Alteration of *Escherichia coli* Murein During Amino Acid Starvation

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We have studied the mechanism by which amino acid starvation of *Escherichia coli* induces resistance against the lytic and bactericidal effects of penicillin. Starvation of *E. coli* strain W7 of the amino acids lysine or methionine resulted in the rapid development of resistance to autolytic cell wall degradation, which may be effectively triggered in growing bacteria by a number of chemical or physical treatments. The mechanism of this effect in the amino acid-starved cells involved the production of a murein relatively resistant to the hydrolytic action of crude murein hydrolase extracts prepared from normally growing *E. coli*. Resistance to the autolysins was not due to the covalently linked lipoprotein. Resistance to murein hydrolase developed most rapidly and most extensively in the portion of cell wall synthesized after the onset of amino acid starvation. Lysozyme digests of the autolysin-resistant murein synthesized during the first 10 min of lysine starvation yielded (in addition to the characteristic degradation products) a high-molecular-weight material that was absent from the lysozyme-digests of control cell wall preparations. It is proposed that inhibition of protein synthesis causes a rapid modification of murein structure at the cell wall growth zone in such a manner that attachment of murein hydrolase molecules is inhibited. The mechanism may involve some aspects of the relaxed control system since protection against penicillin-induced lysis developed much slower in amino acid-starved relaxed controlled (relA) cells than in isogenic stringently controlled (relA<sup>+</sup>) bacteria.

It has been known for a long time that non-growing bacteria are protected against the lytic and bactericidal effects of penicillins (12, 26). In *Escherichia coli*, starvation of amino acid auxotrophs for the required amino acid was shown to make the cells resistant to penicillin-induced lysis (3, 17). The biochemical basis of this penicillin "tolerance" in nongrowing bacteria is not well understood. However, the interaction between penicillin molecules and the primary biochemical targets of the antibiotic does not appear to be involved, since radioactive penicillin has the same access and affinity to the penicillin-binding proteins of growing and chloramphenicol-inhibited pneumococci (36). Thus, the requirement for "growth" (protein synthesis) is more likely to be some feature of the coupled secondary mechanisms such as autolytic activity that in several bacterial species were proposed to be directly responsible for the lysis and loss of viability of the penicillin-treated cells (33, 34).

There are several lines of evidence suggesting the role of autolytic enzymes (murein hydrolases) in the penicillin-induced lysis of *E. coli* (15, 16, 29, 35). Therefore, we have examined the effect of amino acid starvation on the activity of the autolytic system of *E. coli*. The major findings that we report here are that the murein synthesized during amino acid starvation was resistant to the autolysins (murein hydrolases) of the bacterium and that over the first 30 to 60 min of amino acid starvation the murein synthesized before starvation was also converted to a resistant form.

MATERIALS AND METHODS

**Bacteria and culture conditions.** The strains of *E. coli* used were W7 (lys<sup>+</sup> dap<sup>+</sup>) (27), CP79 (thr his arg thi relA<sup>+</sup>) and the isogenic (relA<sup>−</sup>) strain CP78 (5). The bacteria were cultured with vigorous aeration at 37°C in M9 medium (23) supplemented with glucose (2 mg/ml) and, where required, amino acids (40 μg/ml), 2,6-diaminopimelic acid (DAP, 4 μg/ml), and thiamine (1 μg/ml). Culture turbidity was measured with a Coleman Neph-Colorimeter (24).

Murein was radioactively labeled by adding (N<sup>1</sup> + meso)-2,6-diamino-[U-<sup>14</sup> C]pimelic acid (1.5 Ci/mmol; Amersham Corp., Arlington Heights, Ill.) to the medium; 10 μCi of [H]DAP was added per ml of culture medium, giving a final DAP concentration of 5.2 μg/ml.

To starve the cells for lysine, they were filtered onto a membrane filter (0.45-μm pore size; Millipore Filter Corp., Bedford, Mass.), washed three times with 2.5 volumes of prewarmed M9 basal salts, and then resuspended in fresh, prewarmed medium lacking lysine.

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Murein isolation. Samples from cell cultures were immediately placed into an equal volume of boiling 1% sodium dodecyl sulfate. After boiling for 10 min, each sample was centrifuged (35,000 × g, 60 min, 25°C) to pellet the "sacculi," which consist of murein and lipoprotein material (28). This material will be referred to as murein or cell wall material throughout the paper. The pellet was washed three times with 10 ml of 0.01 M Tris buffer (pH 7.5) containing 0.02 M NaCl. If trypsin treatment was required to remove the lipoprotein, the pellet was suspended in 1 ml of 0.01 M Tris buffer (pH 8.1) containing 0.01 M CaCl₂, 0.1 ml of trypsin (1 mg/ml; Worthington Biochemical Corp., Freehold, N.J.) was added, and the sample was incubated overnight at 37°C (1). The residual murein was then boiled in sodium dodecyl sulfate and washed with Tris buffer as described above.

Murein degradation in situ. Samples (1 to 3 ml) from a culture of E. coli W7 (10⁴ to 2 × 10⁶ cells per ml) grown in M9 medium containing [¹⁴C]HDAF were pipetted into 5 ml of precooled (0°C) M9 basal salts, and the cells were collected by centrifugation (12,000 × g, 10 min). The cells were then treated in one of four ways known to trigger hydrolyses. (i) In the trichloroacetic acid treatment, cells were resuspended in 0.5 ml of 0.05 M Tris buffer (pH 7.4, 0°C) containing 0.08 M KCl, 7 mM MgCl₂, 2 mM ethylene glycol-bis-(β-aminoethyl ether)-N,N-tetraacetic acid, and 1 mM mercaptoethanol. To this buffer, 20 μl of trichloroacetic acid (50%, 0°C) was added, and the suspension was incubated at 0°C for 10 min. The cells were then washed twice with 10 ml of the above Tris buffer (0°C) and finally resuspended in 0.5 ml of 0.01 M Tris-maleate buffer (pH 6.2, 0°C) containing 0.01 M Mg²⁺ (10). (ii) In the EDTA-Triton X-100 treatment, cells were suspended in 0.5 ml of 0.01 M Tris buffer (pH 7.8) containing 5 mM EDTA and 2% Triton X-100 (Beckman Instruments, Fullerton, Calif.) and incubated at 37°C for 30 min to solubilize the cell membranes (4). The samples were cooled to 0°C, and Mg²⁺ (15 mM, final concentration) was added. (iii) For freeze-thawing, bacteria were suspended in 0.5 ml of 0.01 M Tris-maleate buffer (pH 6.2) containing 0.01 M Mg²⁺ and subjected to three cycles of freezing (−70°C, 2 min) and thawing (37°C, 2 min) (7). (iv) For mechanical disruption, bacterial envelopes were prepared by suspending the bacteria in 3.5 ml of the same 0.05 M Tris buffer (0°C) used in the trichloroacetic acid treatment, adding 3.5 g of glass beads (0.17-mm diameter; Minnesota Mining & Manufacturing Co.), and shaking them for 10 min at 60 Hz (The Mickel Laboratory Engineering Co., Gomshall, England). The envelopes were collected by centrifugation (35,000 × g, 60 min) and resuspended in 0.5 ml of 0.01 M Tris-maleate buffer (pH 6.2) containing 0.01 M Mg²⁺.

These treatments are known to initiate cell wall degradation in E. coli grown in complex media. However, autolytic phenomena are known to be influenced by growth conditions, and it was important to test the effectiveness of these triggering methods in our system with bacteria grown in the minimal medium. Control experiments of this type (data not shown) have reproduced essentially the same results as those obtained with cells grown in complex media.

Murein degradation was measured at 37°C. In most of the experiments, the amount of undegraded murein present at any given time was measured by removing 50-μl samples and precipitating them on 2.5-cm-diameter 3 MM Whatman filter disks (W.R. Balston, England) with cold trichloroacetic acid (21). In some experiments (see Fig. 5, for instance), an alternative assay was used; radioactively labeled murein and murein hydrolyses were incubated, and 200-μl samples were removed at intervals into centrifuge tubes containing formaldehyde (final concentration, 10%) and albumin (final concentration, 100 μg/ml) and radioactivity released into the supernatants was determined after centrifugation at 10,000 × g for 20 min in an Eppendorf microcentrifuge (13).

Preparation of crude extract of murein hydrolase. E. coli cells from 50-ml cultures (3 × 10⁸ cells per ml) were collected by centrifugation and disrupted, and the envelopes were pelleted as described in the previous section; however, after pelleting, murein hydrolases adsorbed to the envelopes were solubilized in 1 ml of 0.01 M Tris buffer, pH 7.8) containing 5 mM EDTA and 2% Triton X-100 at 0°C (8).

Enzyme assays. The murein hydrolase activity in the solubilized envelope preparation was assayed in 0.01 M Tris-maleate buffer (pH 6.2) containing 0.01 M Mg²⁺ and 1% Triton X-100 at 37°C with [³H]murein as a substrate (0.05 mg/ml, final concentration). Lysozyme activity was measured with the same system. The rate of murein degradation was measured as described in Höltje et al. (13).

Protein determination. Protein concentration was determined as described by Lowry et al. (20) with bovine serum albumin as the standard.

RESULTS

Penicillin-induced lysis and amino acid starvation. Addition of a high concentration of penicillin to a growing culture of E. coli is known to cause rapid lysis (Fig. 1). Lysis was extensive after 30 min (see control culture; viable cells per milliliter had decreased 30-fold) (data not shown). A portion of the cells was transferred by rapid filtration into lysine-free growth medium and distributed into a number of separate culture tubes which were incubated at 37°C. Individual tubes received benzylpenicillin at various times after resuspension in the lysine-free medium. Figure 1 shows the rapid drop in the rate of penicillin-induced lysis after the onset of amino acid starvation; cells starved for 15 min or more before addition of penicillin showed virtually no lysis, and most of the cells (70%) were still viable after 60 min of penicillin treatment.

Effect of amino acid starvation on murein degradation. A possible cause for decreased
INHIBITION OF AUTOLYSIS IN E. COLI

FIG. 1. Development of resistance to penicillin-induced lysis during lysine starvation. Cultures of E. coli W7, growing in minimal medium supplemented with DAP and lysine, were filtered and reincubated in lysine-free medium for 20 min (■), 15 min (□), 5 min (○), and 0 min (△) before addition of penicillin G (1 mg/ml) to the medium (t = 0 min). One control culture (x − x) received penicillin in full growth medium. Change in the light scattering (nephelometric value) of the cultures was followed after the addition of penicillin.

FIG. 2. Effect of lysine starvation on the rate of murein hydrolysis. After several generations of growth in minimal medium containing [3H]DAP and lysine, the bacteria were filtered and reincubated into fresh medium containing [3H]DAP but no lysine. Periodically, samples were removed, the cells were mechanically disrupted, and the envelopes were isolated. The envelopes were suspended in buffer and incubated at 37°C. (A) The amount (per cent) of undigested murein was plotted against incubation time for each envelope preparation: growing cells (○), cells after 10 min of lysine starvation (●), 20 min of starvation (□), 30 min of starvation (■), 60 min of starvation (△), 90 min of starvation (▲), and 120 min of starvation (▽). From these data, the rate of murein digestion by each envelope preparation, relative to that of the envelopes from growing cells, was estimated (B).

Analysis of lysine-starved E. coli W7 in the presence of penicillin was a reduced rate of cell wall (murein) breakdown. The capacity of these cells to degrade their own murein after lysine starvation was tested by several methods (see Materials and Methods) that are known to initiate ("trigger") autolytic cell wall degradation in E. coli (10). The cells, after 5 to 120 min of lysine starvation, were mechanically disrupted, and the envelopes were isolated and incubated under conditions that led to autolytic wall degradation in envelopes isolated from unstarved bacteria. Figure 2 shows the decline in the rate of wall degradation in the envelopes from the amino acid-starved cells. Similar results were obtained with the trichloroacetic acid activation method (data not shown). In both cases, during the initial 30 min of lysine starvation, there was a rapid decline in the rate at which the cells were able to degrade their murein.

The effect was not unique to lysine starvation. Methionine starvation can be produced in E. coli W7 by adding excess threonine to the normal growth medium (30); methionine starvation also resulted in a decrease in the rate of murein degradation (Fig. 3). Similar, although less marked, effects were observed after the addition of α-methylglucoside (glucose starvation) (9) or chloramphenicol (100 µg/ml) to the medium (data not shown).

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The effect of amino acid starvation could be reversed. Cells were given [³H]DAP during lysine starvation and then returned to a medium containing lysine. Samples were then removed and treated with cold trichloroacetic acid (Fig. 4). After these cells were returned to a medium with lysine, their capacity to degrade murein increased slowly during the first 30 min; after 60 min, however, the rate of murein degradation was equal to that of typical exponentially growing cells.

Mechanism of the autolytic defect in the amino acid-deprived bacteria. The lower rate of murein hydrolysis in lysine-starved cells could be due either to a change in the murein, making it less sensitive to the murein hydrolases in the cell envelope, or to decreased activity of the murein hydrolases in the cell envelope.

Specific activity of murein hydrolases during amino acid starvation. Amino acid starvation of E. coli is known to induce protein turnover in many strains (25), and such a mechanism could cause a loss of murein hydrolase activity during amino acid starvation. The specific activities of crude murein hydrolase preparations (cell envelopes solubilized by EDTA-Triton X-100 treatment) obtained from lysine-starved cells were tested with [³H]DAP-labeled murein from log-phase E. coli cells (Table 1). There was no significant drop in the specific activity of the murein hydrolases for the first 30 min of lysine starvation; subsequently (60 to 180 min), there was an apparent decline. Thus, the rapid response of strain W7 to lysine starvation cannot be explained by a decline in murein hydrolase activity.

Decline in the autolysin sensitivity of murein isolated from bacteria starved of amino acids. A bacterial culture was grown in full medium supplemented with radioactive DAP to label the murein. The cells were transferred to fresh medium free of lysine and containing fresh radioactive DAP. Samples were removed for the preparation of murein at various times after the transfer to the lysine-free medium. The murein samples were then treated with crude murein hydrolase extract prepared from normally growing E. coli, and the rate of enzymatic cell wall degradation was determined.

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**Fig. 3.** Effect of methionine starvation on the rate of murein hydrolysis. E. coli W7 was grown in minimal medium supplemented with lysine and [³H]DAP; after several generations of growth, threonine (400 μg/ml) was added to the culture. Samples were removed just before threonine addition (O), and 5 min ( ●), 15 min ( □), 30 min ( ■), 60 min ( △), and 90 min ( ▲) thereafter. The cells were treated with cold trichloroacetic acid (to trigger murein degradation [10]) washed, and incubated in buffer at 37°C; the amount (per cent) of undigested murein was plotted against incubation time. The cells recover somewhat from the addition of excess threonine and commence growing at a slower rate; this is perhaps why the rate of murein hydrolysis in the 60-min and 90-min samples was higher than that in the 30-min sample.

**Fig. 4.** Rate of murein hydrolysis after readdition of lysine to a lysine-starved culture. After several generations of growth in minimal medium, the cells were washed and placed in fresh medium which contained [³H]DAP and lacked lysine. After 60 min, the cells were washed again, placed in medium containing unlabeled DAP and no lysine, and incubated for another hour. Lysine was then added to the culture, and samples were removed after 0 min (O), 5 min ( ●), 15 min ( □), 30 min ( ■), 60 min ( △), and 90 min ( ▲), treated with cold trichloroacetic acid, washed, and incubated at 37°C. The percentage of undigested murein in these samples has been plotted against the time of incubation.
TABLE 1. Effect of lysine starvation on the activity of the murein hydrolases of the cell envelope

<table>
<thead>
<tr>
<th>Lysine starvation (min)</th>
<th>Murein hydrolase activity (mg of murein digested per h per mg of protein)</th>
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</thead>
<tbody>
<tr>
<td>Control (growing cells)</td>
<td>0.338</td>
</tr>
<tr>
<td>5</td>
<td>0.320</td>
</tr>
<tr>
<td>15</td>
<td>0.324</td>
</tr>
<tr>
<td>30</td>
<td>0.335</td>
</tr>
<tr>
<td>60</td>
<td>0.283</td>
</tr>
<tr>
<td>120</td>
<td>0.220</td>
</tr>
<tr>
<td>180</td>
<td>0.203</td>
</tr>
</tbody>
</table>

* Envelopes, prepared from growing bacteria and from lysine-starved bacteria, were suspended in 10 mM Tris (pH 7.8) containing 5 mM EDTA and 2% Triton X-100 at a protein concentration of 1 mg/ml. The autolysin test mixtures (500 μl) contained 100 μl of each envelope preparation.

(Fig. 5). There was a rapid and progressive drop in the hydrolase sensitivity of the murein samples isolated from the culture undergoing amino acid starvation; however, the murein remained sensitive to lysozyme.

After the onset of amino acid starvation, murein synthesis continued but at a much slower rate than in growing cells (14). To determine whether this murein was especially autolysin resistant, the experiment described above was repeated with bacteria that were labeled with [3H]DAP immediately after the start of lysine starvation. The change in murein synthesized prior to lysine starvation was also measured by giving a second culture a 60-min exposure of [3H]DAP immediately after the start of lysine starvation. The third culture received [3H]DAP continuously both before and during lysine starvation (Fig. 5A and B). Murein samples were prepared, and the rates of hydrolysis of these samples by crude murein hydrolase were compared. Invariably, lysine starvation was found to cause a progressive decline in the hydrolase-sensitivity of the murein samples. However, loss of sensitivity was most rapid and most extensive for the murein synthesized after the onset of lysine starvation (culture 1). The resistance of murein synthesized before lysine starvation slowly increased until, after 30 min, it appeared to be equal to that of murein synthesized during starvation. Figure 5B shows the decline in hydrolase sensitivity expressed as relative rates of murein degradation (relative to the rate of degradation in the appropriately labeled control murein sample).

The treatment used to isolate murein from E. coli cells, boiling in 1% sodium dodecyl sulfate, removes all other cell components except the covalently bound lipoprotein (1). To determine whether the lipoprotein contributed to the resistance of murein from lysine-starved cells, the lipoprotein was removed by trypsin treatment (1). Subjecting this murein to murein hydrolase digestion showed that the trypsin pretreatment increased the sensitivity of murein from growing cells to autolysins, but not that of murein from lysine-starved cells (Table 2). This result indicates that it is the structure of the murein itself from lysine-starved cells which makes it autolysin resistant.

FIG. 5. Digestion of murein from lysine-starved cells by murein hydrolases from growing cells. Three 20-ml cultures of E. coli were used; each was grown to mid-log phase in the presence of lysine and then the lysine was removed. Culture 2 was labeled continuously with [3H]DAP; culture 3 was labeled with [3H]DAP only before the start of lysine starvation; culture 1 was labeled with [3H]DAP only after the start of lysine starvation. Samples (2.5 ml) were removed periodically from all three cultures after the start of lysine starvation; the murein was isolated and then resuspended in 0.9 ml of 0.01 M Tris-maleate buffer (pH 6.2, with 0.01 M MgCl2 and 1% Triton X-100). Approximately 2.5 μg of murein was obtained from each sample; 50 μg of unlabeled murein from a log-phase culture of W7 was added to ensure a constant substrate concentration. Each sample was treated with 100 μl of a crude murein hydrolase preparation obtained by treating cell envelope from log-phase E. coli cells with detergent. (A) The rate of hydrolysis of the murein samples taken from the continuously labeled culture (3) is given; the percent of murein which has been hydrolyzed is plotted against the time of incubation. Symbols: ○, no lysine starvation; ●, 5-min lysine starvation; □, 15-min starvation; ■, 30-min starvation; Δ, 60-min starvation; ▲, 90-min starvation. Similar data were collected (not shown) for cultures 1 and 2 as well. From these data relative hydrolysis rates were calculated and plotted against the time of lysine starvation (B). The hydrolysis rate of each murein sample is given as a fraction of the hydrolysis rate of a murein from cells not starved for lysine. Symbols: △, culture 3; □, culture 2; ○, culture 1. In the latter, the drop in murein hydrolysis sensitivity was extremely rapid (see earliest time point).
strain also responded to amino acid starvation as W7 had, similar experiments were performed with strain CP 78. Cultures were labeled for 2 h with \[^{3}H\]DAP either during exponential growth or during valine-induced isoleucine starvation. The isolated murein was treated with trypsin to remove the covalently bound lipoprotein since these strains of \textit{E. coli} convert \[^{3}H\]DAP into \[^{3}H\]lysine which can be incorporated into the lipoprotein. Each murein sample was digested with a murein hydrolase preparation obtained from a growing culture of the same strain. In CP 78, the murein isolated from isoleucine-starved cells was digested about half as rapidly as the murein from growing cells (Table 2).

Analysis of the lysozyme digest of murein synthesized by growing and by lysine-starved \textit{E. coli}. We examined the nature of cell wall degradation products solubilized during treatment of control and lysine-starved murein with egg white lysozyme. Analysis by gel filtration showed that both preparations of murein yielded the expected major lysozyme products (C3 and C6 in reference 29, corresponding to peaks D and E, respectively, in Fig. 6). However, the digest of murein from the amino acid-starved cells contained higher amounts of C3 (bis-disaccharide tetrapeptide) relative to C6 and showed the presence of a material of relatively high molecular weight (distributed in at least three

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**Fig. 6.** Chromatography of lysozyme digests of murein isolated from lysine-starved cells and from growing cells. Two 100-ml cultures were labeled with \[^{3}H\]DAP for 2 h. Culture 1 (•) was labeled during exponential growth; culture 2 (○) was labeled during lysine starvation. The murein was isolated, treated with trypsin to remove the lipoprotein, washed, and then digested overnight with lysozyme (1 mg of lysozyme per ml in 0.01 M ammonium acetate, pH 6.5) at 37°C. After hydrolysis, the sample was placed on a column (1.5-cm diameter, 30-cm long) packed with Sephadex G50 (superfine) and eluted with 0.05 M Tris buffer (pH 7.5 containing 0.2 M NaCl) at a rate of 2 ml/hr. Four unlabeled controls were run with both samples: blue dextran (●), C3 (peak D), C6 (peak E), and DAP (▲). Peaks representing higher-molecular-weight material were labeled A, B, and C. The disaccharide tetrapeptide (C6) and its dimer (C3) were prepared from \textit{E. coli} W7 cell wall digests by a published procedure (29).
TABLE 2. Decreased autolysin susceptibility of murein isolated from amino acid-starved cultures of E. coli strains W7 and CP 78

<table>
<thead>
<tr>
<th>Cells</th>
<th>Strain W7</th>
<th>Strain CP 78</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>25</td>
<td>14</td>
</tr>
<tr>
<td>Pretreated with trypsin</td>
<td>34</td>
<td>14 14 7</td>
</tr>
</tbody>
</table>

*[^3]H*murein from approximately 10^6 cells (ca. 2.5 µg) was mixed with 450 µl of 10 mM Tris-maleate buffer (pH 6.2) containing 10 mM Mg^2+*, 1% Triton X-100, and 25 µg of unlabeled murein obtained from growing strain W7 cells; 50 µl of a murein hydrolase (autolysin) preparation (1 mg of protein per ml) was added to the test mixture. Release of acid-soluble counts was measured as described in Høltje et al. (13).

overlapping size classes—A, B and C in Fig. 6). Approximately 30% of the label from the lysine-starved cells was in fraction A; whereas very little, if any, of the label from exponentially growing cells was in this fraction.

Effect of amino acid starvation on the bactericidal and lytic effects of penicillin in stringently controlled (relA*) and in relaxed (relA) strains of E. coli. To test whether or not the mechanism of protection against penicillin-induced lysis involved the relaxed control system (2), we have carried out simple culture lysis experiments with the isogenic pair of E. coli strains CP 78 (relA*) and CP 79 (relA). Figure 7 shows that in the relA strain the protective effect of amino acid starvation against penicillin-induced lysis and loss of viability has developed only after much longer periods of amino acid starvation as compared with the relA+ strain.

DISCUSSION

Inhibition of protein synthesis or, generally, inhibition of bacterial growth during penicillin treatment is known to inhibit the irreversible antibacterial effects of penicillin (3, 12, 17, 26). This antagonistic effect may even have clinical consequences in chemotherapy (18). This phenomenon has often been interpreted to represent an "unbalanced" growth of the cytoplasmic mass generating the mechanical and osmotic pressure needed to rupture the "weakened" cell wall of penicillin-treated bacteria. However, an increasing body of evidence indicates that the uncontrolled activity of autolytic enzymes plays an important role in the penicillin-induced lysis of E. coli (6, 7, 15, 16, 19, 26, 29, 35) and other bacteria as well (31–33). Thus, it appeared plausible that conditions specifically suppressing the irreversible effects of penicillin do so by interfering in some manner with the expression of uncontrolled hydrolase activity. The observations described in this paper indicate that this is indeed the case. Apparently, E. coli cells have a mechanism that can rapidly modify the structure of newly made murein in bacteria that have stopped making protein. We suggest that attachment of murein hydrolase molecules to such a murein is blocked, causing a decreased susceptibility to autolytic degradation. The resistance of biosynthetically "old" portions of murein, also observed in these experiments, may be explained by assuming that most of the hypothetical murein hydrolase attachment sites reside within newly made murein. There is evidence suggesting that the incorporation of new wall material and initiation of autolytic wall degradation may be localized to the equatorial area of E. coli (11, 28). It is tempting to speculate that the hydrolase attachment site also reside in this equatorial region of the murein sacculi.

The chemical nature of the structural change that occurs in the murein of amino acid-starved bacteria remains to be elucidated. Lysozyme digests of murein synthesized during amino acid starvation contained a higher proportion of fraction C3 and substantially more higher-molecular-weight material (peaks A, B and C in Fig. 6) than digests of murein synthesized in normally growing bacteria. A crude estimate of the molecular size of these materials (based on their elution from the Sephadex column) would suggest the range of 3,000 to 10,000. A possible structure
for this material would be cross-linked oligomers (3 to 10 U) of the normal disaccharide-peptide units of murein. A recent report indicates a substantially increased degree of crosslinking in the cell walls of *E. coli* undergoing amino acid starvation (R.E. Harkness and E.F. Ishiguro, Abstr. Annu. Meet. Am. Soc. Microbiol., 1980, K199 p. 159). Another specific alternative would involve O-acetylation of the muramic acid residues. Significant change in the degree of murein O-acetylation has been noted in the cell walls of a proteus L-form (22).

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LITERATURE CITED


