Effects of Moenomycin on *Escherichia coli*

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(Received 16 June 1986; revised 10 September 1986)

The antibiotic moenomycin is a valuable biochemical tool for studying the metabolism of peptidoglycan and the autolytic system in *Escherichia coli*, since as a specific inhibitor of peptidoglycan polymerases it can efficiently promote cell lysis. In liquid media the bacteriolytic effect on *E. coli* K12 was dependent on the concentration of moenomycin, on growth phase and on growth rate. Before lysis cells underwent major morphological alterations. In sucrose-containing medium complete transformation to osmotically sensitive spheroplasts was easily achieved by addition of moenomycin. The minimum inhibitory concentration of the antibiotic varied with the strain of *E. coli* and was highly dependent on the growth medium. A tritiated derivative of moenomycin, \[^{3}H\]decahydromoenomycin A, was prepared and found to have the same inhibiting efficiency. Its binding to *E. coli* membranes and membrane proteins was investigated. The absence of irreversible binding suggested that moenomycin might be a competitive inhibitor of the peptidoglycan polymerases. Spontaneous moenomycin resistant variants were isolated at a frequency of about 10\(^{-9}\).

INTRODUCTION

Antibiotics of the moenomycin group are phosphoglycolipid compounds produced by various species of *Streptomyces* (Huber, 1979). They are mostly obtained as complexes of very similar components. Moenomycin A is the main constituent of the product isolated from *Streptomyces bambergiensis* (Huber, 1979). Its structure was determined by Welzel et al. (1981, 1983). A very similar structure was reported for the related antibiotic pholipomycin (Takahashi et al., 1983).

Moenomycin interferes with the synthesis of the bacterial cell wall (Huber, 1979). With cell-free systems from *Escherichia coli*, moenomycin inhibits the formation of the linear glycan strands of peptidoglycan when the membrane intermediate N-acetyl-glucosaminyl-N-acetylmuramyl-(pentapeptide)-pyrophosphoryl-undecaprenol is used as substrate (van Heijenoort et al., 1979; van Heijenoort & van Heijenoort, 1980). More specifically, penicillin-binding protein 1b (PBP 1b) is the polymerase responsible for this reaction (Suzuki et al., 1980); as a purified protein it can catalyse in vitro a transglycosylation reaction with the same membrane substrate and this reaction is inhibited by moenomycin (Suzuki et al., 1980; Nakagawa et al., 1984). With both the cell-free system and purified PBP 1b, moenomycin has an inhibitory effect at concentrations between 10\(^{-8}\) and 10\(^{-7}\) M (van Heijenoort et al., 1979; Suzuki et al., 1980; van Heijenoort & van Heijenoort, 1980; Nakagawa et al., 1984). The in vitro polymerization catalysed by two other peptidoglycan polymerases of *E. coli*, PBP 1a and PBP 3, is also sensitive to moenomycin, or at least to a related antibiotic like macarbomycin (Ishino & Matsuhashi, 1981; Matsuhashi et al., 1981). Furthermore, moenomycin can very efficiently promote the autolysis of *E. coli* cells (Leduc et al., 1982, 1985). Owing to its specific action on essential

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*Abbreviations*: MIC, minimum inhibitory concentration; MBC, minimum bactericidal concentration.
peptidoglycan polymerases, moenomycin should be a valuable biochemical tool in the study of peptidoglycan metabolism and its correlations with the autolytic process. It is therefore important to know the general effects of moenomycin on E. coli. Some of these effects are investigated in this paper.

METHODS

Bacterial strains and growth conditions. Most experiments were done with E. coli K12 HfrH (Leduc et al., 1982). E. coli K12 C600 was kindly provided by R. Devoret (CNRS, Gif-sur-Yvette, France) and E. coli W7 (A,p-lys-) by C. Lazdunski (CNRS, Marseille, France). E. coli B and E. coli K235 were strains from the American Type Culture Collection (Rockville, Md., USA). E. coli 0111:B4 was from the Pasteur Institute Collection (Paris, France). E. coli JA200(pLC 19-19) was from Dr Clarke (Clarke & Carbon, 1976). Bacteria were grown either in M63 minimal medium supplemented with 0.2% (w/v) glucose and thiamin (0.5 mg 1−1), in rich medium containing (g 1−1) pasteone (Institut Pasteur Production) (10), yeast extract (Institut Pasteur Production) (10), KH2PO4 (5-6) and K2HP04 (29), supplemented with thiamin (0.5 mg 1−1), or in Penassay AM2 broth (Difco). Cultures (40 ml of medium in 200 ml Erlenmeyer flasks) were incubated at 37 °C with vigorous agitation on a gyrotrary shaker. Growth was followed by measuring the optical density of cultures at 600 nm with a Gilford spectrophotometer (model 240). The first order rate constant of autolysis (k) was calculated as described by Leduc et al. (1982). [3H]Penicillin G (880 GBq mmol−1) was kindly provided by Dr L. Gutman (Laboratoire de Microbiologie Médicale, Université Paris VI, France).

Spheroplast formation. Early exponential phase cells grown in minimal or rich medium (40 ml) up to a cell concentration of about 108 cells ml−1 were harvested at room temperature by centrifugation at 10000 g for 10 min. Cells were resuspended in warm growth medium (20 ml) containing 20% (w/v) sucrose and incubated at 37 °C with slow agitation. After 5 min 200 µl moenomycin solution (1 mg ml−1) was added. Samples were taken at different times for examination by phase-contrast microscopy, and optical density determination before and after dilution (100 µl in 900 µl of water).

Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values. The MIC values of moenomycin against various E. coli strains were determined by use of serial twofold dilutions of the antibiotic. The inoculum was from an overnight culture in the relevant liquid medium. Liquid medium (2 ml) contained about 106 bacteria ml−1 plus moenomycin at concentrations ranging from 2 to 512 µg ml−1. These tubes, together with controls, were incubated for 24 h at 37 °C. The lowest concentration that prevented visible growth was taken as the MIC. The MBC of moenomycin against E. coli K12 HfrH was determined by use of serial twofold dilutions of the antibiotic. The inoculum was from an overnight culture in liquid rich medium. Rich medium (2 ml) contained about 106 bacteria ml−1 plus moenomycin at concentrations ranging from 1 to 8 × MIC. These tubes, together with controls, were incubated for 18 h at 37 °C. A sample (10 µl) from each tube was transferred onto an antibiotic-free Penassay medium agar plate, which was incubated at 37 °C overnight. The MBC was defined as the lowest concentration at which the count was reduced to less than 99.9% of that of the bacterial inoculum.

Isolation of moenomycin resistant mutants. Two different procedures were followed for the isolation of spontaneous moenomycin resistant variants. In both cases E. coli K12 strain C600 was used since its auxotrophy for leucine and threonine was convenient for the control of the isolated variants. In the first procedure an overnight culture (40 ml) was centrifuged (for 15 min at 10000 g at room temperature), the cell pellet was suspended in 0.85% (w/v) NaCl (10 ml), and samples (0-1 ml) were spread on agar plates (rich medium) containing moenomycin (20 µg ml−1). After incubation at 37 °C for 36 to 48 h resistant colonies were picked and purified on plates containing moenomycin. In the second procedure an early exponential phase culture (40 ml) grown in rich medium up to 2 × 108 cells ml−1 was treated with moenomycin (25 µg ml−1). This led to a rapid decrease in optical density and viability. After 1 h the culture was centrifuged and the pellet was suspended in fresh medium (40 ml) devoid of antibiotic. Growth was allowed to proceed at 37 °C up to 2 × 108 cells ml−1 and moenomycin was added (25 µg ml−1); 1 h later the culture was centrifuged, the pellet suspended in 0.85% (w/v) NaCl (2 ml), and 0-1 ml samples of various dilutions (10−1, 10−2, 10−3, 10−4) were spread on agar plates (rich medium) containing moenomycin (20 µg ml−1). Only plates with dilution 10−4 were countable.

Preparation of [3H]decahydromoenomycin A. Moenomycin A, kindly provided by Dr Huber (Hoechst, Frankfurt, FRG), was tritiated by the Service des Molécules Marquées of the CEA (Saclay, France) according to the procedure developed for the preparation of unlabelled decahydromoenomycin A (Welzel et al., 1983). The labelled compound was purified by reverse-phase TLC on plates precoated with a 0.25 mm layer of RP18-F254S (Merck) in methanol/acetonitrile/water (6:3:1, by vol.). Under these conditions, RF values of 0.5 for moenomycin A and 0.25 for decahydromoenomycin A were determined. A sample of authentic unlabelled decahydromoenomycin A had been kindly provided by Dr Welzel (Bochum, FRG). Products were detected by UV light (254 nm) and by autoradiography (Kodak Kodirex X-ray film). In the crude radioactive material four products were detected at RF values of 0, 0.25, 0.6 and 0.9, recovered and found to account for 21, 41, 5 and 21% respectively of the radioactivity on the plate. The material recovered at RF 0.25 was considered to be [3H]decahydromoenomycin A.
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Its phosphorus content was determined (Chen et al., 1956) and its specific radioactivity was calculated to be 205 GBq mmol⁻¹. When tested in the in vitro assay for peptidoglycan polymerization (van Heijenoort et al., 1979), it was found to inhibit as efficiently as moenomycin A.

**Particulate fraction from E. coli.** Cells of E. coli [K12 HfrH or JA(200-pLC 19-19)] were grown at 37 °C with vigorous aeration in 2 l flasks containing 0.5 ml Penassay AM₂ medium (17.5 g l⁻¹), harvested at mid-exponential phase (about 4 × 10⁸ cells ml⁻¹; 1.3 g wet wt l⁻¹), washed with 0.02 M-Tris/HC1 buffer, pH 8, and disrupted by grinding with alumina as described by van Heijenoort et al. (1979). The mixture was then suspended in 0.05 M-Tris/HC1 buffer, pH 8.5, containing 10⁻³ M-2-mercaptoethanol and 10⁻⁴ M-MgCl₂, and centrifuged for 1 h at 100000 g. The pellet was suspended in the same buffer at a protein concentration of 24 mg ml⁻¹ and the suspension was kept at -20 °C.

**Assay for binding to particulate fractions.** Preliminary experiments were done to determine conditions (protein concentration, incubation time, final volume, etc) suitable for a binding assay. The following conditions were used: varying amounts (5 to 50 pmol) of [³H]decahydromoenomycin A (205 GBq mmol⁻¹), or a fixed amount of [³H]decahydromoenomycin A (11000 Bq) and varying amounts (0 to 500 pmol) of moenomycin A, were incubated at 37 °C for 15 min with 60 μg protein of the particulate fraction from E. coli K12 HfrH in a final volume of 200 μl 0.1 M-Tris/HC1 buffer, pH 8.5, containing 10⁻³ M-2-mercaptoethanol and 10⁻⁴ M-MgCl₂. Three samples (50 μl) of each reaction mixture were centrifuged for 15 min at 130000 g in a Beckman Airfuge and the pellets washed three times with the same buffer. The pellets were suspended in 0.5 ml of Soluene (Packard, USA), and after incubation at 37 °C for 10 min 5 ml of Lipofluor (Baker Chemical, Holland) were added. Suitable samples of the supernatants were added to 5 ml of Aqualyte (Baker). All samples were counted in an Intertechnique scintillation spectrometer. The blank value, which was obtained by adding at time zero 125 nmol unlabelled moenomycin A to an assay, accounted for less than 2% of the total radioactivity put in. It was subtracted from the values for each assay. Bound radioactivity was chased out by adding, after the 15 min incubation period, 125 nmol of unlabelled antibiotic to the reaction mixture.

**Assay for binding to E. coli membrane proteins.** The binding of [³H]penicillin to membrane proteins present in the particulate fraction from JA200(pLC 19-19) was determined as described by Spratt (1977). The same procedure was followed to evaluate the binding of [³H]decahydromoenomycin. Samples of the particulate fraction (100 μg protein) were incubated for 10 min at 37 °C with [³H]decahydromoenomycin (370 KBq; approximately 2 nmol) in a total volume of 50 μl 50 mM-sodium phosphate buffer, pH 6.8. After the addition of 5 μl unlabelled moenomycin A (40 mg ml⁻¹) to the samples, treatment followed the procedure used for [³H]penicillin (Spratt, 1977).

**RESULTS**

**Effect of moenomycin on E. coli in liquid media**

The effect of moenomycin on early exponential phase cells of E. coli K12 HfrH growing in liquid medium was greatly dependent on antibiotic concentration. With fast growing cells (generation time 35 min) a bacteriostatic effect was observed at about 1 μg ml⁻¹ and the greatest rate of autolysis at and above 10 μg ml⁻¹ (Fig. 1). The lag between the addition of the antibiotic and the onset of lysis (decrease in optical density) varied with the concentration (5 min at 25 μg ml⁻¹, 10 min at 10 μg ml⁻¹ etc). Under conditions of rapid lysis (10 μg moenomycin ml⁻¹) the viability decreased quickly and extensively (Fig. 2). Less than 1% of cells remained viable 30 min after the onset of lysis. Mg²⁺ ions have been shown to inhibit autolysis in E. coli (Leduc et al., 1982). Their addition at various times after treatment with moenomycin had an inhibitory effect on the rate of autolysis but none on the loss of viability (Fig. 2). Surprisingly, when cells were grown in a very rich medium such as Penassay broth (generation time 25 min) the effect of moenomycin was greatly reduced and concentrations higher than 50 μg ml⁻¹ were needed to obtain the same effect as with the rich medium usually used. With cells growing in glucose minimal medium (generation time 1 h) a bacteriostatic effect was observed at 5 to 10 μg ml⁻¹ and the greatest rate of autolysis at and above 50 μg ml⁻¹ (Fig. 3). The lag between addition of moenomycin and the onset of lysis was far greater than with fast growing cells. The effect of moenomycin on E. coli strains other than K12 HfrH was also examined. With strains C600, B and W7 growing in rich medium and the greatest rates of autolysis were also observed at 10 μg moenomycin ml⁻¹. With strain 0111: B4 a concentration greater than 25 μg ml⁻¹ was needed in rich medium and greater than 50 μg ml⁻¹ in Penassay broth.

The effect of moenomycin on E. coli K12 was highly dependent on the time of its addition. This was clearly exemplified by comparing early exponential phase cells (5 min lag before lysis and k = 90 × 10⁻³) and late ones (1 h lag and k = 25 × 10⁻³) after treating both with 10 μg
Fig. 1. Effect of moenomycin on growth of *E. coli* K12 in rich medium. To a culture (40 ml) of early exponential phase cells (10^8 ml^{-1}) of *E. coli* K12 HfrH growing in rich medium moenomycin (200 μl of an aqueous solution of appropriate concentration) was added at the time indicated by the arrow. Untreated cells; ○, 0.1 μg ml^{-1}; ■, 1 μg ml^{-1}; △, 5 μg ml^{-1}; □, 10 μg ml^{-1}; ■, 25 μg ml^{-1}.

Fig. 2. Effect of Mg^{2+} on moenomycin treated cells of *E. coli* K12. To a culture (40 ml) of early exponential phase cells (10^8 ml^{-1}) of *E. coli* K12 HfrH growing in rich medium moenomycin (10 μg ml^{-1} final concentration) was added at time zero; MgSO_4 (10^{-2} M final concentration) was added at the time indicated by the arrow. The optical density of cultures was determined at various times: ○, culture without Mg^{2+}; ■, culture with Mg^{2+}. The cell viability was determined at various times by the standard procedure of counting colonies (in triplicate) on agar plates: ○, culture without Mg^{2+}; △, culture with Mg^{2+}.

Fig. 3. Effect of moenomycin on growth of *E. coli* K12 in minimal medium. To a culture (40 ml) of early exponential phase cells (10^8 ml^{-1}) of *E. coli* K12 HfrH growing in minimal moenomycin (200 μl of an aqueous solution of appropriate concentration) was added at the time indicated by the arrow. Untreated cells; ○, 5 μg ml^{-1}; □, 10 μg ml^{-1}; △, 25 μg ml^{-1}; ▽, 50 μg ml^{-1}.

Moenomycin ml^{-1} (Fig. 1). Moreover, although a slow rate of lysis was still detectable with early stationary phase cells, no lysis could be promoted in overnight cultures. This dependence on growth phase, as well as on growth rate, was consistent with the results described for other types of autolysis of *E. coli* by Leduc et al. (1982).

**Morphological alterations and spheroplast formation induced by moenomycin**

To follow possible morphological alterations, early exponential phase cells were examined by phase-contrast microscopy at different times after the addition of moenomycin. With fast growing cells both rods and round cells were observed 15 min after addition of 10 μg antibiotic
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Table 1. MIC of moenomycin against various E. coli strains grown in various media

<table>
<thead>
<tr>
<th>Strain</th>
<th>Penassay broth</th>
<th>Rich medium</th>
<th>Minimal medium</th>
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<tbody>
<tr>
<td>K12 HfrH</td>
<td>128</td>
<td>8</td>
<td>16</td>
</tr>
<tr>
<td>K12 3092</td>
<td>64</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>32</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td>K235</td>
<td>128</td>
<td>32</td>
<td>32</td>
</tr>
<tr>
<td>0111:B4</td>
<td>512</td>
<td>32</td>
<td>32</td>
</tr>
</tbody>
</table>

After 30 min the remaining cells were mostly round and after 45 min only ghosts and cell debris were observed. With cells growing in glucose minimal medium, small normal rods were observed 60 min after addition of 25 µg moenomycin ml⁻¹. After 90 min rods had almost totally disappeared, but swollen irregularly shaped cells were present. All these morphological alterations were in agreement with the respective time courses of autolysis (Figs 1 and 3). When early exponential phase cells suspended in fresh medium containing sucrose were treated with 10 µg moenomycin ml⁻¹ they were converted to osmotically sensitive spheroplasts. This change was complete in 15 min with cells grown in rich medium but only after 2 h with cells grown in glucose minimal medium. In the latter case faster spheroplast formation was observed with moenomycin at 25 or 50 µg ml⁻¹.

MIC and MBC values

The MIC of moenomycin against several E. coli strains was determined in three different growth media (Table 1). Values varied from 4 to 512 µg ml⁻¹ and were thus in the range of those previously reported for various E. coli strains (Wasielewski et al., 1965; Slusarchyk, 1971; Huber, 1979). Furthermore, the MIC values against all the strains examined were much higher in Penassay broth than in the rich or minimal medium used (Table 1). This result should be compared with the fact that higher moenomycin concentrations were necessary to obtain a bacteriolytic effect when strains K12 HfrH and 0111:B4 were grown on Penassay broth. The lower efficiency of moenomycin in Penassay broth could perhaps be accounted for by its association with components of this medium. It was also observed that the MIC values of moenomycin against strain 0111:B4 were higher than against strains K12 or B whatever the growth medium. This could, perhaps be due in part to the differences in lipopolysaccharide structure, on which the permeability of the outer membrane of E. coli has been shown to depend (Nikaido, 1979). The MBC of moenomycin against E. coli K12 HfrH was found to be 16 µg ml⁻¹. This value is only twofold higher than the corresponding MIC value.

Binding to particulate fractions of E. coli and to membrane proteins

Moenomycin inhibits the polymerization reaction catalysed by PBP 1b in a cell-free system (van Heijenoort et al., 1979; van Heijenoort & van Heijenoort, 1960). Recently, the same effect was observed with moenomycin A and with various of its derivatives (P. Welzel, unpublished results). In particular, decahydromoenomycin A (Welzel et al., 1983) was found to inhibit as efficiently as moenomycin. Therefore, moenomycin A was tritiated to produce, after purification, [³H]decahydromoenomycin A, which was used to evaluate the extent of binding to particulate fractions and membrane proteins.

A binding assay was developed with the particulate fraction from E. coli K12 HfrH. When the concentration of free antibiotic in the assay was varied from 10⁻⁷ to 10⁻⁵ M, the amount of bound radioactive material increased steadily from 2 to 160 pmol. No well defined plateau was observed. By adding unlabelled antibiotic (5 × 10⁻⁴ M final concentration) the bound radioactivity was rapidly and completely chased in all cases, the remaining activity being identical to the blank value at time zero. This suggests that the radioactive material may bind reversibly to the peptidoglycan polymerases present in the particulate fraction. Assuming a quantitative yield for the preparation of the particulate fraction from cells and about 300
molecules of polymerases PBP la, PBP lb and PBP 3 per cell (Spratt, 1977), each assay should contain 5 to 10 pmol of polymerases. Since the amount of antibiotic effectively bound could greatly exceed these values, most probably a large part of bound [3H]decahydromoenomycin was non-specifically incorporated into membrane material in the same way as an amphipathic phospholipid would be, thus masking the specific binding to peptidoglycan polymerases.

The binding of [3H]penicillin and [3H]decahydromoenomycin to the membrane proteins of \textit{E. coli} JA200 (pLC 19-19) was compared. With the [3H]penicillin treated samples, the well known penicillin-binding protein pattern (Gale \textit{et al.}, 1980) was observed, except that owing to the presence of plasmid pLC 19-19 the overproduction of PBP lb was quite conspicuous as described by Tamura \textit{et al.} (1980). With the [3H]decahydromoenomycin treated samples, examination of the autoradiographs of the electrophoretic gels revealed no radioactive protein band. However, in these samples a wide low molecular mass band, presumably corresponding to the radioactive antibiotic itself, was clearly detectable.

\textit{Moenomycin resistant mutants}

Spontaneous moenomycin resistant mutants of \textit{E. coli} C600 were isolated in two ways. In the first procedure cells from an overnight culture were plated on agar containing moenomycin and colonies were detected at a frequency of about 10^{-9}. When these variants were tested for their susceptibility to moenomycin in liquid medium, all thirty examined were still highly sensitive to the antibiotic. In the second procedure fast growing early exponential cells in rich liquid medium were treated twice with moenomycin. In between the two treatments a period of regrowth without the antibiotic was allowed. After plating on agar containing moenomycin, 1200 colonies were obtained. Owing to the regrowth period between moenomycin treatments, most of these colonies undoubtedly originated from a very limited number of individual variants. Assuming a fixed generation time for all cells, a frequency of about 10^{-9} could be estimated from the time of the regrowth period. This is in agreement with the first procedure. However, in contrast to the first procedure, differences in the susceptibility to moenomycin in liquid medium were observed. Moenomycin led to very slow rates of autolysis with approximately one-third of the colonies, rates equivalent to that of the parental strain with another third and rates of intermediate value with the remaining third.

\textbf{DISCUSSION}

The effect of moenomycin on \textit{E. coli} was followed by measuring the optical density of liquid cultures, by determining viable counts, by microscopy and by determining MIC or MBC values. Although these various approaches were not easily comparable, a certain consistency in the results was observed. In fast growing cells the sharp decrease in optical density correlated well with the rapid loss of viability, the disappearance of whole cells and the rapid formation of spheroplasts in sucrose-containing medium. Similarly, the slower rate of formation of spheroplasts from cells grown in minimal medium was in agreement with the slower rates of cell lysis. Furthermore, the MIC values of moenomycin against cells grown in rich or minimal medium paralleled its effect on the optical density of slow growing liquid cultures.

The alterations in cell morphology, the formation of spheroplasts and the proneness to cell lysis that could be observed with moenomycin were in many respects similar to those described for other types of antibiotics that interfere with the biosynthesis of peptidoglycan in \textit{E. coli} (Gale \textit{et al.}, 1980; Rogers \textit{et al.}, 1980; Leduc \textit{et al.}, 1982). In particular, cephaloridine, which preferentially binds to PBP 1a and PBP 1b at low concentrations, also promotes fast cell lysis (Gale \textit{et al.}, 1980), and in a manner similar to moenomycin (Leduc \textit{et al.}, 1985). Both PBP 1a and PBP 1b appear to be bifunctional enzymes since they also catalyse an \textit{in vitro} transpeptidation reaction sensitive to \beta-lactams (Matsushashi \textit{et al.}, 1981; Nagakawa, 1984). In PBP 1b the transglycosylation and transpeptidation activities are on two separate domains (Kato \textit{et al.}, 1984; Nagakawa, 1984). Presumably the target site for moenomycin is on the domain carrying the transglycosylation activity. The nucleotide sequences coding for PBP 1a and PBP 1b have been published by Broome-Smith \textit{et al.} (1985).
The correlation between peptidoglycan metabolism and autolysis in _E. coli_ has been discussed by Leduc _et al._ (1982) and Weidel & Pelzer (1964). Under conditions of normal growth, the polymerization reactions catalysed by PBP 1a and PBP 1b are undoubtedly closely coupled in a well balanced manner with the rearrangement and recycling processes (Goodell & Schwartz, 1983: Goodell, 1985) that maintain peptidoglycan in a dynamic state during cell growth and division, and that are catalysed by specific endogenous peptidoglycan hydrolases. When the polymerization reactions are abruptly arrested by inhibitors like moenomycin or cephaloridine, certain of these hydrolases are apparently not subject to any rapid control and they continue unabated to catalyse the cleavage of peptidoglycan linkages that will lead to autolysis. Moreover, the coupling between polymerase and hydrolase activities appears to occur at the outer side of the cytoplasmic membrane, since PBP 1a and PBP 1b are located in this membrane (Spratt, 1977), and since after moenomycin or cephaloridine treatment peptidoglycan degradation seems to start in the periplasmic region immediately in contact with the cytoplasmic membrane (Leduc _et al._, 1985). Even after all cells lyse peptidoglycan breakdown does not exceed 30 to 35%, and the rod shape of isolated peptidoglycan sacculi is preserved for some time after the onset of cell lysis (Leduc _et al._, 1985). The alterations in cell morphology observed with moenomycin before lysis are presumably the consequence of this partial breakdown of peptidoglycan. During moenomycin induced formation of spheroplasts breakdown is perhaps more extensive, but this remains to be substantiated.

The assay with the particulate fractions did not permit us to discriminate between specific binding to peptidoglycan polymerases and non-specific incorporation into membrane material. The complete chase of bound radioactivity suggested that both the non-specific and the specific binding was reversible. However, owing to the low amounts of polymerases present in the assay, the binding to these enzymes would have to be studied by developing a more sensitive assay with an antibiotic of higher specific radioactivity. A reversible interaction between the antibiotic and the sensitive polymerases was also suggested by the absence of decahydromoenomycin-binding proteins, at least under the conditions used for the irreversible binding of penicillin. Perhaps it would be appropriate to examine the binding under the conditions of the polymerization assay (van Heijenoort _et al._, 1979). Ultimately, the specific binding will have to be studied with the purified polymerases. A reversible binding would be in agreement with the postulated competitive inhibition based on the structural analogy between their membrane substrate and moenomycin (Linnett & Strominger, 1973; P. Welzel, unpublished results). Presumably moenomycin competes in _vitro_ with the membrane substrate by insertion into the cytoplasmic membrane via its moenocin moiety (Welzel _et al._, 1981). This raises the questions of how it penetrates this membrane. The use of radioactive decahydromoenomycin for the determination of its location in the envelope and the detailed study of the spontaneous resistant variants isolated here could perhaps help to bring some insight into this matter.

This work was supported by grants from the Centre National de la Recherche Scientifique (UA 1131 and ATP Microbiologie).

**REFERENCES**


