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Subcellular Distribution of the Soluble Lytic Transglycosylase in *Escherichia coli*

BRIGITTE WALDERICH AND JOACHIM-VOLKER HÖLTJE*

Abteilung Biochemie, Max-Planck-Institut für Entwicklungsbiologie, Spemannstrasse 35, 7400 Tübingen, Germany

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The localization of the major autolytic enzyme, the soluble lytic transglycosylase, in the different cell compartments of *Escherichia coli* was investigated by immunoelectron microscopy. Ultrathin sections were labeled with a specific antiserum against purified soluble lytic transglycosylase, and the antibody-enzyme complexes were visualized with colloidal protein A-gold. A preferential localization of the lytic transglycosylase in the envelope was observed, with only 20 to 30% of the enzyme left in the cytoplasm. Most of the enzyme associated with the cell wall was tightly bound to the murein sacculus. Sacculi prepared by boiling of cells in 4% sodium dodecyl sulfate could be immunolabeled with the specific antiserum, indicating a surprisingly strong interaction of the lytic transglycosylase with murein. The enzyme-substrate complex could be reconstituted in vitro by incubating pronase-treated, protein-free murein sacculi with purified lytic transglycosylase at 0°C. Titration of sacculi with increasing amounts of enzyme indicated a limiting number of binding sites for about 1,000 molecules of enzyme per sacculus. Ruptured murein sacculi obtained after penicillin treatment revealed that the enzyme is exclusively bound to the outer surface of the sacculus. This finding is discussed in the light of recent evidence suggesting that the murein of *E. coli* might be a structure of more than one layer expanding by inside-to-outside growth of patches of murein.

The bag-shaped polymer murein that completely encloses the cell maintains both the mechanical stability and the specific shape of a bacterium (47). Enzymes cleaving covalent bonds in the murein sacculus are ubiquitous among bacteria (9), although they represent potential autolysins for the cell (34, 39, 42, 47). These murein hydrolases have been proposed to fulfill fundamental functions as "spacemaker" enzymes (43) during general murein enlargement and as specific cutting enzymes during cell septum formation (25, 35, 37, 43, 47). It is very likely that at least some of these autolytic enzymes are absolutely essential for growth and division of the cell. Strict control of the murein hydrolases is required, on the one hand to avoid autolysis of the cells and on the other hand to allow an orderly enlargement of the murein sacculus with the specific shape maintained (20, 21). Unfortunately, detailed information on the regulation and topological organization of these enzymes is still lacking.

In *Escherichia coli*, as many as nine different murein hydrolases have been identified (21). Four of these, two endopeptidases and two lytic transglycosylases, are capable of degrading murein sacculi to low-molecular-weight muropeptides. These enzymes, therefore, represent the autolytic system of the cell.

The prevailing autolysin of the soluble fraction is the soluble lytic transglycosylase (Sl-transglycosylase). This muramidase catalyzes the cleavage of the β-1,4-glycosidic bond between *N*-acetylmuramic acid and *N*-acetylglucosamine with concomitant intramolecular transglycosylation that results in the formation of 1,6-anhydromuramic acid (19). The enzyme has been extensively characterized biochemically (3, 19, 22, 31) and genetically (4, 5). The structural gene (*slt*) of the enzyme (molecular mass, 65 kDa) has been mapped at 99.7 min upstream of the *trpR* gene on the *E. coli* chromosome (5).

Although present in the soluble fraction after mechanical breakage of the cells, the enzyme cannot be released by the osmotic shock procedure (19) known to release periplasmic proteins. This finding seems to be in conflict with the fact that the target of the enzyme, the murein sacculus, is located in the periplasm. To obtain reliable information on the subcellular distribution of the Sl-transglycosylase in situ, we performed a series of immunogold labeling experiments with *E. coli*.

MATERIALS AND METHODS

Bacterial strains and growth conditions. *E. coli* HB101 and W3110 were grown aerobically in complete medium (Luria broth) at 37°C. The growth of the cultures was monitored by measuring the optical density at 578 nm (OD_{578}) in an Eppendorf photometer. Cells were harvested during the logarithmic growth phase at an OD_{578} of 0.5.

Purification of the Sl-transglycosylase. For purification of the Sl-transglycosylase, *E. coli* HB101 was transformed with plasmid pAB58, which contains the *slt* gene under the control of the *lac* promoter (5). In this strain, the enzyme is overproduced by about 30-fold (5). All enzyme purification steps were carried out at 4°C. Enzyme activity was determined with labeled murein sacculi as described below. The bacteria were suspended in 10 mM Tris-maleate buffer, pH 6.8, containing 10 mM $MgSO_4$ and 0.1 mM dithioerythriol at a concentration of 0.5 g (wet weight) of cells per ml. After filtration through a gauze filter, the cells were washed three times with the same buffer. Mechanical breakage of the cells was done in a precooled French pressure cell at 15,000 lb/in^2. The cell envelopes were spun down at 100,000 × *g* for 60 min at 4°C, and the supernatant was used to isolate the enzyme. A first enrichment was accomplished by 40% ammonium sulfate precipitation (19). This was followed by Biogel A 1.5 m gel filtration according to the method of Höltje et al. (19). Further purification was obtained by affinity chromatography, first on Blue Sepharose CL-6B (Pharmacia, Uppsala, Sweden) and then on poly(U)-Sepharose 4B (Pharma-

* Corresponding author.

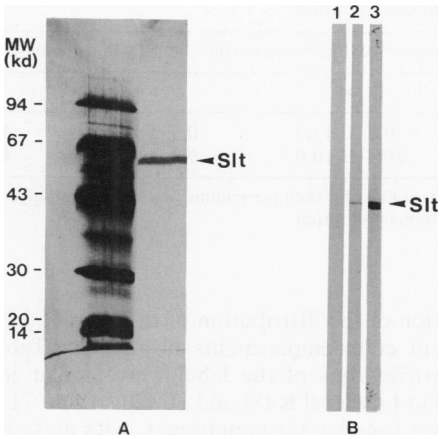


FIG. 1. (A) Gel electrophoresis of purified Sl-transglycosylase (Slt). Electrophoresis was carried out on an ultrathin (0.1-mm-thick) SDS-polyacrylamide (10%) gel, which was stained with silver as described in Materials and Methods. About 5 μg of Slt purified as described in Materials and Methods was applied to the right lane. Low-molecular-weight proteins (Pharmacia electrophoresis calibration kit) were applied to the left lane. The numbers on the left show molecular mass (MW) in kilodaltons. (B) Specificity of anti-Slt serum. Crude cell extracts of *E. coli* were separated by SDS-polyacrylamide (7.5 to 15%) gel electrophoresis on standard 1-mm-thick gels, and the Slt was visualized by the Western blot technique. Lanes: 1, soluble proteins (150 μg) of wild-type *E. coli* HB101 labeled with preimmune serum; 2, same as lane 1 but labeled with specific anti-Slt serum (diluted 1:2,000); 3, soluble proteins (150 μg) of *E. coli* HB101(pAB58) labeled as in lane 2.

cia) according to the method of Kusser and Schwarz (27). Biochemical homogeneity was achieved by preparative sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) (30) on a 7.5 to 15% polyacrylamide gradient gel (thickness, 1 mm) stained with Coomassie blue. The main band at a molecular mass of 65 kDa was cut out, and the protein was regained by electroelution (Biometra). The preparative SDS-PAGE procedure was repeated twice after the first time.

Analytical SDS-PAGE of the final product was performed essentially as described by Neukirchen et al. (33). Ultrathin gels (0.1 mm thick) that were covalently bound to glass plates consisted of a 10% acrylamide resolving gel and a 4% acrylamide stacking gel. After silver staining of the gel (32), only one band corresponding to a molecular mass of 65 kDa was visible, indicating that the enzyme was sufficiently pure (Fig. 1a).

Preparation of polyclonal antibodies against Sl-transglycosylase. Pure enzyme (1 mg of protein in a mixture of 1 ml of phosphate-buffered saline [PBS] and 2 ml of complete Freund's adjuvant) was injected into a rabbit, the preimmune serum of which showed no reaction with *E. coli* proteins (Fig. 1b). The rabbit was injected four times subcutaneously and two times intramuscularly. This procedure was repeated twice at intervals of 4 weeks with incomplete Freund's adjuvant. The rabbit was bled weekly starting 2 weeks after the last injection, and the serum was obtained by centrifugation at 1,000 × *g* for 10 min at 4°C. The supernatant was tested by Western blotting (immunoblotting) (45) of crude total cell extracts of both wild-type *E. coli* HB101 and the overproducer harboring plasmid pAB58 (150 μg of protein per slot) separated electrophoretically on a 7.5 to 15% polyacrylamide-SDS gel (30). The antiserum obtained 8 weeks after the last booster dose reacted specifically with the Sl-transglycosylase at dilutions of up to 1:2,000 (Fig. 1b) and was used in the experiments presented.

Preparation of murein sacculi. Sacculi were prepared as described previously (14); however, both α-amylase and pronase treatments were omitted unless stated otherwise.

Binding of purified Sl-transglycosylase to isolated murein sacculi. Murein sacculi were prepared as described elsewhere, with the preparation including α-amylase and pronase treatment (14), from *E. coli* W3110 grown in Luria broth to an OD_{578} of 0.6 (4.8×10^8 cells per ml). The concentration of the sacculus suspension was estimated according to the titer of the original culture, ignoring losses during preparation. Sacculi (6.4×10^9) were incubated with purified enzyme (3.7 to 10.25 pmol; 83 μg/ml) for 30 min in an ice bath to prevent enzymatic degradation. After centrifugation for 20 min at 200,000 × *g* and 4°C, the amount of enzyme remaining in the supernatant was determined enzymatically with [^{3}H]diaminopimelic acid-labeled sacculi as the substrate (see below). The pellet was resuspended in 4% SDS, boiled for 30 min, washed twice, and subjected to immunolabeling as described below.

Murein sacculi partially digested to allow an inspection of the inner surface were obtained by treating exponentially growing *E. coli* W3110 (OD_{578} = 0.25) with penicillin G at a concentration of 100 U/ml. Cells were harvested 20 min after the onset of lysis (drop in OD readings), and sacculi were prepared as described above.

Enzyme assay of Sl-transglycosylase. Murein sacculi labeled with [^{3}H]A_2pm (0.5 mg of murein per ml; 10^6 cpm/ml) were used as a substrate for the Sl-transglycosylase as described previously (27).

Electron microscopic methods. Bacteria were plasmolyzed and fixed as described previously (46). The cells, suspended in 1 ml of PBS, were washed three times in an Eppendorf centrifuge to remove the fixative, embedded in 2% agarose, dehydrated with ethanol at temperatures decreasing to −35°C, and finally infiltrated first with Lowicryl K4M and then with Lowicryl HM20 (Lowi, Waldkraiburg, Germany). After polymerization under indirect UV light for 24 h and then under direct UV light for 48 h, the samples were thin sectioned with an LKB IV ultramicrotome and used for immunolabeling experiments.

For both sacculi and thin sections, hexagonal copper grids (mesh size, 200 μm; SCI; Science Services) covered with a carbon-coated Formvar film were used. The grids were laid on water drops containing sacculi, incubated for 15 min at room temperature, and finally dried for 30 min at room temperature.

Immunolabeling procedure. Ultrathin sections and sacculi were labeled with specific anti-Sl-transglycosylase serum and colloidal gold (10 nm)-protein A complex (40) according to the method of Tommassen et al. (44). A 1:10 dilution of the antiserum was found to be most suitable. All steps were performed on water drops on parafilm, and all solutions were centrifuged at least for 2 min at 10,000 × *g* to get rid of contaminating particles. The grids were first incubated with 50 mM glycine in PBS for 15 min and then incubated two times for 10 min each time with PBS containing 0.2% gelatin (Merck) and 0.5% bovine serum albumin (Sigma, Munich, Germany) (PBG) to block nonspecific binding. Incubation with the specific antibody was done for 1 to 2 h. The grids were washed six times for 2 min each with PBG and then incubated with the protein A-gold solution (1:50 dilution in PBG) for 1 h. Afterwards, the samples were again washed six times for 2 min each time with PBG, two times for 2 min

TABLE 1. Distribution of gold particles in cell sections

Embedding material[a]	No. of cells analyzed	Avg no. of gold particles/cell section[b]	Relative distribution of gold particles (mean % ± SD) in cell compartments[c]			
			CP	IM	P	OM
HM20	64	22.2 (2.2)	19.7 ± 12.4	30.7 ± 11.4	0.9 ± 2.9	48.7 ± 16.8
K4M	47	34.1 (4.2)	28.4 ± 12.1	26.4 ± 10.6	0.1 ± 1.0	45.1 ± 15.6

[a] Background labeling was 1 to 2 gold grains per mm^2 on HM20 and 2 to 4 gold grains per mm^2 on K4M for both preimmune and anti-Sl-transglycosylase sera.
[b] Values in parentheses are average numbers of gold particles per cell section treated with preimmune serum.
[c] CP, cytoplasm; IM, inner membrane; P, periplasm; OM, outer membrane.

each with PBS, and finally four times for 1 min each with distilled water. The grids were stained with 1% uranyl acetate in distilled water for 5 to 10 min and carefully rinsed with distilled water. The samples were examined and micrographs taken with a Zeiss 902 electron microscope at 80 kV.

The relative distribution of the Sl-transglycosylase among the cell compartments was analyzed in three independent experiments by determining the number of gold particles per compartment in random samples (about 50 per experiment) of thin sections showing an almost circular geometry to exclude extreme tangential sectionings. A bordering area of the width of the gold particles (10 nm) on both sides of the membranes was included in the respective membrane compartment. The counts were made with photographic prints of the electron micrographs. In cases of incomplete separation of inner and outer membranes or blurred appearance, only the legible half of the section was analyzed.

The labeling density of isolated murein sacculi was determined by counting the number of gold particles on the photographic prints in defined fields representing 1-μm^2 surface areas of the sacculus. The average surface area of sacculi was calculated by measuring the width and length of all sacculi analyzed and assuming that the rod shape can be described by a cylinder with two hemispheres.

RESULTS

Specific immunolabeling of Sl-transglycosylase. Purification of the antigen, the Sl-transglycosylase, was performed until biochemical homogeneity according to a silver staining procedure of the enzyme separated by SDS-PAGE was achieved (Fig. 1a). The specificity of the antiserum against the Sl-transglycosylase was demonstrated on Western blots of crude cell extracts. No unspecific cross-reactivity with proteins other than the transglycosylase could be detected (Fig. 1b). Two basic requirements for successful specific labeling were met. First, the preserum showed a low background of not more than 2 to 4 gold grains per cell section, and second, a satisfactory labeling of 20 to 30 grains per cell section was achieved with the specific antiserum (Table 1).

Localization of the Sl-transglycosylase on ultrathin sections. Since it is known that the antigenicity of proteins in specimens prepared for electron microscopy may be affected by the embedding material (23), we tested two different materials, the polar metacrylate resin Lowicryl K4M and the nonpolar Lowicryl HM20. It turned out that the antigenicity of the Sl-transglycosylase was preserved more effectively in specimens embedded in Lowicryl K4M than in those embedded in Lowicryl HM20. This is shown in Table 1 and the electron micrographs in Fig. 2.

The soluble enzyme was mostly present in the cell envelope (Fig. 2). Differentiation into the outer membrane, inner membrane, and periplasm was possible with cells plasmolyzed in 30% sucrose before fixation. A summary of a quantification of the distribution of the specific label among the different cell compartments is given in Table 1. The relative distributions of the label were similar in samples embedded in Lowicryl K4M and HM20. About 71 to 80% of the label was found in the membranes, with most of the label (45 to 49%) associated with the outer membrane. Thus, almost twice the amount present in the cytoplasmic membrane was found in the outer membrane. The periplasm was almost without any label (<1%), and only 20 to 28% of the label was found in the cytoplasm.

Immunochemical determination of Sl-transglycosylase on isolated murein sacculi. The preferential association of the Sl-transglycosylase with the outer membrane was surprising because of the hydrophilic character of the enzyme present in the soluble fraction after fractionation of the cells. It seemed more likely to be due to binding to the murein layer, which in plasmolyzed cells stays with the outer membrane (41), than due to an interaction with the membrane. Therefore, we decided to find out whether or not the enzyme could be cross-linked chemically to the murein. Similar experiments using the cleavable bifunctional cross-linker dithiobis-(succinimidylpropionate) to covalently link murein-associated proteins to the sacculus have been reported previously (29). Surprisingly, we observed a strong immunolabel signal in control sacculi that were not treated with the cross-linker (Fig. 3a and b). It seems that the binding of Sl-transglycosylase to murein sacculi survives boiling in 4% SDS. As shown in Fig. 3, the distribution of the enzyme is random, with no indication of an accumulation in specific areas such as the equatorial zone or the site of constriction (Fig. 3b). With 36 sacculi analyzed, a mean labeling density of 46.7 (±10.7 [standard deviation]) gold particles per μm^2 was determined. This means that about 434 grains were present on an average sacculus. The same results were obtained with two *E. coli* wild-type strains (HB101 and W3110).

The astonishing tight binding of the enzyme to murein prompted us to attempt to bind isolated Sl-transglycosylase onto protein-free, i.e., pronase-treated, sacculi in vitro. The amount of enzyme bound to the murein sacculi at 0°C was determined by immunogold labeling after the sacculi were boiled in 4% SDS. Figure 3c shows that a formation of a surprisingly stable enzyme-substrate complex in vitro is indeed possible. An accurate determination of the average number of gold particles per sacculus was difficult because of the fact that even at a low temperature, some degradation of the sacculi did occur. A value of roughly 1,000 grains per sacculus, which is about twice the value for isolated non-proteinase-treated sacculi, was found (see above). The results are consistent with the number obtained by calculating the loss of enzyme from the supernatant as follows. Binding of increasing amounts of purified enzyme (3.7 to 10.25 pmol) to a limited amount of sacculi showed the characteristics of a saturation curve. With 6.4×10^9 sacculi, saturation was achieved with about 9.25 pmol of enzyme. Thus, on the

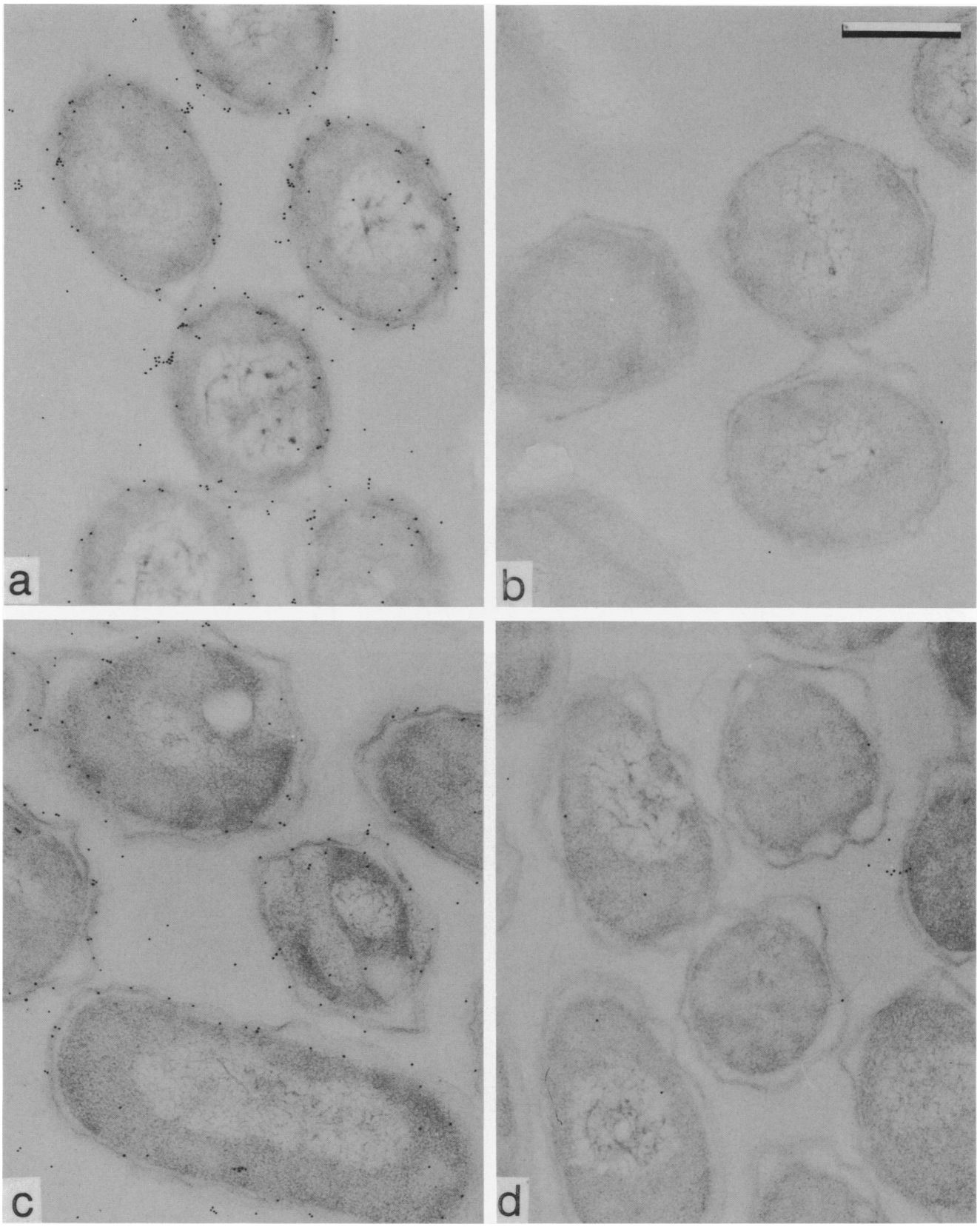
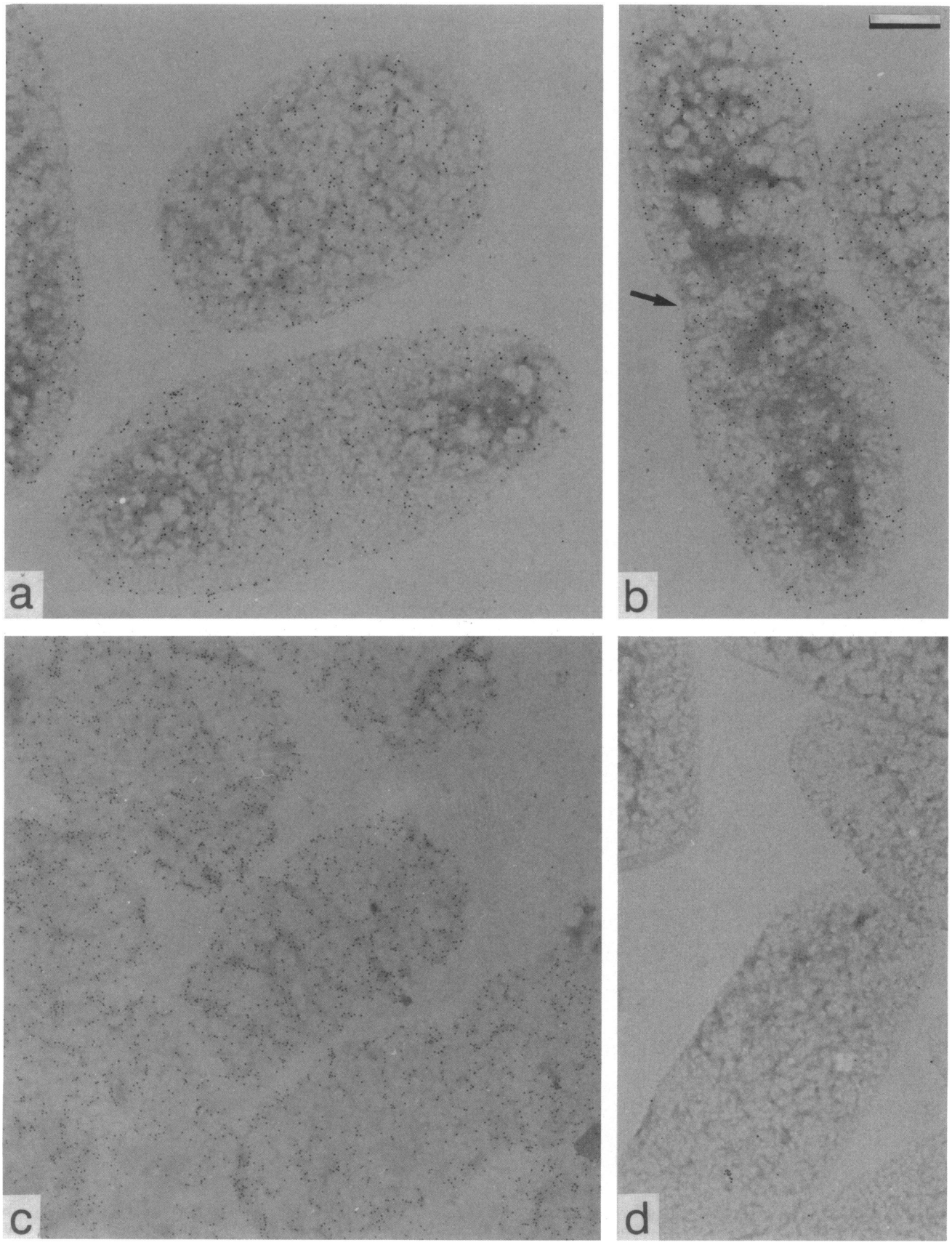


FIG. 2. Immunogold labeling of SI-transglycosylase of ultrathin sections of *E. coli* HB101. Plasmolyzed cells from the exponential growth phase were embedded in Lowicryl K4M (a and b) or Lowicryl HM20 (c and d) and incubated first with anti-SI-transglycosylase serum (a and c) or preimmune serum (b and d) and then with protein A-gold (10 nm) as described in Materials and Methods. Bar, 0.5 μm.


a
b
c
d

average, a maximum of about 870 enzyme molecules [(6.023 $\times 10^{23}$ molecules per mol) $\times$ (9.25 $\times 10^{-12}$ mol)/(6.4 $\times 10^{9}$ sacculi)] could be calculated to have been bound to one sacculus. We conclude that the labeling efficiency is quite high.

Specific binding of Sl-transglycosylase to the outer surface of murein sacculi. Murein hydrolases involved in murein turnover might operate exclusively in the outer parts of the murein sacculus that are no longer needed to bear the surface stress caused by the cellular osmotic pressure (24). Immunoelectron microscopy of broken murein sacculi that allows the inspection of both sides of a sacculus seemed a straightforward approach to investigate this hypothesis with respect to the Sl-transglycosylase. In order to gently rupture sacculi in vivo, we used penicillin G to induce bacteriolysis (see Materials and Methods). As shown in Fig. 4, at a concentration of 100 U of penicillin G per ml, restricted degradation of the murein, preferentially along the equatorial zone of the rod-shaped sacculus, could be achieved, thus allowing a clear determination regardless of whether the inner or the outer side of the sacculus was looked at. As had been expected, the inner surface of the sacculi isolated from lysed cells remained free from immunolabel when these sacculi were labeled with the enzyme-specific antiserum and protein A-gold. The apparent increased labeling along the fringes of the holes in the sacculi (Fig. 4) could be due to either some curling of the rims during preparation of the specimen or binding of cytoplasmic Sl-transglycosylase leaking out at the sites of cellular lysis. In addition, in vitro reconstitution of isolated sacculi degraded by penicillin that had been freed from proteins by pronase treatment specifically spared the inner surface from binding of the Sl-transglycosylase (not shown).

DISCUSSION

The specific binding of the Sl-transglycosylase to the outer surface of the murein sacculus of *E. coli*, as shown in this report, is further evidence for a growth mechanism by which the synthesizing enzymes add new murein from the cytoplasmic membrane to the sacculus while murein-hydrolyzing enzymes degrade the outermost parts of the murein sacculus at the outer membrane site (17, 24, 25). It seems unlikely that the preferential binding to one side of the murein is an artifact produced by penicillin, since recent results (26) show that the overall chemistry of the murein remains almost unchanged, with the exception of a strictly localized degradation of the glycan strands along the equatorial zone of the rod. At the moment, it is not clear whether a specific structural binding site for the enzyme exists at the outside of the sacculus or whether binding to the inner side of the sacculus is sterically hindered by the bulky peptide moieties of the murein (1). Some kind of inside-to-outside growth mechanism has been suggested to take place, even in this gram-negative bacterium, to explain the finding of murein turnover (12, 13) and a number of biochemical results (11, 15, 17, 28, 38) indicating that the murein sacculus of *E. coli* is likely to be a structure containing more than one layer. According to a proposed patching mechanism, small sheets of murein are first hooked underneath the stress-supporting murein layer (17), and then bonds in the murein protected by the newly added murein patches are cleaved precisely. In this way, the new material is pulled into the layer under stress. Material no longer part of the layer under stress is hydrolyzed and reinstalled by a recycling process (12). To avoid uncontrolled cellular lysis, operation of the murein hydrolases exclusively at the outermost surface of the murein sacculus has been postulated (17, 18, 25). For the membrane-bound lytic transglycosylase (31), it has been shown that the enzyme is active only when residing in the outer membrane, although it is also present in the cytoplasmic membrane (18). Preferential binding of the Sl-transglycosylase to the outer side of the murein sacculus seems to represent another way of protecting the cell from incidental autolysis.

A limiting number of binding sites for the Sl-transglycosylase on murein sacculi have been demonstrated in the present report. The total number of gold particles that could be bound to one average sacculus in vitro was about 1,000. By determining the amount of enzyme remaining in the supernatant, we showed that the labeling efficiency seems to be quite high. Being an exomuramidase, the enzyme has been shown to bind to the *N*-acetylglucosamine terminus of the sugar chains of murein (3). The number of bound enzyme molecules is therefore expected to reflect the number of glycan strands occupied by Sl-transglycosylases. On the basis of the finding that the anhydro ends of the glycan strands represent about 4% of the total murein subunits (11) and that one sacculus contains about 2.7 $\times 10^{6}$ molecules of diaminopimelic acid (7), which is equal to the total number of subunits, a calculation reveals that one murein sacculus is made up of roughly 10.8 $\times 10^{4}$ glycan strands. Thus, the Sl-transglycosylase binds to only 4 to 10% of the total glycan strands of one sacculus. This is a reasonable value, since the preferred binding of the enzyme to the outside of the murein sacculus suggests that the enzyme binds specifically to the relaxed outer parts that are no longer withstanding the internal pressure and are actively involved in murein turnover.

The tight binding of the Sl-transglycosylase to its substrate, the murein sacculus, in the periplasm explains a puzzling apparent contradiction about the subcellular localization of the Sl-transglycosylase in *E. coli*: the failure to release this soluble enzyme by an osmotic shock despite its expected localization in the periplasm (19). The extremely strong binding of the enzyme, which quite efficiently survives boiling in 4% SDS, raises the question of whether the interaction could be a covalent one. Being a transferase reaction, the enzymic reaction follows a two-step mechanism. First, the glycosidic bond between *N*-acetylmuramic acid and *N*-acetylglucosamine is cleaved and a muramyl-substrate enzyme intermediate is formed; this intermediate further reacts with an acceptor, leading to the formation of a new glycosidic bond concomitant with the release of the enzyme. In this case, the acceptor for the glycosyl transferase reaction is the free C-6 hydroxyl group of the muramyl group, whose binding results in the formation of an intramo-

FIG. 3. Immunogold labeling of Sl-transglycosylase on isolated murein sacculi of *E. coli* W3110. Murein sacculi, prepared by boiling cells from the exponential growth phase in 4% SDS, were adsorbed onto grids and incubated with specific anti-Sl-transglycosylase serum (a through c) or preimmune serum (d) as described in Materials and Methods. (a and b) Native murein sacculi, i.e., isolated sacculi not treated with pronase; (c) reconstituted murein sacculi, i.e., sacculi treated with pronase to remove all proteins and incubated with purified Sl-transglycosylase at 0°C as described in Materials and Methods. The arrow indicates the site of septum formation. Bar, 0.5 μm.

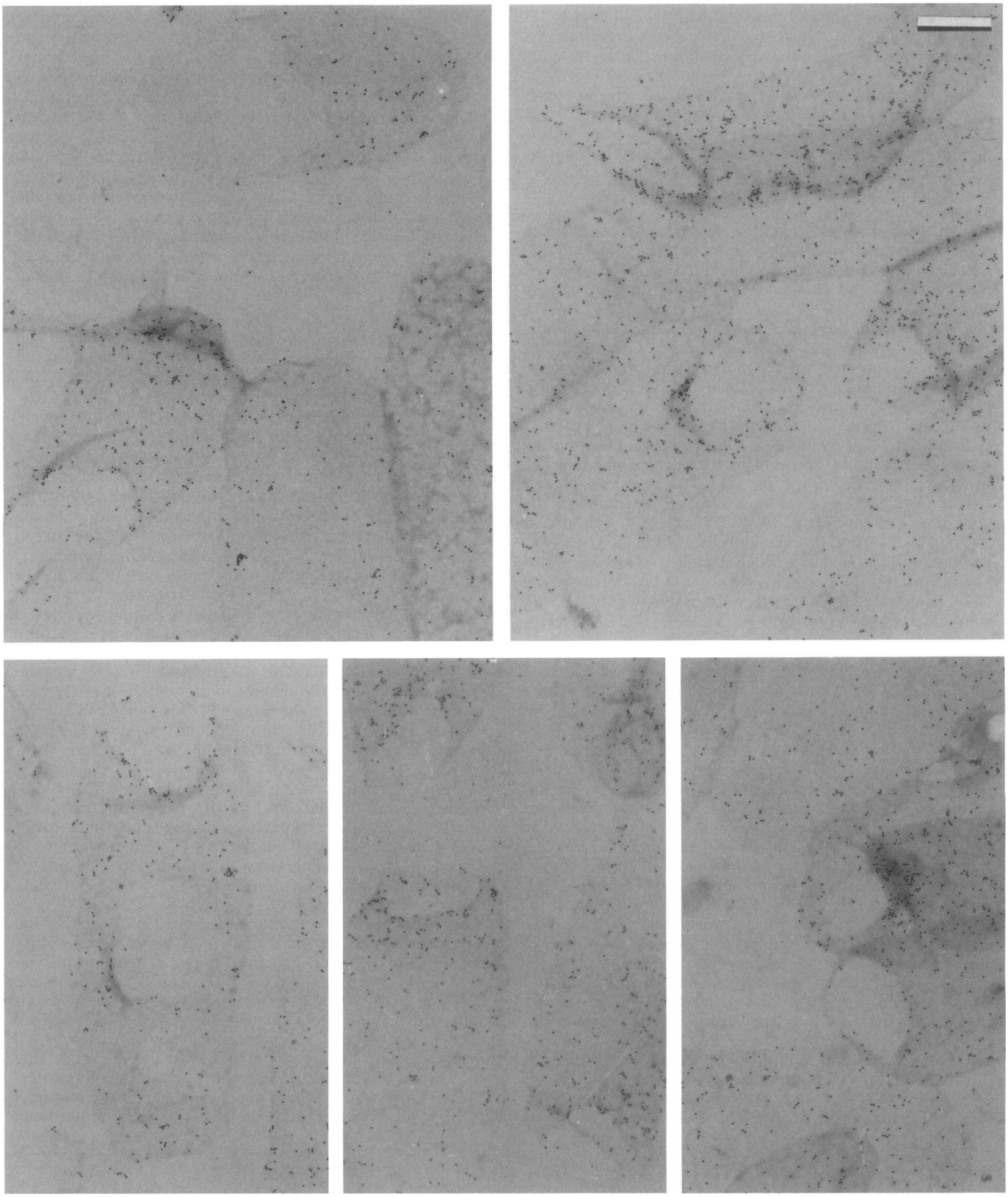


FIG. 4. Immunogold labeling of Sl-transglycosylase on penicillin-damaged murein sacculi. *E. coli* W3110 was treated with 100 U of penicillin G per ml. Murein sacculi were prepared from samples withdrawn at the onset of cellular lysis. Sacculi were adsorbed onto grids and labeled with anti-Sl-transglycosylase serum and protein A-gold as described in Materials and Methods. Bar, 0.5 μm.

lecular 1,6-anhydro ring at the muramic acid (19). As is the case for the penicilloyl-transpeptidase intermediates (6, 10), an analogous muramyl–Sl-transglycosylase intermediate could well be stable enough to be trapped during the isolation. We conclude that, by employing the immunogold labeling technique, we have visualized an enzyme-substrate complex with an electron microscope.

On one hand, binding of the potentially autolytic enzyme to murein sacculi seems extremely risky for the cell, but on the other hand, it allows a steady control of the immobilized exoglycosidase that progressively degrades the glycan strands in an orderly fashion (2, 26). It has, therefore, been speculated that this enzyme is an ideal candidate for a cutting system that cleaves the septum precisely along its middle line during cell division (26, 36). Another quite stable murein-muramidase complex has been described for an analogous exomuramidase in *Streptococcus faecium* (2). This enzyme has been shown to remain bound to the terminal disaccharide fragment as long as it has no chance to switch to a new murein glycan strand. Quite recently, some labeling of wall-bound amidase was also found for SDS-treated walls of *Bacillus subtilis* in an investigation of the intracellular distribution of the autolytic amidase by immunoelectron microscopy (16). Interestingly, septa of dividing cells showed the highest concentration of gold particles, indicating an involvement of the amidase in the process of cutting the septum.

According to the DNA sequence of the *slt* gene (8), the enzyme is expected to be a typical exported protein that is synthesized with a leader peptide and processed by a signal peptidase. It is tempting to speculate that the cell takes advantage of the fact that the synthesis of this autolytic enzyme occurs in a cellular compartment different from the site of the substrate for the enzyme. Control of the export of the enzyme into the periplasm could very well be envisaged. Consistent with this view, it was found that the enzyme could be overproduced in an active form by almost 30-fold without harming the cell (4, 5). Immunoelectron microscopic studies are in progress to find out whether regulation of the action of the Sl-transglycosylase by an export control occurs.

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