

Purification and Properties of a Membrane-Bound Lytic Transglycosylase from *Escherichia coli*

ASTRID URSINUS AND JOACHIM-VOLKER HÖLTJE*

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

Received 13 September 1993/Accepted 13 November 1993

A membrane-bound lytic transglycosylase (Mlt) has been solubilized in the presence of 2% Triton X-100 containing 0.5 M NaCl from membranes of an *Escherichia coli* mutant that carries a deletion in the *slt* gene coding for a 70-kDa soluble lytic transglycosylase (Slt70). The enzyme was purified by a four-step procedure including anion-exchange (HiLoad SP-Sepharose and MonoS), heparin-Sepharose, and poly(U)-Sepharose 4B column chromatography. The purified protein that migrated during denaturing sodium dodecyl sulfate-polyacrylamide gel electrophoresis as a single band corresponding to an apparent molecular mass of about 38 kDa is referred to as Mlt38. Optimal activity was found in buffers with a pH between 4.0 and 4.5. The enzyme is stimulated by a factor of 2.5 in the presence of Mg^{2+} at a concentration of 10 mM and loses its activity rapidly at temperatures above 30°C. Besides insoluble murein sacculi, the enzyme was able to degrade glycan strands isolated from murein by amidase treatment. The enzymatic reaction occurred with a maximal velocity of about 2.2 mg/liter/min with murein sacculi as a substrate. The amino acid sequences of four proteolytic peptides showed no identity with known sequences in the data bank. With Mlt38, the number of proteins in *E. coli* showing lytic transglycosylase activity rises to three.

One of the best-characterized murein hydrolases is lysozyme, a muramidase which is widely found in bacteria and higher organisms (5). In *Escherichia coli*, an analogous enzyme specificity is present that cleaves the very same β -1,4-glycosidic bond between *N*-acetylmuramic acid and *N*-acetylglucosamine that is hydrolyzed by lysozyme (12). Unlike lysozyme, the *E. coli* enzyme does not simply hydrolyze the glycosidic bond. Cleavage of the bond is followed by an intramolecular transglycosylation with the C₆ hydroxyl group of the muramic acid serving as an acceptor. By this mechanism, the high-molecular-weight, insoluble murein is degraded into soluble muropeptides carrying 1,6-anhydro muramic acid residues (12). The biological meaning of this type of reaction, which partially preserves the chemical-bond energy of the glycosidic bond, is not known. As a matter of fact, all of the reducing ends of the glycan strands in the murein of *E. coli* are blocked by the presence of a 1,6-anhydro ring structure at the terminal muramic acid (9, 12, 22). Uncontrolled action of this type of endogenous enzyme causes autolysis from within (2). Therefore, this enzyme activity is called lytic transglycosylase.

Two lytic transglycosylases had been isolated so far from the soluble fraction of *E. coli*, a soluble lytic transglycosylase (Slt) with a molecular mass of 70 kDa, called Slt70 (7, 12, 16), and recently a second one with a molecular mass of 35 kDa, called Slt35 (8). In a mutant with a deletion in the *slt* gene encoding Slt70 (27), we detected in the membrane fraction lytic transglycosylase activity that could only be solubilized by means of detergents (23). The membrane enzyme differed from the known soluble enzymes by its activity towards isolated murein glycan strands [poly(MurNAc-GlcNAc)]. Here we describe the purification, characterization, and partial amino acid sequence of this membrane-bound lytic transglycosylase (Mlt), which will be referred to as Mlt38.

MATERIALS AND METHODS

Bacterial strain and growth conditions. *E. coli* MUF16 (27), a mutant of *E. coli* MC1061 (4) carrying a deletion in the *slt* gene, was used for the isolation of a membrane-bound murein hydrolase. Cultures were grown aerobically at 37°C in a 200-liter fermentor in LB medium (20) and harvested at the beginning of the stationary phase of growth. For the preparation of radioactively labeled murein sacculi, *E. coli* W7 (*dap lys*) was used (10).

Enzyme assays. Two different assays were used, one determining the degradation of insoluble [³H]A₂pm-labeled murein sacculi into soluble muropeptides (10, 16) and the other one measuring the breakdown of [³H]GlcNAc-labeled poly(MurNAc-GlcNAc)_{n > 30} glycan strands (23).

(i) **Assay for hydrolysis of murein sacculi.** Labeled murein sacculi were prepared from *E. coli* W7 cells grown in the presence of *meso*-2,6-diamino-[3,4,5-³H]pimelic acid (0.85 TBq/mmol; Centre D'Etudes Nucleaires de Saclay Service des Molecules Marquées, Gif-Sur-Yvette, France) as described elsewhere (16). Briefly, cells were boiled in 4% sodium dodecyl sulfate (SDS) and washed free of detergent by repeated high-speed centrifugation (60 min, 100,000 × *g*). Glycogen associated with the sacculi was removed by digestion with α -amylase and covalently bound lipoprotein was removed by digestion with pronase as described elsewhere (11).

Enzyme aliquots in a total volume of 100 μ l of 10 mM sodium acetate, pH 4.5, containing 10 mM MgCl₂ were incubated at 30°C in the presence of 5 μ g of [³H]A₂pm-labeled murein sacculi (about 10,000 cpm). After incubation, the assay mixture was placed on ice, 100 μ l of 1% *N*-cetyl-*N,N,N*-trimethylammonium bromide was added, and the samples were left on ice for 15 min. Precipitated material was pelleted by centrifugation (15 min, 10,000 × *g*), and the radioactivity in 100 μ l of the supernatant was measured by liquid scintillation counting (27). One unit of enzyme activity was defined as the amount of enzyme that solubilizes 1 μ g of murein in 10 min at 30°C under the conditions described above.

(ii) **Assay for hydrolysis of poly(MurNAc-GlcNAc) glycan strands.** Radioactively labeled murein glycan strands were

* Corresponding author. Mailing address: Max-Planck-Institut für Entwicklungsbiologie, Abteilung Biochemie, Spemannstrasse 35, Postfach 2109; 72011 Tübingen, Germany. Phone: (7071) 601 412. Fax: (7071) 601 447.

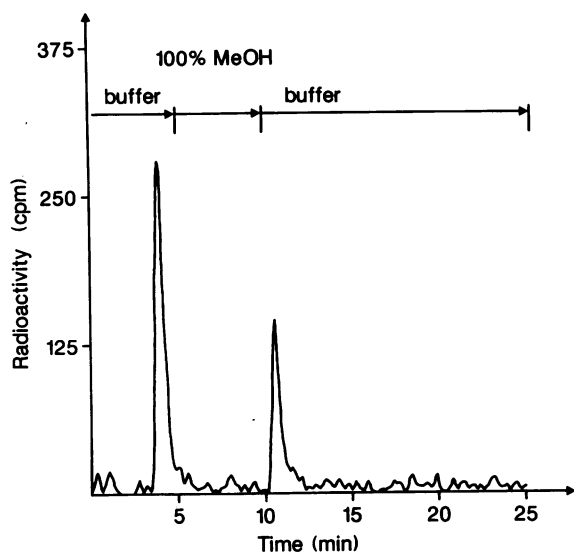


FIG. 1. Hydrolysis of isolated glycan strands followed by HPLC. Glycan strands, poly(MurNAc-GlcNAc)_n > 30, were incubated with enzyme samples as described in Materials and Methods. The reaction was stopped by boiling, and the assay sample was applied to a 5- μ m Nucleosil 300 C₁₈ column (125 by 4.6 mm). Elution was first with 0.1 M sodium phosphate buffer, pH 2.0, containing 5% acetonitrile for 5 min, and then with 100% methanol for 5 min before switching back to starting buffer. The radioactivity in the eluent was monitored. Isolated anhydrodisaccharide eluted at 4 min.

isolated from murein sacculi labeled with D-[1-³H]-N-acetylglucosamine (Amersham Buchler, Braunschweig, Germany) by digestion with human serum amidase following the procedure of Harz et al. (11). The solubilized glycan strands were fractionated in the range of 1 to 30 disaccharide units by reversed-phase high-performance liquid chromatography (HPLC) as described elsewhere (11, 23). Glycan strands longer than 30 disaccharide units were finally eluted in one fraction by a stepwise increase of the elution solvent to 100% methanol (23). The material was desalted by HPLC as described elsewhere (11) and used as substrate for the enzyme.

Enzyme aliquots were incubated in the presence of 0.5 μ g of [³H]GlcNAc-labeled poly(MurNAc-GlcNAc)_n > 30 (about 4,000 cpm) in a total volume of 100 μ l of 10 mM sodium acetate buffer, pH 4.2, containing 10 mM MgCl₂ for 30 min at 37°C. The reaction was stopped by boiling the samples for 5 min. The separation of product and substrate was accomplished by reversed-phase HPLC on a 5- μ m Nucleosil 300 C₁₈ column (125 by 4.6 mm; Bischoff, Leonberg, Germany). The pH of the sample was adjusted to 3.5 by the addition of about 10 μ l of 20% phosphoric acid. The sample was applied onto the column and fractionated at room temperature with 0.1 M sodium phosphate buffer, pH 2.0, containing 5% acetonitrile at a flow rate of 1 ml/min (Fig. 1). The material that elutes after 4 min consists of the disaccharide GlcNAc- β -1,4-anhydro-MurNAc (23). After 5 min, elution was continued with 100% methanol, causing unreacted substrate and the intermediate oligosaccharides to elute in a single peak at 11 min. The radioactivity in the eluent was monitored by a flow-through scintillation counter (model A-100; Canberra/Packard, Downers Grove, Ill.).

Enzyme purification. All preparations were carried out at 4°C.

(i) **Enzyme solubilization.** Cells of *E. coli* MUF16 (wet

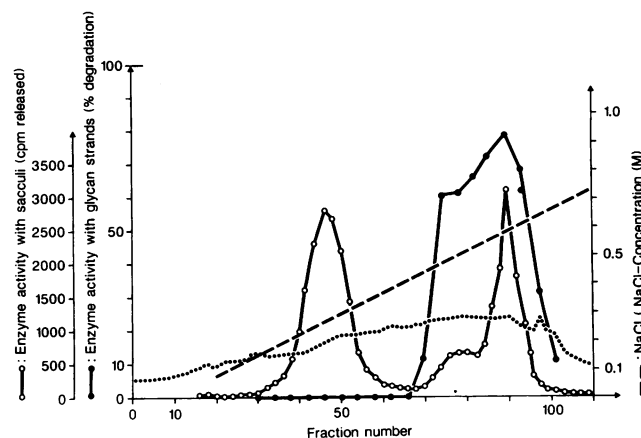


FIG. 2. Elution of murein hydrolase activity from an SP Sepharose HP ion-exchange column. Membrane proteins (916 mg in 300 ml) from *E. coli* MUF16 solubilized by 2% Triton X-100 and 0.5 M NaCl were applied onto a HiLoad 26/10 SP Sepharose column and eluted with a linear NaCl gradient in buffer II. Enzyme activity was determined as described in Materials and Methods. Activity with murein sacculi as substrate (open circles) was measured in 20- μ l aliquots of the fractions. The release of radioactivity in 100 μ l of the supernatant after precipitation of high-molecular-weight murein is shown. Enzyme activity with isolated glycan strands as substrate (closed circles) was assayed in 20- μ l aliquots of the fractions. The dashed line indicates salt molarity, and the dotted line indicates UV A₂₈₀.

weight, 100 g) were suspended in 250 ml of 10 mM Tris-maleate buffer, pH 5.2, containing 10 mM MgCl₂ and 1 mM dithiothreitol (DTE) (buffer I). After the addition of 1 μ g of DNase per ml and 1 mM phenylmethylsulfonyl fluoride, the cells were broken by passage through a French pressure cell (American Instrument Company, Silver Spring, Md.) at a pressure of 18,000 lb/in². Cell envelopes were collected by centrifugation at 100,000 \times g for 90 min. The pellet was washed once with buffer I supplemented with 1 M NaCl and once with simple buffer I. Membrane proteins were extracted with 2% Triton X-100 and 0.5 M NaCl. Accordingly, the envelopes were suspended in 250 ml of 10 mM Tris-maleate, pH 5.2, containing 20 mM EDTA, 0.5 M NaCl, 0.1 mM DTE, 1 mM phenylmethylsulfonyl fluoride, and 2% Triton X-100 and stirred gently overnight. The suspension was cleared by centrifugation at 100,000 \times g for 60 min, and the supernatant was dialyzed against four changes of 2 liters of buffer I, first with buffer I containing 0.25 M NaCl (2 h), next with buffer I containing 0.125 M NaCl (2 h), and finally with two changes of buffer I with no NaCl added (overnight). To remove precipitated material, the preparation was centrifuged at 100,000 \times g for 30 min. The solubilized enzyme was then subjected to the enzyme purification procedure described below.

(ii) **SP Sepharose HP ion-exchange fast-protein liquid chromatography (FPLC).** The solubilized enzyme (300 ml, 916 mg of protein, 4,030 U) was applied at a flow rate of 10 ml/min to a HiLoad 26/10 SP Sepharose HPLC column (Pharmacia, Freiburg, Germany) equilibrated with buffer I containing 2% Triton X-100. The column was washed until the UV baseline was stable, and then it was washed with one column volume of buffer I containing 0.2% Triton X-100 (buffer II). The proteins were eluted with a linear gradient of buffer II to 1 M NaCl in 10 mM Tris-maleate, pH 6.8, containing 10 mM MgCl₂, 1 mM DTE, and 0.2% Triton X-100 (1,000 ml) (Fig. 2). Fractions 83 to 96 (5 ml each) showing enzymatic activity with both murein

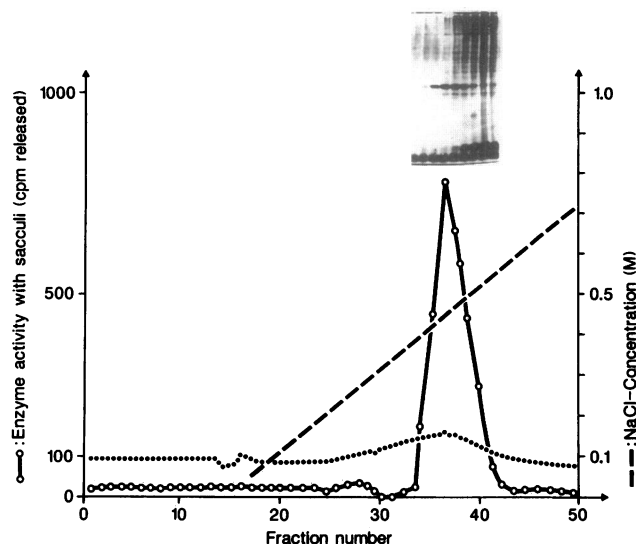


FIG. 3. Elution of Mlt38 of *E. coli* MUF16 from a MonoS ion-exchange FPLC column. Enzyme activity was determined with [^3H]A₂₈₀-labeled murein sacculi as described in Materials and Methods. The dashed line indicates salt molarity, and the dotted line indicates the UV A₂₈₀. Aliquots of each fraction were analyzed by SDS-12.5% PAGE and stained with silver nitrate (21) as shown in the inset. Fractions 35 to 39 were combined and subjected to preparative SDS-PAGE (see Fig. 4).

sacculi and glycan strands were combined (70 ml); concentrated by dialysis against polyethylene glycol 20,000 (Merck, Darmstadt, Germany) to about 20 ml; and dialyzed against 10 mM potassium phosphate, pH 6.8, containing 10 mM MgCl₂, 0.1 mM DTE, and 0.2% Triton X-100 (buffer III) (SP Sepharose HP fraction; 60.7 mg of protein, 904.4 U).

(iii) **Heparin-Sepharose affinity chromatography.** The dialyzed SP Sepharose HP fraction (18 ml) was applied to a heparin-Sepharose column (Pharmacia; 5 ml, 5.5 by 1 cm) equilibrated with buffer III at a flow rate of 12 ml/h. The column was washed with buffer III before elution with a linear molarity gradient from 10 to 500 mM buffer III (200 ml). The fractions (2 ml each) containing enzymatic activity were pooled (42 ml) and dialyzed against buffer III (heparin-Sepharose pool; 15.3 mg of protein, 680.9 U).

(iv) **Poly(U)-Sepharose 4B affinity chromatography.** The heparin-Sepharose pool was pumped at a flow rate of 8.4 ml/h onto a poly(U)-Sepharose 4B column (Pharmacia; 7 ml, 3.5 by 1 cm) equilibrated with buffer III. The column was washed with buffer III before elution with a linear gradient of 10 to 500 mM buffer III (200 ml). The fractions (1.4 ml each) showing enzymatic activity were combined (26 ml), concentrated by dialysis against polyethylene glycol 20,000, and dialyzed against buffer II [poly(U)-Sepharose pool; 12.5 ml, 4.1 mg of protein, 291.9 U].

(v) **MonoS ion-exchange FPLC.** The poly(U)-Sepharose pool was applied at a flow rate of 1 ml/min to a MonoS preppacked HR 5/5 column (Pharmacia; 1 ml, 0.5 by 5 cm) equilibrated with buffer II. The column was washed with equilibration buffer, and the proteins were eluted with a linear gradient of 0 to 750 mM NaCl in buffer II (Fig. 3). Fractions 35 to 39 (0.5 ml each) containing enzymatic activity were combined (2.5 ml) and dialyzed against buffer II (MonoS pool; 0.59 mg of protein, 174.6 U).

SDS-polyacrylamide gel electrophoresis (PAGE). The gel system described by Lugtenberg et al. (17) was used. A

gradient of polyacrylamide from 10 to 18% turned out to be best for the separation of the 38-kDa enzyme protein. Analytical gels were stained with silver nitrate (21), and preparative ones were stained with Coomassie blue.

Determination of the amino acid sequence. The protein band corresponding to Mlt38 was cut out from an SDS-PAGE gel and digested with endoproteinase LysC (10 μg) in 200 μl of 25 mM Tris-HCl, pH 8.5, for 6 h at 37°C. The peptides were extracted with two portions of 400 μl of 60% CH₃CN in 0.1% trifluoroacetic acid (TFA) overnight. The peptides were fractionated on Supersher 60RP select B (Merck) by using a linear gradient of 0.1 to 0.15% TFA in acetonitrile (1%/min) at a flow rate of 200 $\mu\text{l}/\text{min}$. The N-terminal amino acid sequence of selected peptides was determined by using an automatic Sequencer Porton 3600 (Beckman Instruments, Fullerton, Calif.) and a Microphore-HPLC System Gold (Beckman Instruments) basically according to the method of Edman and Begg (6).

Analytical methods. Proteins in solution were quantified according to the method of Bradford (3) by using bovine serum albumin as a standard.

RESULTS

Purification of an Mlt. Analysis of murein hydrolase activity in the Slt70 deletion mutant MUF16 had revealed the presence of a lytic transglycosylase in the membrane fraction that could only be released with detergents (23). Efficient solubilization of the enzyme needed 2% Triton X-100 combined with 0.5 M NaCl. However, a lower amount of Triton X-100, namely, 0.2%, was sufficient to keep the enzyme in solution. It was important to reduce the concentration of the detergent in a stepwise fashion to avoid precipitation of the bulk of proteins. It had been shown that this membrane enzyme is able to degrade isolated murein glycan strands (23). Therefore we used two different enzyme assays, the common test for murein hydrolases with murein sacculi as a substrate and the more specific one with murein glycan strands, to look for the presence of Mlt activity in column eluates.

Most of the contaminating soluble enzyme activity, probably due to Slt35 (8), could be removed by washing the membranes with 1 M NaCl (23). Remaining activity could finally be separated during the first purification step by HiLoad SP Sepharose FPLC (Fig. 2). Two activity peaks in the murein sacculi assay were found, with one also showing activity in the glycan strand assay. This fraction, eluting between 0.53 and 0.63 M NaCl, was further purified. Similar to the Slt, the membrane-bound enzyme binds both to heparin-sulfate and to poly(U)-Sepharose. Consequently, both materials were used for additional purification of the Mlt by affinity chromatography. From the heparin-Sepharose column, the enzyme eluted between 0.1 and 0.25 M phosphate buffer, and from the poly(U)-Sepharose column it eluted at a concentration in phosphate buffer from 0.1 and 0.15 M.

A major enrichment of the enzyme was finally achieved by cation-exchange FPLC on MonoS (Fig. 3). The specific activity of the enzyme eluting between 0.4 and 0.5 M NaCl was increased to 295.9 U/mg. The final yield of the purified enzyme activity was about 4%, and the purification factor was about 67-fold (Table 1). The degree of purification of the enzyme was determined by denaturing SDS-PAGE of the activity-containing fractions of the MonoS column chromatography. As shown in Fig. 3, one protein, migrating between ovalbumin and carbonic anhydrase, was coeluting with the enzyme activity. This protein was subjected to amino acid sequence determination (see below).

TABLE 1. Purification of Mlt38 from the envelope fraction of *E. coli* MUF16^a

Purification step	Total protein (mg)	Total activity (U)	Yield (%)	Sp act (U/mg)	Purification (fold)
Membrane extract	916	4,030	100	4.4	1
SP Sepharose	60.7	904.4	22.4	14.9	3.4
Heparin-sulfate	15.3	680.9	16.9	44.5	10.1
Poly(U)-Sepharose	4.1	291.9	7.2	71.2	16.2
MonoS	0.59	174.6	4.3	295.9	67.3

^a Enzyme activity was determined with murein sacculi as substrate according to the assay described in Materials and Methods.

Substrate specificity of the enzyme. An interesting feature of Mlt38 is its activity with isolated murein glycan strands (23). This particular property allowed a clear-cut distinction of the membrane enzyme from the soluble one. The assay uses poly(MurNAc-GlcNAc) longer than 30 disaccharide units. By an exomuramidase-like action, anhydrodisaccharide units, which can be separated from the oligosaccharides by reversed-phase HPLC as described in detail in Materials and Methods and shown in Fig. 1, are split off (23). In addition, the purified enzyme activity also degrades insoluble, high-molecular-weight murein sacculi, a reaction catalyzed also by the other known lytic transglycosylases (1, 8, 23).

Determination of molecular mass. The molecular mass of the enzyme was determined by SDS-PAGE on a 10 to 18% polyacrylamide gradient gel (Fig. 4). From a plot of the R_f values for the standard proteins phosphorylase, bovine serum albumin, ovalbumin, carbonic anhydrase, trypsin inhibitor, and α -lactalbumin and the purified Mlt, an apparent molecular mass of 38 kDa can be calculated. The presence of a detergent such as Triton X-100 that is absolutely necessary to keep the enzyme in solution does not allow a determination of the native molecular mass by gel filtration procedures. Nondenaturing gel electrophoresis was not tried.

Biochemical properties of the enzyme. The effect of pH on

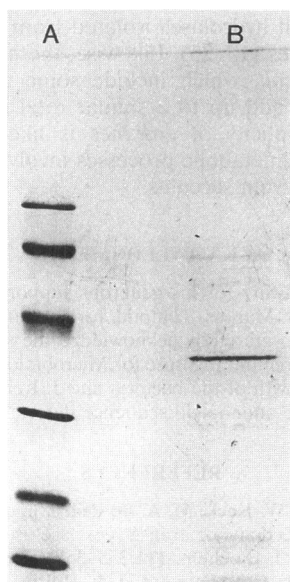


FIG. 4. SDS-PAGE of purified Mlt38. Electrophoresis was performed on a 10 to 18% polyacrylamide gel (17). Lane A, molecular mass standards (from top to bottom, phosphorylase *b*, 94 kDa; albumin, 67 kDa; ovalbumin, 43 kDa; carbonic anhydrase, 30 kDa; trypsin inhibitor, 20.1 kDa; α -lactalbumin, 14.4 kDa); lane B, 1 μ g of purified Mlt38. The gel was stained with Coomassie brilliant blue.

the enzymatic reaction with murein sacculi and with murein glycan strands was determined. For both reactions, a maximum was reached at about pH 4.0 to 4.5 (Fig. 5). Magnesium at a concentration of 10 mM stimulated the activity by a factor of 2.5, whereas 10 mM EDTA did not affect the enzymatic reaction. The isolated enzyme shows a pronounced temperature dependency (Fig. 6). With sacculi, the optimal temperature is between 28 and 30°C, and with glycan strands, it is between 34 and 36°C. A rapid decline in activity was found at temperatures beyond the optimal one.

Kinetic properties of the enzyme. The enzymatic reaction with murein sacculi at a concentration of 50 μ g/ml was linear with time up to 20 min at 30°C. Determination of a Michaelis constant (K_m) is not possible, since the substrate is insoluble. However, the apparent maximum velocity (V_{max}) of the reaction can be determined. Accordingly, initial velocity measurements were done at various substrate concentrations in the range of 2.5 to 50 mg/liter. At higher substrate concentrations, a decrease of the reaction velocity was observed (Fig. 7). From a double-reciprocal Lineweaver-Burk plot, an apparent K_m of about 52.6 mg/liter can be calculated. V_{max} was determined to be about 2.2 mg/liter/min.

Partial amino acid sequences. Attempts to determine the amino-terminal amino acid sequence of the protein, separated by SDS-PAGE and blotted onto Immobilon polyvinylidene difluoride membranes (Millipore Corp., Bedford, Mass.) failed because the N terminus was found to be blocked. Therefore, the Coomassie blue-stained protein band corresponding to

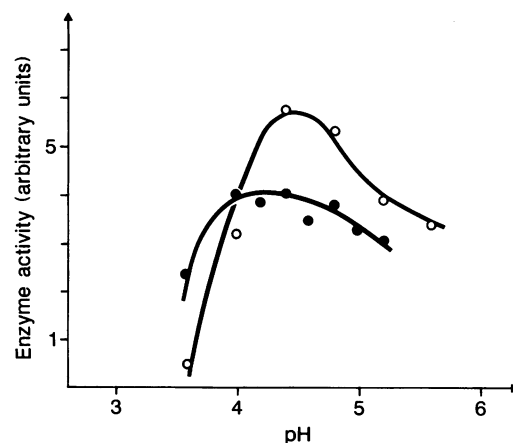


FIG. 5. Effect of pH on the lytic transglycosylase reaction with murein sacculi (open circles) and isolated glycan strands (closed circles). Purified Mlt38 (296 U/mg) was incubated in 10 mM Tris-maleate containing 10 mM $MgCl_2$ and 1 mM DTE for 30 min at 30°C. The pH of the buffer was adjusted to the indicated values. The enzyme activity was determined as described in Materials and Methods.

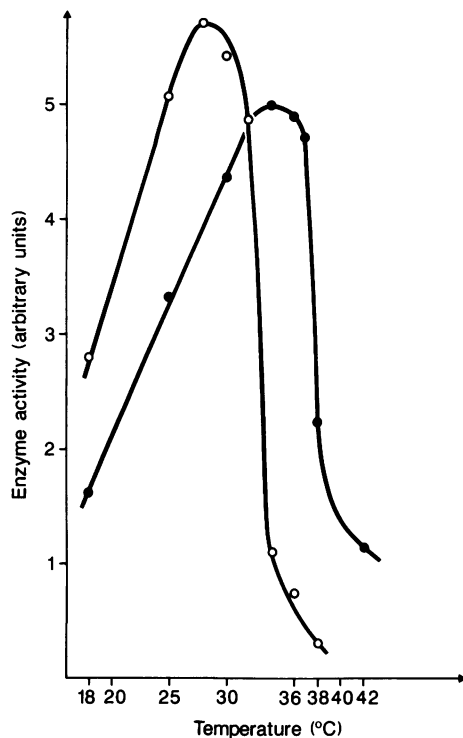


FIG. 6. Temperature dependency of the Mlt reaction with either murein sacculi (open circles) or isolated glycan strands (closed circles). Purified Mlt38 (296 U/mg) was incubated at the indicated temperatures for 10 min with either murein sacculi or isolated glycan strands as described in Materials and Methods.

Mlt38 was eluted from the gel and subjected to endoproteinase LysC digestion. The resulting peptides were separated by reversed-phase HPLC, and the amino acid sequences of four of the peptides were determined as described in Materials and Methods. The following sequences were obtained (as shown by the amino acids in parentheses, a few positions are uncertain): Asn Gly His Ala Tyr Arg Ser Ile Gly Lys (peptide 1), Arg (Ser

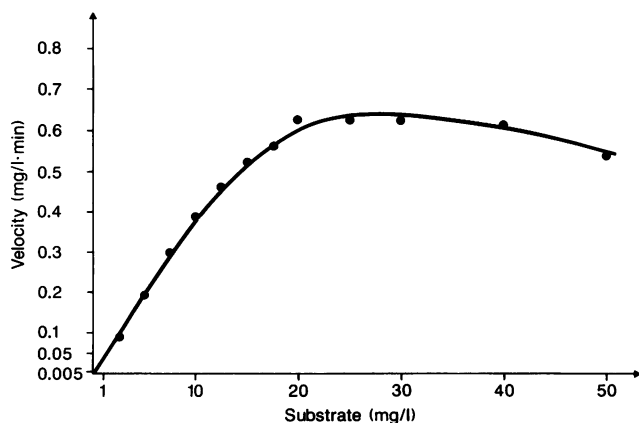


FIG. 7. Dependency of the reaction velocity on the concentration of murein sacculi. The indicated amounts of [^3H]A₂pm-labeled murein sacculi were incubated at 30°C for 20 min with purified Mlt38 (296 U/mg) as described in Materials and Methods.

Gly Arg Leu Pro Ser Arg Ala Glu Ile Tyr Ala Gly Ala Leu (peptide 2), Pro Gln Ser Phe Ala Pro Val Lys (peptide 3), and Gly (Thr/Ser) Ala Ser Ala Val Pro Leu Val Gly Arg Ala Ser Val Ala Ser (peptide 4). A computer-aided comparison with the GenBank data base, done by using the GCG software package (Genetics Computer Group, Wis.), revealed no identity with any of the sequences in the data base, including the published sequence of the gene incorrectly presumed to be the *mlt* gene (8), *msbB* (14) (see below).

DISCUSSION

An Mlt had already been reported to exist and to have been purified from *E. coli* (15, 19). However, it was later found that the purified "Mlt" protein was contaminated by a novel soluble, but membrane-associated, lytic transglycosylase with a molecular mass of 35 kDa (8). This enzyme has recently been purified and is referred to as Slt35 (8). The nucleotide sequence of the presumed *mlt* gene (8) turned out to be identical to *msbB*, a protein totally unrelated to murein hydrolases (14). Nevertheless, a true Mlt exists and is shown in the present report to have a molecular mass of 38 kDa. Thus, *E. coli* is equipped with three lytic transglycosylases: two soluble ones, Slt70 and Slt35, and the membrane-bound Mlt38.

Whereas lysozyme is an endomuramidase (5), all three lytic transglycosylases are exoenzymes that processively degrade murein (1, 8, 23). Another difference in the biochemical properties of the lytic transglycosylases compared with lysozyme is their rather low pH optimum (5). Since the three-dimensional structure of Slt70 has already almost been solved (24, 28), the details of the catalytic mechanism of the lytic transglycosylation reaction may be elucidated soon.

Mlt38 differs from the two Slt's by its capacity to degrade isolated poly(MurNAc-GlcNAc) glycan strands and may therefore have a unique function. It is expected that our current attempt to clone the gene and to construct a mutant will shed some light on the specific role of this enzyme.

With the isolation of a third lytic transglycosylase, Mlt38, the number of murein hydrolases isolated from *E. coli* rises to a total of 10 enzymes (13, 25). Likewise, the number of murein synthases in *E. coli*, which include some of the penicillin-binding proteins, add up to a similar total (18, 26, 29). The astonishing multiplicity of enzymes is likely to reflect the complexity of the metabolic processes involved in growth and division of the murein sacculus.

ACKNOWLEDGMENTS

We thank U. Schwarz for his generous support, Tina Romeis for helpful advice, and Markus Templin for a critical reading of the manuscript. We also gratefully acknowledge the service of P. Schütz and D. Neubauer from the Institute for Microbiology at the University of Tübingen for growth of the bacteria and J. Kellermann (Beckman Instruments) for the amino acid sequence determinations.

REFERENCES

1. Beachey, E. H., W. Keck, M. A. de Pedro, and U. Schwarz. 1981. Exoenzymatic activity of transglycosylase isolated from *Escherichia coli*. *Eur. J. Biochem.* **116**:355-358.
2. Betzner, A. S., L. C. S. Ferreira, J.-V. Höltje, and W. Keck. 1990. Control of the activity of the soluble lytic transglycosylase by the stringent response in *Escherichia coli*. *FEMS Microbiol. Lett.* **67**:161-164.
3. Bradford, M. M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **72**:248-254.

4. Casabadan, M. J., and S. N. Cohen. 1980. Analysis of gene control signals by DNA fusion and cloning in *Escherichia coli*. *J. Mol. Biol.* **138**:179–207.
5. Chipman, D. M., and N. Sharon. 1969. Mechanism of lysozyme action. *Science* **165**:454–465.
6. Edman, P., and G. Begg. 1967. A protein sequenator. *Eur. J. Biochem.* **1**:80–91.
7. Engel, H., B. Kazemier, and W. Keck. 1991. Murein-metabolizing enzymes from *Escherichia coli*: sequence analysis and controlled overexpression of the *slt* gene, which encodes the soluble lytic transglycosylase. *J. Bacteriol.* **173**:6773–6782.
8. Engel, H., A. J. Smink, L. van Wijngaarden, and W. Keck. 1992. Murein-metabolizing enzymes from *Escherichia coli*: existence of a second lytic transglycosylase. *J. Bacteriol.* **174**:6394–6403.
9. Glauner, B., J.-V. Höltje, and U. Schwarz. 1988. The composition of the murein of *Escherichia coli*. *J. Biol. Chem.* **263**:10088–10095.
10. Hartmann, R., J.-V. Höltje, and U. Schwarz. 1972. Targets of penicillin action in *Escherichia coli*. *Nature (London)* **235**:426–429.
11. Harz, H., K. Burgdorf, and J.-V. Höltje. 1990. Isolation and separation of the glycan strands from murein of *Escherichia coli* by reversed-phase high-performance liquid chromatography. *Anal. Biochem.* **190**:120–128.
12. Höltje, J.-V., D. Mirelman, N. Sharon, and U. Schwarz. 1975. Novel type of murein transglycosylase in *Escherichia coli*. *J. Bacteriol.* **124**:1067–1076.
13. Höltje, J.-V., and E. I. Tuomanen. 1991. The murein hydrolases of *Escherichia coli*: properties, functions and impact on the course of infections in vivo. *J. Gen. Microbiol.* **137**:441–454.
14. Karow, M., and C. Georgopoulos. 1992. Isolation and characterization of the *Escherichia coli msbB* gene, a multicopy suppressor of null mutations in the high-temperature requirement gene *htrB*. *J. Bacteriol.* **174**:702–710.
15. Keck, W., F. B. Wientjes, and U. Schwarz. 1985. Comparison of two hydrolytic murein transglycosylases of *Escherichia coli*. *Eur. J. Biochem.* **148**:493–497.
16. Kusser, W., and U. Schwarz. 1980. *Escherichia coli* murein transglycosylase—purification by affinity chromatography and interaction with polynucleotides. *Eur. J. Biochem.* **103**:277–281.
17. Lugtenberg, B., J. Meyers, R. Peters, P. van der Hoek, and A. L. Van Alphen. 1975. Electrophoretic resolution of the major outer membrane protein of *Escherichia coli* K 12 into four bands. *FEBS Lett.* **58**:254–258.
18. Matsuhashi, M., J. Nakagawa, F. Ishino, S. Nakajima-Iijima, S. Tomioka, M. Doi, and S. Tamaki. 1981. Penicillin-binding proteins: their nature and functions in the cellular duplication and mechanism of action of β -lactam antibiotics in *Escherichia coli*, p. 203–223. In S. Mitsuhashi (ed.), *Beta-lactam antibiotics*. Japan Scientific Societies Press, Tokyo.
19. Mett, H., W. Keck, A. Funk, and U. Schwarz. 1980. Two different species of murein transglycosylase in *Escherichia coli*. *J. Bacteriol.* **144**:45–52.
20. Miller, J. H. 1972. *Experiments in molecular genetics*. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
21. Morrissey, J. H. 1981. Silver stain for proteins in polyacrylamide gels: a method with enhanced uniform sensitivity. *Anal. Biochem.* **117**:307–310.
22. Obermann, W., and J.-V. Höltje. Alterations of murein structure and of penicillin-binding protein pattern in minicells from *Escherichia coli*. *Microbiology*, in press.
23. Romeis, T., W. Vollmer, and J.-V. Höltje. 1993. Characterization of three different lytic transglycosylases in *Escherichia coli*. *FEMS Microbiol. Lett.* **111**:141–146.
24. Rozeboom, H. J., B. W. Dijkstra, H. Engel, and W. Keck. 1990. Crystallization of the soluble lytic transglycosylase from *Escherichia coli* K12. *J. Mol. Biol.* **212**:557–559.
25. Shockman, G. D., and J.-V. Höltje. Microbial peptidoglycan (murein) hydrolases. In J.-M. Ghuysen and R. Hakenbeck (ed.), *New comprehensive biochemistry*, vol. 27. Bacterial cell wall, in press. Elsevier, Amsterdam.
26. Spratt, B. G. 1975. Distinct penicillin binding proteins involved in the division, elongation, and shape of *Escherichia coli* K12. *Proc. Natl. Acad. Sci. USA* **72**:2999–3003.
27. Templin, M. F., D. H. Edwards, and J.-V. Höltje. 1992. A murein hydrolase is the specific target of bulgecin in *Escherichia coli*. *J. Biol. Chem.* **267**:20039–20043.
28. Thunnissen, A.-M., and B. W. Dijkstra. Personal communication.
29. Waxman, D. J., and J. L. Strominger. 1983. Penicillin-binding proteins and the mechanism of action of β -lactam antibiotics. *Annu. Rev. Biochem.* **52**:825–869.