

Role of the Penicillin-Sensitive Transpeptidation Reaction in Attachment of Newly Synthesized Peptidoglycan to Cell Walls of *Micrococcus luteus*

(bacterial cell-wall biosynthesis/transglycosylation/ β -lactam antibiotics)

DAVID MIRELMAN, RIVKA BRACHA, AND NATHAN SHARON

Department of Biophysics, Weizmann Institute of Science, Rehovoth, Israel

Communicated by W. Z. Hassid, August 25, 1972

ABSTRACT Cell-wall preparations of *Micrococcus luteus* (*lysodeikticus*) catalyze *in vitro* peptidoglycan synthesis from UDP *N*-acetyl-D-glucosamine, UDP *N*-acetylmuramic acid-pentapeptide, and glycine. Newly synthesized peptidoglycan is partially cross-linked by a transpeptidation reaction with concomitant release of C-terminal D-alanine. Penicillin not only strongly inhibits release of D-alanine (98% at 1 μ g/ml), but also markedly inhibits incorporation of acetylglucosamine and *N*-acetylmuramic acid-pentapeptide into the preformed cell-wall peptidoglycan. The simplest explanation for the results is that incorporation of newly synthesized strands of peptidoglycan and their attachment to "older" cell-wall peptidoglycan proceeds mainly by transpeptidation and that transglycosylation is responsible only for part of the elongation of the pre-existing peptidoglycan. Another possibility is that incorporation occurs by transglycosylation, but it cannot continue without concurrent formation of peptide cross-bridges.

Use of UDP-*N*-acetyl-D-glucosamine (GlcNAc) and UDP-*N*-acetylmuramic acid (MurNAc)-L-Ala-D-isoGlu-L-Lys-D-Ala-D-Ala (UDP-MurNAc-pentapeptide) by a particulate enzyme fraction from *Micrococcus luteus* (*lysodeikticus*) cells for synthesis of a chromatographically immobile, or acid-precipitable, product has been reported (1, 2). Incorporation of the precursors into the product was not affected by penicillin G (1300 μ g/ml), and it was assumed that it proceeds by transglycosylation of the activated disaccharide-pentapeptide to the growing end of the linear peptidoglycan. Moreover, no evidence for transpeptidation has been reported with extracts of *M. luteus* (3, 4).

A new system for studying the last stages of peptidoglycan biosynthesis was described (5). Cell-wall preparations of *Staphylococcus aureus* incorporated nucleotide precursors into the preformed peptidoglycan. Incorporation was accompanied by the penicillin-sensitive release of C-terminal D-alanine from the MurNAc-pentapeptide. In line with current concepts of peptidoglycan biosynthesis (3, 6, 7), we assumed that the release of D-alanine is the result of the terminal biosynthetic step *i.e.*, the transpeptidation reaction between two adjacent peptide chains that results in formation of a cross-linked, three-dimensional polysaccharide-peptide network.

We now report that cell-wall preparations of *M. luteus* incorporate GlcNAc, MurNAc-pentapeptide, and glycine

into pre-existing cell-wall peptidoglycan with concomitant release of C-terminal D-alanine, from the MurNAc-pentapeptide. Penicillin not only inhibited the release of D-alanine but also the incorporation of GlcNAc and MurNAc-pentapeptide into the preformed cell-wall peptidoglycan.

METHODS AND MATERIALS

M. luteus ATCC 2665 was grown to early log phase at 32° in a medium containing 0.5% bacto-peptone, 0.2% yeast extract, 0.5% NaCl, and 0.5% glucose. Crude cell walls, used as source of enzyme and as acceptor for peptidoglycan biosynthesis, were prepared as described for *S. aureus* (5). Such preparations contained tightly attached fragments of the cell membrane (8). In the crude cell-wall preparations Ala, Glu, Lys, and Gly account for about 88% of the total amino acids present; the remaining 12% were accounted for by small amounts of other amino acids. The protein content, estimated by a modification of the Lowry *et al.* method (8), was 55 μ g/mg of cell-wall. These preparations also contain about 0.12 μ mol Pi/mg cell wall. Purified cell walls, after heating and extensive washings with detergents, contained only 0.038 μ mol Pi/mg cell wall; they did not contain any protein, and more than 98% of the total amino acids are from peptidoglycan.

UDP-MurNAc-pentapeptide, UDP-MurNAc-[L-¹⁴C]Lys-pentapeptide, and UDP-MurNAc-L-Ala-D-isoGlu-L-Lys (UDP-MurNAc-tripeptide) were prepared (9, 10). D-Ala-[D-¹⁴C]Ala was chemically synthesized (Burstein and Patchornik, paper in preparation) and was used for enzymatic synthesis of UDP-MurNAc-pentapeptide with C-terminal [D-¹⁴C]Ala (10). UDP-MurNAc-[D-¹⁴C]Ala-pentapeptide was purified by high voltage paper electrophoresis (pH 3.5, 50 V/cm, 45 min) followed by paper chromatography (48 hr) in solvent I (isobutyric acid-1 M ammonia, 5:3). Upon amino-acid analysis of the product, the following molar ratios were found: Ala_{2.9}, Glu_{1.0}, Lys_{0.92}, Mur_{0.89}; the specific activity was 26 cpm/pmol.

UDP-[¹⁴C]GlcNAc (260 Ci/mol) and labeled amino acids were obtained from the Radiochemical Centre, Amersham, England. *Streptomyces* amidase was a gift from Dr. J. M. Ghuysen.

Aminoacid analyses of UDP-MurNAc-pentapeptide and of cell-wall peptidoglycan were done with a Beckman 120C aminoacid analyzer (11). Radioactive amino acids and peptides were detected with a Packard Tri-carb flow analyzer scintillation spectrometer 3022 connected directly to a column

Abbreviations: MurNAc, *N*-acetylmuramic acid[2-acetamido-3-O-(D-1-carboxyethyl)-2-deoxy-D-glucose]; GlcNAc, *N*-acetyl-D-glucosamine.

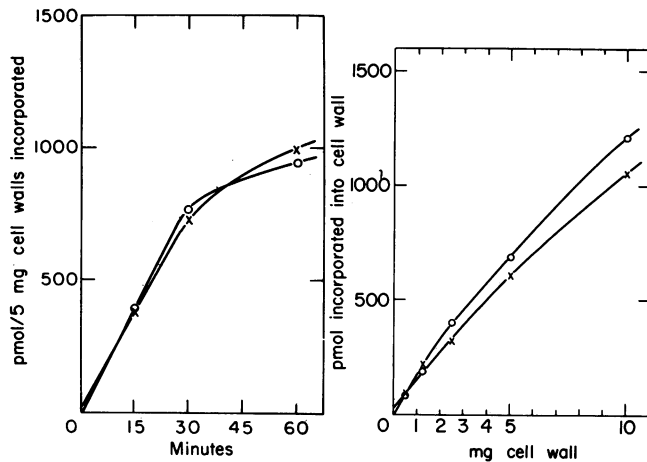


FIG. 1. (left) Effect of time on incorporation of [^{14}C]GlcNAc and MurNAc-[^{14}C]Lys-pentapeptide into the purified cell-wall. Incorporation was measured in separate incubation mixtures.

FIG. 2. (right) Incorporation of [^{14}C]GlcNAc and MurNAc-[^{14}C]Lys-pentapeptide into purified cell walls. Different amounts of cell-wall preparations were used and the determination was done in separate incubation mixtures. O, GlcNAc; X, MurNAc-pentapeptide.

(19 cm) of the amino acid analyzer and run with the first buffer (citrate, pH 3.25). [^{14}C]alanine is eluted after 27 min and D-Ala-[^{14}C]Ala after 60 min.

Assay of Enzymatic Activities. Incubation mixtures, in a final volume of 180 μl , contained: 50 mM Tris buffer, pH

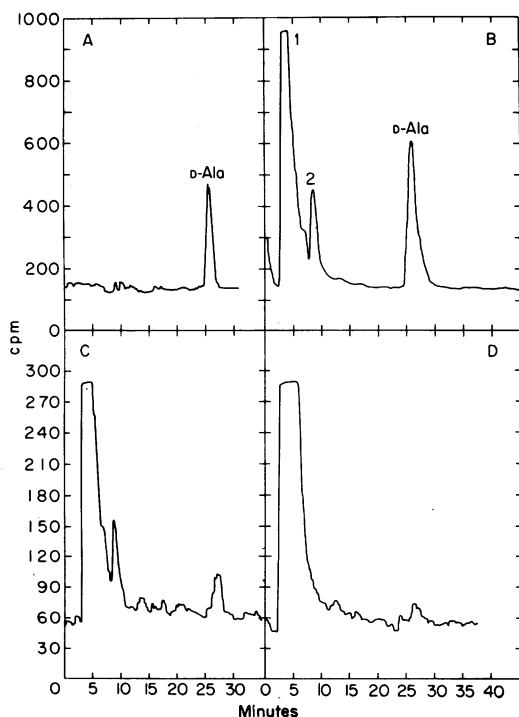


Fig. 3. Flow scintillation charts of the effluent from the amino acid analyzer (see text). (A) [^{14}C]Ala standard. (B) Complete incubation mixture containing UDP-MurNAc-[^{14}C]Ala-pentapeptide as the labeled substrate together with UDP-GlcNAc and glycine. Peak 1, unmodified substrate; peak 2, degradation product. (C) Complete incubation mixture as in B containing penicillin G (0.1 $\mu\text{g}/\text{ml}$). (D) Incubation mixture without the addition of UDP-GlcNAc.

7.8, 100 mM NH_4Cl , 20 mM MgCl_2 , 15 mM ATP, 1 mM 2-mercaptoethanol, and about 5 mg (dry weight) of crude cell walls. Complete reaction mixtures also contained UDP-GlcNAc, UDP-MurNAc-pentapeptide, and glycine, either radioactively labeled or unlabeled. Reaction mixtures were routinely incubated at 25° for 30 min after which 0.5 ml of cold water was added and the suspension was centrifuged for 4 min at $8000 \times g$ in an Eppendorf 3200 microcentrifuge. The clear supernatant was carefully removed, and, in experiments where UDP-MurNAc-[^{14}C]Ala-pentapeptide was used, the supernatant fluid was analyzed on the amino acid analyzer for [^{14}C]alanine. The remaining cell-wall pellet was resuspended in cold TMM buffer [50 mM Tris buffer, pH 7.8, 20 mM MgCl_2 , and 1 mM 2-mercaptoethanol containing 1% sodium dodecyl sulfate (0.5 ml)], and washed four times with the same buffer solution. The pellet was resuspended in TMM buffer (0.5 ml) and heated at 100° for 15 min. The heated cell-walls were washed in TMM buffer (0.5 ml) and finally washed in water (0.5 ml). The purified cell-walls were then resuspended in water (0.5 ml), and their radioactivity was determined in a Packard Tri-carb liquid scintillator 3003 with a scintillation fluid for aqueous solutions (12). For paper chromatographic analysis of the reaction products, separate tubes were incubated as described above, and 40 μl of their contents were chromatographed on Whatman No. 1 paper. After chromatography in solvent I, radioactive compounds were located by autoradiography (Kodak, Royal Blue x-ray film), and the radioactive spots were cut and counted.

Purified cell-walls were digested with lysozyme, *Streptomyces* amidase, and Pronase (11). The digests were chromatographed on Whatman No. 1 paper in solvent II (*n*-butanol-acetic acid-water, 3:1:1, v/v). Free amino groups were estimated by dinitrophenylation (13). The configuration of the alanine released by transpeptidation was determined with D-amino acid oxidase (Worthington Co.) (13).

RESULTS

Incorporation of UDP-[^{14}C]GlcNAc and of UDP-MurNAc-[^{14}C]Lys-pentapeptide into Cell-Walls and Its Inhibition by Penicillin. Complete incubation mixtures contained crude cell-walls as enzyme source, together with UDP-[^{14}C]GlcNAc (10 nmol, 15 cpm/pmol), UDP-MurNAc-pentapeptide (100 nmol), and glycine (500 nmol) or with UDP-MurNAc-[^{14}C]Lys-pentapeptide (50 nmol, 4.2 cpm/pmol), UDP-GlcNAc (200 nmol), and glycine (500 nmol). Upon analysis

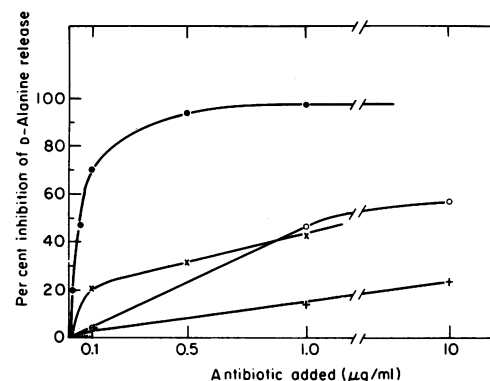


FIG. 4. Inhibitory effect of different β -lactam antibiotics on release of [^{14}C]Ala by the transpeptidation reaction. ●, penicillin G; ○, 6-amino penicillanic acid; X, methicillin; +, cephaloridine.

TABLE 1. Incorporation of labeled peptidoglycan precursor into *Micrococcus luteus* cell-walls*

¹⁴ C-labeled substrate	Unlabeled substrate added	Isotope incorporated into purified cell walls (pmol/5 mg of cell walls)			Molar ratio*	Isotope incorporated into cell walls in the presence of penicillin G (pmol/5 mg of cell walls)			Molar ratio†	Inhibition of incorporation into cell walls‡ (%)
		Re-leased	At-tached to cell wall	Sum		Re-leased	At-tached to cell wall	Sum		
UDP-MurNAc-L-Ala-D-Glu- [L- ¹⁴ C]Lys-D-Ala-D-Ala	UDP-GlcNAc; glycine	620	232	852	1.03	<10	248	248	0.93	None
UDP-[¹⁴ C]GlcNAc	UDP-MurNAc-pentapeptide; glycine	38	63	101		<10	54	54		
UDP-MurNAc-L-Ala-D-Glu- [L- ¹⁴ C]Lys-D-Ala-D-Ala	—									
UDP-[¹⁴ C]GlcNAc	—									
		[D- ¹⁴ C]alanine				[D- ¹⁴ C]alanine				
		Re-leased	At-tached to cell wall	Sum		Re-leased	At-tached to cell wall	Sum		
UDP-MurNAc-L-Ala-D-Glu- L-Lys-D-Ala-[D- ¹⁴ C]Ala	UDP-GlcNAc; glycine	620	232	852	1.03	<10	248	248	0.93	None
UDP-MurNAc-L-Ala-D-Glu- L-Lys-D-Ala-[D- ¹⁴ C]Ala	—	38	63	101		<10	54	54		

* Incubation mixtures in a final volume of 180 μ l contained: Tris buffer pH 7.8 (50 mM), NH₄Cl (100 mM), MgCl₂ (20 mM), ATP (15 mM), 2-mercaptoethanol (1 mM), and crude cell walls (about 5 mg dry weight) containing 275 μ g of protein. Labeled and unlabeled substrates were added as indicated. Incubations were done at 25° for 30 min.

† Molar ratio incorporated is calculated relative to 1.0 for the incorporation of MurNAc-[L-¹⁴C]Lys pentapeptide.

‡ By penicillin G (1 μ g/ml).

by paper chromatography in solvent I, radioactivity was found in "origin material" ($R_F = 0$) and in lipid intermediates ($R_F = 0.9$). Formation of these radioactive products required the presence of the two uridine derivatives and, as shown earlier (1, 2), was not inhibited by penicillin G (1 μ g/ml).

In parallel experiments, also with complete incubation mixtures, incorporation of either [¹⁴C]GlcNAc or MurNAc-[L-¹⁴C]Lys-pentapeptide into cell-wall peptidoglycan was measured. Incorporation of GlcNAc paralleled incorporation of MurNAc-pentapeptide, was time-dependent (Fig. 1), and was proportional to the amount of cell-wall preparation added (Fig. 2). The K_m for UDP-GlcNAc was 5.6×10^{-5} M, and the K_m for UDP-MurNAc-pentapeptide was 9.0×10^{-5} M. These incorporations were unaffected by the presence or absence of ATP. Penicillin G (1 μ g/ml) strongly inhibited (65–70%) incorporation of both [¹⁴C]GlcNAc and MurNAc-[L-¹⁴C]Lys-pentapeptide into purified cell walls (Table 1). Only slightly higher inhibition (75–80%) was observed when the concentration of penicillin G was raised to 100 μ g/ml.

In experiments in which UDP-[¹⁴C]GalNAc replaced UDP-[¹⁴C]GlcNAc, no incorporation of [¹⁴C]GalNAc into cell walls or lipid intermediates was observed.

Incorporation of UDP-MurNAc-L-Ala-D-isoGlu-L-Lys-D-Ala-[D-¹⁴C]Ala. Analysis by paper chromatography (solvent I) of complete incubation mixtures in which the labeled precursor was UDP-MurNAc-[D-¹⁴C]Ala-pentapeptide, revealed radioactivity in "origin material" and in the lipid-bound intermediates. In addition, free [D-¹⁴C]Ala ($R_F = 0.67$) re-

leased into the medium by transpeptidation, was detected both on paper chromatograms and by amino acid analysis (Fig. 3B). In the absence of UDP-GlcNAc in the reaction mixture, the amount of radioactivity incorporated into "origin material" was only 10–15% of that found in the complete mixture and only traces of free [D-¹⁴C]Ala were detected (Fig. 3D).

Minute amounts of penicillin G strongly inhibited the release of free [D-¹⁴C]Ala (Fig. 3C). About 50% inhibition was observed at 0.06 μ g/ml of penicillin G, and 98% at 1 μ g/ml (Fig. 4). Other β -lactam antibiotics tested had a considerably smaller effect on the release of D-alanine (Fig. 4). Inhibition of the release of D-alanine by penicillin G was irreversible. Crude cell-wall preparations were incubated for 5 min with penicillin (10 μ g/ml) at 20°, after which the penicillin was thoroughly washed off (four times with TMM buffer) and any excess was destroyed by penicillinase (100 units, 10 min at 25°). Upon incubation of the penicillin G-pretreated cell-walls with UDP-MurNAc-[D-¹⁴C]Ala-pentapeptide, UDP-GlcNAc, and glycine, only minute amounts of [D-¹⁴C]Ala were released (43 pmol/5 mg of cell walls), compared to 620 pmol/5 mg of cell walls in the uninhibited preparation. Crude cell-wall preparations that were pretreated with penicillin also exhibited a lower incorporation of [¹⁴C]GlcNAc into the peptidoglycan (30%), compared to the untreated cell walls, but the incorporation of MurNAc-[D-¹⁴C]Ala-pentapeptide was only slightly affected. The same results were obtained in the presence of penicillin with crude cell walls that had not been pretreated with the antibiotic.

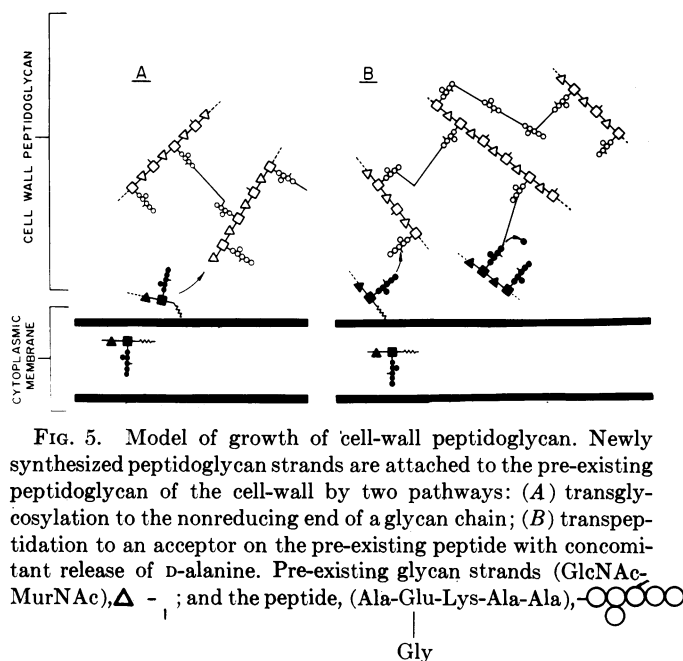


Fig. 5. Model of growth of cell-wall peptidoglycan. Newly synthesized peptidoglycan strands are attached to the pre-existing peptidoglycan of the cell-wall by two pathways: (A) transglycosylation to the nonreducing end of a glycan chain; (B) transpeptidation to an acceptor on the pre-existing peptide with concomitant release of *D*-alanine. Pre-existing glycan strands (GlcNAc-MurNAc), Δ - ; and the peptide, (Ala-Glu-Lys-Ala-Ala), \circ - \circ - \circ - \circ - \circ .

Newly synthesized peptidoglycan is in *black*. The repeating unit, single or polymerized, that is incorporated by transpeptidation, is depicted in *B* as lipid-linked, although it is possible that the lipid moiety is removed before incorporation.

The molar ratio of [^{14}C]GlcNAc to MurNAc-[^{14}C]Lys-pentapeptide incorporated either in the presence or absence of penicillin G (1 $\mu\text{g}/\text{ml}$), is about one (Table 1). The molar proportion of MurNAc-[^{14}C]Ala-pentapeptide incorporated into the peptidoglycan was much lower (about 0.3 mol of [^{14}C]Ala/1.0 mol of [^{14}C]GlcNAc). However, when the amount of [^{14}C]Ala that was released by transpeptidation is added to the [^{14}C]Ala that remains attached to the wall, the molar amount obtained is about equal to the value obtained for the other two nucleotides. In the presence of penicillin G, release of [^{14}C]Ala was completely inhibited, but the amount of [^{14}C]Ala that was incorporated into the peptidoglycan remained the same as that observed in the absence of penicillin, and was similar to the molar amounts found for the other two nucleotides under the same conditions.

The incorporation of MurNAc-[^{14}C]Ala-pentapeptide and the release of [^{14}C]Ala into the medium, increased with time (up to 2 hr), were proportional to the amount of crude cell wall added (0.5–10 mg of cell wall), and were unaffected by the presence or absence of ATP. Mg^{++} was required (optimal concentration 20–30 mM), and the optimal temperature for release of *D*-Ala was 30°, whereas maximal incorporation of *D*-Ala into the cell-wall peptidoglycan was at 37°. The optimal pH range of the incorporation was between pH 7.8 and 8.6.

Aminoacid analysis of incubation products from experiments in which only unlabeled substrates were added revealed that the total amount of alanine released by the reaction was 1.8 nmol/10 mg cell wall. No free alanine (<0.3 nmol/10 mg) was obtained when cell-wall preparations were incubated in the absence of the two nucleotide precursors. The amount of alanine released was higher than that calculated from the release of [^{14}C]Ala under the same con-

ditions (1.2 nmol/10 mg), thus suggesting that some dilution with unlabeled alanine occurred. This dilution may originate from peptide chains in the pre-existing peptidoglycan that still contain the *D*-Ala-*D*-Ala terminal structure and serve as donors for the transpeptidation reaction (see Fig. 5B).

Incorporation of [^{14}C]Glycine. Paper chromatographic analysis (solvent I) of incubation mixtures that contained 200 nmol of UDP-GlcNAc, 100 nmol of UDP-MurNAc-pentapeptide, and 50 nmol of [^{14}C]glycine (8 cpm/pmol) revealed that radioactivity was incorporated into both lipid intermediates ($R_F = 0.9$) and "origin material" ($R_F = 0$). [^{14}C]Glycine (520 pmol/5 mg of walls) was also found in the purified cell-walls isolated from the incubation mixtures. In the presence of penicillin G (10 $\mu\text{g}/\text{ml}$), incorporation of [^{14}C]glycine into cell-wall peptidoglycan was inhibited 57%. Lysozyme digests of purified cell walls labeled with [^{14}C]glycine, contained about 92% of the radioactivity in a soluble form. No Dnp-[^{14}C]glycine was found in acid hydrolysates of dinitrophenylated cell walls labeled with [^{14}C]glycine.

Enzymatic Digestion of Purified Cell Walls. Purified cell walls were almost completely solubilized (>95%) with either lysozyme or *Streptomyces* amidase, but were resistant to Pronase digestion. *Streptomyces* amidase digests of [^{14}C]GlcNAc-labeled cell walls afforded two major radioactive compounds that were identified by their migration on paper electrophoresis (pH 6.5) and paper chromatography (solvent II). They were identical with the disaccharide, GlcNAc β (1 \rightarrow 4)MurNAc, and the corresponding tetrasaccharide (11). Lysozyme digests of cell walls labeled with [^{14}C]GlcNAc contained several radioactive compounds. Two of the compounds obtained were identical in their migration to the authentic disaccharide-pentapeptide, GP-2, and its dimer, the crosslinked bis-disaccharide-decapeptide, GP-1 (11). In addition, small amounts of radioactive disaccharide were present in the lysozyme digests.

Paper electrophoretic comparison of lysozyme digests of cell-walls labeled with [^{14}C]glycine in the presence or absence of penicillin G (10 $\mu\text{g}/\text{ml}$) showed different patterns. The glycopeptide fragments, which contain a higher degree of peptide crosslinks, migrate faster (11). Digests of walls labeled in the presence of penicillin G contain a considerably smaller proportion of the faster moving glycopeptide fragments, compared to the digests of walls labeled in the absence of penicillin.

DISCUSSION

Our results show that cell-wall preparations of *M. luteus* serve both as source of enzyme and as acceptor for newly synthesized peptidoglycan, and thus provide us with a system for the study of the terminal stages of cell-wall biosynthesis. In this respect, these crude cell-wall preparations are similar to those obtained from *S. aureus* (5).

MurNAc-pentapeptide, labeled either in the lysine or in the terminal *D*-alanine, was found attached to the cell-wall peptidoglycan, although in different molar ratios. This difference could be accounted for by the amount of *D*-alanine released by transpeptidation so that the molar sum of [^{14}C]alanine found in the purified walls, together with the free [^{14}C]alanine released into the medium, was equivalent to that of the [^{14}C]lysine found in the walls (Table 1). Incorporation of UDP-MurNAc-pentapeptide was dependent

on addition of UDP-GlcNAc, and only traces of D-alanine were released in the absence of UDP-GlcNAc. These findings strongly suggest that a transpeptidase is present in the crude cell-wall preparations and that these preparations are devoid of D-carboxypeptidase activity. Although D-carboxypeptidase activity is commonly found in rod-like organisms containing diaminopimelic acid (7, 14–16), it has not yet been detected in spherical organisms, which contain lysine in their peptidoglycan (5, 17).

It is generally assumed that cell-wall biosynthesis from the two nucleotide precursors proceeds by formation of linear peptidoglycan strands by a transglycosylation reaction, followed by the crosslinking of the linear strands by a specific transpeptidase, to form a two- or three-dimensional network (3, 7). These two reactions differ in their sensitivity to penicillin, the transpeptidase being highly sensitive to the antibiotic whereas the transglycosylation reaction is presumably insensitive.

The data presented here show that small concentrations of penicillin inhibit both the release of D-alanine and the incorporation of labeled nucleotides into the pre-existing cell-wall peptidoglycan (Table 1). This finding is in contrast to the earlier observations of Anderson *et al.* (1, 2). The reason for this discrepancy becomes clear if the nature of the product obtained with their membrane system (1, 2) is compared to that obtained with the crude cell-walls we used. With isolated membranes only linear, non-crosslinked peptidoglycan is synthesized, apparently by the penicillin-insensitive transglycosylation reaction. However, with crude cell-walls an additional event takes place by which newly synthesized linear strands are attached to the pre-existing cell wall. This attachment is accompanied by release of the terminal D-alanine and is strongly inhibited by penicillin (Table 1, Fig. 4). Part of the attachment, however, proceeds by transglycosylation, as can be deduced from the extent of incorporation of radioactive precursors into the preformed cell wall in the presence of penicillin (Fig. 5A).

The simplest explanation for these results is that the role of the transpeptidase is not only for the formation of interpeptide crosslinks, subsequent to the transfer and attachment of linear glycan strands to pre-existing wall, but mainly for the attachment of new strands to "older" ones (Fig. 5B). This explanation agrees with the observation that penicillin exerts a marked inhibitory effect on incorporation of glycine into purified cell-walls of *S. aureus* (5). Further support for the proposal that penicillin interferes with attachment of newly synthesized peptidoglycan to pre-existing cell wall is derived from observations (18, 19) that small concentrations of penicillin G (5 µg/ml) interfere in the *in vivo* incorporation of L-lysine or glycine into cell walls of *S. aureus*. The dramatic killing effect of penicillin at very low concentrations (2) may be more clearly understood if it affects attachment of newly synthesized strands to existing ones and not only formation of interpeptide bridges. A new role may also be envisaged for the cell-wall endopeptidases that act, in many bacteria, on the peptide moiety of the cell-wall peptidoglycan (20). One function of these enzymes may be to liberate free amino groups to serve as acceptors for attachment of newly syn-

thesized strands of peptidoglycan by transpeptidation. That such a cleavage by an amidase may occur in *M. luteus* cell-walls (11, 21, 22) is supported by our finding of the radioactive disaccharide in lysozyme digests of purified cell walls that had been labeled with [¹⁴C]GlcNAc.

The simple assumption that the main function of the transpeptidase is to attach linear peptidoglycan strands to pre-existing ones is very appealing. However, since little is known about the formation of the three-dimensional structure of the cell wall and its orderly controlled growth, we cannot overlook another, more complex explanation for our results. It is possible that the organization of the enzymes involved in the last stages of cell-wall biosynthesis is such that extensive elongation of peptidoglycan chains by transglycosylation cannot proceed if concomitant formation of peptide crossbridges is prevented. Thus, inhibition of transpeptidation by penicillin would result in inhibition of transglycosylation.

We thank Mr. N. Kaldor and his collaborators for growing the *M. luteus* cells and Mr. D. Levanon for preparation of the UDP-MurNAc-tripeptide.

- Anderson, J. S., Matsushashi, M., Haskin, M. A. & Strominger, J. L. (1965) *Proc. Nat. Acad. Sci. USA* **53**, 881–889.
- Anderson, J. S., Meadow, P. M., Haskin, M. A. & Strominger, J. L. (1966) *Arch. Biochem. Biophys.* **116**, 487–515.
- Strominger, J. L. (1970) in *The Harvey Lectures* (1968–69) (Academic Press, New York) Series 64, pp. 179–213.
- Katz, W., Matsushashi, M., Dietrich, C. P. & Strominger, J. L. (1967) *J. Biol. Chem.* **242**, 3207–3217.
- Mirelman, D. & Sharon, N. (1972) *Biochem. Biophys. Res. Commun.* **46**, 1909–1917.
- Wise, E. M. & Park, J. T. (1965) *Proc. Nat. Acad. Sci. USA* **54**, 75–81.
- Strominger, J. L., Izaki, K., Matsushashi, M. & Tipper, D. J. (1967) *Fed. Proc.* **26**, 9–22.
- Mirelman, D., Shaw, D. R. D. & Park, J. T. (1971) *J. Bacteriol.* **107**, 239–244.
- Chatterjee, A. N. & Park, J. T. (1964) *Proc. Nat. Acad. Sci. USA* **51**, 9–16.
- Neuhaus, F. C. & Struve, W. G. (1965) *Biochemistry* **4**, 120–131.
- Mirelman, D. & Sharon, N. (1967) *J. Biol. Chem.* **242**, 3414–3427.
- Bray, A. G. (1960) *Anal. Biochem.* **1**, 279–285.
- Ghuysen, J. M., Tipper, D. J. & Strominger, J. L. (1966) in *Methods in Enzymology*, eds. Neufeld, E. F. & Ginsburg, V. (Academic Press, New York), Vol. **8**, pp. 685–699.
- Lawrence, P. J. & Strominger, J. L. (1970) *J. Biol. Chem.* **245**, 3653–3659.
- Wickus, G. & Strominger, J. L. (1971) *Fed. Proc.* **30**, 707.
- Ghuysen, J. M., Leyh-Bouille, M., Bonaly, R., Nieto, M., Perkins, H. R., Schleifer, K. H. & Kandler, O. (1970) *Biochemistry* **9**, 2955–2960.
- Matsushashi, M., Dietrich, C. P. & Strominger, J. L. (1967) *J. Biol. Chem.* **242**, 3191–3206.
- Park, J. T. (1958) *Biochem. J.* **70**, 2P.
- Mandelstam, J. & Rogers, H. J. (1959) *Biochem. J.* **72**, 654–662.
- Strominger, J. L. & Ghuysen, J. M. (1967) *Science* **156**, 213–221.
- Schleifer, K. H. & Kandler, O. (1967) *Biochem. Biophys. Res. Commun.* **28**, 965–972.
- Ghuysen, J. M., Bricas, E., Lache, M. & Leyh-Bouille, M. (1968) *Biochemistry* **7**, 1450–1460.