Structure, Biosynthesis, and Function of Teichoic Acids

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Received November 17, 1969

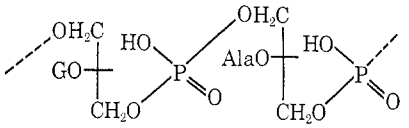
The walls of gram-positive bacteria have received much attention in recent years, due to an increasing awareness of their fundamental importance in many aspects of the study of bacteria and to the greatly improved techniques available for their preparation and study. Bacteria often comprise 10–20% of the dry weight of the cell and thus represent a significant part of the cell's metabolic products. Moreover, they usually have attached to them the characteristic immunologically active components of the cell. Of medical and biochemical significance is the fact that many antibiotics, including the penicillins, bacitracin, vancomycin, ristocetin, and cycloserine, act by interfering with the processes of wall biosynthesis; this is not only clinically important but has been valuable as a specific tool in establishing the mechanism of wall biosynthesis. The membrane situated immediately beneath the wall is perhaps of even greater interest, as this fragile structure is not only an osmotic barrier but is also the part of the cell to which are attached many enzymes and specific proteins associated with the transport of metabolites, biosynthesis, electron transport, and other vital activities.

As teichoic acids are major components of both walls and membranes of many bacteria, advances in understanding the behavior of the outer regions of the cell must depend upon corresponding advances in our knowledge of the properties and function of these interesting biopolymers. Moreover, the nature and function of the membranes of cells from higher forms of life are subjects of outstanding importance and increasing activity, and this growing field of biology at the molecular level should be assisted by the more detailed knowledge already available and currently being assembled from work on bacterial membranes.

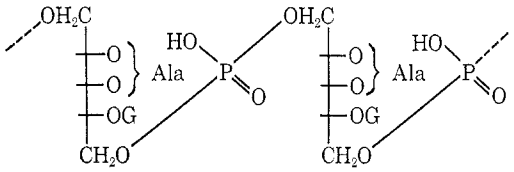
Recent interest in teichoic acids includes knowledge of their structure, the nature of their linkage to the other main wall material and to the membrane, and details of the mechanism of their biosynthesis, function, and immunological properties. Although these substances exhibit important serological reactions and are significant in bacterial taxonomy, and doubtless in host–parasite relationships, these immunological and biochemical aspects are not discussed in this article; attention is confined to structure, biosynthesis, and function.

Structure

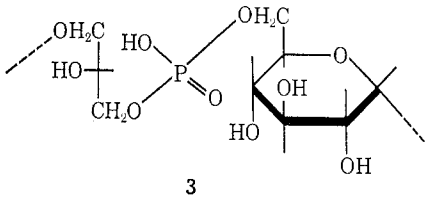
Polymers of Glycerol Phosphate and of Ribitol Phosphate. The term teichoic acid[1] was first applied to a group of polymers in the walls of many gram-positive bacteria, and included structurally related polymers described as "intracellular" teichoic acids from elsewhere in the cell. It is now known that the latter polymers are associated with the cytoplasmic membrane,[2,3] and the term "membrane teichoic acid" is preferred for this group. The membrane teichoic acids are remarkably uniform in structure. They all possess poly(glycerol phosphate) chains in which linkage is through phosphodiester groups involving positions 1 and 3 on adjacent glycerol units; in addition, glycosyl substituents are often found on some or all of the 2 positions, and D-alanine ester residues occur on either glycerol or glycosyl hydroxyl groups (*e.g.*, **1**). They are believed to occur associated with the membrane of all gram-positive bacteria, and structural differences are confined to the nature and number of glycosyl substituents.



1, G = glycosyl; Ala = D-alanyl



2, G = glycosyl; Ala = D-alanyl



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The first wall teichoic acids resembled the membrane teichoic acids but, in addition to glycerol phosphate polymers, examples were found of poly(ribitol phosphate) in which phosphodiester linkages joined positions 1 and 5 on adjacent ribitol residues; glycosyl substituents and D-alanine ester residues were again present (*e.g.*, **2**). The length of the chains has not been determined accurately in view of their ready hydrolysis during extraction, but they probably range from 6 to 20 repeating units. In most of the walls where these polymers occur they represent between 20 and 60% of the dry weight; the rest of the wall is mainly pep-

(1) J. J. Armstrong, J. Baddiley, J. G. Buchanan, B. Carss, and G. R. Greenberg, *J. Chem. Soc.*, 4344 (1958).

(2) J. B. Hay, A. J. Wicken and J. Baddiley, *Biochim. Biophys. Acta*, **71**, 188 (1963).

(3) G. D. Shockman and H. D. Slade, *J. Gen. Microbiol.*, **37**, 297 (1964).

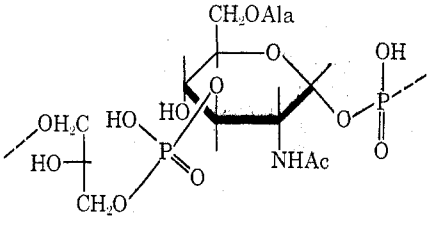
tidoglycan, an insoluble polymer comprising polysaccharide chains of regularly repeating *N*-acetylglucosamine and *N*-acetylmuramic acid (an ether of D-lactic acid and the 3-hydroxyl group of *N*-acetylglucosamine) cross-linked with peptide chains attached through the carboxyl groups of some or all of the muramic acid residues. The proportion of teichoic acid is somewhat dependent upon growth conditions, and higher values are sometimes encountered.

Sugar Residues in the Polymer Chain: What is a Teichoic Acid? An interesting finding arising from work on a wider range of bacterial species is the structural diversity now recognized among the wall teichoic acids.[4] Examples are known in which sugar residues form a part of the polymer chain, and the presence of sugar 1-phosphate linkages has been found in an increasing number of cases. The breadth of structural variation is so large that discussion of teichoic acids must now include many bacterial wall, membrane, and capsule polymers that lack one or more of the features contained in the original poly(glycerol phosphate) and poly(ribitol phosphate) structures. In fact, there is no obvious criterion for the definition of teichoic acids on purely structural grounds, and it is arguable that one might include all wall and membrane polymers that possess phosphodiester groups, polyol, and/or sugar residues and usually but not always D-alanine ester residues. Wall, membrane, and capsule polymers are now known to include structural varieties extending in a broad range from conventional teichoic acids through sugar 1-phosphate polymers, phosphorylated polysaccharides, polysaccharides, and lipopolysaccharides, to phospholipids, in addition to the structural peptidoglycans and proteins. Thus, the problem of definition of teichoic acids must include both general structural features and regard to their location in the cell.

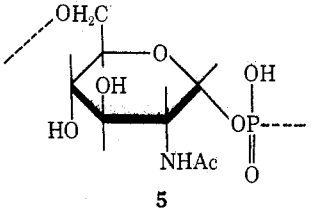
The first examples of wall teichoic acids in which the glycosyl residues constitute a part of the polymer chain were found in bacilli,[5] *e.g.*, **3**, and a similar galactosyl derivative occurring in *Bacillus licheniforms* ATCC 9945. It was known earlier, however, that related but more complex polymers, with repeating units comprising several different sugars attached to ribitol phosphate, occur as type-specific substances in the capsules of some pneumococci.[6-8] An interesting feature of the pneumococcal compounds is the frequent occurrence in them of galactofuranosyl residues; these organisms also possess in their walls a unique ribitol teichoic acid (C substance) containing choline phosphate.[9]

The structure of hydrolysis products of teichoic acids from certain bacilli and actinomycetes suggests that 1,2-phosphodiesters of glycerol may be present in these polymers. The evidence is, however, not conclusive, and it remains possible that these teichoic acids are related to those of **3** in which the phosphate linkage is between a terminal hydroxyl on glycerol and a sugar hydroxyl on a neighboring repeating unit.

Polymers Containing Sugar 1-Phosphate Linkages. The walls of *Staphylococcus lactis* I3 and *Micrococcus* Sp24 contain a polymer in which *N*-acetylglucosamine 1-phosphate is attached through phosphodiester linkage to glycerol phosphate (**4**). This polymer differs from those encountered previously in that some of its sugar residues are destroyed (to give saccharinic acid) in alkali.[10] This arises through hydrolysis of phosphodiester linkages, involving the usual cyclic phosphate mechanism, to give glycerol diphosphates and thereby exposing the alkali-sensitive reducing groups of *N*-acetylglucosaminyl residues. Although phosphodiester linkages involving the 1 position of sugar residues were known in phosphomannans of yeast cell walls, such linkages had not previously been found in teichoic acids or other bacterial polymers. More recently other examples of sugar 1-phosphate groups in bacterial wall polymers have been described.[11] These include a poly(*N*-acetylglucosamine 1-phosphate) (**5**) and the related poly[D-glucopyranosyl(1→3 or 4)-*N*-acetylgalactosamine 1-phosphate][12] from micrococci.



4, Ac = acetyl; Ala = D-alanyl



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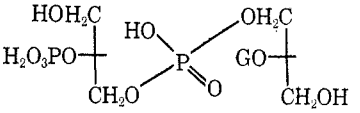


6, G = α-D-glucopyranosyl

Distribution of Glycosyl Substituents along the Chain. Some wall teichoic acids and most membrane teichoic acids possess fewer glycosyl substituents than polyol phosphate units. This has raised the question whether these polymers consist of uniformly but in-

(4) A. R. Archibald and J. Baddiley, *Advan. Carbohyd. Chem.*, **21**, 323 (1966).
(5) L. Glaser and M. Burger, *J. Biol. Chem.*, **239**, 3187 (1964).
(6) Z. A. Shabarova, J. G. Buchanan, and J. Baddiley, *Biochim. Biophys. Acta*, **57**, 146 (1962).
(7) P. A. Rebers and M. Heidelberger, *J. Am. Chem. Soc.*, **83**, 3056 (1961).
(8) W. K. Roberts, J. G. Buchanan, and J. Baddiley, *Biochem. J.*, **88**, 1 (1963).
(9) D. E. Brundish and J. Baddiley, *ibid.*, **110**, 573 (1968).
(10) A. R. Archibald, J. Baddiley, and D. Button, *ibid.*, **110**, 543 (1968).
(11) A. R. Archibald, J. Baddiley, D. Button, S. Heptinstall, and G. H. Stafford, *Nature*, **219**, 855 (1968).
(12) M. D. Partridge, A. L. Davison, and J. Baddiley, unpublished work.

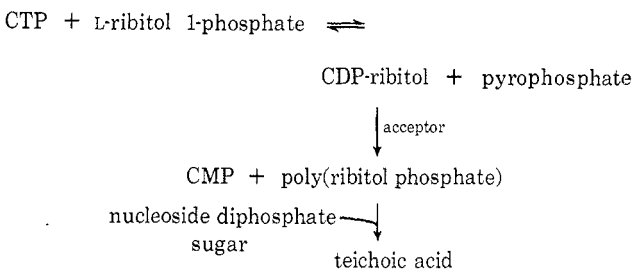
completely glycosylated chains or whether they are mixtures of unsubstituted and fully substituted chains. Serological methods were used to study this in a bacillus possessing incompletely glucosylated ribitol teichoic acids in their walls.[13] In this case a specific antiserum was used to separate a polymer in which one glucosyl substituent was present on each ribitol from that which was unsubstituted. This is analogous to the serological demonstration[14] that the ribitol teichoic acid from strains of *Staphylococcus aureus* is a mixture of two homogeneous molecular species, one having only α-*N*-acetylglucosaminyl substituents and the other being entirely β substituted.

The problem can be studied in glycerol teichoic acids by chemical degradation with alkali. A 1,3-poly(glycerol phosphate) would be hydrolyzed by alkali through intermediate cyclic phosphate mainly to glycerol and its mono- and diphosphates. Howev r, if there is a glycosyl substituent on position 2 of each glycerol residue, cyclic phosphate formation would be prevented and no hydrolysis would occur. Consequently, a mixture of such polymers would yield on hydrolysis in alkali no degradation products containing glycosyl substituents, whereas incompletely substituted polymer chains would be hydrolyzed to give small molecules containing glycosyl substituents, *e.g.*, glycosylglycerol. The matter was examined on intact walls of *Lactobacillus bulgaricus*,[15] and it was found that alkali gave glucosylglycerol, glycerol phosphates, and a small amount of a phosphodiester with structure **6**. These products, which were also formed in similar proportions by hydrolysis of extracted teichoic acid with alkali, indicate that the polymer in this case is composed of partially glucosylated glycerol phosphate chains. This result agrees with similar studies on isolated glycerol teichoic acids from the walls and membranes of other bacteria; thus it seems that in some organisms a mixture of fully substituted and unsubstituted polymer chains occurs, whereas in others the chains are all partially substituted.

Biosynthesis

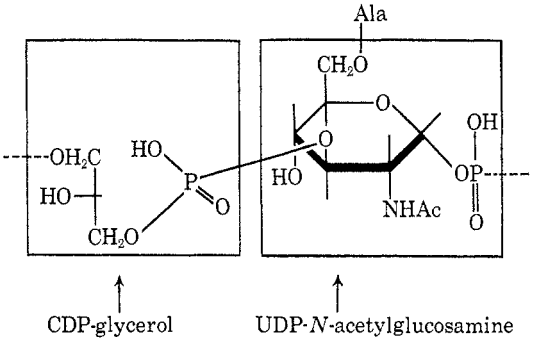
Nucleotide Precursors. At the time of their discovery[1] it had been assumed that the biosynthesis of teichoic acids occurs through a stepwise addition of polyol phosphate residues to chains by transfer from the nucleotides cytidine diphosphate glycerol (CDP-glycerol) or cytidine diphosphate ribitol (CDP-ribitol), both of which were already known to occur in bacteria;[16,17] the stereochemistry of polyol phosphate residues in the nucleotides and polymers supported this view. In fact, it was this biosynthetic argument that led to the discovery of teichoic acids. The correctness of this mechanism was confirmed experimentally with cell-free enzyme systems that were shown to constitute a part of the cytoplasmic membrane.[18,19] The attachment of glycosyl substituents occurs through the intermediary nucleoside diphosphate sugars either following polyol phosphate chain synthesis or in step with it; no information is yet available about the mechanism of attachment of alanine ester residues. These general features are outlined in Scheme I for a ribitol teichoic acid.

Scheme I



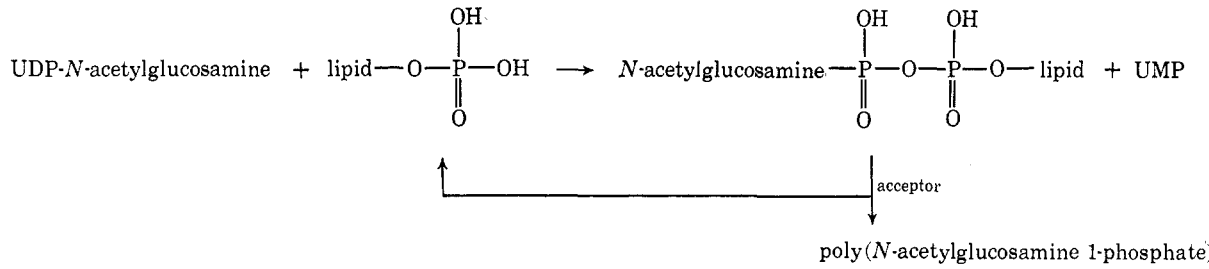
In this synthesis chain extension can occur in the absence of the nucleoside diphosphate sugar, whereas the corresponding synthesis of teichoic acids in which sugar residues form a part of the polymer chain, *e.g.*, **3**, must require the presence of both nucleotide precursors.[20] On the other hand, the synthesis of the polymer with structure **4**, in which the chain contains a repeating unit comprising glycerol, *N*-acetylglucosamine, and two phosphate groups, presents a particularly interesting case. Although it seemed likely that glycerol and one phosphate would be transferred from CDP-glycerol, and the amino sugar might well come from a nucleotide precursor, the origin of the second phosphate was by no means clear. The mechanism of synthesis was established by using a fragmented membrane preparation with which it was shown that both CDP-glycerol and uridine diphosphate *N*-acetylglucosamine (UDP-*N*-acetylglucosamine) were required simultaneously for polymer synthesis.[21] The CDP-glycerol contributed a glycerol phosphate unit, in agreement with the known stereochemistry shown in

Scheme II



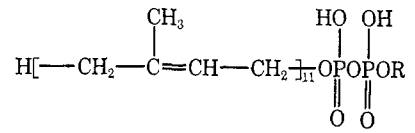
(13) T. Chin, M. Burger, and L. Glaser, *Arch. Biochem. Biophys.*, **116**, 358 (1966).
(14) M. Torii E. A. Kabat, and A. E. Bezer, *J. Exp. Med.*, **120**, 13 (1964).
(15) A. R. Archibald, J. Baddiley, and S. Heptinstall, *Biochem. J.*, **111**, 245 (1969).
(16) J. Baddiley and A. P. Mathias, *J. Chem. Soc.*, 2723 (1954).
(17) J. Baddiley, J. G. Buchanan, B. Carss, A. P. Mathias, and A. R. Sanderson, *Biochem. J.*, **64**, 599 (1956).
(18) M. Burger and L. Glaser, *J. Biol. Chem.*, **239**, 3168 (1964).
(19) L. Glaser, *ibid.*, **239**, 3178 (1964).
(20) M. Burger and L. Glaser, *ibid.*, **241**, 494 (1966).
(21) J. Baddiley, N. L. Blumsom, and L. J. Douglas, *Biochem. J.*, **110**, 565 (1968).

Scheme III

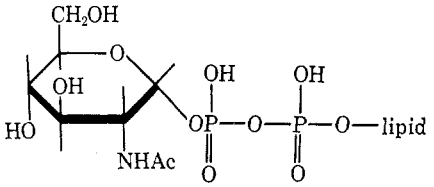


Scheme II, and the uridine derivative contributed an intact *N*-acetylglucosamine 1-phosphate unit. The transfer of sugar 1-phosphate units from nucleotides to polymers had not been observed hitherto in a biosynthetic process but is now believed to be a general route for the synthesis of sugar 1-phosphate residues in biopolymers.

Lipid Intermediates. A better understanding of the mechanism of synthesis of teichoic acids comes from the recent discovery that the process occurs through lipid intermediates. This type of biosynthetic route was first observed in the formation of polysaccharide chains in lipopolysaccharides[22] and in the glycan chains of peptidoglycan.[23] In the latter case, amino sugar derivatives that constitute the structurally rigid highly cross-linked polysaccharide (peptidoglycan) of bacterial walls are transported from nucleotide precursors to final wall polymer through lipid-soluble intermediates in which the sugar residues are attached through a pyrophosphate group to a C-55 polyisoprenoid alcohol, *e.g.*, **7**.



7, R = repeating unit of peptidoglycan



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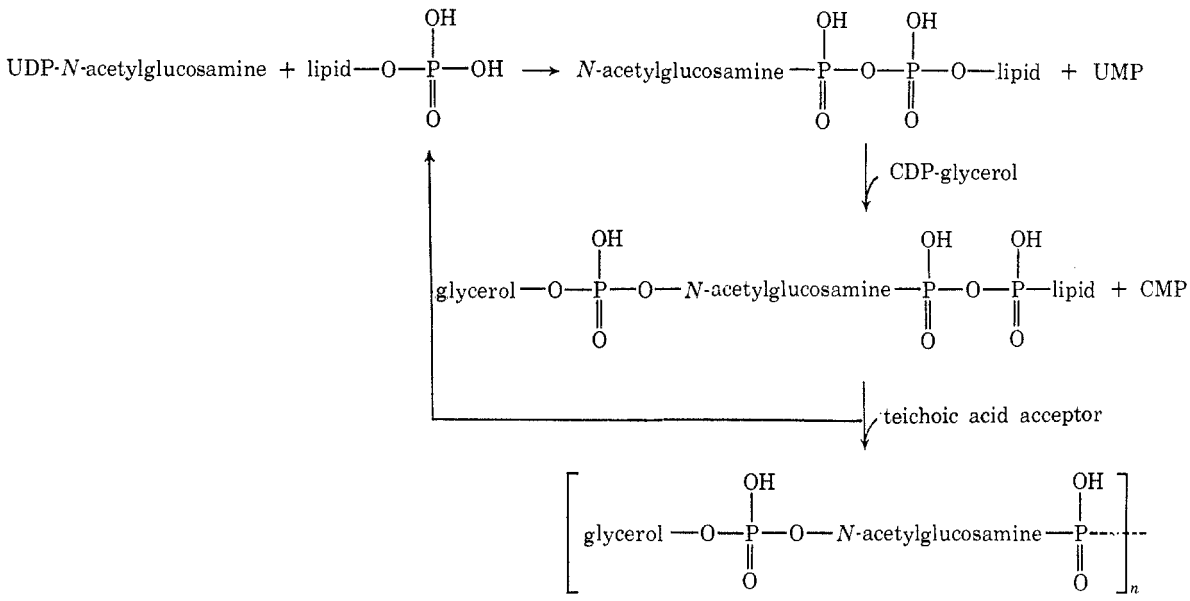
The simplest case studied so far of the participation of a lipid intermediate in the formation of a polymer broadly classified as a teichoic acid is the poly(*N*-acetylglucosamine 1-phosphate) (**5**) from the walls of *Staphylococcus lactis* 2102. It was found that the cytoplasmic membrane synthesizes polymer from UDP-*N*-acetylglucosamine; no other precursors are required, and the *N*-acetylglucosamine 1-phosphate residues are transferred to polymer as intact units.[24] By using labeled nucleotide it was shown that a lipid component of the membrane becomes labeled during the synthesis. The amount of lipid intermediate is extremely small (probably only a few micrograms per milliliter of wet sedimented membrane), and technical difficulties have so far prevented its isolation in sufficient quantity for characterization of the lipid part of the molecule. However, with ^{14}C labeling in the *N*-acetylglucosamine, purification has been achieved, and hydrolysis studies have established that the intermediate is a derivative of α-*N*-acetylglucosamine 1-pyrophosphate (**8**); the high acid lability of the linkage to the lipid part of the molecule is consistent with the pyrophosphate being joined in ester linkage to an isoprenoid structure. Pulse-labeling experiments have established that the lipid derivative is an intermediate between nucleotide and polymer.[25] The proposed biosynthetic route to polymer is given in Scheme III.

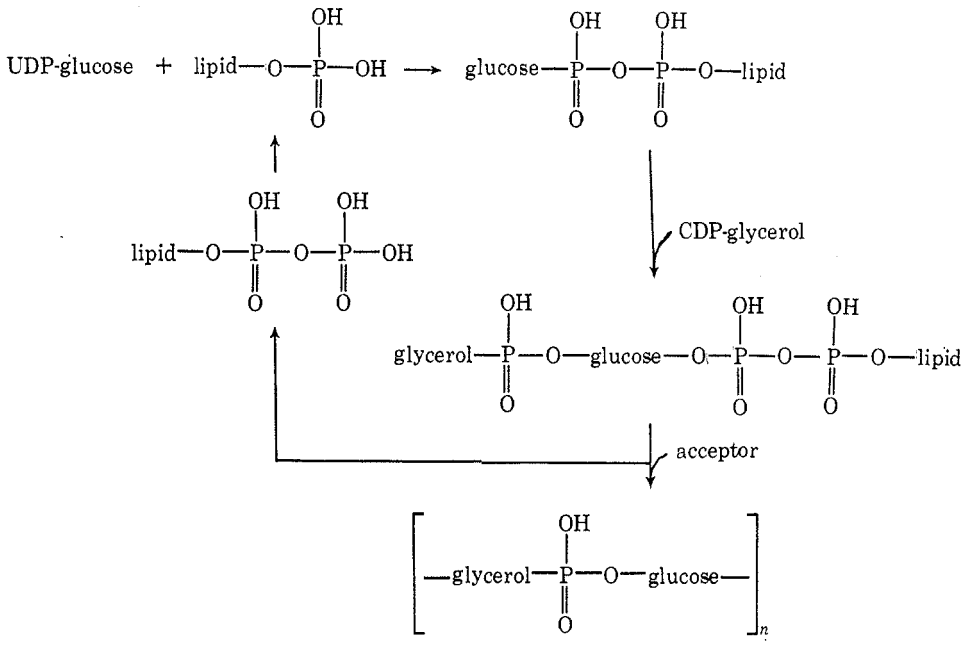
The situation is more complex in the enzymic synthesis of the teichoic acid with structure **4**. Here also, by using UDP-*N*-acetylglucosamine and CDP-glycerol in pulse-labeling experiments,[26] it was found that lipid components in the membrane accept the labeled units from the nucleotides and then transfer them to the growing polymer. At 37° a lipid was formed in about the same amount as in the case described above. It is not known whether this is identical with that already described, but at least it possesses an *N*-acetylglucosamine 1-pyrophosphate–lipid structure. Under these conditions, but with the addition of labeled CDP-glycerol, barely detectable traces of ^{14}C were incorporated into the lipid components of the membrane fragments. At 30°, however, a somewhat enhanced incorporation of label occurred, but this was still only 10% of the corresponding incorporation obtained from UDP-*N*-acetylglucosamine. Nevertheless, when both nucleotides were present a second lipid intermediate was detected; chemical and enzymic degradation showed that it probably contained the complete repeating unit of the teichoic acid and this is, of course, consistent with the labeling pattern. The suggested biosynthetic route for this teichoic acid is given in Scheme IV, where it is seen that UDP-*N*-acetylglucosamine transfers its amino sugar 1-phosphate residue to a lipid monophosphate; the resulting lipid then accepts a glycerol phosphate from CDP-glycerol to give the intermediate that possesses a complete repeating unit (except for the alanine ester group).

(22) A. Wright, M. Dankert, P. Fennessy, and P. W. Robbins, *Proc. Nat. Acad. Sci. U. S.*, **57**, 1798 (1967).
(23) Y. Higashi, J. L. Strominger, and C. C. Sweeley, *ibid.*, **57**, 1878 (1967).
(24) D. Brooks and J. Baddiley, *Biochem. J.*, **113**, 635 (1969).
(25) D. Brooks and J. Baddiley, *ibid.*, **115**, 307 (1969).
(26) L. J. Douglas and J. Baddiley, *FEBS Lett.*, **1**, 114 (1968).

Scheme IV



Scheme V



This unit is transferred to polymer, leaving lipid monophosphate that is available for a second cycle.

The route to the synthesis of wall polymers that contain sugar 1-phosphate residues differs in one important respect from those leading to the synthesis of peptidoglycan and polysaccharide chains in lipopolysaccharide. In the transfer of sugar phosphate residues from the lipid intermediates to polymers the other reaction product is inevitably a lipid monophosphate, whereas in the transfer of sugar or oligosaccharide residues from such intermediates during the synthesis of lipopolysaccharides or peptidoglycans no phosphate groups are transferred; the product is thus a lipid pyrophosphate, and the completion of the cycle requires the hydrolysis by a specific phosphatase of the terminal phosphate, thereby regenerating a lipid monophosphate. It is interesting that the antibiotic bacitracin is a selective inhibitor of this phosphatase and so prevents the completion of the cycle.[27] In agreement with this proposed mechanism of action, bacitracin has no significant inhibitory effect on teichoic acid biosynthesis.[24]

An especially interesting feature of teichoic acid biosynthesis arises in the case of the glycerol phosphate–glucosyl polymer[20] in the walls of *Bacillus licheniformis* ATCC 9945 (**3**). It has been found recently[28] that, like the other teichoic acids, this is synthesized by membrane-bound enzymes and lipid intermediates are involved in the synthesis. Whereas in the polymer itself glycerol phosphate and glucose residues were

(27) M. Matsuhashi, C. P. Dietrich, and J. L. Strominger, *J. Biol. Chem.*, **242**, 3193 (1967).
(28) I. Hancock and J. Baddiley, unpublished work.

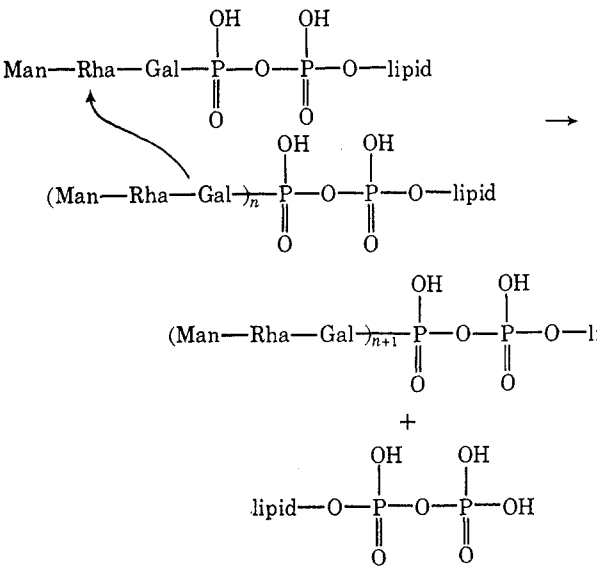
incorporated in equimolar amounts from CDP-glycerol and UDP-glucose, labeling of the unfractionated lipids was much greater from the glucose nucleotide than it was from CDP-glycerol. Fractionation of the lipids on thin-layer chromatography showed that the most abundant and first-formed lipid contained glucose but not glycerol, and probably has the structure indicated in Scheme V. Although structural work is incomplete, there is evidence for the formation of much smaller amounts of a lipid containing both glycerol phosphate and glucose residues, and a possible course of biosynthesis is shown in Scheme V. This route differs fundamentally from the proposed routes for the synthesis of other teichoic acids; in the latter cases chain extension of the polymer occurs through transphosphorylation, *i.e.*, the formation of a new phosphodiester linkage between polymer and the newly donated repeating unit, whereas in the case of the polymer from the *B. licheniformis* chain extension occurs through a process of transglucosylation, *i.e.*, the formation of a glucosyl linkage between the glycerol phosphate–glucosyl unit in the lipid and a hydroxyl group on the terminal glycerol of the polymer. Unfortunately, inhibitory experiments with bacitracin were inconclusive, and further work is required in order to establish the details of this biosynthesis.

During the course of studies on the effect of antibiotics on the cell-free synthesis of teichoic acid in *B. licheniformis*, it was noted that chloramphenicol inhibits teichoic acid synthesis in concentrations similar to those required for inhibition of protein synthesis. Other inhibitors of protein synthesis are ineffective and, in fact, it was shown that protein synthesis was not occurring during the experiment. Apparently this new action of chloramphenicol is unrelated to its well-established role in the inhibition of protein synthesis.

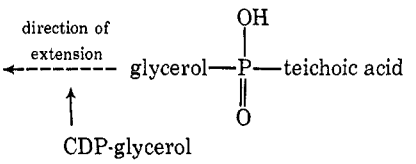
Direction of Chain Extension. The synthesis of a macromolecule in nature can occur in one of two ways. It can be built up from monomer units through reactive monomeric intermediates (*e.g.*, nucleotides) that transfer individual units in succession to the growing end of the polymer chain. Examples of this type of chain elongation include the transfer of glucose units from nucleoside diphosphate glucose to nonreducing ends of polysaccharide chains in the synthesis of starch and glycogen. Alternatively, a chain may be built up by transfer of the activated growing chain to an activated monomer. This is the mechanism that operates in the synthesis of polypeptide chains of proteins on the ribosomes; the growing peptide chain is transferred at its ester linkage with RNA to the amino group of a simple aminoacyl-RNA, and growth thereby occurs at the carboxyl end of the polypeptide.

Pulse-labeling techniques have been used to study this problem in the extension of chains of lipopolysaccharides and of teichoic acids. In the case of polysaccharide chains of lipopolysaccharides extension occurs from the reducing end in a manner analogous to that for the polypeptides.[29] Growth takes place on the lipid intermediates by the transfer of a growing chain from its lipid pyrophosphate to the monomeric unit that is being added (Scheme VI). In the synthesis of a teichoic acid of the poly(glycerol phosphate) type (1) incubation of the enzyme system with labeled CDP-glycerol, followed by unlabled CDP-glycerol, indicated that a labeled glycerol phosphate unit was first added to the glycerol terminus and this later appeared within the newly extended chain.[30] Thus, extension occurs at the glycerol terminus according to Scheme VII by analogy with glycogen chain extension and in contrast to lipopolysaccharide and protein synthesis. Pulse-labeling experiments[24,31] were also used to show that chain extension in teichoic acids containing sugar 1-phosphate residues takes a similar course to that observed with the poly(glycerol phosphate). It seems then that chain growth involving the transfer of a phosphate to form a phosphodiester linkage occurs in a direction similar to that for glycogen, even though lipid intermediates may be involved.

Scheme VI



Scheme VII



Attachment of Wall Polymers

Although teichoic acids can be removed from walls by extraction with dilute acid,[1] alkali,[32,33] or 0.1 *M*

(29) P. W. Robbins, D. Bray, M. Dankert, and A. Wright, *Science*, **158**, 1536 (1967).
(30) L. D. Kennedy and D. R. D. Shaw, *Biochem. Biophys. Res. Commun.*, **32**, 861 (1968).
(31) H. Hussey, D. Brooks, and J. Baddiley, *Nature*, **221**, 665 (1969).
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dimethylhydrazine solution at pH 7[34] under gentle conditions, they are removed by extensive washing with salt solutions, and attachment to the other wall material (peptidoglycan) is by covalent linkage. The removal of these polymers from the wall thus requires the fission of covalent bonds, and consequently many preparations of extracted teichoic acids have suffered some degree of chemical degradation. For this reason estimates of chain length on such material can sometimes be lower than that of the undegraded polymer in the wall. It is interesting that during recent studies on the extraction of teichoic acids with alkali it was observed[33] that the walls of many, but not all, gram-positive bacteria dissolve in 0.5 *M* NaOH solution during 30 hr at 22°. This facile degradation is associated with the presence of glycine residues in the peptide chains of the peptidoglycan in the wall,[35] and it is found that peptide linkages involving glycine are much more labile toward alkali than are those involving other amino acids. This lability is not a function of peptidoglycan structure as such, and it is concluded that glycine peptide linkages in proteins would be similarly labile.

The nature of the linkage between teichoic acid and peptidoglycan is indicated from the action of enzymes on walls. Enzymic hydrolysis of the peptidoglycan yields teichoic acid to which is still attached some degraded peptidoglycan. These soluble preparations still retain the covalent linkage and, as they do not possess phosphomonoester groups, it is assumed that the linkage involves the terminal phosphate of the teichoic acid.[36] A detailed chemical degradative study has been made with *Staphylococcus lactis* I3 where this conclusion has been confirmed, and it is established that the phosphodiester linkage is attached to a muramic acid residue in the peptidoglycan.[37] In fact, muramic acid phosphate is a minor product of acid hydrolysis of the walls of many bacteria (ref 38 and unpublished work from this laboratory), and it is likely that it represents the point of attachment not only of teichoic acids but also of other wall polysaccharides.[39]

Function of Teichoic Acids

It is useful to distinguish between the true biological functions of wall polymers and those of their properties that cause walls to behave in certain ways. For example, teichoic acids and wall polysaccharides frequently possess characteristic serological properties that are responsible for the agglutination of bacteria in the presence of antisera. This property of the polymers is presumably to the disadvantage of the organism and is thus not the purpose for which they were designed. Similarly, the connection between phage receptor sites on the surface of bacilli and cocci and the presence and nature of wall teichoic acids are probably best regarded as manifestations of a property of teichoic acids, because phage infection is mainly a disadvantageous phenomenon.

The importance of teichoic acids to bacteria is indicated by their widespread distribution in the bacterial kingdom. Wall teichoic acids are common among the gram-positive bacteria but are not always found; in those walls that lack teichoic acid, however, acidic polysaccharides containing uronic acid residues are normally present. On the other hand, as far as is known, membrane teichoic acids are present in the ill-defined region between wall and membrane of all gram-positive bacteria. Among the many species examined in the author's laboratory no exception has been found. Although these polymers are not present in typical gram-negative bacteria, it is nevertheless significant that these organisms possess related acidic wall polymers, the lipopolysaccharides, and it is reasonable to assume that many of the aspects of the function of teichoic acids discussed below could be applied to lipopolysaccharides. Direct evidence for the importance of acidic polymers in the outer regions of bacteria comes from the demonstration that when cells are grown under conditions where the availability of inorganic phosphate in the medium limits the growth rate, wall teichoic acid is not produced, but in its place is substituted a polysaccharide rich in uronic acid residues;[40] even under these growth conditions, however, the cells continue to synthesize membrane teichoic acid, thus confirming the essential role of that cellular component.

The original suggestion[41] that these polymers might be concerned in ion exchange and control of the access of ions to the cell has gained much weight recently. It has been shown that monovalent and particularly bivalent cations have a powerful affinity for the acidic groups in bacterial walls,[42,43] and the assumption that this is normally due to the phosphate groups of teichoic acids is established by experiments on the ability of walls to bind Mg^{2+} before and after modification or removal of teichoic acids. In the walls studied[44] about 0.5 equiv of Mg^{2+} was bound for each phosphate in the wall. The influence of the alanine residues was studied by their removal by reaction with dilute hydroxylamine under gentle neutral conditions. When walls were treated in this way, alanine was removed but the rest of the teichoic acid remained; such walls showed a greatly increased affinity for Mg^{2+}. One of the walls studied contained a ribitol teichoic acid,

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and this could be removed by oxidation with periodate; the binding capacity was thereby reduced considerably. These experiments confirm in a quantitative manner that wall teichoic acids, and presumably also membrane teichoic acids, are responsible for binding bivalent cations, and it is probable that this is a major function of these polymers. It is also possible that the cell can control its binding of cations through control of the amount of alanine ester in its wall.

There are several reasons why it is important for the cell to be able to maintain a high concentration of bivalent cations in the region of its membrane. It is known, for example, that the membrane has bound to it many enzymes,[42] including those required for the synthesis of peptidoglycan, teichoic acids, and capsular polysaccharides and those concerned with electron transport; many of these[13,24,43,45] have been shown to require high concentrations of bivalent cations for optimal activity. The physical integrity[46] of the membrane requires Mg^{2+}, as does the association of membrane and ribosomes during protein synthesis.[47] The membraneous convoluted structures known as mesosomes that are frequently seen in association with the cell membrane also require Mg^{2+} for this association.[48] If the function of teichoic acids is to provide a suitable environment for the correct functioning of the membrane, then it follows that membrane teichoic acid is of fundamental importance to the cell and is thus much more important biologically than is wall teichoic acid. This conclusion is supported by the observation discussed above that in unfavorable circumstances organisms can survive by attaching other acidic polymers to the wall, but nevertheless are obliged to produce membrane teichoic acid. It is also significant that cells grown under conditions of magnesium deficiency contain exceptionally large amounts of teichoic acid in their walls; this would represent an attempt by the cells to scavenge as much bivalent cation as possible.

Specialized functions have been attributed to specific wall teichoic acids. In the *Pneumococcus* the wall teichoic acid (C substance) is a complex polymer containing sugars, ribitol phosphate, and choline phosphate residues.[9] When the organism is grown in a medium lacking choline but containing ethanolamine, it is possible to substitute the choline residues in the teichoic acid by ethanolamine. Although the cells continue to grow and reproduce under these conditions, the normal characteristic autolysis of older cells is prevented and cell separation is impaired. The bacteria thus grow as long chains of unseparated cells.[49] A relationship between teichoic acid structure and lysis has also been observed in *Streptococcus zymogenes;*[50] the walls of this organism are immune toward the powerful lytic enzymes that it excretes, but if the alanine ester residues of its wall teichoic acid are removed autolysis can occur. The nature of the effect of teichoic acids on these apparently rather specialized aspects of action of autolytic enzymes has not yet been estabished, and it remains possible that their influence on the enzymes might be connected with their cation binding properties. Another obvious property of teichoic acids is associated with their overall negative charge. Their presence in walls should cause mutual repulsion of cells because of their surface charge, thereby ensuring equitable distribution of organisms throughout the nutrient medium. It can be shown experimentally that walls that have had their teichoic acid removed chemically exhibit a marked tendency to aggregate.[44]

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The Structure of Porphyrins and Metalloporphyrins

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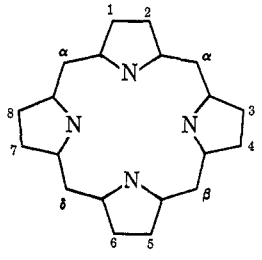
Received May 15, 1969

Understanding of the porphyrin system has advanced appreciably in recent years;[1] determination of detailed structures for porphyrin and metalloporphyrin molecules by X-ray diffraction has contributed importantly to comprehension of the chemistry and the physical properties of porphyrin molecules. This Account reviews some of these structural studies on porphyrins and discusses their implications.

Porphyrins are a class of tetrapyrrole macrocycles with a skeleton as shown in **1**. The porphine (the parent compound) free base **2** has 11 double bonds and



1, porphyrin skeleton

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