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The Biosynthesis of the Wall Teichoic Acid in *Staphylococcus lactis* I3

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1. The biosynthesis of the wall teichoic acid in *Staphylococcus lactis* I3 was studied. Cell-free particulate enzyme preparations, probably representing fragmented membrane, were isolated and used for the synthesis of polymer. 2. By using appropriately labelled CDP-glycerol and UDP-*N*-acetylglucosamine it was shown that the former contributes a glycerol phosphate residue and the latter contributes an *N*-acetylglucosamine 1-phosphate residue to the repeating unit. 3. No polymer was synthesized unless both nucleotides were present, and no other substrates were required. 4. The properties of the enzyme system were studied. 5. Although attempts to fractionate the system failed, the biosynthesis is believed to be complex and its mechanism is considered.

Considerable interest has been centred recently on the biosynthetic pathways leading to the formation of the various polymers in the cell walls of bacteria. The general mechanism of biosynthesis of teichoic acids from nucleotide precursors was apparent from the time of their discovery, as it was biosynthetical reasoning after the discovery of CDP-glycerol and CDP-ribitol that led to the discovery of teichoic acids themselves (Armstrong, Baddiley, Buchanan, Carss & Greenberg, 1958). The suggestion that these nucleotides participated in an enzymic transfer of polyol phosphate groups to the teichoic acid chain was strengthened by the fact that the stereochemistry of these groups in the nucleotides and in the polymers is identical. More recently, it has been shown experimentally in cell-free systems that CDP-ribitol and CDP-glycerol are the precursors of the phosphodiester-linked polyol 'backbones' of teichoic acids (Burger & Glaser, 1964; Glaser, 1964; Ishimoto & Strominger, 1966). Moreover, the addition of either glucose or *N*-acetylglucosamine, through their uridine nucleotide derivatives, to the preformed chain has also been shown (Glaser & Burger, 1964; Chin, Burger & Glaser, 1966; Nathenson & Strominger, 1963). Teichoic acids in which sugar residues form part of the polymer chain have also been synthesized in cell-free systems from the appropriate nucleotides (Burger & Glaser, 1966).

The teichoic acid from the wall of *Staphylococcus lactis* I3 contains alanine, *N*-acetylglucosamine, glycerol and two phosphate groups, and has a structure (I) in which *N*-acetylglucosamine 1-phosphate is an integral part of the polymer chain (Archibald, Baddiley & Button, 1968*a*). As this structure differed considerably from earlier teichoic acids, and in particular the origin of the phosphate attached to the 1-position on amino sugar residues was obscure, a study of the substrate requirements for the biosynthesis of this polymer was undertaken in an attempt to understand the mechanism involved in its biosynthesis. A preliminary report of this work has been published (Blumsom, Douglas & Baddiley, 1966).

MATERIALS AND METHODS

Analytical methods. Protein was determined by the method of Lowry, Rosebrough, Farr & Randall (1951).

Radioactive products on chromatograms were located on a chromatogram strip scanner (Baird–Atomic Inc., Cambridge, Mass., U.S.A.). Quantitative measurements of radioactive products were made by cutting out the radioactive areas and counting the paper directly in a Packard 4000 series Tri-Carb scintillation counter. The liquid scintillant used had the following composition: xylene, 385ml.; dioxan, 385ml.; ethanol, 230ml.; naphthalene, 80g.; 2,5-diphenyloxazole, 4g.; 1,4-bis-(4-methyl-5-phenyloxazol-2-yl)benzene, 0·2g. (Kinard, 1957).

Paper chromatography. The following solvent systems were used: *A*, ethanol–M-ammonium acetate, pH3·8 (5:2, v/v) (Paladini & Leloir, 1951) with Whatman no. 1 or 3MM paper and descending development; *B*, propan-2-ol–aq. NH_3 (sp.gr. 0·88)–water (7:1:2, by vol.) (Le Cocq & Ballou, 1964) with Whatman no. 4 paper and descending development. The products were detected with the following spray reagents: alkaline $AgNO_3$ for sugars and polyols (Trevelyan, Procter & Harrison, 1950); molybdate for phosphoric esters (Hanes & Isherwood, 1949); ninhydrin for amino acids (Consden & Gordon, 1948).

Cultural conditions. Cultures of the organism (250ml.) (Davison & Baddiley, 1963), inoculated from a nutrient agar slope, were grown overnight at 37° with shaking in a

medium of the following composition: nutrient broth no. 2 (Oxoid), 12·5g.; yeast extract (Difco), 2·5g.; K_2HPO_4, 2·5g.; trisodium citrate, 0·5g.; glucose, 1g.; water (demineralized), 1000ml. Four of these cultures were used to inoculate a batch (10l.) of medium with an increased glucose content (10g./l.). Incubation was continued with forced aeration at 37° for 3–4hr. (late exponential growth phase), then the organisms were harvested by using the continuous-flow attachment on a Sorvall refrigerated centrifuge and washed with cold 0·6% NaCl solution.

Preparation of enzyme. Cells (10g. wet wt.), suspended in 0·05M-tris–HCl buffer (10ml.), pH7·5, containing sodium thioglycollate (5mM), were shaken in a Braun cell disintegrator with Ballotini no. 11 beads (30ml.) at 4000rev./min. for 2·5min. Beads were removed by filtering with a no. 1 sintered-glass funnel and the filtrate was centrifuged at 15000*g* in a Sorvall refrigerated centrifuge for 20min. The cloudy supernatant fluid was decanted and the sedimented walls and unbroken cocci were washed once with a further amount of the suspending buffer. The combined supernatant and washings were centrifuged at 105000*g* in a Spinco model L2 ultracentrifuge for 1hr. The sedimented gel, consisting of membrane fragments and ribosomes, was washed once with 0·15M-sodium pyrophosphate buffer, pH7·5 (Burger & Glaser, 1964), and then washed and resuspended in 0·1M-tris–HCl buffer, pH8·0, 0·01M with respect to $MgCl_2$ and 1mM with respect to EDTA. The protein content of the preparation was approx. 20mg./ml. The enzyme could be kept frozen for several weeks with essentially no loss of activity.

Preparation of substrates. UDP-*N*-acetylglucosamine was purchased from Sigma Chemical Co., St Louis, Mo., U.S.A. CDP-glycerol was synthesized from the morpholidate of CMP by the general procedure described by Roseman, Distler, Moffatt & Khorana (1961).

CDP-[^{14}C]glycerol was prepared enzymically. [1-^{14}C]-Glycerol (The Radiochemical Centre, Amersham, Bucks.) was phosphorylated by using ATP and crystalline glycerokinase (Bublitz & Kennedy, 1954). The product, D-[^{14}C]-glycerol 1-phosphate, was then treated with CTP and a fractionated soluble enzyme preparation (Shaw, 1962) obtained from *Lactobacillus buchneri*. This enzyme preparation contains the synthetases for both CDP-glycerol and UDP-*N*-acetylglucosamine. A typical incubation mixture contained 50μmoles of tris–HCl buffer, pH8·0, 5μmoles of $MgCl_2$, 0·5μmole of EDTA, 5μmoles of D-[^{14}C]glycerol 1-phosphate, 13μmoles of CTP, 20units of inorganic pyrophosphatase (Sigma Chemical Co.) and 0·1ml. of enzyme in a total volume of 1·0ml. After incubation at 37° for 1hr. protein was destroyed by boiling for 3min. after the addition of an equal volume of ethanol, followed by immediate cooling in ice. The protein precipitate was washed once with aq. 50% (v/v) ethanol and the combined supernatant and washings were concentrated to a small volume *in vacuo*. The mixture was applied as a band to Whatman 3MM paper and chromatographed in solvent *A* for 16hr. After careful drying, the paper was washed in ethanol to remove traces of ammonium acetate, and CDP-glycerol was eluted from the appropriate area with water and rechromatographed in the same solvent. The yield was 4μmoles with specific radioactivity about 2×10^5counts/min./μmole.

UDP-[*acetyl*-^{14}C]*N*-acetylglucosamine or [^{32}P]UDP-[*acetyl*-^{14}C]*N*-acetylglucosamine was prepared from UTP and [*acetyl*-^{14}C]*N*-acetylglucosamine 1-phosphate or [*acetyl*-^{14}C]*N*-acetylglucosamine 1[^{32}P]-phosphate by using the soluble enzyme preparation from *L. buchneri* described above. The procedure was essentially that used for the preparation of CDP-[^{14}C]glycerol. A typical incubation mixture contained 50μmoles of tris–HCl buffer, pH8·0, 5μmoles of $MgCl_2$, 0·5μmole of EDTA, 5μmoles of [*acetyl*-^{14}C]*N*-acetylglucosamine 1-phosphate, 13μmoles of UTP, 40units of inorganic pyrophosphatase and 0·25ml. of enzyme in a total volume of 1·2ml. The yield was 3μmoles with specific radioactivity $1\cdot6\times10^6$counts/min./μmole. The potassium salt of glucosamine 1-phosphate was prepared chemically by a method developed by Dr R. Cherniak (unpublished work). This technique was also used in the synthesis of glucosamine 1[^{32}P]-phosphate. Acetylation with [^{14}C]acetic anhydride under the conditions described by Distler, Merrick & Roseman (1958) yielded [*acetyl*-^{14}C]*N*-acetylglucosamine 1[^{32}P]-phosphate.

RESULTS

Enzymic synthesis of teichoic acid and its identification from radioactive products of degradation. To determine the requirements for biosynthesis of the teichoic acid different substrates were incubated with the enzyme preparation as shown in Table 1. Areas of radioactivity were located with the chromatogram strip scanner, and the amount of polymeric product was determined by measuring the area under the peak corresponding to material that did not move from the origin of the chromatogram. The extent of polymer formation was expressed as percentage incorporation of the radioactive substrate initially present.

It was found that the only substrates required for biosynthesis of polymer were CDP-glycerol and UDP-*N*-acetylglucosamine. Addition of either ATP or D-alanine did not increase the synthesis of polymer. In experiments with added D- or DL-[^{14}C]alanine, no incorporation of amino acid into the polymer could be demonstrated. Apparently, the polymer can be synthesized without a concomitant incorporation of alanine.

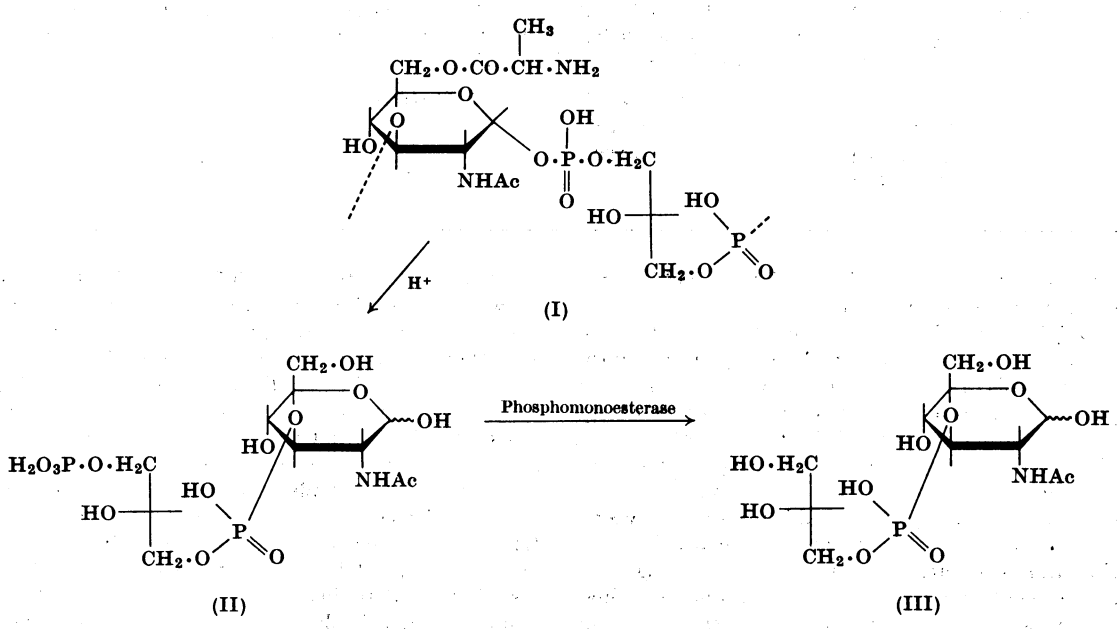
To show that the reaction product was, in fact, teichoic acid it was subjected to one of the degradation procedures described in work on the chemical structure of this polymer (Archibald *et al.* 1968*a*). Hydrolysis in 0·1N-hydrochloric acid at 100° for 8min. depolymerizes the teichoic acid by fission of the sugar 1-phosphate linkages to yield the fragment of structure (II), as indicated in Scheme 1. This compound, when chromatographed in solvent *B*, had $R_{\alpha GP}$ ($R_{\text{glycerol 1-phosphate}}$) 0·8. Dephosphorylation of this product with calf intestinal phosphomonoesterase yielded a fragment (III) with $R_{\alpha GP}$ 3·4 in solvent *B*.

Samples of the polymer were synthesized from each of the radioactive precursors, as indicated in Table 2, and products were separated by chromatography on Whatman 3MM paper in solvent *A*. The radioactive material at the origin was subjected to

Table 1. *Substrate requirements for polymer synthesis*

The complete reaction mixture (Expt. 1) contained 10 μl. of CDP-[^{14}C]glycerol solution (50 μmoles/ml.; 1 μmole $\equiv$ 1·8 $\times$ 10^5 counts/min.), 10 μl. of UDP-*N*-acetylglucosamine solution (50 μmoles/ml.), 20 μl. of ATP solution (100 μmoles/ml.), 20 μl. of D-alanine solution (50 μmoles/ml.) and 150 μl. of enzyme (suspended in 0·1 M-tris–HCl–0·01 M-$MgCl_2$–1 mM-EDTA, pH 8·0). In Expts. 6 and 7 10 μl. of UDP-[*acetyl*-^{14}C]*N*-acetylglucosamine (50 μmoles/ml.; 1 μmole $\equiv$ 6·7 $\times$ 10^5 counts/min.) was used. Reaction mixtures were incubated for 1 hr. at 37° and then applied as bands to Whatman no. 1 paper and chromatographed in solvent *A*, when the polymer remained at the origin.

Expt. no.	Substrates	Polymer formed (% incorporation of labelled substrates)
1	CDP-[^{14}C]glycerol + UDP-*N*-acetylglucosamine + ATP + D-alanine	12·3
2	CDP-[^{14}C]glycerol + ATP + D-alanine	0·2
3	CDP-[^{14}C]glycerol + UDP-*N*-acetylglucosamine + D-alanine	15·4
4	CDP-[^{14}C]glycerol + UDP-*N*-acetylglucosamine + ATP	12·4
5	CDP-[^{14}C]glycerol + UDP-*N*-acetylglucosamine	12·6
6	CDP-glycerol + UDP-[*acetyl*-^{14}C]*N*-acetylglucosamine	18·6
7	UDP-[*acetyl*-^{14}C]*N*-acetylglucosamine	2·4
8	CDP-[^{14}C]glycerol + UDP-*N*-acetylglucosamine (enzyme boiled)	0·0



Scheme 1. Degradation of teichoic acid by acid and phosphatase. Ac, Acetyl.

the controlled acid hydrolysis followed by chromatography in solvent *B*; Expts. A and B both yielded a radioactive product with the $R_{\alpha GP}$ of fragment (II).

Material corresponding to compound (II) was eluted with water, the solution evaporated to dryness *in vacuo* and the residue dissolved in 1·0 ml. of 0·05 M-ammonium carbonate buffer, pH 9·4, containing calf intestinal phosphomonoesterase (0·5 mg./ml.). After incubation at 37° for 2 hr. fragment (II) derived from either Expt. A or B gave compound (III) with $R_{\alpha GP}$ 3·4, identical with that of the expected product (Table 2). When fragment (III) from Expt. A was subjected to further hydrolysis with 2 N-hydrochloric acid for 3 hr. at 100° in a sealed tube, [^{14}C]glycerol phosphates were obtained, as shown by comparative paper chromatography in solvent *B*. These degradation experiments indicate that the polymer being synthesized has the

Table 2. *Characterization of labelled teichoic acid by hydrolysis*

Details of biosynthesis from radioactive nucleotides, with 150 μl. of enzyme, are given with Table 1, and the acid hydrolysis and enzymic dephosphorylation of the hydrolysis product are described in the text. For biosynthesis, each incubation mixture contained the substrates indicated in a final volume of 0·18 ml. Chromatography is described in Table 1.

Substrate for biosynthesis	Polymer formed (counts/min.)	Acid-hydrolysis product (II) (counts/min.)	Phosphomonoesterase product (III) (counts/min.)	Glycerol phosphate (counts/min.)
Expt. A UDP-*N*-acetylglucosamine (0·5 μmole) + CDP-[^{14}C]glycerol (0·1 μmole; 1 μmole $\equiv$ 6·0 $\times$ 10^5 counts/min.)	1532	1283	1140	800
Expt. B UDP-[*acetyl*-^{14}C]*N*-acetylglucosamine (0·5 μmole; 1 μmole $\equiv$ 6·7 $\times$ 10^5 counts/min.) + CDP-glycerol (1·0 μmole)	2500	2138	1650	—

Table 3. *Biosynthesis of teichoic acid from* [^{32}P]*UDP-*[*acetyl*-^{14}C]*N-acetylglucosamine*

Polymer was formed enzymically from [^{32}P]UDP-[*acetyl*-^{14}C]*N*-acetylglucosamine (0·2 μmole; 1 μmole $\equiv$ 1·6 $\times$ 10^6 counts/min. of ^{14}C and 1·1 $\times$ 10^5 counts/min. of ^{32}P), CDP-glycerol (1·0 μmole) and enzyme (0·15 ml.) in a total volume of 0·18 ml. under the conditions given in Table 1.

	^{14}C (counts/min.)	^{32}P (counts/min.)	^{14}C/^{32}P ratio
Nucleotide	12465	1111	11·2
Fragment (III)	4920	—	9·8
Inorganic orthophosphate	—	504	

same structure as that of the teichoic acid isolated from the walls of this organism.

Origin of the phosphate group attached to the sugar 1-position. A particularly noteworthy feature in the biosynthesis of this teichoic acid is the origin of the phosphate group that joins the 1-position of the sugar residue to glycerol in the repeating unit. Since CDP-glycerol and UDP-*N*-acetylglucosamine are the only substrate requirements, it seemed likely that CDP-glycerol would supply the glycerol phosphate unit that is attached to the 3-position of the amino sugar, whereas UDP-*N*-acetylglucosamine would donate *N*-acetylglucosamine 1-phosphate as an intact unit to the other end of the glycerol. This view is consistent with the known stereochemistry of the glycerol phosphate residues in the teichoic acid (Archibald *et al.* 1968*a*), i.e. the glycerol phosphate residue attached to the 3-position of the hexosamine has the same configuration as that in CDP-glycerol, namely D-glycerol 1-phosphate.

To establish this, UDP-*N*-acetylglucosamine was prepared in which the phosphate group attached to the 1-position of the hexosamine contained ^{32}P and the *N*-acetyl group on the hexosamine contained ^{14}C. The teichoic acid obtained from this doubly labelled UDP-*N*-acetylglucosamine and unlabelled CDP-glycerol contained both ^{32}P and ^{14}C; this was shown by scanning the paper first with the unmasked collimator slit, giving both ^{14}C and ^{32}P, and then masking the slit with aluminium foil, which excludes the soft β-particle emission of ^{14}C.

The polymer was hydrolysed in dilute acid as described above, and the product (II) was shown to contain both isotopes. Treatment of this with calf intestinal phosphomonoesterase yielded inorganic [^{32}P]orthophosphate and the fragment (III); the latter was radioactive owing to the presence of [*acetyl*-^{14}C]*N*-acetylglucosamine. The distribution of radioactivity in the fragment (III) and in the organic phosphate was determined in the Packard scintillation counter by using the channels-ratio method. The ^{14}C/^{32}P ratio in the nucleotide was 11·2:1 and the ^{14}C/^{32}P ratio in fragment (III) and inorganic orthophosphate was 9·8:1 (Table 3).

From these results it was concluded that UDP-*N*-acetylglucosamine donates its *N*-acetylglucosamine 1-phosphate moiety as an intact unit to the polymer.

Properties of the enzyme system. The effect of varying the pH of the reaction mixture on teichoic acid synthesis was studied and the result is shown in Fig. 1; the curve shows a sharp optimum at pH 8·0. Further, the amount of polymer formed is propor-

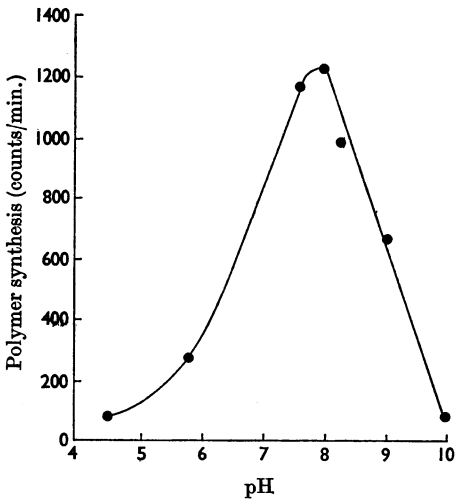


Fig. 1. Effect of pH on teichoic acid biosynthesis. The incubation mixtures contained 0·4 μmole of UDP-*N*-acetylglucosamine, 0·05 μmole of CDP-[^{14}C]glycerol (1 μmole$\equiv$1·3$\times$10^6 counts/min.) and 0·15 ml. of enzyme in buffer as indicated below (total volume 0·17 ml.): pH 4·5–5·8, 0·05 M-succinic acid–NaOH, 0·01 M with respect to $MgCl_2$ and 1 mM with respect to EDTA; pH 7·6–8·3, 0·1 M-tris–HCl, 0·01 M with respect to $MgCl_2$ and 1 mM with respect to EDTA; pH 9·0–10·0, 0·05 M-glycine–NaOH, 0·01 M with respect to $MgCl_2$ and 1 mM with respect to EDTA. Reaction mixtures were incubated at 37° for 60 min. and treated as described in Table 1, and the polymer was measured directly in the Packard scintillation counter.

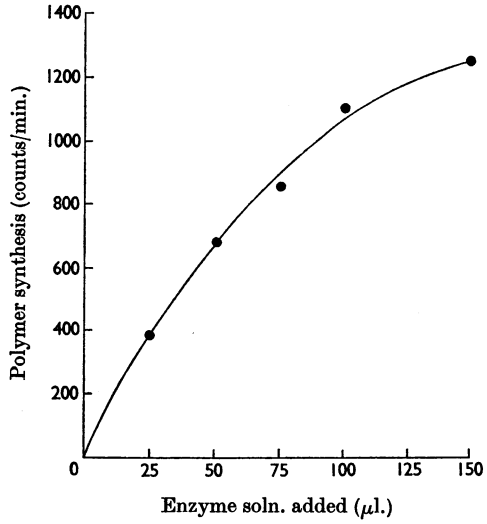


Fig. 2. Effect of enzyme concentration on teichoic acid biosynthesis. The incubation mixtures contained 0·4 μmole of UDP-*N*-acetylglucosamine, 0·035 μmole of CDP-[^{14}C]-glycerol (1 μmole$\equiv$1·5$\times$10^6 counts/min.) and enzyme (in 0·1 M-tris–HCl buffer, pH 8·0, 0·01 M with respect to $MgCl_2$ and 1 mM with respect to EDTA) in a total volume of 0·17 ml., assayed as described in Fig. 1.

tional to the amount of enzyme present (Fig. 2), and to the time of incubation (Fig. 3), although the amount formed does not increase much after 30 min.

Although the reaction is dependent on a high concentration of Mg^{2+} (Fig. 4) the system shows a sharp optimum, and concentrations above 20 mM rapidly bring about an inhibition in synthesis. Mn^{2+} is as effective as Mg^{2+} at 20 mM concentration; Ca^{2+} is slightly less effective. The amount of radioactivity incorporated into the polymer is proportional to the concentration of UDP-[*acetyl*-^{14}C]*N*-acetylglucosamine and a plot of $1/v$ against $1/s$ gave a straight line (Fig. 5) from which a K_m value of $1{\cdot}25\times10^{-3}$ M was obtained. When similar experiments were performed with various concentrations of CDP-glycerol a similar proportional relationship was not observed.

The specificity of the nucleotides in the enzyme system was examined by replacing UDP-*N*-acetylglucosamine in the complete system with either UDP-glucose, ADP-glucose, TDP-glucose or TDP-mannose (where TDP refers to thymidine 5′-diphosphate); none of these nucleotide–sugar

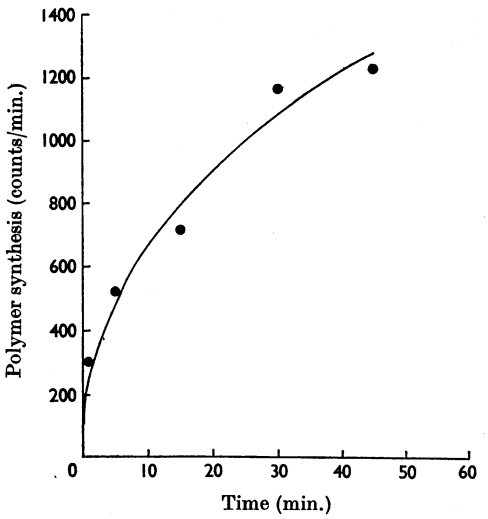


Fig. 3. Rate of teichoic acid biosynthesis. The incubation mixture contained 1·6 μmoles of UDP-*N*-acetylglucosamine, 0·15 μmole of CDP-[^{14}C]glycerol (1 μmole$\equiv$5·5$\times$10^5 counts/min.), 4 μmoles of $MgCl_2$ and 0·3 ml. of enzyme (in 0·1 M-tris–HCl buffer, pH 8·0, 1 mM with respect to EDTA) in a total volume of 0·35 ml. Samples (0·05 ml.) were removed at times indicated and boiled for 1 min., and product was determined as described in Fig. 1.

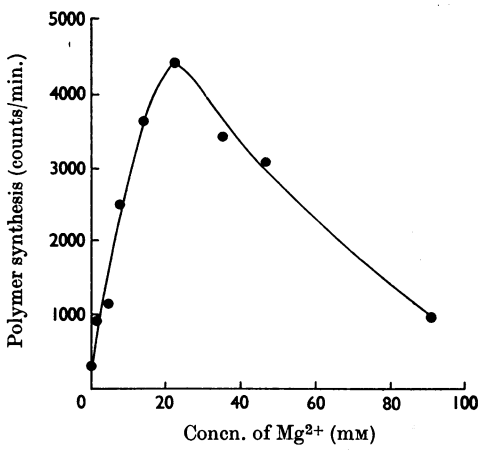


Fig. 4. Effect of Mg^{2+} on teichoic acid biosynthesis. Incubation mixtures contained 0·4 μmole of UDP-*N*-acetylglucosamine, 0·05 μmole of CDP-[^{14}C]glycerol (1 μmole≡6·5 × 10^5 counts/min.), 0·15 ml. of enzyme (in 0·05 M-tris–HCl buffer, pH 8·0, 1 mM with respect to EDTA). $MgCl_2$ was present as indicated and the total volume was 0·22 ml. Product was determined as described in Fig. 1.

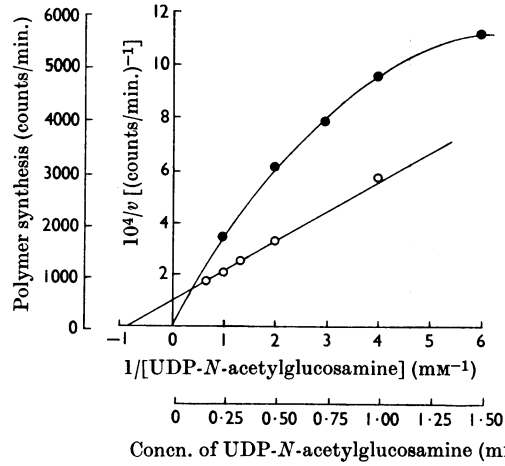


Fig. 5. Effect of concentration of UDP-*N*-acetylglucosamine on polymer synthesis. Incubation mixtures contained 1 μmole of CDP-glycerol, 4 μmoles of $MgCl_2$, 0·15 ml. of enzyme (in 0·1 M-tris–HCl buffer, pH 8·0) and labelled UDP-*N*-acetylglucosamine as shown in a total volume of 0·2 ml. Product was determined as described in Fig. 1. The results are plotted both as polymer synthesis versus concentration of UDP-*N*-acetylglucosamine (●) and as the double-reciprocal plot (○).

compounds substituted for UDP-*N*-acetylglucosamine. Similarly, no polymer was formed when CDP-glycerol was replaced by CDP-ribitol in the presence of UDP-[*acetyl*-^{14}C]*N*-acetylglucosamine.

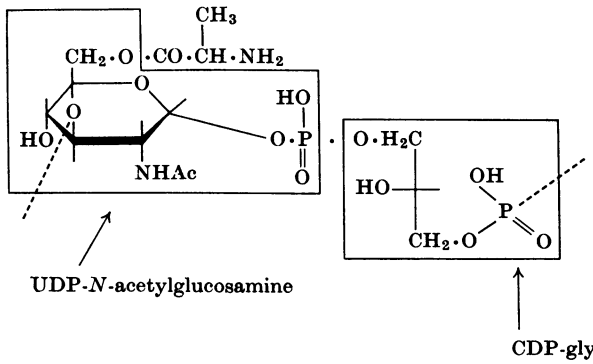
Attempts to fractionate or solubilize the enzyme. Attempts were made to fractionate the particulate preparation through a sucrose density gradient in the ultracentrifuge. The gradient ranged from 1 M- to 3 M-sucrose in tris–hydrochloric acid buffer, pH 8·0, with 0·01 M-magnesium chloride and 1 mM-EDTA. Zoning of the preparation was observed and some increase in specific activity was achieved, but the results were not reproducible, presumably because of the different extent to which the membrane was fragmented. Attempts to obtain a soluble enzyme preparation by treatment of the particulate material with Triton X-100 (0·1% or 1·0%) or sodium deoxycholate (0·5%) were unsuccessful. Paper-chromatographic and chemical analysis of an enzyme preparation that had been hydrolysed with acid indicated the presence of both wall and membrane teichoic acids and peptidoglycan. Since it was not possible to remove the teichoic acid from the enzyme system the requirement for primers has not been established.

DISCUSSION

These studies on the biosynthesis of the wall teichoic acid from *Staph. lactis* I3 show that the only substrates required for the formation of the polymer are UDP-*N*-acetylglucosamine and CDP-glycerol. The possibility that the phosphate attached to the sugar 1-position might be derived from the terminal phosphate group of ATP has been eliminated. Conclusive evidence was obtained from double-labelling experiments that the UDP-*N*-acetylglucosamine donates its *N*-acetylglucosamine 1-phosphate moiety as an intact unit, and that CDP-glycerol donates its glycerol phosphate moiety to the polymer as shown in Scheme 2.

Attempts were made to incorporate D-alanine into the polymer, either during its biosynthesis or after chain formation, but these failed. The mechanism whereby D-alanine is incorporated into teichoic acid has been studied in several Laboratories without success, but analogy with other biosynthetic routes suggests that a transfer RNA derivative might be involved. The formation of membrane teichoic acid by the enzyme system was not observed. The reason for this is not clear, as this polymer possesses the usual simple structure in which the glycerol phosphate units form a linear chain (Archibald, Baddiley & Button, 1968*b*), and presumably the only substrate requirement would be CDP-glycerol. The enzyme system shows a fairly sharp pH optimum of 8·0 and requires a high concentration of Mg^{2+}.

The resistance of the particulate enzyme preparation towards attempts to fractionate it has made



Scheme 2. Origin of the components of the wall teichoic acid from *Staph. lactis* I3. Ac, Acetyl.

difficult further work on the details of the biosynthetic process. Thus it is not known whether the components from the two nucleotides are transferred as a preformed repeating unit or whether alternative transfer occurs from the separate nucleotides; nor is it known in which direction chain extension occurs. It is significant that newly formed teichoic acid can be centrifuged with the enzyme particles and adheres strongly to them. This is consistent with the fact that the teichoic acid in the wall is attached through a terminal glycerol phosphodiester to a muramic acid residue associated with the peptidoglycan of the wall (Button, Archibald & Baddiley, 1966), and it is therefore likely that the teichoic acid in the particles is attached to fragments of peptidoglycans. Attempts to dissociate the teichoic acid from the particles by the action of lysozyme, trypsin, Pronase or papain, separately or combined, were unsuccessful.

Recently we have shown (Douglas & Baddiley, 1968) that the biosynthetic system must be complex, as a lipid intermediate participates in the biosynthesis. In this respect, the synthesis of the teichoic acid shows some similarity to the synthesis of peptidoglycan, lipopolysaccharide and probably other wall polysaccharides. Although the full structure of the lipid intermediate is not yet known, it contains an *N*-acetylglucosamine 1-phosphate residue.

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