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Analysis of Murein and Murein Precursors during Antibiotic-Induced Lysis of *Escherichia coli*

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Lysis of *Escherichia coli* induced by either D-cycloserine, moenomycin, or penicillin G was monitored by studying murein metabolism. The levels of the soluble murein precursor UDP-*N*-acetylmuramyl-L-alanyl-D-glutamyl-*m*-diaminopimelyl-D-alanyl-D-alanine (UDP-MurNAc-pentapeptide) and the carrier-linked MurNAc-(pentapeptide)-pyrophosphoryl-undecaprenol as well as *N*-acetylglucosamine-β-1,4-MurNAc-(pentapeptide)-pyrophosphoryl-undecaprenol varied in a specific way. In the presence of penicillin, which is known to interfere with the cross-linking of murein, the concentration of the lipid-linked precursors unexpectedly decreased before the onset of lysis, although the level of UDP-MurNAc-pentapeptide remained normal. In the case of moenomycin, which specifically blocks the formation of the murein polysaccharide strands, the lipid-linked precursors as well as UDP-MurNAc-pentapeptide accumulated as was expected. D-Cycloserine, which inhibits the biosynthesis of UDP-MurNAc-pentapeptide, consequently caused a decrease in all three precursors. The muropeptide composition of the murein showed general changes such as an increase in the unusual DL-cross bridge between two neighboring *meso*-diaminopimelic acid residues and, as a result of uncontrolled DL- and DD-carboxypeptidase activity, an increase in tripeptidyl and a decrease in tetrapeptidyl and pentapeptidyl moieties. The average length of the glycan strands decreased. When the glycan strands were fractionated according to length, a dramatic increase in the amount of single disaccharide units was observed not only in the presence of penicillin but also in the presence of moenomycin. This result is explained by the action of an exo-muramidase, such as the lytic transglycosylases present in *E. coli*. It is proposed that antibiotic-induced bacteriolysis is the result of a zipperlike splitting of the murein net by exo-muramidases locally restricted to the equatorial zone of the cell.

Synthesis of the extracellular polymer murein, the scaffold structure of most bacterial walls, occurs by prefabrication of soluble, activated precursors, UDP-*N*-acetylglucosamine (UDP-GlcNAc) and UDP-*N*-acetylmuramyl-L-alanyl-D-glutamyl-*m*-diaminopimelyl-D-alanyl-D-alanine (UDP-MurNAc-pentapeptide), in the cytoplasm of the cell (for a review, see reference 20). These are hooked one after the other to a lipid carrier, undecaprenyl-phosphate (bactoprenol-phosphate), an isoprenoid residing in the cytoplasmic membrane. First, MurNAc-pentapeptide is translocated onto the bactoprenol-phosphate, yielding an intermediate (lipid I), followed by GlcNAc to complete the final murein precursor, the lipid-linked disaccharidepentapeptide (lipid II). Membrane-bound enzymes, the penicillin-binding proteins, polymerize the subunits to form the netlike murein sacculus. The bifunctional penicillin-binding proteins PBP 1A, 1B's, 2, and 3 catalyze transglycosylation to yield polysaccharide chains as well as transpeptidation to cross-link the glycan strands (17). Enlargement and division of the covalently closed bag-shaped sacculus, prerequisites for bacterial growth, call for an interplay of the synthesizing enzymes, i.e., the penicillin-binding proteins, with enzymes cleaving bonds in the murein network to allow the expansion of the existing sacculus and finally to split the septum along its middle line. Such murein hydrolases, although potential autolytic enzymes, are considered pacemaker enzymes for murein growth (11, 26, 33). At least some of them are likely to be essential for bacterial growth.

Inhibition of murein synthesis results in lysis of the cells irrespective of the site of blockage along the biosynthetic pathway (25). The biochemical basis for this therapeutically important bacteriolytic effect of murein synthesis inhibitors, including penicillin, is still not understood. One attempt to explain the lysis response as a consequence of a halt in murein synthesis is the postulation of a critical balance between murein-synthesizing and murein-hydrolyzing enzymes that becomes disturbed when the rate of synthesis is slowed, for example, by the action of antibiotics (33). This hypothesis, however, fails to explain the phenomenon of antibiotic tolerance (25, 27), which is characterized by normal inhibition of murein synthesis, and thereby of growth, but without lysis of the culture.

By taking advantage of the sensitive high-pressure liquid chromatography (HPLC)-based analytical methods established recently (5, 7, 8, 14), we tried to gain some insight into the molecular events of bacteriolysis induced by inhibition of murein synthesis. The intracellular concentrations of the soluble murein precursor in the cytoplasm and the membrane-bound lipid precursors, as well as the structure of the murein during the course of cellular lysis, were analyzed in detail. D-Cycloserine was used to inhibit an early step in murein biosynthesis, namely, the formation of D-alanyl-D-alanine (19). Moenomycin was used to inhibit the transglycosylation reaction (31), and penicillin was used to inhibit the final cross-linking reaction (24).

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MATERIALS AND METHODS

Bacterial strain and growth conditions. *Escherichia coli* W7 (*dap lys*) was grown with agitation in M9 medium, supplemented with *meso*-diaminopimelic acid (A_2pm; 2 μg/ml), L-lysine (20 μg/ml), and 0.4% glucose at 28°C. A rather low growth temperature was chosen to slow the rate of antibiotic-induced lysis. Growth was followed by optical density readings at 578 nm (OD_{578}) by use of an Eppendorf photometer.

Incorporation of [^{3}H]diaminopimelic acid into murein. Pulse-labeling of murein was performed with logarithmically growing cultures of *E. coli* W7 in M9 minimal medium at 28°C. At the indicated time points, culture samples (1 ml) were added to 10 μCi of *meso*-2,6-diamino[3,4,5-^{3}H]pimelic acid (23 Ci/mmol; CEA, Gif-sur-Yvette, France) in an Eppendorf cup and incubated for 3 min at room temperature. The reaction was stopped by incubation of the samples at 100°C for 5 min. To include un-cross-linked, soluble murein in the measurements, the samples were subjected to paper chromatography instead of membrane filtration. The pellet obtained after centrifugation (10,000 × *g* for 5 min) was suspended in 20 μl of H_2O and applied to chromatogram paper (3MM; Whatman, Maidstone, United Kingdom). Descending paper chromatography was done for 18 h in isobutyric acid–1 M ammonium hydroxide (5:3) (12). The chromatograms were dried, and the radioactivity which remained at the origin was determined. A piece of the chromatogram (2 by 3 cm) was cut out and placed into a scintillation vial. A 300-μl volume of H_2O and 3 ml of scintillation cocktail (Hydroluma; Baker Chemicals, Deventer, Netherlands) were added, and the samples were measured in a Packard Tri-Carb scintillation spectrometer.

Determination of murein precursors. *E. coli* W7 was grown in M9 medium containing [^{3}H]A_2pm (25 μCi/ml) for at least four generations, resulting in precursor labeling with a constant specific radioactivity. At an OD_{578} of about 0.3, the indicated antibiotic was added (D-cycloserine, 50 μg/ml [Serva, Heidelberg, Federal Republic of Germany]; moenomycin, 500 μg/ml [Hoechst AG, Frankfurt/Main, Federal Republic of Germany]; penicillin G, 641 μg/ml equal to 1,000 IU/ml [Serva]). OD readings were done with an identical monitor culture lacking radioactivity. Culture samples (1 ml) were withdrawn at the indicated time points for the analysis of murein precursors by a method described previously (14). Briefly, the cells were extracted with ice-cold *n*-butanol–6 M pyridinium acetate (pH 4) (4:1) by shaking with glass beads (0.17-mm diameter) in a vibrator for 30 min at 4°C. UDP-MurNAc-pentapeptide in the aqueous phase was separated by reversed-phase HPLC on a 5-μm Hypersil ODS column. The column was eluted at 35°C at a flow rate of 1 ml/min with 50 mM NaOH adjusted to pH 4.0 with phosphoric acid. The lipid precursors in the organic extraction phase were hydrolyzed with 0.1 M HCl for 15 min in a boiling-water bath to yield MurNAc-pentapeptide and GlcNAc-β-1,4-MurNAc-pentapeptide, respectively. Before HPLC separation on Hypersil ODS with 50 mM NaOH adjusted to pH 4.5 with phosphoric acid, the muramyl residues were reduced to the muramitol derivatives with sodium borohydride (7).

Analysis of the muropeptide composition. *E. coli* W7 was grown, labeled (for five generations), and treated as described above. At the indicated time points, culture samples (2 ml) were added to 8 ml of boiling 5% sodium dodecyl sulfate (SDS) solution with vigorous stirring. The suspension was kept boiling for 30 min and finally allowed to cool to room temperature. The murein was collected on membrane filters (24-mm diameter, 0.22-μm pore size; Millipore, Molsheim, France). α-Amylase digestion (100 μg/ml for 2 h at 37°C) and pronase digestion (100 μg/ml for 1 h at 60°C) of the murein was done directly on the membrane filters in 0.01 M Tris-HCl buffer (pH 7.4) containing 0.01 M NaCl. After extensive washing of the filter-bound murein sacculi, the material was digested at 37°C overnight in the presence of muramidase (20 μg/ml) isolated from *Streptomyces coelicolor* (Cellosyl; kindly provided by Hoechst AG) in 0.02 M sodium phosphate buffer (pH 4.8) containing 0.02% NaN_3. More than 98% of the radioactivity was solubilized in this manner. HPLC separation of the muropeptides, which were reduced with sodium borohydride prior to column application, was done at 55°C on a 3-μm Hypersil ODS column with a linear gradient from 50 mM sodium phosphate (pH 4.31) to 75 mM sodium phosphate (pH 4.95) as described in detail by Glauner et al. (7).

Analysis of the length distribution of the glycan strands. To label the glycan strands of the murein, *E. coli* W7 was grown in M9 medium containing *N*-acetyl-D-[1-^{3}H]glucosamine (50 μCi/ml; 5.6 Ci/mmol; Amersham, Braunschweig, Federal Republic of Germany) for six generations. Antibiotic-induced lysis of the cultures was done as described above. At the indicated time points, culture samples (1.5 ml) were added to 1.5 ml of boiling 8% SDS. Boiling was continued for 45 min. To minimize losses during the preparation of sacculi, unlabeled "carrier sacculi" (150 μg of A_2pm) of *E. coli* W7 (grown in LB medium at 37°C to an OD_{578} of 0.75) were added to each sample. The α-amylase- and pronase-digested sacculi (8) were digested for 12 h at 37°C in 50 mM Tris-HCl buffer (pH 7.9) containing 5 mM $MgCl_2$ and 0.02% NaN_3 with human serum amidase (8.5×10^{-3} U/ml), and the released glycan strands were fractionated by HPLC on a 5-μm Nucleosil 300 ODS column. Elution was done at a flow rate of 0.5 ml/min at 50°C with a convex gradient of 5 to 11% acetonitrile in 100 mM sodium phosphate (pH 2.0) as described previously (8).

RESULTS

Inhibition of murein synthesis and induction of lysis. To correlate the changes observed in both murein precursor concentrations and murein chemistry with the primary effect of murein synthesis inhibitors, the rate of [^{3}H]A_2pm incorporation into high-molecular-weight murein was determined (Fig. 1). The incorporation of radioactivity in the control cultures was not exactly proportional to the cell concentration. This result was due to a growth-dependent decrease in the low amount of unlabeled A_2pm (2 μg/ml) added to allow high incorporation of label. The growth rate of the A_2pm-auxotrophic *E. coli* W7, however, remained undisturbed. When the drug was added, the inhibitory effect on murein synthesis could be detected almost instantly. The fastest response was seen with D-cycloserine (50 μg/ml), which inhibited the rate of A_2pm incorporation by about 86% at 8 min after the addition, whereas only about 55% inhibition was found with penicillin (641 μg/ml) at the same time point. The lag between the addition of the antibiotic and the onset of lysis of the culture correlated with the effect on murein synthesis. Lysis occurred by 15 min after the addition of D-cycloserine and 25 min after the addition of penicillin or penicillin together with moenomycin, but not before 50 min when moenomycin (500 μg/ml) was added alone.

Intracellular concentration of murein precursors. Murein precursors were labeled by growth of the A_2pm-auxotrophic strain *E. coli* W7 in the presence of [^{3}H]A_2pm as described

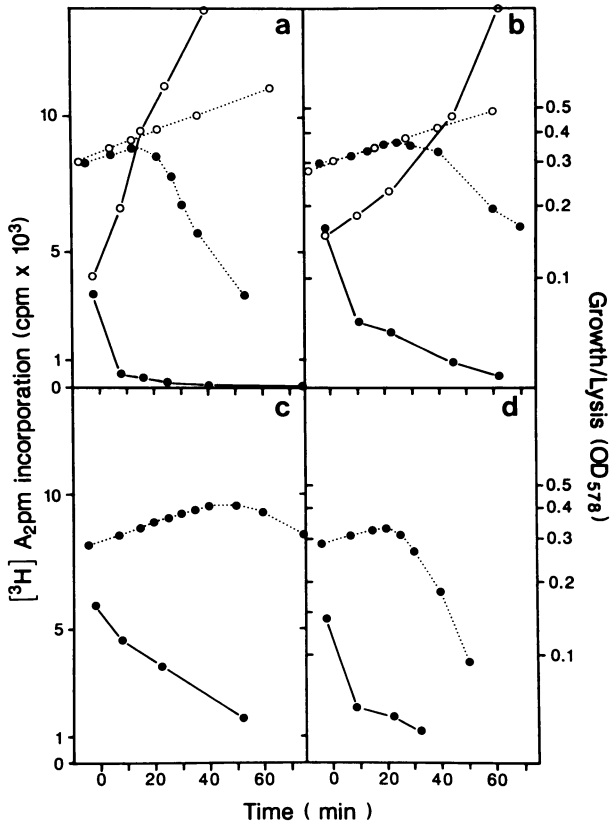

FIG. 1. Inhibition of murein synthesis. The rate of incorporation of [^{3}H]A_2pm into high-molecular-weight murein was determined in the presence of D-cycloserine (50 μg/ml) (a), penicillin (641 μg/ml) (b), moenomycin (500 μg/ml) (c), or penicillin (641 μg/ml) and moenomycin (500 μg/ml) (d). The antibiotics were added at time point 0 min. Culture samples (1 ml) were added to 10 μCi of [^{3}H]A_2pm and incubated for 3 min at room temperature. The radioactivity remaining at the origin after descending paper chromatography of the samples was determined as described in Materials and Methods. Symbols: ○, control culture; ●, antibiotic-treated culture; dotted line, OD_{578}; solid line, incorporation of [^{3}H]A_2pm in 3 min at room temperature by 1 ml of culture sample.

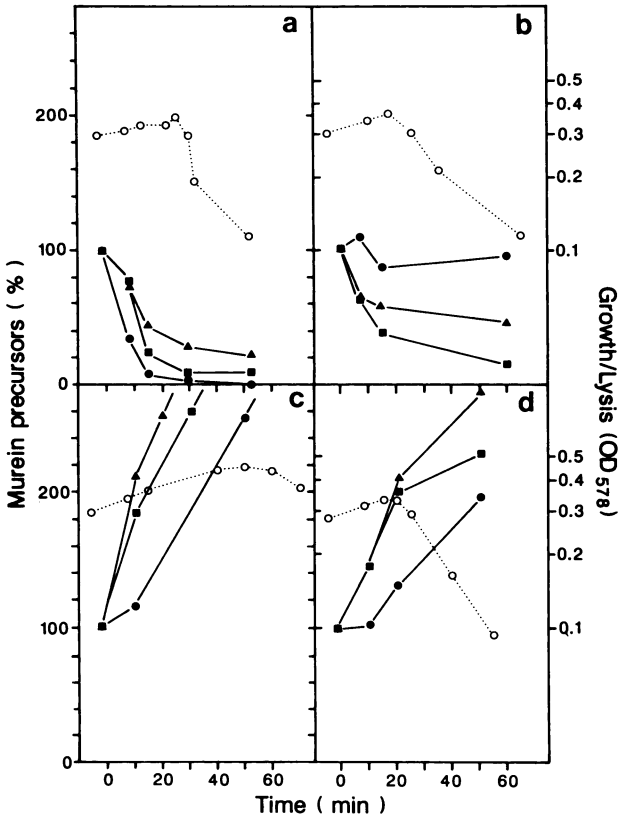

FIG. 2. Changes in the concentration of murein precursors during antibiotic-induced lysis. UDP-MurNAc-pentapeptide (●), MurNAc-(pentapeptide)-pyrophosphoryl-undecaprenol (▲), and GlcNAc-β-1,4-MurNAc-(pentapeptide)-pyrophosphoryl-undecaprenol (■) were determined in the aqueous and organic phases after extraction of 1-ml culture samples with *n*-butanol–pyridinium acetate. Separation of the precursors was done by reversed-phase HPLC on a Hypersil ODS column as described previously (10). At time point 0 min, either D-cycloserine (50 μg/ml) (a), penicillin (641 μg/ml) (b), moenomycin (500 μg/ml) (c), or penicillin (641 μg/ml) and moenomycin (500 μg/ml) (d) were added. The dotted line represents the OD_{578} of the culture.

in Materials and Methods. The soluble murein precursor UDP-MurNAc-pentapeptide and the lipid-linked precursors MurNAc-(pentapeptide)-pyrophosphoryl-undecaprenol (lipid I) as well as GlcNAc-β-1,4-MurNAc-(pentapeptide)-pyrophosphoryl-undecaprenol (lipid II) were determined by reversed-phase HPLC. Intensive shaking of the culture samples with *n*-butanol–pyridinium acetate extracted the bactoprenol precursors into the lipid phase and UDP-MurNAc-pentapeptide into the aqueous phase. The lipid-linked precursors were hydrolyzed with 0.1 N HCl to yield MurNAc-pentapeptide and GlcNAc-β-1,4-MurNAc-pentapeptide, respectively, prior to HPLC fractionation as described previously (14). Samples were withdrawn from the culture at the indicated time points before and during antibiotic-induced bacteriolysis.

Inhibition of murein synthesis by D-cycloserine (50 μg/ml) caused an immediate dramatic decrease in the concentration of UDP-MurNAc-pentapeptide after the addition of the antibiotic. Similarly, although at a lower rate, both lipid-linked precursors decreased, lipid II faster than lipid I (Fig. 2a). A different change in the intracellular concentrations of the precursors was observed during moenomycin-induced lysis (500 μg/ml). All three precursors analyzed accumulated with increasing OD after the addition of the antibiotic until the culture started to lyse about 50 min later (Fig. 2c). In the case of penicillin G-induced lysis (641 μg/ml), the intracellular concentration of UDP-MurNAc-pentapeptide remained unchanged from the time of penicillin addition (Fig. 2b). In contrast, however, the concentration of both lipid-linked precursors started to drop immediately after penicillin addition, and, thus, clearly before lysis occurred. Lipid I was decreased to a smaller extent than lipid II. The rapid drop of the lipid precursors during penicillin-induced lysis was not observed when penicillin was added together with moenomycin, as shown in Fig. 2d. The changes in the intracellular murein precursor concentrations followed those of moenomycin-induced lysis, although the lag between the addition of the drugs and the commencement of lysis was greatly reduced to 20 min.

Changes in muropeptide composition. Lysis induced by inhibition of murein synthesis is due to the activity of murein hydrolases (25). Although the participation of lytic transgly-

cosylases and endopeptidases has been proposed (13), clear-cut results identifying the enzyme(s) involved and its sequence of action during the lysis process have not yet been presented for *E. coli*. Therefore, detailed analyses were performed of the muropeptide composition of the murein that should reflect the action of specific murein hydrolases.

At different time points (i.e., immediately before the addition of the antibiotic, halfway between addition of the drug and onset of lysis, and when the drop in OD of the culture had reached 75% of the value before onset of lysis) during antibiotic-induced lysis of *E. coli* W7 growing in the presence of [^{3}H]A_2pm, samples were taken and the murein was isolated. Muropeptide analysis was performed after complete muramidase (Cellosyl) digestion and reversed-phase HPLC by the methods of Glauner (5).

Figure 3 summarizes the results obtained for the determination of the muropeptide composition. Most of the changes detected were observed for all three lysis processes, although they were less pronounced in the case of moenomycin, which showed the lowest lysis rate (Fig. 1c and 2c). The prominent increase in cross-linkage via the unusual DL-A_2pm-A_2pm cross-bridge, especially of the bis(disaccharide) dimer consisting of a tetrapeptidyl side chain cross-linked to a tripeptidyl side chain (Fig. 3, tetra-tri-A_2pm), was most dramatic in the case of D-cycloserine-induced lysis (about 7-fold) as compared with penicillin (about 4-fold)- and moenomycin (about 2.5-fold)-induced lysis. The amount of pentapeptide (L-Ala-D-Glu-*m*-A_2pm-D-Ala-D-Ala) carrying disaccharide monomers was decreased in all three cases. The increase in tripeptide (L-Ala-D-Glu-*m*-A_2pm) monomers was by far higher during moenomycin-induced lysis. Another distinct change was an increase in the amount of chain ends, that is, the 1,6-anydro muramic acid-containing anhydro-muropeptides (10), indicating a shortening in the average chain length of the glycan strands. Interestingly, the rise in dianhydro-muropeptides was much higher than the rise in monoanhydro-muropeptides. The changes in anhydro compounds during moenomycin-induced lysis were not significant.

Determination of the length distribution of the murein glycan strands. Recently, we established an HPLC-based method that allows the determination of the different lengths of the glycan strands in murein (8), an important parameter of murein structure that could not be analyzed until now. After amidase digestion of the isolated ^{3}H-amino sugar-labeled murein, the released polysaccharide chains were fractionated according to their degree of polymerization by reversed-phase HPLC on a wide-pore Hypersil ODS column. By this method, only the glycans between 1 and 30 disaccharide units long can be separated, making up about 50 to 60% of the total murein. It turned out that lysis did not interfere with the length distribution in these size classes with one important exception, namely, a pronounced increase in the relative number of the shortest glycan strand observed, the disaccharides. The increase in disaccharides was about fourfold when lysis was due to the action of moenomycin (Fig. 4a) and about sixfold in the case of penicillin-induced lysis (Fig. 4b).

DISCUSSION

That inhibition of murein synthesis generally results in bacteriolysis independent of the specific site of interaction of the inhibitory compound with the multistep biosynthetic pathway suggests the existence of a common molecular event that finally derepresses the autolytic system of the cell. It has been speculated that changes in the relative amounts of the murein precursors, for example, in the proportion of bactoprenol-linked precursors to free bactoprenol, are such a general effect caused by murein synthesis inhibition (28, 29). Here we show that changes in the levels of the murein precursors indeed occur when murein synthesis is inhibited. However, the changes are quite different not only quantitatively but also qualitatively for the different antibiotics tested and therefore cannot represent the postulated common lysis-triggering signal.

For penicillin-induced lysis, an unexpected decrease in the lipid-linked precursors was detected rather than the increase one would expect when further metabolism was blocked, such as in the case of moenomycin. Either the translocation of the soluble UDP-linked precursors onto the lipid carrier is inhibited by an unknown feedback control or the bactoprenol is not recycled properly by the formation of a kind of dead-end complex; for example, the bactoprenol may remain bound to the high-molecular-weight murein. Unfortunately, fractionation and quantification of bactoprenol is a difficult task and could not be performed. At the moment, we have no explanation for the decrease in the amount of lipid-linked precursors. This finding may prove to be important in understanding the mode of action of penicillin.

The changes observed in the muropeptide composition give no clear hint as to the mechanism of bacteriolysis. They may simply reflect the perturbation of murein synthesis and cellular attempts to overcome it. A typical change in the muropeptide composition that occurs during lysis is an increase in the amount of tripeptide moieties and a reduction of the amount in tetrapeptide and pentapeptide side chains. This is likely to be the result of an uncontrolled action of an DL-carboxypeptidase known to reside in the periplasmic space (3) and the membrane-bound DD-carboxypeptidases (22). As a consequence of lysis, some regulatory control circuits that adjust the activity of these enzymes to the cellular needs become disturbed.

As seen already with other lysis processes such as phage-induced lysis (32) or lysis due to an overproduction of a lytic enzyme (4, 15), it was found that the cross-linkage via the unusual DL-A_2pm-A_2pm cross-bridge was increased. It appears as if the bacterium tries to counteract the destruction of the murein by increasing the degree of cross-linkage by a transpeptidation independent of the presence of pentapeptide moieties in the murein. These pentapeptides that normally function as energy donors for the transpeptidation reaction have a very short half-life (6) and are therefore present in mature murein in amounts insufficient to enable the observed compensation of the decrease in normal DD-A_2pm-Ala cross-linkage. The synthesis of DL-peptide bridges, a kind of salvage transpeptidation reaction induced in lysis-prone cells, is assumed to use a tetrapeptide side chain as the donor for the formation of a DL-peptide bond between two neighboring diaminopimelic acid residues with the release of D-Ala (9). The degree of total cross-linkage did not change significantly.

The average length of the glycan strands was reduced by almost 50% in the case of penicillin-induced lysis. Surprisingly, the length distribution of the glycan strands between 1 and 30 disaccharide units did not change during cellular lysis, with the only exception being a major increase in disaccharides. This increase, however, cannot account for the observed decrease in the average chain length as determined by muropeptide analysis. It seems, therefore, that minor changes, not evident from the length distribution, in many of the fractionated length classes of the glycan strands

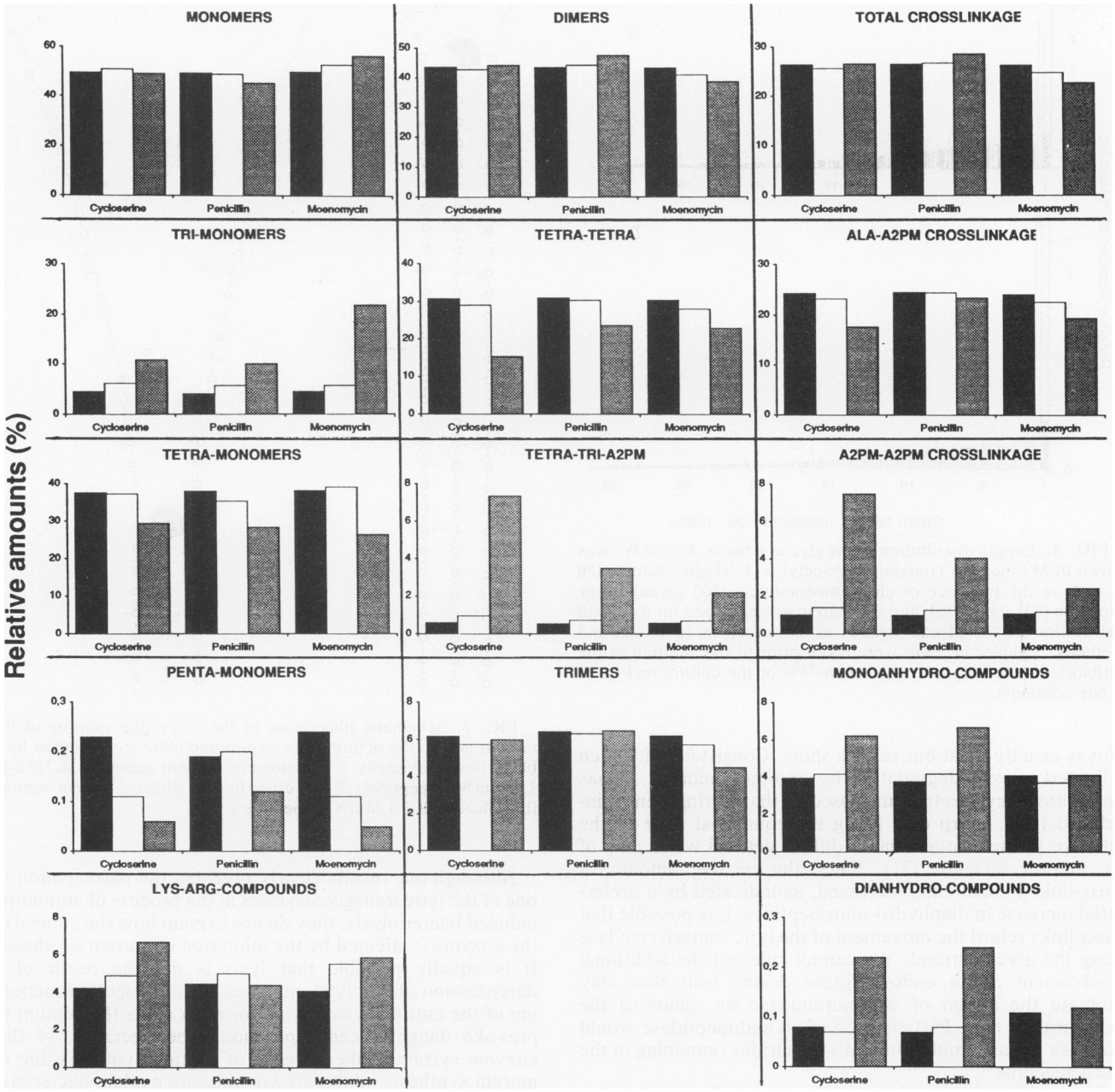

FIG. 3. Muropeptide analysis. *E. coli* W7 was labeled with [^{3}H]A_2pm (25 μCi/ml) in M9 minimal medium. Samples (2 ml) of cultures growing in the presence of D-cycloserine (50 μg/ml), moenomycin (500 μg/ml), or penicillin (641 μg/ml) were subjected to muropeptide analysis as described in Materials and Methods. Samples were taken prior to the addition of the antibiotic (black column), midway between the addition of the antibiotic and the onset of lysis (white column), and when 75% of the cultures had lysed (gray column). Abbreviations for muropeptides: monomer, GlcNAc-β-1,4-MurNAc-peptide; dimer, bis(GlcNAc-β-1,4-MurNAc-peptide); trimer, tris(GlcNAc-β-1,4-MurNAc-peptide). Abbreviations for stem peptides: tri, L-Ala-D-Glu-*m*-A_2pm; tetra, L-Ala-D-Glu-*m*-A_2pm-D-Ala; penta, L-Ala-D-Glu-*m*-A_2pm-D-Ala-D-Ala. Other abbreviations: Lys-Arg-compounds, muropeptides carrying L-Ala-D-Glu-*m*-A_2pm-L-Lys-L-Arg peptide moieties; anhydro-compounds, 1,6-anhydromuramic acid-containing muropeptides from the end of the glycan strands.

and changes in the longer glycans that cannot be fractionated add up to the overall decrease in average chain length.

The dramatic increase in the shortest glycan strands possible, the disaccharides, points to the action of an exo-muramidase, such as the lytic transglycosylases present in *E. coli* (2, 10, 18). Which one of the two enzymes is involved cannot be determined until proper mutants in these enzymes become available. As illustrated in Fig. 5, the exo-muramidase would progressively remove the disaccharides one by one, starting at one end of the glycan strands. Such a processive exo-disaccharidase mechanism has been demonstrated for a muramidase present in *Streptococcus faecium* (1). However, at the points of cross-linkage, the disaccharides would remain covalently bound to the murein. Such a zipperlike action of exo-muramidases would therefore cause an accumulation of disaccharides in the ruptured sacculus.

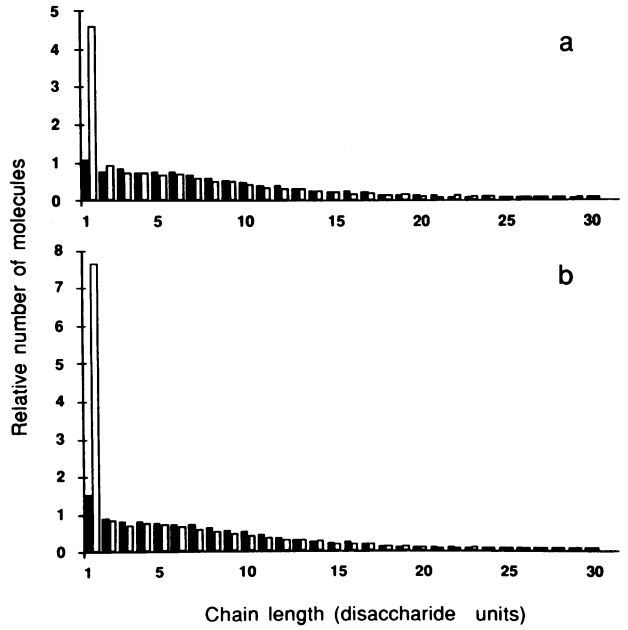

FIG. 4. Length distribution of the glycan strands. *E. coli* W7 was grown in M9 medium containing *N*-acetyl-D-[1-^{3}H]glucosamine (50 μCi/ml) in the presence of either moenomycin (500 μg/ml) (a) or penicillin (641 μg/ml) (b), and the murein was analyzed for its length distribution of the glycan strands as described in Materials and Methods. Samples (1.5 ml) were taken prior to the addition of the antibiotic (black columns) and when 75% of the culture had lysed (white columns).

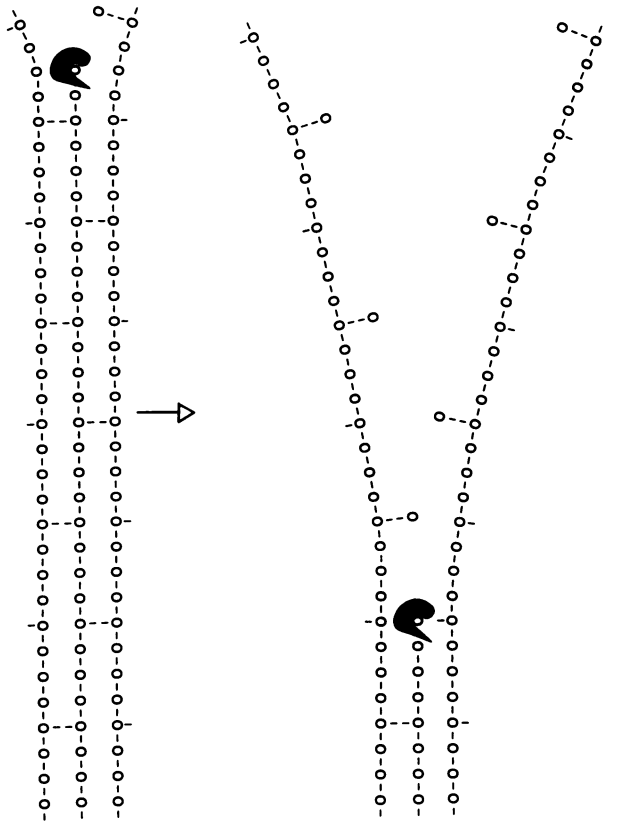

FIG. 5. Schematic illustration of the zipperlike splitting of the murein net by the action of an exo-muramidase (represented by a black form). Symbols: O, monomeric murein subunit (GlcNAc-β-1,4-MurNAc-peptide); O--O, cross-linked, dimeric murein subunit [bis(GlcNAc-β-1,4-MurNAc-peptide)].

This is exactly what our results show. Consistent with such a limited murein degradation by exo-muramidases, it has been shown by electron microscopy that during penicillin-induced lysis, sharp cuts along the equatorial zone of the cells are formed rather than a diffuse general weakening of the murein sacculus (21). Since the relative amount of cross-linked chain ends increased, as indicated by a preferential increase in dianhydro-muropeptides, it is possible that cross-links retard the movement of the lytic transglycosylase along the glycan strand. We cannot rule out the additional involvement of an endopeptidase during lysis that may facilitate the action of the muramidase by removing the cross-bridges (13). Participation of an endopeptidase would decrease the accumulation of disaccharides remaining in the lysed sacculus.

Some of the changes in murein structure observed during antibiotic-induced lysis were expressed to a minor extent only, or even not at all, in the case of moenomycin-induced lysis. A major difference between the mode of action of moenomycin on the one hand and that of penicillin G and D-cycloserine on the other was the retarded inhibitory effect of moenomycin on the rate of *m*-diaminopimelic acid incorporation into murein and the lower rate of lysis under the given experimental conditions. This somewhat slower manifestation of the inhibition of murein synthesis by moenomycin may explain the observed differences in some of the changes of the murein structure, which are likely to be irrelevant for the lytic response. Only the degree of increase in disaccharides was comparable to the penicillin-induced lysis process. This is consistent with our conclusion that this accumulation of disaccharides reflects the action of the cutting mechanism by a lytic exo-muramidase that results in bacteriolysis, the final outcome of all three antibiotics tested.

Although our results clearly revealed the participation of one of the lytic transglycosylases in the process of antibiotic-induced bacteriolysis, they do not explain how the control of the enzyme is affected by the inhibition of murein synthesis. It is equally possible that lysis is not the result of a derepression of autolytic enzymes but of a regular functioning of the cutting system that normally splits the septum to provoke daughter cell separation. The operation of this enzyme system in the absence of septum synthesis due to murein synthesis inhibition would easily explain bacteriolysis. It seems that the cell has no control mechanism that could curtail the splitting system when murein synthesis is blocked by antibiotics. Only in the case of a more natural inhibition of murein synthesis, that is, after amino acid starvation, is the cell able to control the autolytic system by means of the stringent response (4, 16). The bacteria are even tolerant to the action of penicillin under such conditions (16, 30). Controllable expression systems and suitable mutants in both lytic transglycosylases present in *E. coli* (18) are needed to study the endogenous regulatory mechanisms of the autolytic system of *E. coli*. Construction of such mutants is in progress, with a deletion mutant in the soluble lytic transglycosylase (*slt*) already available (23).

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