The Mechanism of Biosynthesis and Direction of Chain Extension of a Poly-(*N*-Acetylglucosamine 1-Phosphate) from the Walls of *Staphylococcus lactis* N.C.T.C. 2102

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1. The synthesis of a polymer of *N*-acetylglucosamine 1-phosphate, occurring in the walls of *Staphylococcus lactis* N.C.T.C. 2102, was examined by using cell-free enzyme preparations. The enzyme system was particulate, and probably represents fragmented cytoplasmic membrane. 2. Uridine diphosphate *N*-acetylglucosamine was the only substrate required for polymer synthesis and labelled substrate was used to show that *N*-acetylglucosamine 1-phosphate is transferred as an intact unit from substrate to polymer. 3. The properties of the enzyme system were studied. A high concentration of Mg^{2+} or Mn^{2+} was required for optimum activity, and the pH optimum was about 8.5. 4. End-group analysis during synthesis *in vitro* showed that newly formed chains contain up to about 15 repeating units. Pulse-labelling indicated that chain extension occurs by transfer from the nucleotide to the 'sugar-end' of the chain, i.e. to the end that is not attached to peptidoglycan in the wall.

Although the general nature of the enzymic synthesis of teichoic acids from the nucleotide precursors CDP-glycerol and CDP-ribitol was apparent from the time of their discovery (Armstrong, Baddiley, Buchanan, Carss & Greenberg, 1958), further details of the mechanism of formation of the phosphodiester linkages and the attachment of sugar residues were obtained from studies with cell-free enzyme preparations. Thus in poly(glycerol phosphate) teichoic acid and poly(ribitol phosphate) teichoic acid the polyol phosphate residues are transferred to the teichoic acid chains and the sugar residues are introduced independently from the appropriate nucleoside diphosphate sugars (Burger & Glaser, 1964; Glaser, 1964; Glaser & Burger, 1964; Chin, Burger & Glaser, 1966; Ishimoto & Strominger, 1966; Nathenson & Strominger, 1963). In those teichoic acids where the sugar residues constitute a part of the polymer chain [phosphodiester groups joining glycerol to sugar in a poly(glucosylglycerol phosphate)] the presence of both CDP-glycerol and nucleoside diphosphate sugar are required for synthesis to occur (Burger & Glaser, 1966).

Staphylococcus lactis I3 contains in its wall an unusual teichoic acid in which the repeating unit is a D-alanine ester comprising D-glycerol 1-phosphate attached through its phosphate to the 3-position of *N*-acetylglucosamine 1-phosphate; the repeating units are joined together through the sugar 1-phosphate group to the 3-position of a neighbouring

glycerol (Archibald, Baddiley & Button, 1968a). The enzymic synthesis of this polymer occurs by successive transfer of glycerol phosphate from CDP-glycerol and *N*-acetylglucosamine 1-phosphate from UDP-*N*-acetylglucosamine, with lipid pyrophosphate intermediates participating in the transfer (Baddiley, Blumsom & Douglas, 1968; Douglas & Baddiley, 1968).

The wall of *Staphylococcus lactis* 2102 contains a polymer (I) of *N*-acetylglucosamine 1-phosphate in which the repeating units are joined together through phosphodiester linkages involving the 1- and 6-positions of *N*-acetylglucosamine (Archibald, Baddiley, Button, Heptinstall & Stafford, 1968b). No alanine ester residues are found in this polymer, and the absence of polyol phosphate residues suggests that although it resembles a teichoic acid in a number of its features it should not be classified with them. The synthesis of this polymer is examined in the present work, particularly to establish whether, as with the teichoic acid from *S. lactis* I3, sugar 1-phosphate residues are transferred as intact units from a nucleotide precursor. The direction of chain extension has also been established.

MATERIALS AND METHODS

UDP-*N*-acetylglucosamine, *N*-acetylglucosamine, diphenyl phosphate and calf intestinal phosphomonoesterase were purchased from Sigma Chemical Co., St Louis, Mo.,

U.S.A. [^{14}C]Acetic anhydride and diphenyl [^{32}P]phosphate were purchased from The Radiochemical Centre, Amersham, Bucks. A sample of *N*-acetylglucosamine 6-phosphate was given by Dr D. E. Brundish.

Analytical methods. Protein was determined by the method of Lowry, Rosebrough, Farr & Randall (1951). Radioactive material was located on chromatograms with a radiochromatogram scanner (Baird-Atomic Inc., Cambridge, Mass., U.S.A.); quantitative measurements were carried out with an integrator attached to the strip scanner or by cutting out appropriate areas of paper and examining in a Packard 4000 Series Tri-Carb Liquid Scintillation Counter with a scintillant of the following composition: toluene (A.R.), 2l.; 2,5-diphenyloxazole, 8g.; 1,4-bis-(4-methyl-5-phenyloxazol-2-yl) benzene, 0.2g. For soluble samples the scintillant was mixed with Triton X-100 (2:1, v/v) (Patterson & Greene, 1965).

Paper chromatography. This was carried out on Whatman nos. 1 and 3 MM papers in the following descending solvent systems: *A*, *m*-ammonium acetate-ethanol, pH 3.6 (5:2, v/v) (Paladini & Leloir, 1951); *B*, propan-2-ol-aq. NH_3 (sp.gr. 0.88)-water (7:1:2, by vol.) (LeCocq & Ballou, 1964); *C*, propan-1-ol-ethyl acetate-water (7:1:2, by vol.) (Baar & Bull, 1953). Compounds were detected with the alkaline AgNO_3 reagent for sugars and polyols (Trevelyan, Procter & Harrison, 1950) and the molybdate reagent for phosphates (Hanes & Isherwood, 1949).

Growth of organism. A culture of *Staphylococcus lactis* N.C.T.C. 2102 was kindly supplied by Dr H. R. Perkins. The organism was grown for 17 hr. at 37° with shaking in a medium (500 ml.) with 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.; distilled water to a final volume of 1l. Two such cultures were grown from an inoculum prepared on a nutrient-agar slope, and these were used to inoculate a batch (10l.) of medium with an increased content of glucose (10g./l.). Growth was continued with forced aeration for 3–4 hr. (late exponential phase) and cells were collected in a continuous-flow attachment on a Sorvall RC2 refrigerated centrifuge; they were washed once with 0.6% NaCl solution.

Preparation of enzyme. Cells were suspended in 0.05 *M*-tris-HCl buffer, pH 7.5, containing 5 *mM*-sodium thioglycollate (1:1, w/v) and shaken in a Braun cell disintegrator with Ballotini no. 11 beads (30 ml.) at 4000 rev./min. for 3 min. Beads were removed by filtering through a no. 1 sintered-glass funnel. The filtrate was centrifuged at 15000 *g* for 20 min. with refrigeration, the supernatant decanted and the pellet of unbroken cells and walls washed once with the tris buffer. The combined supernatant and washing were then centrifuged at 100000 *g* for 1 hr. in a Beckman Spinco model L-2 ultracentrifuge. The resultant pellet, consisting mainly of fragmented membranes and ribosomal material, was washed once with 0.15 *M*-sodium pyrophosphate buffer, pH 7.5 (Burger & Glaser, 1964), and once with 0.1 *M*-tris-HCl buffer, pH 8.5. The pellet was finally suspended in 0.1 *M*-tris-HCl buffer, pH 8.5, to give a final protein content of about 40 mg./ml. This enzyme suspension was kept at –15°, and at this temperature activity was retained for several months.

Preparation of substrates. UDP-[^{14}C]N-acetylglucosamine and [^{32}P]UDP-[^{14}C]N-acetylglucosamine were prepared by the method of Baddiley *et al.* (1968), except that the enzyme used for coupling UTP with *N*-acetylglucos-

amine 1-phosphate was prepared from the supernatant remaining after centrifuging the membrane particles. This enzyme was precipitated from the supernatant by the slow addition of $(\text{NH}_4)_2\text{SO}_4$ at 0° to 0.8 saturation. The mixture was kept for 1 hr. at this temperature before the precipitate was removed by centrifugation at 20000 *g* for 20 min. in a refrigerated centrifuge. The pellet was dissolved in the minimum amount of 0.05 *M*-tris-HCl buffer, pH 7.5, containing sodium thioglycollate (5 *mM*) and dialysed for 24 hr. against 4l. of the same buffer at 0–4°. The non-diffusible portion was stored at –15°; this was used directly in the coupling reaction.

RESULTS

Substrate requirements for polymer synthesis. The substrate requirements for the synthesis of the polymer are given in Table 1. Polymer formation was indicated by the detection of a labelled compound that did not migrate from the origin of a paper chromatogram in the solvent *A*. This method of observing the formation of polymer has been used previously in work on the synthesis of teichoic acids (Nathenson & Strominger, 1963; Baddiley *et al.* 1968). The amount of polymer formed is expressed as a percentage incorporation of the radioactive substrate present.

Table 1. *Substrates required for polymer synthesis*

The complete reaction mixture (Expt. 1) contained: UDP-[^{14}C]N-acetylglucosamine, 0.1 μmole (1 $\mu\text{mole} \equiv 4 \times 10^5$ c.p.m.); ATP, 4.0 μmoles ; particulate enzyme preparation suspended in 0.1 *M*-tris-HCl buffer, pH 8.5, 0.15 ml.; water to a final volume of 0.17 ml. In Expts. 4, 5, 6 and 7 the mixture contained: [^{14}C]N-acetylglucosamine 1-phosphate, 0.01 ml. (1 ml. $\equiv 32 \times 10^5$ c.p.m.); MgCl_2 , 4 μmoles ; nucleoside triphosphate, 1 μmole ; particulate enzyme suspended in 0.1 *M*-tris-HCl buffer, pH 8.5, 0.15 ml.; water to a final volume of 0.18 ml. Mixtures were incubated for 1 hr. at 37°, then heated for 2 min. at 100°, applied as bands to Whatman no. 3 MM paper and chromatographed in solvent *A*. The polymer remained at the origin.

Expt. no.	Substrates	Polymer formed (% incorporation of labelled substrates)
1	UDP-[^{14}C]N-acetylglucosamine + ATP	3.4
2	UDP-[^{14}C]N-acetylglucosamine	4.5
3	UDP-[^{14}C]N-acetylglucosamine (enzyme boiled)	0.0
4	[^{14}C]N-Acetylglucosamine 1-phosphate + UTP	8.2
5	[^{14}C]N-Acetylglucosamine 1-phosphate + ATP	0.0
6	[^{14}C]N-Acetylglucosamine 1-phosphate + CTP	0.0
7	[^{14}C]N-Acetylglucosamine 1-phosphate	0.0

Fig. 1. Effect of enzyme concentration on polymer synthesis. Incubation mixtures contained: UDP-[acetyl- ^{14}C]N-acetylglucosamine ($0.1\text{ }\mu\text{mole}$) ($1\text{ }\mu\text{mole} \equiv 4 \times 10^5\text{ c.p.m.}$); MgCl_2 ($4\text{ }\mu\text{moles}$); particulate enzyme system suspended in 0.1 M -tris-HCl buffer, pH 8, and the same buffer to give a final volume of 0.2 ml. Mixtures were incubated for 1 hr. at 37° and treated as described for Table 1; the product was determined as described in the text.

Fig. 3. Effect of Mg^{2+} on polymer synthesis. Incubation mixtures contained: UDP-[acetyl- ^{14}C]N-acetylglucosamine ($0.1\text{ }\mu\text{mole}$) ($1\text{ }\mu\text{mole} \equiv 4 \times 10^5\text{ c.p.m.}$); particulate enzyme system (0.15 ml. suspended in 0.1 M -tris-HCl buffer, pH 8.5); MgCl_2 as indicated; water to give a final volume of 0.2 ml. Mixtures were incubated for 1 hr. at 37° and the product was determined as described in the text.

Fig. 2. Rate of polymer synthesis. Incubation mixtures contained: UDP-[acetyl- ^{14}C]N-acetylglucosamine ($0.1\text{ }\mu\text{mole}$) ($1\text{ }\mu\text{mole} \equiv 4 \times 10^5\text{ c.p.m.}$); MgCl_2 ($4\text{ }\mu\text{moles}$); particulate enzyme system (0.15 ml. , suspended in 0.1 M -tris-HCl buffer, pH 8); final volume 0.17 ml. Mixtures were incubated for the times indicated and the product was determined as described in the text.

Fig. 4. Effect of concentration of UDP-N-acetylglucosamine on polymer synthesis. Incubation mixtures contained: MgCl_2 ($14\text{ }\mu\text{moles}$); particulate enzyme system (0.15 ml. , suspended in 0.1 M -tris-HCl buffer, pH 8); UDP-[acetyl- ^{14}C]N-acetylglucosamine as indicated; water to give a final volume of 0.2 ml. Mixtures were incubated for 1 hr. at 37° and the product was determined as described in the text.

interfere with the enzymic synthesis of teichoic acids and peptidoglycans in cell-free systems. Novobiocin, vancomycin and crystal violet inhibit the synthesis of poly(glycerol phosphate) (Burger & Glaser, 1964) and of poly(ribitol phosphate) (Glaser, 1964; Ishimoto & Strominger, 1966). A larger

number of compounds, e.g. butan-1-ol, deoxycholate, Triton X-100 and the antibiotics novobiocin, bacitracin, vancomycin and ristocetin, inhibit the synthesis of the cell-wall peptidoglycans

Table 2. *Effect of antibiotics, detergents and solvents on polymer synthesis*

Incubation mixtures contained: UDP-[acetyl- ^{14}C]*N*-acetylglucosamine, 0.1 μmole (1 $\mu\text{mole} \equiv 2.7 \times 10^6$ c.p.m.); MgCl_2 , 10 μmoles ; particulate enzyme preparation suspended in 0.1 M-tris-HCl buffer, pH 8.5, 0.15 ml.; the addition as required and water to a final volume of 0.22 ml. Mixtures were incubated for 1 hr. at 37° and then the reactions were stopped by heating for 2 min. at 100°. The polymer was extracted from the mixtures with 80% (w/v) phenol (Burger & Glaser, 1964), the mixture was centrifuged, the aqueous layer removed and the remaining phenol washed with water. The combined aqueous portions were extracted once with chloroform and then chromatographed on Whatman no. 1 in solvent *A*. The polymer remaining at the origin was counted in the scintillation counter.

Inhibitor	Concentration	Polymer synthesis (% of control)
None	—	100
D-Cycloserine	500 $\mu\text{g.}/\text{ml.}$	93
	100 $\mu\text{g.}/\text{ml.}$	103
Bacitracin	500 $\mu\text{g.}/\text{ml.}$	109
	100 $\mu\text{g.}/\text{ml.}$	97
Novobiocin	500 $\mu\text{g.}/\text{ml.}$	83
	100 $\mu\text{g.}/\text{ml.}$	106
Triton X-100	0.25%	56
	0.05%	105
Sodium deoxycholate	0.25%	76
	0.05%	107
Cetyltrimethylammonium bromide	0.25%	77
	0.05%	111
Butan-1-ol	4.00%	8
	1.20%	61
Octan-2-ol	4.00%	49
	1.20%	56
UMP	25 $\mu\text{moles}/\text{ml.}$	35
	5 $\mu\text{moles}/\text{ml.}$	72

(Matsushashi, Dietrich & Strominger, 1967). The mechanism of the inhibition of synthesis of teichoic acid is not yet understood, but inhibition of synthesis of peptidoglycan by the antibiotics has been more fully investigated and the steps in the synthetic pathway at which they interfere have been determined (Matsushashi *et al.* 1967).

The effect of several antibiotics, detergents and solvents on the synthesis of poly(*N*-acetylglucosamine 1-phosphate) is shown in Table 2; only octan-2-ol, butan-1-ol and Triton X-100 inhibited polymer formation significantly. UMP also had an inhibitory effect.

Chain length of the polymer. The average apparent chain length of synthesized polymer was determined by incubation for various times of a mixture of enzyme preparation and labelled nucleotide, hydrolysis of the product under the controlled conditions and measurement of the ratio of the label in *N*-

Fig. 5. Chain length of polymer after various times of incubation. Incubation mixtures contained: UDP-[acetyl- ^{14}C]*N*-acetylglucosamine (0.1 μmole) (1 $\mu\text{mole} \equiv 2.7 \times 10^6$ c.p.m.), MgCl_2 (10 μmoles); particulate enzyme system (0.15 ml., suspended in 0.1 M-tris-HCl buffer, pH 8.5); final volume 0.17 ml. Mixtures were incubated at 37° for the times indicated, then heated at 100° for 2 min. The polymer was extracted by shaking with an equal volume of 80% (w/v) phenol (Burger & Glaser, 1964) for 40 min. at 0°. The mixture was centrifuged, the aqueous layer removed and the phenol layer washed once with water. Combined aqueous portions were washed once with an equal volume of chloroform and products were chromatographed on Whatman no. 1 paper in solvent *A*. The polymer remaining at the origin was hydrolysed as described in the text, and the *N*-acetylglucosamine and its 6-phosphate were chromatographed in solvent *A*; they were located by the strip scanner, appropriate areas of paper cut out and radioactivity was determined in the scintillation counter. The ratio of radioactively labelled *N*-acetylglucosamine to its 6-phosphate was used to calculate the chain length (see the Discussion section).

acetylglucosamine to the label in the 6-phosphate. Results are given in Fig. 5. Chain length increased rapidly with time and reached a constant value of 14–15 units.

Pulse-labelling of polymer during synthesis. The direction of chain extension during polymer synthesis was studied in pulse-labelling experiments with labelled and unlabelled nucleotide precursor. The results given in Table 3 show that the percentage of total radioactivity that represents the 'sugar-end' of chains (measured as *N*-acetylglucosamine) fell after incubation with unlabelled nucleotide, whereas the percentage within the chain (measured as 6-phosphate) rose.

DISCUSSION

The enzyme preparation used in these studies probably represents fragmented cytoplasmic membrane; no attempt was made to purify the particulate preparation and ribosomal material was present

Table 3. *Direction of chain extension*

Incubation mixture contained: UDP-[acetyl- ^{14}C]N-acetylglucosamine (0.2 μmole) (1 $\mu\text{mole} \equiv 2.7 \times 10^6$ c.p.m.), MgCl_2 (20 μmoles) and particulate enzyme suspended in 0.1M-tris-HCl buffer, pH 8.5 (0.3 ml.). The mixture was incubated for 2 min. at 37° and then diluted with cold 0.1M-tris-HCl buffer, pH 8.5, to about 25 ml. The enzyme was centrifuged at 184000 g for 15 min. and then washed once in the buffer before being resuspended in it to give the original volume. A sample (0.15 ml.) was heated for 2 min. at 100°. The remainder was incubated with UDP-N-acetylglucosamine (0.1 μmole) and MgCl_2 (10 μmoles) for 30 min. at 37°. The enzyme was inactivated as before, the polymer was extracted from the mixtures by the use of phenol, purified by paper chromatography and subjected to mild acid hydrolysis, and the products were separated and counted as has been described. The amounts of each present were calculated as a percentage of the total radioactivity.

Time of incubation (min.)	Amount of (II) formed		Amount of (III) formed	
	(c.p.m.)	(% of total)	(c.p.m.)	(% of total)
0.0	5553	93.0	415	6.9
30.0	6219	97.0	199	3.1

The only substrate required for synthesis of poly-(N-acetylglucosamine 1-phosphate) was UDP-N-acetylglucosamine. Polymer was formed from N-acetylglucosamine 1-phosphate, but only in the presence of UTP, and it is concluded that this arises through the presence in the system of UDP-N-acetylglucosamine synthetase; the presence of this and related synthetases in fragmented membrane preparations has been observed on many occasions with other bacteria in this laboratory. By the use of doubly labelled UDP-N-acetylglucosamine, with ^{14}C in acetyl and ^{32}P in the phosphate attached to the 1-position of the N-acetylglucosamine residue, it was shown that N-acetylglucosamine 1-phosphate is transferred as an intact unit from the nucleotide to the polymer. In this respect the mechanism of synthesis resembles markedly the synthesis of the wall teichoic acid from *S. lactis* I3 (Baddiley *et al.* 1968), where UDP-N-acetylglucosamine also transfers an intact N-acetylglucosamine 1-phosphate unit. It is likely that this is a general mechanism for the introduction of sugar 1-phosphate residues into this increasingly encountered group of microbial polymers (Archibald *et al.* 1968b).

The enzyme system has a requirement for an unusually high concentration of bivalent cations (Mg^{2+} or Mn^{2+}), but very high concentrations are inhibitory. The optimum pH was about 8.5. These properties are comparable with those previously observed for the particulate system from *S. lactis* I3, although the optimum concentration of cation is much larger in the present case.

The average chain lengths of teichoic acids have

been determined by several methods, all involving end-group analysis, with material that was synthesized enzymically or extracted from cells or walls. For the poly(glycerol phosphate) types, values for the number of repeating units of 30–32 (synthesized) (Burger & Glaser, 1964), 18 (extracted) (Critchley, Archibald & Baddiley, 1962) and 20 (extracted) (Wicken & Baddiley, 1963) have been reported. For the poly(ribitol phosphate) types values are 5–9 (synthesized) (Glaser, 1964), 8–12 (extracted) (Archibald, Baddiley & Buchanan, 1961) and 7–9 (extracted) (Hay, Archibald & Baddiley, 1965). Thus, although the values for synthesized and extracted ribitol teichoic acids are comparable, it seems that synthesized glycerol teichoic acids are longer than those extracted from cells. The significance of the values for extracted polymers is uncertain, as the process of extraction with trichloroacetic acid is accompanied by, and possibly requires, the hydrolysis of a limited number of phosphodiester linkages. On the other hand, in most cases the extracted material was reprecipitated by use of organic solvents and this would remove much material with low molecular weight.

For the poly(N-acetylglucosamine 1-phosphate), chemical studies on polymer present in walls indicate a chain of about 80 units (G. H. Stafford & A. R. Archibald, unpublished work). In the present study the apparent chain length reached a maximum of about 14–15 units. The presence in the enzyme preparation of partly formed polymer, that would accept further units, could result in the observed average chain length being shorter than the actual average chain length. A sample (0.15 ml.) of the enzyme suspension was treated according to the procedure given with Fig. 5, and the hydrolysate was found to contain N-acetylglucosamine and its 6-phosphate, thereby confirming the presence of polymer. Thus, although no quantitative values were obtained, it is likely that this explains, at least in part, the low value for the chain length of the enzymically synthesized polymer. Partial hydrolysis of N-acetylglucosamine 6-phosphate to N-acetylglucosamine during the controlled degradation of the polymer would also decrease the apparent chain length, but independent experiments showed that this was not an important factor.

The apparent chain length increased rapidly with time up to about 10 min. (Fig. 5) and then remained almost constant, although polymer synthesis continued at a decreased rate (Fig. 2). This suggests that the rapid initial synthesis of polymer occurs by further extension of existing partly formed chains; when these are approaching, or have approached, their maximum length the rate decreases due to a decrease in the number of available chains and the absence of new acceptor sites.

The direction of chain extension during the

formation of bacterial wall polymers is of some interest, and has been examined recently for the polysaccharide chains of lipopolysaccharides and for teichoic acids of the poly(glycerol phosphate) type. A feature of these studies is the demonstration that the polysaccharide chains of a lipopolysaccharide are built up on the polyisoprenoid pyrophosphate intermediates by the transfer of trisaccharide residues to the 'reducing end' of the growing polymer (Bray & Robbins, 1967; Robbins, Bray, Dankert & Wright, 1967), in a manner analogous to amino acid transfer in protein synthesis on ribosomes. This contrasts with glycogen synthesis, where sugar residues are transferred to the 'non-reducing' ends of chains. It also differs from the mechanism of chain extension in a poly(glycerol phosphate) type of teichoic acid, where glycerol phosphate units are transferred from CDP-glycerol to the glycerol-terminal end of chains (Kennedy & Shaw, 1968). The occurrence of sugar 1-phosphate residues in certain bacterial wall polymers and teichoic acids raises the question whether these are formed by a route analogous to that for the synthesis of polysaccharide chains in lipopolysaccharides, or whether they follow a route similar to that for the poly(glycerol phosphate). With the wall teichoic acid from *S. lactis* I3, it has been found by using CDP-[^{14}C]glycerol and UDP-[acetyl- ^{14}C]*N*-acetylglucosamine that chain extension follows a similar course to that for poly(glycerol phosphate), i.e. in pulse-labelling experiments ^{14}C initially present in the terminal repeating unit at the 'glycerol end' of the chain is found later in units deeper in the chain (Hussey, Brooks & Baddiley, 1969; H. Hussey & J. Baddiley, unpublished work); the present work establishes a similar mechanism for the synthesis of the poly(*N*-acetylglucosamine 1-phosphate).

The controlled acid hydrolysis (Scheme 1) of the polymer provides a simple method for distinguishing between the '*N*-acetylglucosamine end' of the polymer and repeating units deeper in the chain. Thus, in the experiments given in Table 3, isotope from UDP-[acetyl- ^{14}C]*N*-acetylglucosamine appeared after a short period of incubation both in end groups (represented by *N*-acetylglucosamine) and deeper in the chain (represented by the 6-phosphate). When the particulate material containing this labelled polymer was washed and then incubated with unlabelled nucleotide, the label in end groups decreased by a little more than 50%, and there was a corresponding increase in label deeper in the chain. It follows that new residues are being attached at the '*N*-acetylglucosamine end' of the chains. In the experiments described in Table 3 relatively little chain extension had occurred during incubation of the enzyme system with unlabelled nucleotide. Nevertheless, the direction of chain extension

was clearly demonstrated, as the addition of a single repeating unit to, say, half of the chains would produce a decrease of 50% in labelled chain ends.

The synthesis of polymer is markedly inhibited by UMP (Table 2), and this is consistent with a mechanism involving the step:

The nature of this acceptor is not yet fully established, although it appears to be a lipid phosphate bound in the membrane and is probably similar to that involved in the synthesis of peptidoglycans (Higashi, Strominger & Sweeley, 1967), lipopolysaccharides (Wright, Dankert, Fennessy & Robbins, 1967) and mannan (Scher, Lennarz & Sweeley, 1968). The existence of a similar lipid intermediate in the biosynthesis of the teichoic acid in *S. lactis* I3 has also been reported (Douglas & Baddiley, 1968), although the small amounts available have so far prevented adequate characterization of the lipid part of the molecule.

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