Turnover of Murein in a Diaminopimelic Acid Dependent Mutant of *Escherichia coli*

J. Chaloupka and M. Sternadová

Department of General Microbiology, Institute of Microbiology, Czechoslovak Academy of Sciences, Prague 4

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**ABSTRACT.** *Escherichia coli* 173-25, whose cell wall was labelled with $^{14}$C-diaminopimelic acid, was found to lose about 15% radioactivity during growth in a fresh medium, two thirds or more being lost during the first two generations. Degradation products of the cell wall were mostly of low-molecular type. About 5% of the cells lysed as a result of transfer associated with filtration, washing and resuspension of the bacterial population. No additional lysis could be observed during cultivation. Murein was also degraded during incubation in a diaminopimelic acid (DAP) deficient medium. The degradation was very low during the first 20 min. The amount of wall material released from the cells increased between 20—30 min and a sudden decrease of viability of the population was observed. The degradation of murein triggered by starvation for DAP continued when supplementing the deficient medium with DAP and when growth was resumed. About one-half of the cell wall material released into the medium under these conditions was macromolecular. However, lysis of the cells and release of proteins into the medium were rapidly interrupted after DAP was added to the starving culture and the differential rate of synthesis of the cell wall increased. Turnover of murein was not associated with protein turnover.

The cell wall and particularly its rigid component, mucopeptide (murein), determine the shape and size of the bacterial cell. It was suggested previously that certain linkages in the murein structure are hydrolyzed during growth of the cell, thus facilitating the insertion of new building units and enlargement of the cell surface (Weidel and Pelzer, 1964). Enzymes hydrolyzing murein were found in the cell walls of many bacteria (Ghuysen, 1968). It was found in this laboratory that a gradual decrease of radioactivity of the cell wall takes place in the growing population of *Bacillus megaterium* prelabelled with $^{14}$C-diaminopimelic acid. The results were interpreted as symptoms of turnover of the cell wall (Chaloupka et al., 1962). It was found in further work that some products of murein degradation are released into the medium during growth and a relationship between the rate of growth and the turnover of murein was suggested (Chaloupka et al., 1964; Chaloupka, 1967).

Turnover of various components of the cell wall of different species of Gram-positive bacteria was also described by Rogers (1967a), Ellwood and Tempest (1969) and by Mauck and Glaser (1970).

Leutgeb and Schwarz (1967) found certain changes in distribution of internal linkages of murein during the first phase of growth of a DAP-dependent *Escherichia coli* mutant in a DAP-deficient medium. However, the degradation of murein as well as a decrease of viable cell count were observed only after prolonged DAP starvation. Similar degradation changes in the wall were also detected during conversion of *Escherichia coli* to spheroplasts by means of penicillin (Schwarz and Weidel, 1965). Schwarz *et al.* (1969) were able to relate the activity of murein hydrolases to replication of chromosome and cell division.

Some data concerning the degradation and turnover of murein of a DAP-dependent *Escherichia coli* will be referred to in the present communication.
MATERIALS AND METHODS

**Bacterial strain.** Escherichia coli 173 — 25 daps — mutant kindly supplied by Dr. Elisabeth Work of Twyford Laboratories, London, was used throughout.

**Labelling and cultivation.** The culture was maintained in medium 56 with $1 \times 10^{-4}$M DAP and lysine. When labelling the cell wall the culture was grown $2\frac{1}{2}$ to 3 h in medium 56 (Cohen and Monod, 1951) with glycerol, supplemented with casamino-acids Difco (5 mg/ml) and containing $2 \times 10^{-4}$M-diaminopimelic acid (0.05 μCi/ml, $2.5 \times 10^{-5}$M) and nonradioactive lysine ($1 \times 10^{-5}$M). This quantity of DAP was sufficient to maintain the logarithmic growth for the required time interval. 80% of incorporated $^{14}$C was found in DAP, 20% in lysine. Cultivation was performed in a shaken water bath at 37°C. The labelled population was filtered through a membrane filter Synpor 6 (Synthesia, pore size 0.4 μm) and transferred to the same medium without DAP. Nonradioactive DAP ($1 \times 10^{-4}$M) was then added after various intervals of starvation (0—60 min).

Labelling the cells with $^{14}$C-leucine (universally labelled, 0.1 μCi/ml, 18.8 mCi/mm) was performed as above but the content of casamino acids was decreased to 1 mg/ml. The quantity of casamino acids was increased to 5 mg/ml after 120 min growth.

All cultivations were performed in a shaken water bath at 37°C. Samples taken at various time intervals were precipitated with trichloracetic acid (TCA) containing excess non-radioactive DAP or leucine. The degradation of murein and proteins was calculated from a decrease of TCA-precipitable radioactivity collected on membrane filters Synpor (pore size 0.4 μm) and an increase of radioactivity in the TCA supernatant. When determining the amount of radioactive proteins released from cells into the medium, 2 mg/ml caseine were added to the supernatant after centrifugation and the proteins were precipitated with 5% TCA. The precipitate was centrifuged, washed, dissolved in 5% ammonia and placed on planchets. The samples were air-dried and the radioactivity assayed.

Starvation was performed in medium 56 containing lysine ($10^{-3}$M), casamino acids, but without DAP.

**Measurement of growth and autolysis.** Absorbance was measured in 1 cm cuvettes in a spectrophotometer Specol at 600 nm. The value of $A_{600}$ of 0.500 corresponded to $1 \times 10^{9}$ cells/ml. Autolysis of the cell suspension resuspended in Tris buffer (0.01M) pH 8.6 containing 2 mg/ml NaCl and 100 μg/ml chloramphenicol was measured by decrease of absorbance during incubation in a water bath at 45°C.

**Assay of radioactivity.** Radioactivity in TCA precipitates collected on membrane filters or in samples evaporated on planchets was assayed in a Tracerlab low background counter. Radioactivity of TCA supernatants was assayed in a Bray solution (Bray, 1960) using the Mark I liquid scintillation system (Nuclear Chicago).

**RESULTS**

A DAP-dependent mutant of *Escherichia coli* grew exponentially even in the presence of very low concentrations of DAP ($5 \times 10^{-6}$M) — Fig. 1A. A decrease of density occurred very rapidly after the exhaustion of DAP. A DAP concentration of $2.5 \times 10^{-5}$M was found to be satisfactory in our experiments as under these conditions the population reached an absorbance of 1.30—1.60 corresponding to 2.6 to $3.2 \times 10^{9}$ cells/ml. This concentration made possible effective labelling of the cells with $^{14}$C-DAP. The logarithmic growth was restored immediately after transfer of the population grown in the medium with $2.5 \times 10^{-5}$M DAP to an absorbance of 0.5—0.6 into the medium containing $1 \times 10^{-4}$M DAP. After a 5 min starvation without DAP, the growth of the bacteria continued normally upon supplementation of