. Hussey and J. Baddiley, *Biochem. J.* **127**, 11–25 (1972).
[24] Y. Higashi, G. Siewert and J. L. Strominger, *J. Biol. Chem.* **245**, 3683–3690 (1970).
[25] J. Mauck and L. Glaser, *Proc. Nat. Acad. Sci. USA*, **69**, 2386–2390 (1972).
[26] I. C. Hancock and J. Baddiley, *FEBS Letters*, **34**, 15–18 (1973).
[27] R. G. Anderson, L. J. Douglas, H. Hussey and J. Baddiley, *Biochem. J.* **136**, 871–876 (1973).
[28] L. P. Kozak and R. K. Bretthauer, *Biochemistry*, **9**, 1115–1122 (1970).
[29] K. O. Lloyd, *Biochemistry*, **11**, 3884–3890 (1972).
[30] J. E. Gander, N. H. Jentoft, L. R. Drewes and P. D. Rick, *J. Biol. Chem.* **249**, 2063–2072 (1974).