Membrane-Bound Lytic Endotransglycosylase in Escherichia coli

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The gene for a novel endotype membrane-bound lytic transglycosylase, emtA, was mapped at 26.7 min of the E. coli chromosome. EmtA is a lipoprotein with an apparent molecular mass of 22 kDa. Overexpression of the emtA gene did not result in bacteriolysis in vivo, but the enzyme was shown to hydrolyze glycan strands isolated from murein by amidase treatment. The formation of tetra- and hexasaccharides, but no disaccharides, reflects the endospecificity of the enzyme. The products are characterized by the presence of 1,6-anhydrodimeric acid, indicating a lytic transglycosylase reaction mechanism. EmtA may function as a formatting enzyme that trims the nascent murein strands produced by the murein synthesis machinery into proper sizes, or it may be involved in the formation of tightly controlled minor holes in the murein sacculus to facilitate the export of bulky compounds across the murein barrier.

Lysozymes are widely distributed among living organisms, including both pro- and eukaryotes (29). They cleave the β-1,4-glycosidic bond between N-acetylmuramic acid and N-acetyl-glucosamine in the murein (peptidoglycan) sacculus of bacteria. By degrading the sacculus, which serves as a bacterial exoskeleton (37, 43, 51), they cause bacteriolysis. Hence, lysozymes are among the most powerful antibacterial enzymes. Despite their potential bacteriolytic power, lysozymes and other murein hydrolizing enzymes, including glucosaminidases, amidases, and endopeptidases, are ubiquitous in bacteria themselves (43). This is due to the net-like structure of murein, which can only be enlarged by cleaving meshes to allow the insertion of new building blocks into the preexisting sacculus (23, 24, 43, 48, 51). Thus, murein hydrolases are likely to be involved in the enlargement and division of the sacculus during bacterial growth. In addition, murein hydrolases are believed to be needed for a localized opening of the murein net during the export of bulky compounds such as DNA, toxins, flagella, and fimbrial proteins (13).

The muramidases found in Escherichia coli differ from lysozymes in that they catalyze an intramolecular transglycosylation onto the C6 hydroxyl group following cleavage of the β-1,4-glycosidic bond. As a result of this reaction the products are marked by a 1,6-anhydrodimeric acid (22, 45). The meaning of this peculiar reaction mechanism is still not understood. In a way similar to the action of lysozyme, murein is completely degraded by these enzymes. They are therefore called lytic transglycosylases. Four different proteins with this activity have been identified in E. coli (12, 39). Three of them, MltA, MltB, and MltC, each with a mass of about 38 kDa, are lipoproteins residing in the outer membrane (12, 39). The fourth, Slt70, is a soluble enzyme with a mass of 70 kDa (6, 22). Although not a lipoprotein, Slt70 preferentially binds to the outer face of the murein sacculus (12, 39). Three of them, MltA, MltB, and MltC, each with a mass of about 38 kDa, are lipoproteins residing in the outer membrane (12, 39). The fourth, Slt70, is a soluble enzyme with a mass of 70 kDa (6, 22). Although not a lipoprotein, Slt70 preferentially binds to the outer face of the murein sacculus (12, 39). Hence, a specific topological localization of these enzymes in the cell membrane is realized.

Unlike lysozyme, these lytic transglycosylases are exoglycosylases, which by starting at one end processively degrade murein strands releasing anhydrodisaccharide units. Unfortunately, it is not clear at which end these enzymes start. The crystal structure of the Slt70 molecule suggests that binding occurs at the 1,6-anhydrodimeric acid end (47), whereas biochemical data show that the enzymes start at the nonreducing end, i.e., the N-acetylglucosamine terminus of the polysaccharide (4, 39), and release their products in the form of anhydromuropeptides. If true, the latter reaction mechanism raises a question: where are the anhydro groups present in the murein sacculus (18, 24) coming from?

Here we show that a lytic endotransglycosylase exists in E. coli that, by cleaving glycosidic bonds within the murein strands, produces shorter strands with 1,6-anhydrodimeric acid ends in the murein sacculus.

MATERIALS AND METHODS

Bacterial strains, bacteriophages, and plasmids. E. coli MC1061 (10) and the mltA mutant LT12 (mltA::crl) (32) were used as indicated below. The general cloning host was E. coli XLI-1-Blue (Stratagene, La Jolla, Calif.). Phage X245 (7C10) from the Kohara phage library (30) was used as a DNA source for cloning the gene of interest. In order to construct an inducible expression system, the gene was cloned into plasmid pMK118EH (7).

Media and growth conditions. The bacteria were cultivated aerobically at 30°C in Luria-Bertani (LB) medium (34). The medium was supplemented when required with antibiotics at the following concentrations (per milliliter): kanamycin, 50 µg; tetracycline, 12.5 µg; and chloramphenicol, 20 µg. For strains carrying pAKS, overnight cultures and plates were supplemented with 0.2% glucose and 10 mM MgCl2.

DNA manipulations and PCR. Standard techniques were used for manipulating the plasmid DNA (41), and E. coli was transformed as described by Inoue et al. (12). Restriction endonucleases were purchased from Boehringer (Mannheim, Germany), shrimp alkaline phosphatase was obtained from U.S. Biochemicals (Cleveland, Ohio), and the oligonucleotides came from Pharmacia (Uppsala, Sweden) or MWG-Biotech (Ebersberg, Germany).

PCR (40) was performed in a volume of 50 µl of 20 mM Tris·HCl (pH 8.8) containing 2 mM MgSO4, 10 mM KCl, 10 mM (NH4)2SO4, 0.1% Triton X-100, 100 µg of bovine serum albumin per ml, 400 µM deoxynucleoside triphosphates, and 1.25 U of Pfu polymerase (Stratagene) with 0.5 µM concentrations of each primer and 2 µl of Kohara lambda phage 245 lysate (kindly provided by Guido Schiffer) as a template. After initial denaturation for 5 min at 92°C, touchdown PCR (14) was performed with 0.5 min of annealing, 1 min of extension at 72°C, and 0.5 min of denaturation at 92°C. The annealing temperatures were 65, 63, 61, and 59°C for 3 cycles each and 55°C for another 15 cycles.

Construction of an inducible expression system. The gene encoding the lytic endotransglycosylase was cloned into the plasmid pFK118EH (7) to get an inducible expression system under the control of the tac promoter. The coding region was amplified from Kohara phage X245 DNA by touchdown PCR by using the primers 5'-TAAAGAATCTCAAGAAATGAAATTGAGATGTTGCC-3' and 5'-GAAGTGACGCGCCGAGGTATCACTG-3', introducing an EcoRI restriction site and a SalI restriction site at the ends. The first primer changed the start codon of the amplified coding sequence from the chromosomally encoded GTO to ATG. The amplified product was purified and digested with EcoRI and SalI and ligated into pFK118EH cut with these enzymes and dephosphorylated with shrimp alkaline phosphatase. The resulting construct.

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pAK5, was amplified in E. coli XL1-Blue and transformed into E. coli LT12 for biochemical characterization of the protein overproduced upon induction with 1 mM isopropyl-β-D-thiogalactopyranoside (IPTG).

Preparation of membrane extracts. To prepare membrane extracts, cells carrying either pPFK18EH or pAK5 were grown to an optical density at 578 nm (OD$_{578}$) of 0.2 as described above. The expression of the cloned gene was induced by the addition of 1 mM IPTG, and the cells were cultivated for an additional 4 h. Cells from a 25-ml culture were harvested by centrifugation, washed twice with 1 ml of ice-cold buffer A (10 mM Tris-malate, pH 6.8; 10 mM MgCl$_2$; 0.1 mM dithioerythritol), and resuspended in 150 μl of buffer A containing 20 mg of DNase I (Boehringer). After breakage of the cells by shaking them with glass beads at 4°C, the beads were removed by low-speed centrifugation and the suspension was diluted with 800 μl of buffer A. The membranes were sedimented by ultracentrifugation (300,000 × g, 20 min, 4°C), extracted with 1 ml of buffer A containing 1 M NaCl for 90 min at 4°C, and sedimented again as described above. The salt-washed membranes were then resuspended in buffer A containing 500 mM NaCl and 2% Triton X-100 and extracted three more times at 4°C with a total volume of 1.4 ml of this buffer. The pooled Triton X-100 extracts were used for enzyme assays.

Murein hydrolase assays. In one assay, isolated murein glycan chains labeled with N-acetyl-[1-3H]glucosamine (259 GBq/mmol; Amersham) were used as a substrate to determine lytic transglycosylase activity (49). The glycan chains were separated by reversed-phase high-pressure liquid chromatography (HPLC) (20), and the fractions containing glycans with a defined length were desalted by gel filtration chromatography on Bioigel P2 (Bio-Rad Laboratories, Hercules, Calif.). Membrane extracts were incubated with labeled glycan chains (10,000 cpm) in 110 μl of 10 mM Tris-malate (pH 6.8) containing 10 mM MgCl$_2$, and 0.1% Triton X-100 at 30°C for various times. The reaction was stopped by the addition of 4 μl of 2% phosphoric acid, and the samples were boiled for 3 min. After the addition of 55 μl of 100 mM sodium phosphate (pH 2.0), insoluble material was removed by centrifugation (15,000 × g, 5 min, 4°C), and the breakdown products were separated by reversed-phase HPLC as described by Harz et al. (20). Radioactivity in the eluate was detected with a Flu-Ome/Beta liquid scintillation counter (Canberra Packard, Frankfurt, Germany). The breakdown products were identified by comparison with standards of HPLC-separated murein glycan chains (20).

In a more general murein hydrolase assay, the enzyme activity was determined with murein sacculi (10,000 cpm, 5 μg) radiolabeled with meso-2,6-diamino-3,4,5-[3H]pimelic acid (18.5 MBq/mmol; CEA, Gif-sur-Yvette, France) as a substrate (31). The same buffer as described for the assay with glycan strands was used.

Zymogram analysis. The detection of cell wall hydrolase activities after separation of the proteins by denaturing sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed essentially as described by Pottin et al. (36). The SDS-12% polyacrylamide gel contained 0.2% (wt/vol) lipophilized Micrococcus hydrosolitus cells (Sigma, St. Louis, Mo.) and only 0.03% SDS. Prior to PAGE, the samples were boiled for 7 min in sample buffer (100 mM Tris-HCl, pH 6.8; 1% SDS; 6% sucrose; 10 mM dithioerythritol; 0.0025% bromophenol blue) (5). After PAGE, the gel was briefly rinsed with water and soaked for 30 min each with water and renaturation buffer (25 mM sodium phosphate [pH 7.0] containing 10 mM MgCl$_2$, and 0.1% Triton X-100) with gentle agitation at room temperature. The gel was then incubated overnight in 250 ml of renaturation buffer at 30°C and for another 10 h at 37°C. To obtain better contrast, the sacculi were stained with 0.1% methylene blue in 0.01% KOH as described by Berndasky et al. (5). After they were destained with water, the unstained autolysin bands appeared on the blue background.

Palmitate labeling. To label the lipoproteins, cells were grown aerobically in LB medium in the presence of 5 μCi of [1-14C]palmitate per ml (54 Ci/mmol; Amersham Buchler) from an OD$_{578}$ of 0.08 to an OD$_{578}$ of 0.4. Expression of the lytic endo-transglycosylase was induced for 30 min by the addition of 1 mM IPTG in the presence or absence of 100 μg of globomycin per ml (Sankyo Co., Ltd., Tokyo, Japan) (25). Cells from a 10-ml culture were harvested by centrifugation, washed twice with 1 ml of ice-cold phosphatase-buffered saline, and lysed by boiling in 120 μl of 20 mM Tris-HCl (pH 8.0)–1 mM EDTA–1% SDS for 10 min. After centrifugation (13,000 × g, 10 min, 4°C), the proteins in the supernatant were precipitated by the addition of 1.2 ml of ice-cold acetone, left overnight at 4°C, and pelleted by centrifugation as described above. The protein pellet was resuspended in 250 μl of buffer A containing 1 M NaCl for 90 min at 4°C, and sedimented again as described above. The salt-washed membranes were then resuspended in buffer A containing 500 mM NaCl and 2% Triton X-100 and extracted three more times at 4°C with a total volume of 1.4 ml of this buffer. The pooled Triton X-100 extracts were used for enzyme assays.

Identification and cloning of the gene encoding a novel lytic transglycosylase. Of the four studied lytic transglycosylases of E. coli, SlT70, MltA, MltB, and MltC (24), only MltA accepts unsubstituted murein glycan chains as a substrate (39). Nevertheless, membrane extracts of an mltA mutant, E. coli LT12 harboring pAK5 (b) or pPFK18EH as a control (a and c) were induced by the addition of 1 mM IPTG, and membrane extracts were prepared as described in Materials and Methods. Extracts corresponding to 1 μl (a and b) or 100 μl (c) of culture were incubated for 30 min at 30°C with radiolabeled murein glycan chains with a length of seven disaccharide units terminating with a 1,6-anhydromuramic acid residue, were analyzed by reversed-phase HPLC. The numbers 2 to 7 indicated the degree of polymerization in the disaccharide units. Thus, for example, the “3” indicates a 1,6-anhydrohexasaccharide consisting of (GlcNAc-MurNAc)–GlcNAc(1,6-anhydro)MurNAc.
which is consistent with the information obtained from the partial purification. Therefore, the coding region was amplified by PCR and cloned behind the tac promoter of the pBR322 derivative pJFK118EH (7), resulting in plasmid pAK5. Induction of expression of the cloned gene in a mutant with a deletion of mltA (LT12) gave rise to a dramatic increase in glycan chain-degrading activity in membrane extracts (Fig. 1a and b). Therefore, the cloned gene encodes a novel membrane-bound lytic transglycosylase of E. coli.

Nucleotide sequence analysis. The novel lytic transglycosylase is encoded by an open reading frame of 609 bp starting with a GTG codon (Fig. 2). The coding sequence is found inside the reading frame o241 (GenBank entry AE000217), which predicts a 38-amino-acid-longer protein. Like the genes for MltA (32), MltB (15), and MltC (12), the coding region starts with a signal peptide followed by a lipoprotein processing site (9) and therefore is expected to encode a lipoprotein with the calculated molecular mass of 21 kDa. The residue following the modified cysteine of the bacterial lipoproteins determines the localization of the lipoprotein (52); the serine found in this protein predicts a localization in the outer membrane of E. coli. The deduced amino acid sequence shows significant similarity to other lytic transglycosylases. An alignment of protein sequences which score high in a database search (PSI-Blast algorithm, e values of \( < 10^{-30} \) after one iteration) that was prepared with the BLOCKS program shows the conservation of three sequence motifs. These stretches (see Fig. 2) are actually found in the active center of the lytic transglycosylases, as shown for the Slt70 by X-ray crystallography (47).

Controlled overexpression of the cloned lytic transglycosylase. Controlled overexpression of the novel lytic transglycosylase was achieved by the addition of 1 mM IPTG to E. coli LT12 harboring pAK5. Induction of expression at an OD578 of 0.2 caused only a negligible reduction in the growth rate compared to cells carrying the control vector pJFK118EH. Prolonged overexpression (2 to 4 h) led to the accumulation of chains of cells unable to separate properly. Overnight cultures of even uninduced E. coli XL1-Blue carrying pAK5 showed a slight tendency to lyse, probably due to the leakiness of the tac promoter. Therefore, all overnight cultures and plates were supplemented with 0.2% glucose to suppress expression of the

FIG. 2. Nucleotide sequence of the emtA gene and derived amino acid sequence. The consensus sequence of the lipoprotein processing site is shown in bold italics, with an asterisk indicating the modified cysteine. Regions of high similarity to other known lytic transglycosylases are underlined (12, 35).
cloned gene, and 10 mM MgCl₂ was added to stabilize those cells that started to undergo lysis.

Analysis by SDS-PAGE of the cellular proteins in cells induced for the expression of the cloned gene revealed the appearance of a protein band with an apparent molecular mass of 22 kDa. As shown in Fig. 3, this band represents the major protein in Triton X-100 extracts of salt-washed membranes from induced cells. This finding is well in accordance with both the apparent molecular mass of the partially purified novel lytic transglycosylase and the size predicted from the sequence of the cloned gene.

Demonstration of the lipoprotein character of EmtA. To test whether EmtA is indeed lipid modified, as indicated by the presence of a prokaryotic lipoprotein signal sequence and the fact that the protein isolated from the wild type could not be N-terminally sequenced (see above), the protein was expressed in cells grown in the presence of [³H]palmitate. After separation of the proteins by SDS-PAGE, a labeled band running at an approximate molecular mass was detected in induced cells expressing EmtA, but was hardly visible in cells harboring plasmid pJFK118EH (Fig. 4). The intensity of this band was greatly reduced when expression of the cloned gene was induced in the presence of globomycin, an inhibitor of signal peptidase II (25). No labeled band corresponding to a protein with a higher molecular mass could be detected, as one would expect in the presence of globomycin. Therefore, we assume that the unprocessed prolipoprotein is a rather unstable intermediate.

Endospecificity of the novel lytic transglycosylase. Membrane extracts from induced cells of LT12(pJFK118EH) and LT12(pAK5) were used to further characterize the novel lytic transglycosylase activity. Studies on the kinetics of degradation of isolated murein glycan chains (Fig. 5) revealed that the enzyme preferentially cleaves at a distance of more than two disaccharide units from the ends of the glycan chain (it also cleaves at a distance of two disaccharide units, producing the anhydrotetrasaccharide, but prefers cleaving farther away from the ends of glycan chains). The end products of a complete glycan chain degradation by this lytic transglycosylase were fragments with lengths of two and three disaccharide units. Unlike MltA (49), an exospecific lytic transglycosylase, EmtA does not produce monomeric anhydrodisaccharide fragments. This indicates that the novel lytic transglycosylase is an endoglycosylase. We therefore propose to name it EmtA, for endo-type membrane-bound lytic transglycosylase.

Changes in the murein structure upon overproduction of EmtA. The morphological alterations observed when EmtA was overproduced (see above) point to a change in the structure of the murein sacculus. Indeed, analysis of the muropeptide composition of E. coli MC1061(pAK5) revealed a number of changes when noninduced cells were compared with cells overproducing EmtA (Table 1). Two major effects were seen. First, there was a threefold increase in all D,L-A₂pm-A₂pm cross-links and an increase in the relative amount of anhydromuropeptides, indicating a shortening of the glycan strands. The increase in anhydromuropeptides by a factor of about 2 indicates that the glycan strands were cleaved by the overproduced lytic transglycosylase, although without inducing bacteriolysis. Interestingly, only cross-linked anhydromuropeptides were increased, whereas the relative amounts of the monomeric anhydro compounds were not changed. It seems, therefore, that in vivo the enzyme has a preference for cross-linked muropeptide. The increase in the number of D,L-A₂pm-A₂pm cross-links was found for all cross-linked muropeptides, dimers, trimers, and tetramers. By contrast, the cross-linkage via D,D-A₂pm-Ala bonds was hardly changed. Formation of A₂pm-A₂pm cross-links has been reported to take place prior to the onset of bacteriolysis caused by various agents and growth conditions (18, 24). Thus, it has been speculated that an increase in A₂pm-A₂pm cross-bridges represents a kind of defense mechanism to avoid lysis (24). Consistent with this speculation, rapid lysis, even after prolonged expression of emtA, was not observed (see above).

Activity with intact murein sacculi. Radiolabeled murein sacculi of E. coli were used as a substrate to determine in vitro murein degrading activity in membrane extracts from induced cells of LT12(pJFK118EH) and LT12. No increase in the release of murein breakdown products could be detected upon overexpression of the lytic endotransglycosylase.

In contrast, zymogram analysis revealed a double band with autolytic activity at approximately 22 kDa that was present in...
FIG. 5. Kinetics of glycan chain degradation by the lytic endotransglycosylase. E. coli LT12(pAK5) was induced by the addition of 1 mM IPTG, and membrane extracts were prepared as described in Materials and Methods. Extracts corresponding to 1 μl (b and c) or 100 μl (d and e) of culture were incubated with radiolabeled murein glycan chains with a length of seven disaccharide units terminating with 1,6-anhydromuramic acid (a) for 5 min (b and d) or 30 min (c and e) at 30°C. The breakdown products were analyzed by reversed-phase HPLC. Numbers 1 to 7, (GlcNAc-MurNAc)₆₋₇-GlcNAc-(1,6-anhydro) MurNAc (as described in Fig. 1).

**DISCUSSION**

Cooperation between exo- and endoenzymes is well known for the degradation of biopolymers such as chitin and cellulose (11, 16, 28). The advantage of producing more chain ends by endoenzymes for the exoenzymes to start their processive degradation process of the polymers is obvious. Therefore, it is not surprising to find that this strategy also seems to be used in the case of the cross-linked biopolymer murein.

An interesting feature of the endo-type membrane-bound lytic transglycosylase is its lipoprotein character, a feature EmtA shares with most of the other lytic transglycosylases, including MltA, MltB, and MltC (12, 15, 32). As demonstrated for MltA and MltB (15, 32), EmtA is also likely to be inserted into the outer membrane due to the presence of a serine at position 2 in the amino acid sequence (of the processed protein), which has been shown to direct lipoproteins to the outer membrane (52). It is tempting to speculate that the specific localization of the murein hydrolases in the outer membrane is a means to control these potentially suicidal enzymes. Interestingly, the synthetic enzymes, such as the bifunctional transpeptidase-transglycosylases PBP1A, PBP1B, and PBP1C, as well as the transpeptidases PB2P and PB3P, are anchored to the cytoplasmic membrane (2, 44). Thus, hydrolases and synthases are localized in different layers of the complex cell wall. Organization of the murein hydrolases in the outer membrane is, in accordance with the hypothesis of a three-for-one growth strategy of the stress-bearing murein sacculus, a hypothesis which assumes that old murein strands lined with new murein are removed from the side facing the outer membrane (23, 24).

The specific function of EmtA, however, is still uncertain. Although it cleaves isolated poly(MurNAc-GlcNAc) strands, the enzyme was unable to hydrolyze murein sacculi in vitro, and overproduction in vivo did not result in rapid bacteriolysis, although changes in the structure of the murein sacculus could be demonstrated (Table 1). It may well be that the enzyme has to cooperate with other proteins to carry out its specific function on murein sacculi, that is, on peptide-substituted glycan strands. Such a cooperation could also explain why the enzyme shows a preference for cross-linked murein in vivo. MltA, an exo-type lytic transglycosylase that, like EmtA, shows activity with unsubstituted glycan strands (49), also does not cause bacteriolyis when overproduced (32). Preliminary results suggest that MltA may cooperate with a novel amidase (46) that could supply the proper substrate, the murein glycan strands, for the lytic transglycosylase. A concerted action of two other murein specificities in *E. coli* has been demonstrated for the endopeptidase PBP7 and the lytic transglycosylase Slt70 (38).

Being an endo-type enzyme, EmtA would be an ideal enzyme to format the murein strands to proper sizes. As a matter of fact it is still unknown how the length distribution of the murein strands is determined (20, 24). Two main possibilities exist: either the strands are released from the bactoprenol carrier molecule by a specific termination reaction that forms a 1,6-anhydroamuramic acid terminus, or the polymerization of the murein precursors by synthetic transglycosylases is a continuous reaction and the growing strands are formatted by lytic endotransglycosylases such as EmtA.

In addition to a general role in murein metabolism or even as its sole function, EmtA may be important for paving the way for bulky compounds to pass through the murein barrier. Preliminary results by Haigh and Williams at the University of Leicester, who isolated an *emtA* deletion mutant indicating that it is not an essential enzyme, suggest that this endoglycosylase may be involved in the production of pili in enteropathogenic *E. coli* strains (19). Indeed, several examples of the involvement of lytic transglycosylases in the transmurein export have been reported (3, 13, 35). The use of lytic transglycosylases rather than other specificities of murein hydrolases may have an advantage. Repair of the lesions in the murein net could make use of the energy stored in the 1,6-anhydro bond.

The strictly localized action of lytic transglycosylases in facilitating passage through the murein layer may be controlled
by a specific interaction of the enzyme with other proteins of the export apparatus. This could explain the low autolytic activity found for EmtA. Studies of the control mechanisms for this specific function of murein hydrolases seem to be a promising experimental approach to gain more insights into the regulation of these potentially autolytic enzymes.

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REFERENCES


![FIG. 6. Zymogram analysis of overexpressed EmtA. E. coli LT12 harboring pJFK118EH (lane a) or pAK5 (lane b) was induced by the addition of 1 mM IPTG. Cell homogenates were separated by SDS–12% PAGE in gels containing 0.2% lyophilized cells of M. lysodeikticus. Renaturation of the separated proteins and staining of the gel were performed as described in Materials and Methods. The stained gel was scanned and inverted to obtain better contrast. Molecular masses of prestained marker proteins are indicated on the right.](image)

TABLE 1. Muropeptide composition of E. coli MC1061(pAK5)α

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<tr>
<th>Muropeptide</th>
<th>% in noninduced cells</th>
<th>% in induced cells</th>
<th>% Change</th>
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<td>Dimer</td>
<td>2.48</td>
<td>4.23</td>
<td>70.6</td>
<td>2.07</td>
<td>4.41</td>
<td>113.0</td>
</tr>
<tr>
<td>Trimer</td>
<td>0.46</td>
<td>0.91</td>
<td>97.8</td>
<td>0.81</td>
<td>1.61</td>
<td>98.8</td>
</tr>
<tr>
<td>Cross-linked muropeptides</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ala-A2pm</td>
<td>23.47</td>
<td>27.35</td>
<td>16.5</td>
<td>23.57</td>
<td>27.43</td>
<td>16.4</td>
</tr>
<tr>
<td>A2pm-A2pm cross-linkage</td>
<td>2.55</td>
<td>7.28</td>
<td>185.5</td>
<td>1.85</td>
<td>5.81</td>
<td>214.1</td>
</tr>
</tbody>
</table>

a The abbreviations used for the muropeptides are as follows: Tri, GlcNAc-β-1,4-MurNAc-L-Ala-β-Glu-m-A2pm; Tetra, GlcNAc-β-1,4-MurNAc-L-Ala-β-Glu-m-A2pm; Ala-A2pm, cross-linkage via D-Ala and m-A2pm of two different stem peptides; A2pm-A2pm, cross-linkage via m-A2pm and m-A2pm of two different stem peptides; Anhydros, all muropeptides carrying a 1,6-anhydromuramic acid. The relative amounts of the muropeptides and the degree of cross-linkage were calculated as described by Glaser (17).


44. Templin, M. F. Unpublished results.


