

Interference with Murein Turnover Has No Effect on Growth but Reduces β -Lactamase Induction in *Escherichia coli*

ANGELIKA R. KRAFT, JULIA PRABHU, ASTRID URSINUS, AND JOACHIM-VOLKER HÖLTJE*

Abteilung Biochemie, Max-Planck-Institut für Entwicklungsbiologie, 72076 Tübingen, Germany

Received 19 July 1999/Accepted 21 September 1999

Physiological studies of a mutant of *Escherichia coli* lacking the three lytic transglycosylases Slt70, MltA, and MltB revealed that interference with murein turnover can prevent AmpC β -lactamase induction. The triple mutant, although growing normally, shows a dramatically reduced rate of murein turnover. Despite the reduction in the formation of low-molecular-weight murein turnover products, neither the rate of murein synthesis nor the amount of murein per cell was increased. This might be explained by assuming that during growth in the absence of the major lytic transglycosylases native murein strands are excised by the action of endopeptidases and directly reused without further breakdown to muropeptides. The reduced rate of murein turnover could be correlated with lowered cefoxitin-induced expression of β -lactamase, present on a plasmid carrying the *ampC* and *ampR* genes from *Enterobacter cloacae*. Overproduction of MltB stimulated β -lactamase induction, whereas specific inhibition of Slt70 by bulgecin repressed *ampC* expression. Thus, specific inhibitors of lytic transglycosylases can increase the potency of penicillins and cephalosporins against bacteria inducing AmpC-like β -lactamases.

Growth of most bacteria depends on the enlargement and division of the exoskeleton, the murein sacculus that completely encloses the cell (33, 46). Insertion of new building blocks into the sacculus, a latticework made of glycan strands and peptides, is believed to require a concerted action of murein hydrolases and synthases (3, 16, 46). Hydrolases must cleave covalent bonds in the murein netting to allow additional murein subunits to be stitched in by synthases (39). Importantly, cleavage of bonds in the stress-bearing sacculus has to be controlled tightly in order to avoid the risk of bacteriolysis (38, 39, 43, 46).

In most gram-positive rods, as well as in gram-negative bacteria, growth is accompanied by a massive release of soluble muropeptides from the sacculus (8, 11, 12, 31). Such a turnover of murein takes place even during growth of the mostly single-layered murein of *Escherichia coli*. Its rate of about 40 to 50% per generation is comparable with that of gram-positive bacteria. The muropeptides released are marked by a 1,6-anhydro ring structure of the terminal muramic acid due to the action of lytic transglycosylases (LTs) (9, 18, 31). In contrast to the loss of the products to the medium in the case of gram-positive bacteria, the presence of an outer membrane in gram-negative bacteria leads to an accumulation of the turnover products in the periplasm (9, 11).

The 1,6-anhydro muropeptides that are retained in the periplasmic space are quite efficiently recycled for murein biosynthesis after being taken back into the cytoplasm (9, 10, 31, 34). Most of the muropeptides are imported into the cell by the AmpG transporter (27). To a minor extent, turnover products are cleaved in the periplasm by the amidase AmiA into the peptide and sugar parts (44). The released peptides are transported into the cytoplasm by the general oligopeptide permease Opp and by a specific murein peptide permease, Mpp (10, 35). The imported muropeptides are degraded by the cytoplasmic AmpD amidase that is specific for 1,6-anhydro turn-

over products (17, 21). The disaccharide can be cleaved by the β -glucosaminidase NagZ, but nothing is known about the fate of the released 1,6-anhydromuramic acid (12, 34). The peptides are directly linked to UDP-*N*-acetylmuramic acid (UDP-MurNAc) through the action of the specific ligase Mpl, giving rise to the formation of UDP-MurNAc-peptide intermediates (29), which are used for the synthesis of murein precursors. Recently it was shown that the presence of the L,D-carboxypeptidase LdcA prevents the formation of precursors with tetrapeptide substitutions (42). LdcA turned out to be an essential enzyme, since increased incorporation of disaccharide tetrapeptide subunits into the murein sacculus affects the overall cross-linkage, finally leading to autolysis.

The multilayered peptidoglycan of gram-positive bacteria has been shown to grow by an inside-to-outside mechanism (23). According to this strategy, new murein material is hooked underneath the stress-bearing layers before it is gradually forced to take over more and more of the stress due to the hydrolytic dissolution of the outermost layers of the murein shell by the action of murein hydrolases. Murein turnover is thus a consequence of the inside-to-outside growth strategy. Murein turnover in gram-negative bacteria may likewise be the result of the growth mechanism used. A hypothesis, called the three-for-one model (15, 16), claims that in *E. coli* three newly synthesized cross-linked murein strands (see Fig. 5) are first hooked underneath a given strand in the sacculus, called the docking strand, and become inserted into the stress-bearing layer upon the hydrolytic breakdown of the docking strand. The rate of murein turnover determined for *E. coli* is consistent with a replacement of every second murein strand by three new ones per generation (16).

The meaning of murein turnover in *E. coli*, that is, the release of muropeptides from the sacculus, is still a matter of debate. One suggestion is that turnover is the result of the growth strategy used. Indeed, murein turnover is an inherent feature of the three-for-one growth model (16). Another hypothesis claims that turnover represents a signalling device that senses the actual state of the cell-protecting exoskeleton (32, 34). Indeed, murein turnover products have been shown to function as signals for the induction of β -lactamases (19–21).

* Corresponding author. Mailing address: Max-Planck-Institut für Entwicklungsbiologie, Abteilung Biochemie, Spemannstraße 35, 72076 Tübingen, Germany. Phone: 49-7071-601-412. Fax: 49-7071-601-447. E-mail: joachim-volker.hoeltje@tuebingen.mpg.de.

Here it is shown that murein turnover in a mutant lacking three of the major murein hydrolases, the LTs Slt70, MltA, and MltB, is reduced by about 70% compared to that in the isogenic wild-type strain without affecting growth. Although the rate of murein synthesis in the mutant remains unchanged, the amount of murein per cell is not significantly increased, as would be expected. This apparent contradiction may be explained by assuming that by the action of endopeptidases native murein strands are released and recycled without becoming part of the soluble turnover pool. Consequently, the inducibility of the cloned AmpC β -lactamase from *Enterobacter cloacae*, known to depend on soluble muropeptides, was greatly diminished in mutants lacking one or more of the LTs.

MATERIALS AND METHODS

Strains, plasmids, and growth conditions. The LT deletion mutants were derived from *E. coli* MC1061 (4) and included MUF16 Δ slyY::kan (41), LT12 Δ mltA::cat, LT28 Δ slyY::kan Δ mltA::cat, LT60 Tet^r Δ mltA::cat Δ slyY::kan, MUF33 Δ mltB::Tn10, MUF45 Δ mltA::cat Δ mltB::Tn10, MUF49 Δ slyY::kan Δ mltB::Tn10, and MUF61 Δ slyY::kan Δ mltA::cat Δ mltB::Tn10 (28). *E. coli* XL1-Blue (2) was used for plasmid amplification. The plasmids pAK1 (Amp^r) carrying *recA* (28), pBP131 (sulfamethoxazol resistant) carrying *ampC-ampR* (25), and pWSK129 (Kan^r) (45) have been described previously. The strains were grown aerobically in Luria broth (LB) supplemented with the required antibiotics in a shaking water bath at 37 or 30°C as indicated. Growth was monitored by optical density readings at 578 nm (OD₅₇₈) in an Eppendorf (Hamburg, Germany) photometer.

Construction of inducible expression systems. DNA ligations, restriction endonuclease digestions, agarose gel electrophoresis, and transformations were performed according to standard techniques (37). Amplification of plasmids was done in *E. coli* XL1-Blue, and transformations were performed following the method described by Hanahan et al. (13).

In order to have *ampC-ampR* on an inducible expression vector with a suitable resistance marker, these genes present on plasmid pBP131 were transferred onto the vector pWSK129. From pBP131, a 3.5-kb fragment covering *ampC-ampR*, originally taken from *E. cloacae*, could be obtained by digestion with *SalI* and *BamHI*. The fragment was ligated into pWSK129 digested with *SalI* and *BamHI*, yielding pJP1, which, in addition to the *ampC-ampR* region, carries a kanamycin cassette.

Plasmid pAU1 carries the *mltB* gene under the control of the *tac* promoter. It was constructed by removing the kanamycin resistance cassette of pMK360 (28) and replacing it with the chloramphenicol resistance cassette from pBCSK⁺ (Stratagene, Heidelberg, Germany). For that purpose, pMK360 was digested with *NcoI* and *XhoI* and ligated with the Cam^r-containing *HhaI* fragment from pBCSK⁺ after the ends were filled in with the Klenow DNA polymerase I fragment.

Determination of murein turnover. Cells in the exponential growth phase (OD₅₇₈, 0.345) were labeled by growth in the presence of 37 kBq of *N*-acetyl-D-[1-³H]glucosamine (GlcNAc) (259 GBq/mmol) per ml in LB medium without antibiotics at 30°C. After 20 min, when incorporation of the radiochemical had almost reached its maximum, reincorporation of the released radiolabel was suppressed by diluting the cultures 11-fold with prewarmed LB medium containing unlabeled GlcNAc at a final concentration of 300 μ g/ml. At different time points, aliquots (0.5 ml) were withdrawn and added to 1 ml of boiling 8% sodium dodecyl sulfate (SDS). Samples were kept at 100°C for 30 min and filtered through 0.22- μ m-pore-size GS cellulose-nitrate-and-acetate filters (Millipore) (25-mm diameter). The filters were washed once with 3 ml of 0.1 M LiCl and three times with 3 ml of water. The radioactivity on the dried filters was measured in the presence of 1.5 ml of Ultima Gold (Canberra Packard) scintillation cocktail in a TriCarb1500 (Canberra Packard) scintillation counter.

Rates of murein synthesis and murein content of cells. The rates of murein synthesis of the wild type and triple mutant were compared by determining the incorporation of [³H]GlcNAc into murein during short periods of labeling, when the effect of turnover is negligible. Exponentially growing cells (OD₅₇₈, 0.350) were labeled with [³H]GlcNAc (37 kBq/ml; 10 μ g/ml) in LB medium without antibiotics at 30°C. The incorporated radioactivity in 0.5-ml aliquots taken after 5, 10, 15, 20, and 25 min was determined as described above. In contrast, the murein content of cells was determined by continuous labeling with [³H]GlcNAc (74 kBq/ml; 150 μ g/ml) for more than four generation times, starting at an OD₅₇₈ of 0.025. Again, the incorporation into 0.5-ml aliquots was determined as described above.

β -Lactamase induction. To induce β -lactamase, cells (5 ml) from the exponential growth phase were diluted 1:2 with growth medium containing cefoxitin at the indicated concentrations (between 0.8 and 4 μ g/ml) and incubated further at 37°C. Samples (1 ml) were removed at the indicated time points, and the cells were harvested by centrifugation (15 min; 12,000 \times g; 4°C), washed once with 0.05 M sodium phosphate buffer, pH 7.0, and resuspended in 1 ml of the same buffer. The cells (250 μ l) were added to 100 μ l of glass beads (0.17- to 0.18-mm

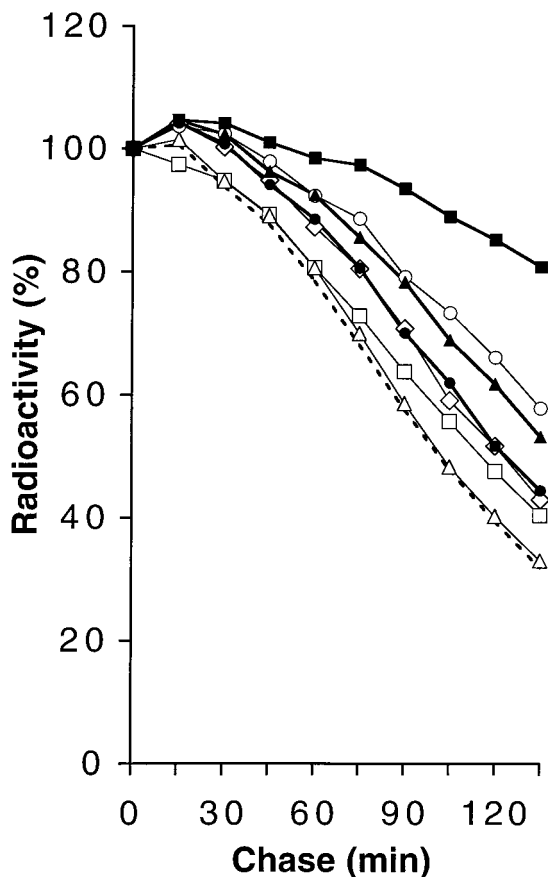


FIG. 1. Murein turnover in mutants with deletions in LTs. Cells were pulse labeled with [³H]GlcNAc, and the loss of radiolabel from SDS-insoluble murein during a chase was determined as described in Materials and Methods. The curves are averages of four independent experiments. The average standard deviation was less than 2%. Dotted line, MC1061 (wild type); open squares, LT12 (Δ MltA); open diamonds, MUF33 (Δ MltB); open triangles, MUF16 (Δ Slt70); solid circles, LT60 (Δ Slt70 Δ MltA); solid triangles, MUF49 (Δ Slt70 Δ MltB); open circles, MUF45 (Δ MltA Δ MltB); solid squares, MUF61 (Δ Slt70 Δ MltA Δ MltB).

diameter) in a microcentrifuge tube. After the addition of DNase (10 μ g/ml), the tube was vigorously shaken on a vortex shaker in the cold room for 30 min. The glass beads were removed by a short centrifugation step, and the supernatant was centrifuged for 20 min at 100,000 \times g at 4°C. The protein concentration in the supernatant was determined by the method of Bradford (1), and the supernatant was used to measure β -lactamase activity.

β -Lactamase enzyme assay. As a substrate for β -lactamase, the chromogenic β -lactam nitrocefin (30) was used. Hydrolysis of nitrocefin was followed by recording the change in the absorbance at 492 nm in an Eppendorf photometer at room temperature. Enzyme assay mixtures consisted of 50 μ l of 1 mM nitrocefin, 3 to 5 μ l of enzyme sample (10 μ g of protein), and 0.05 M sodium phosphate buffer, pH 7.0, to produce a final volume of 1,000 μ l. Under these conditions, the reaction was linear for at least 5 min.

Agar diffusion tests. Agar plates were prepared from 20 ml of LB medium containing 0.7% agar and inoculated with 0.01 ml of an overnight culture at 40°C. Filter strips (0.5 cm wide) were soaked with solutions of the indicated concentrations of cefoxitin or bulgecin. The wet strips were applied to the surface of the agar plates at right angles to each other. Photographs were taken after overnight incubation of the plates at 37°C.

RESULTS

Murein turnover in the absence of Slt70, MltA, and MltB. During growth of *E. coli*, about 40% of the total material is released from the murein sacculus per generation, as can be measured by following the loss of radiolabeled diamino-pimelic acid (A₂pm) from the sacculus in mutants that are

TABLE 1. β -Lactamase induction and murein turnover in LT deletion mutants of *E. coli* harboring pJP1 (*amp^{C+} amp^{R+}*)

Strain (Δ LT)	β -Lactamase activity (pmol/min \times mg of protein) ^a			Murein turnover	
	Uninduced	Induced	Induction factor ^b	Muropeptides released (%) ^c	Decrease in turnover (%) ^d
MC1061 (wild type)	34	400	12	68	0
MUF16 (Δ Slt70)	ND	ND		67	2
LT12 (Δ MltA)	42	330	8	60	13
MUF33 (Δ MltB)	30	180	6	57	16
MUF45 (Δ MltA/MltB)	26	75	3	42	38
LT60 (Δ Slt70/MltA)	ND	ND		56	19
MUF49 (Δ Slt70/MltB)	ND	ND		47	31
MUF61 (Δ Slt70/MltA/MltB)	ND	ND		19	72

^a β -Lactamase activity was determined in crude cell extracts by following the hydrolysis of nitrocefin in a photometer at 492 nm as described in detail in Materials and Methods. The values are averages of four independent experiments. The average standard deviation was less than 2%. ND, not done.

^b Quotient of β -lactamase activity of uninduced and induced cells.

^c Decrease in radioactivity in SDS-insoluble material after growth for 135 min in the absence of label (chase). The cells had been prelabeled with [³H]GlcNAc as described in detail in Materials and Methods.

^d Relative to the turnover of the wild type (100%).

defective in the major recycling pathway via AmpG (34). The turnover products were shown to be mainly the monomeric anhydro-muropeptides GlcNAc- β -1,4-(1,6-anhydro)MurNAc-L-Ala-D-Glu-*m*-A₂pm-D-Ala and GlcNAc- β -1,4-(1,6-anhydro)MurNAc-L-Ala-D-Glu-*m*-A₂pm. Thus, besides endopeptidases, primarily LTs are involved in murein turnover (22, 24). It was therefore of interest to study murein turnover in mutants lacking several of the LTs. To study turnover in strains that do recycle the murein turnover products, we used a label that incorporates into the glycan strands rather than into the peptides, since it has been shown that the amino sugars are recycled at only approximately half the rate of the peptide moiety of murein (12).

E. coli strains containing single or multiple deletions in the LT Slt70, MltA, and/or MltB as well as the corresponding wild-type strain were pulse labeled with [³H]GlcNAc, and the loss of radiolabel from the murein was determined for a chase period of more than three generations (generation time, 42 to 44 min). To our surprise, although growing with the same generation time as the wild type, most of the mutants showed

substantially decreased turnover compared to the wild type (Fig. 1).

A small but significant decrease in murein turnover was observed for the MltA and the MltB deletions, which showed 13 and 16% less turnover than the wild type (Table 1). Deletions of two different LTs showed quite pronounced effects on murein turnover. The turnover in an Slt70-MltA mutant was decreased by 19%, that in an Slt70-MltB double mutant was decreased by 31%, and that in an MltA-MltB mutant was decreased by 38%. A triple mutant showed a reduction by 72% compared to the wild type.

Rate of murein synthesis and amount of murein in the triple mutant. The rate of murein synthesis in the triple mutant was analyzed to find out whether the absence of the murein hydrolases, which are believed to be fundamentally involved in murein synthesis by functioning as spacemaker and pacemaker enzymes, has an effect on murein synthesis. Steady-state incorporation was reached after about 15 min, as shown in Fig. 2. Under these conditions, the rates of incorporation of labeled GlcNAc into SDS-insoluble material was found to be un-

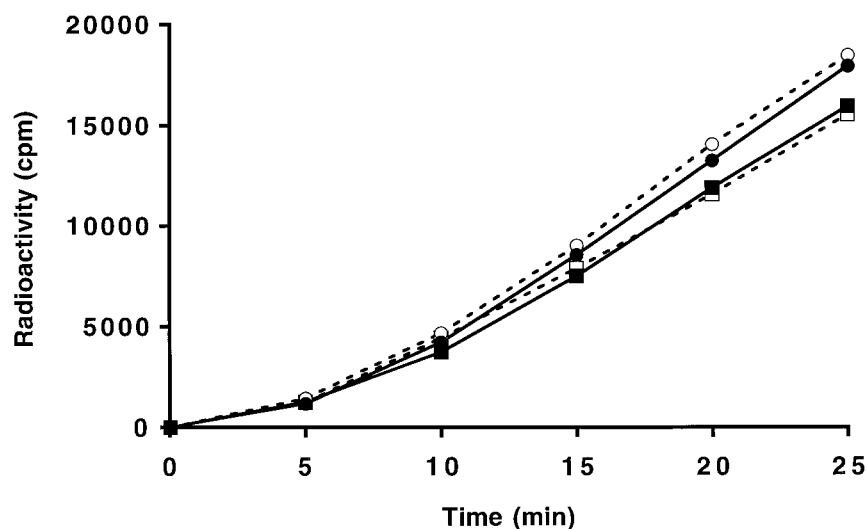


FIG. 2. Rates of murein synthesis in *E. coli* MUF61 and MC1061. The incorporation of [³H]GlcNAc into SDS-insoluble material was determined as described in Materials and Methods. Two independent experiments (circles and squares) are shown. The open symbols represent the wild-type *E. coli* MC1061, and the solid symbols represent the LT triple mutant MUF61.

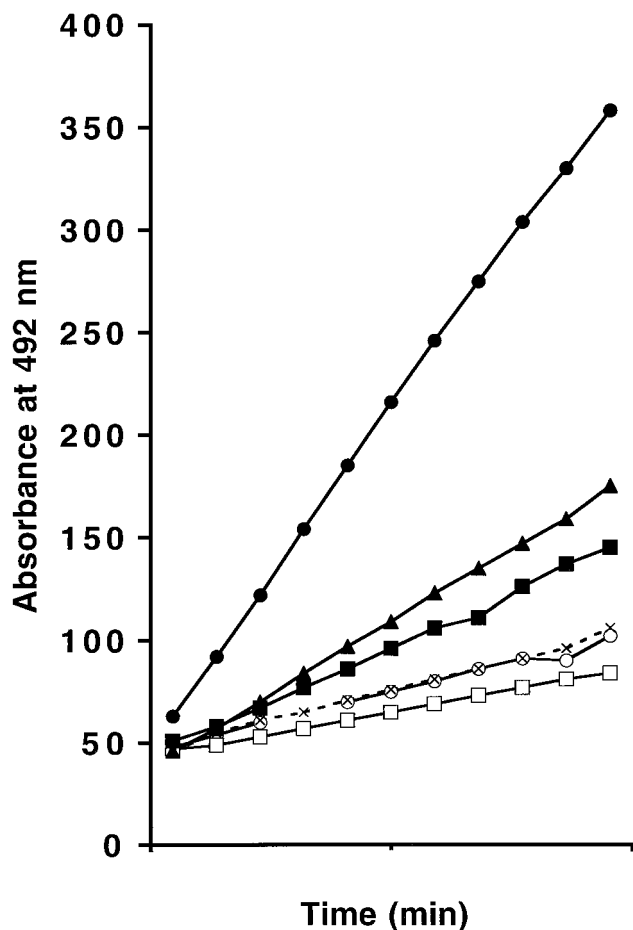


FIG. 3. Dependency of β -lactamase induction on the activity of LTs. Expression of β -lactamase from pJP1 (*ampC ampR*) by cefoxitin (4 μ g/ml) was determined in *E. coli* with different levels of active LTs. To vary the intracellular concentrations of active Slt70 and MltB, respectively, either the Slt70-specific inhibitor bulgecin was added (50 μ g/ml) or MltB was overproduced by induction of expression from plasmid pAU1 by the addition of IPTG (isopropyl- β -D-thiogalactopyranoside) (10 μ g/ml) as described in Materials and Methods. Hydrolysis of nitrocefin by crude cell extracts (10 μ g of protein) was measured as described in Materials and Methods. The change in absorbance at 492 nm is shown. Solid circles, IPTG and cefoxitin; solid triangles, cefoxitin; solid squares, IPTG; crosses, bulgecin and cefoxitin; open circles, control; open squares, bulgecin.

changed when the triple mutant was compared with the wild-type strain MC1061. The rate of incorporation of [3 H]GlcNAc (determined in two independent experiments) was 7,300 cpm/min \times 10 9 cells for MC1061 and 7,700 cpm/min \times 10 9 cells for MUF61/pAK1.

In order to determine the amount of murein per cell, the strains were continuously labeled with [3 H]GlcNAc (150 μ g/ml; 74 kBq/ml) for more than four generation times. At identical cell densities, about 8% more radiolabel was found in the SDS-insoluble material of cultures of the triple mutant MUF61 than in that of cultures of the wild type, reflecting a slightly higher murein content of mutant cells.

Effect of murein turnover on β -lactamase induction. Recently, it has been shown that murein turnover products accumulating in the cytoplasm can function as signals for the induction of AmpC-type β -lactamase (19–21). Therefore, it was interesting to study β -lactamase induction in mutants with a reduced rate of murein turnover. β -Lactamase activity in mutants lacking one or two of the LTs grown in the presence of

cefoxitin was dramatically reduced compared to that in the wild-type strain (Table 1). A calculation of induction factors shows that these values correspond nicely to the degree of reduction in murein turnover determined for these mutants. Whereas single deletions reduced the induction level by 25 to 50%, a double deletion in MltA and MltB resulted in only marginal β -lactamase induction.

Because of the kanamycin resistance cassette present in both the *sltY* deletion mutant and the plasmid pWSK129 carrying *ampC-ampR*, the effect of a mutation in Slt70 on β -lactamase induction could not be studied directly. However, since a specific inhibitor of Slt70, bulgecin, is known (41), we could follow lactamase induction in the presence of this antibiotic. Similar experiments with *E. cloacae* have recently been reported by

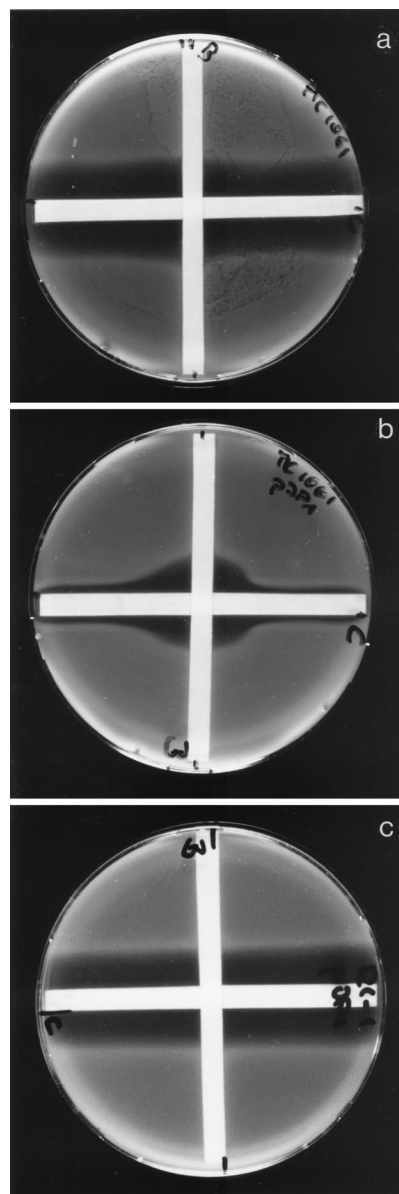


FIG. 4. Resensitization of β -lactamase-inducing *E. coli* by the Slt70 inhibitor bulgecin. An agar diffusion test with a combination of bulgecin (horizontal strip soaked in 500 μ g/ml) and cefoxitin (vertical strip soaked in 500 μ g/ml) is shown. (a) MC1061/pWSK129; (b) MC1061/pJP1 (*ampC ampR* $^+$); (c) 122-1 (*sltY* mutant)/pJP1 (*ampC ampR* $^+$).

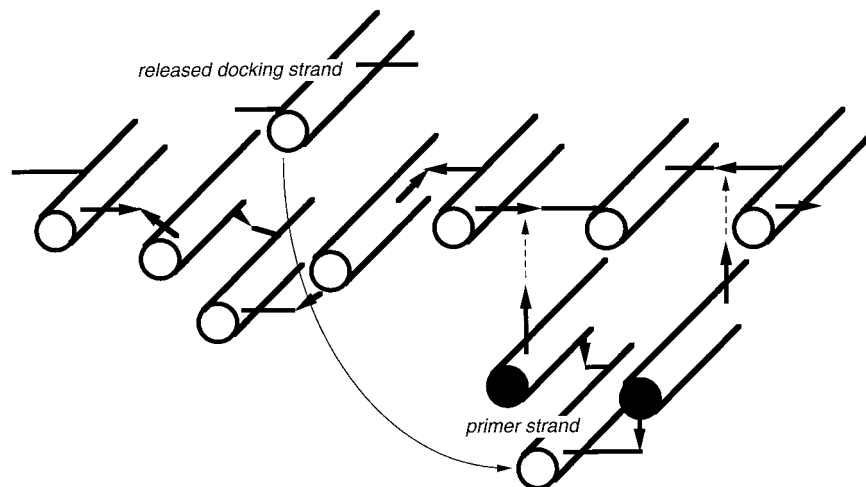


FIG. 5. Schematic representation of a hypothetical growth mechanism as it may occur in multiple-LT-deletion mutants. The murein glycan strands are indicated by rods, and the peptide cross bridges are indicated by bars (acceptor peptide) and arrows (donor peptide). It is proposed that a docking strand released by the action of endopeptidases during the insertion of a murein triplet precursor is directly reused as a central primer strand for the formation of a new murein triplet.

Pfeifle et al. (36). As was expected on the basis of the results with the LT mutant strains, cefoxitin-induced β -lactamase expression was significantly reduced in the presence of bulgecin (Fig. 3). On the other hand, overproduction of MltB from pAU1 greatly stimulated β -lactamase induction.

Suppression of β -lactam resistance due to β -lactamase induction. The observed dependency of β -lactamase induction on the presence of enzymatically active LTs prompted us to study the effect of specific inhibitors of LTs on β -lactamase induction. In order to prove the idea that LT inhibitors would increase the potency of β -lactams on strains carrying *ampC-ampR*, we performed classical agar diffusion tests with paper strips soaked with cefoxitin and the specific inhibitor of Slt70, bulgecin, respectively (Fig. 4). The two strips were applied at a right angle to each other to agar plates inoculated with *E. coli* MC1061 harboring pJP1 or, as a control, pWSK129. The effect of bulgecin on the potency of cefoxitin can be directly observed at the site where the two diffusion gradients cross each other. The zone of growth inhibition at the site where bulgecin acts together with cefoxitin has nearly the same width (21.9 cm) as the cefoxitin-specific inhibition zone (23.0 cm) observed with the wild-type strain. The combination of cefoxitin and bulgecin had no synergistic effect when tested with an Slt70 deletion mutant. The experiments nicely illustrate that the AmpC β -lactamase-inducing and therefore cefoxitin-resistant strain *E. coli* MC1061/pJP1 can be rendered sensitive to cefoxitin by the specific Slt70 inhibitor bulgecin.

DISCUSSION

The LTs Slt70, MltA, and MltB are shown here to be responsible for most of the murein turnover, when measured as the formation of low-molecular-weight murein degradation products. As expected, all three LTs that were investigated participate in this process. The residual turnover observed in the triple mutant is likely to be due to the presence of additional murein hydrolases. These include the LTs EmtA (26) and the recently identified MltC and MltD (6, 7) and the endopeptidases PBP4, PBP7, and MepA (14, 39), as well as the amidase AmiC (40). All of these enzymes are known to be capable of interacting with the growing murein sacculus.

A reduction in murein turnover without any change in the

rate of murein synthesis, as is observed in the LT triple mutant, must result in a thickening of the murein. However, this is not the case, except for a slight increase in the total amount of murein by about 8%. Yet there is a possibility that the turnover material escaped detection. If material is released but is reused without ever becoming part of the pool of low-molecular-weight turnover products, none of it would be diverted into other metabolic pathways or diluted by unlabeled GlcNAc during the chase. From the absence of release of radiolabel we would falsely conclude that turnover did not take place.

Because residual lytic transglycosylase activity in the LT triple mutant is negligible, release of turnover material might be restricted to endopeptidase activity. Hence, it is likely that undegraded, native murein strands are released from the murein net. Such murein strands may be directly reused for the synthesis of nascent murein.

According to the three-for-one growth mechanism model (16), the murein sacculus is enlarged by the replacement of murein strands present in the stress-bearing sacculus by three new strands cross-linked to one another, a murein triplet (Fig. 5). The mode of formation of the murein triplets may differ during septum formation and cell elongation, since it has been shown by pulse-chase labeling experiments that nascent strands are cross-linked to preexisting murein during elongation, whereas newly synthesized murein strands are inserted side by side and cross-linked to one another during cell division (5, 33). Therefore, the three strands of a triplet seem to be synthesized simultaneously during septum formation. By contrast, it is assumed that during cell elongation the murein triplets are synthesized by complementing a preexisting, so-called primer strand with two new strands, one added from the left and one added from the right side to the primer strand in the middle of the triple pack (Fig. 5). It is tempting to speculate that in the LT triple mutant, where probably mostly undegraded murein strands are released by the action of endopeptidases during growth, the cell takes advantage of the accumulation of these strands and uses them as primer strands for the synthesis of new triple packs. Such a direct recycling of released docking strands would explain the apparently drastically reduced murein turnover. It has to be pointed out that release of old murein very likely takes place at a normal rate in

the triple mutant but cannot be detected because the mechanism of turnover differs substantially from that of wild-type cells. The turnover material is probably represented by murein strands, which are formed and directly reused in the periplasm, rather than by muropeptides that are imported into the cytoplasm, as is the case in wild-type cells. The absence of the formation of low-molecular-weight turnover products, however, has another important implication, namely, for the induction of β -lactamases.

Quite ingeniously, murein turnover products can function as signalling compounds. Whether this is used to respond to general changes in the murein structure by adjusting the quantity and/or quality of the murein synthesized, as has been proposed (32), is not yet known. However, in the case of a disturbance of murein synthesis by β -lactam antibiotics, the cell indeed measures the intracellular concentration of certain murein turnover products to induce β -lactamase expression (19–21). Recently, it has been shown that the AmpR regulator specifically interacts with the 1,6-anhydro-muramyl-tripeptide in the periplasm (19).

In agreement with these reports, it is shown here that a reduction in the formation of low-molecular-weight murein turnover products and thereby in the formation of the β -lactamase-inducing compound(s) results in a severe block in inducible β -lactamase expression. This finding suggests a novel strategy to inhibit β -lactamase induction, which can be a major problem in β -lactam therapy. Inhibition of the LTs in *Enterobacteriaceae* may in certain cases increase the efficiency of penicillins. Indeed, we could show that a combination of the specific inhibitor of Slt70, bulgecin, with the β -lactam cefoxitin is a powerful strategy to lyse *E. coli*, even though the bacterium harbors a plasmid-encoded inducible β -lactamase.

ACKNOWLEDGMENTS

We thank Uli Schwarz for his support and interest and B. Wiedemann for supplying the pBP131 plasmid.

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