Effect of Penicillins on the Level of Inorganic Pyrophosphatase in *Escherichia coli* K 12

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The relative rate of DNA synthesis increases over control level and the synthesis of inorganic pyrophosphatase is stimulated when cells of *E. coli* are cultured in the presence of penicillins which inhibit cell division and cause filamentation of the cells. In contrast to that in temperature sensitive cell division mutants filamentation at 42 °C does not change the DNA/cell mass ratio and the synthesis of inorganic pyrophosphatase is not stimulated. This suggests that the change in the synthesis of the enzyme is not correlated with the filamentation but is a consequence of the supernormal DNA/cell mass ratio that appears in the presence of penicillins.

Introduction

Inorganic pyrophosphatase (EC 3.6.1.1) is a constitutive enzyme in *E. coli*, so the specific activity of the enzyme is independent of the composition of the growth medium [1]. However, in our studies concerning the role and regulation of inorganic pyrophosphatase in bacteria we have observed that also its activity can be changed. The specific activity of the enzyme decreased to a new constant level when DNA synthesis was inhibited by transferring a culture of a temperature sensitive *dna* A mutant strain of *E. coli* K 12 to the restrictive temperature [2], whereas stimulation of the synthesis was observed when DNA synthesis was partially arrested by inhibitors of DNA synthesis in the cultures of *E. coli* K 12 [3, 4]. In the presence of the drugs the cells turn into filaments, because cell division does not happen in the absence of DNA synthesis. So we wanted to test if the stimulation of inorganic pyrophosphatase is coupled to the inhibition of cell division. For that purpose we used penicillins, which at low concentrations inhibit cell division, and two mutants of *E. coli* which had temperature sensitive cell division.

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Materials and Methods

Chemicals

Penicillin G was a product of medical factory Leiras (Finland) and mecillinam was kindly supplied by Leo Pharmaceutical Products (Denmark). For reaction mixtures and media commercially available chemical of analytical grade were used. All chemicals were used without further purification.

Bacterial strains and their culture condition

E. coli K 12 a wild type train from the collection of our laboratory, Y 16-mutant of *E. coli* K 12 600 and Bug 6-mutant of *E. coli* AB 1157 were used as test organisms. Y 16-mutant, isolated and described by Santos and De Almeida [5], was kindly donated by Dr. De Almeida, University of Rio de Janeiro, Brazil. Dr. Park, Tufts University, USA, donated Bug 6-mutant, isolated and described by Reeve *et al.* [6].

Cells were transferred with a platinum wire from a nutrient agar slant into 50 ml of the minimal glucose-mineral salts medium [2]. In case of mutant strains Y 16 and Bug 6 the minimal medium was enriched with 0.5% casein hydrolysate (Bacto casamino acids by Difco) and tryptophan 20 mg/l. After overnight growth in a rotary shaker the culture (turbidity 200–240 Klett units) was diluted with fresh similar medium, and immediately devided into 2–4 portions. One served as a control; to the others drugs were added at time zero, as described in the Results and Discussion section. Growth took place in a rotary shaker and was followed by measuring the turbidity of the culture with a Klett-Summerson colorimeter using filter 62 (590–650 nm).

Enzyme assay

The activity of inorganic pyrophosphatase was determined by measuring the liberation of labelled phosphate from (^{32}P)PP_i [7]. The collection of cell samples and their treatment have been described earlier [3].

DNA and protein determination

DNA was determined by measuring the enhancement of the fluorescence of ethidium bromide in the presence of alkali-treated samples [8]. The

amount of DNA is given in the figure as arbitrary units (fluorometer readings). Protein was measured as the turbidity caused by added sulfosalicylic acid [9].

Results and Discussion

When penicillin G (30 µg/ml) was added to the culture of *E. coli* K 12, cell wall synthesis required for septation was inhibited, which caused the formation of aseptate filaments. The growth of the cell mass is only slightly retarded (Fig. 1 A), and the relative rate of net DNA synthesis rose over control level (Fig. 1 B). The differential rate of synthesis of inorganic pyrophosphatase was stimulated about 2-fold (Fig. 1 C). Similar results were obtained with another penicillin derivative [10], the amidino penicillanic acid mecillinam (1 µg/ml), which inhibit cell elongation with the result that cells grow into osmotically stable spheres.

Y 16 is a temperature sensitive cell division mutant of *E. coli* K 12. It is deficient in septum formation at 42 °C, while continuing other cellular functions and maintaining its viability after several hours at the nonpermissive temperature [5]. In our experiment filamentation of cells took place when the mutant was shifted from 30 °C to 42 °C, while the DNA/cell mass ratio did not change. The differential rate of synthesis of inorganic pyrophosphatase was similar at both temperatures. Similar results were obtained by growing another temperature sensitive cell division mutant Bug 6-strain of *E. coli* AB [6] at nonpermissive temperature.

However, the differential rates of synthesis of DNA and pyrophosphatase increased also in temperature sensitive cell division mutants when filamentation was caused by penicillins. Low concentrations of penicillin G (30 µg/ml) at 30 °C resulted in increased DNA/cell mass ratio and a higher differential rate of synthesis of inorganic pyrophosphatase.

We believe that the change in the synthesis of the enzyme is not directly related to the inhibition of cell division, but is a consequence of excessive DNA synthesis that appears in the presence of penicillin. We have earlier observed that when the relative rate of DNA synthesis increases over control level (when DNA synthesis is restored after partial inhibition), the synthesis of inorganic pyrophosphatase is stimulated [3, 4].

Completion of a round of DNA replication begins a sequence of events which in the cell envelope

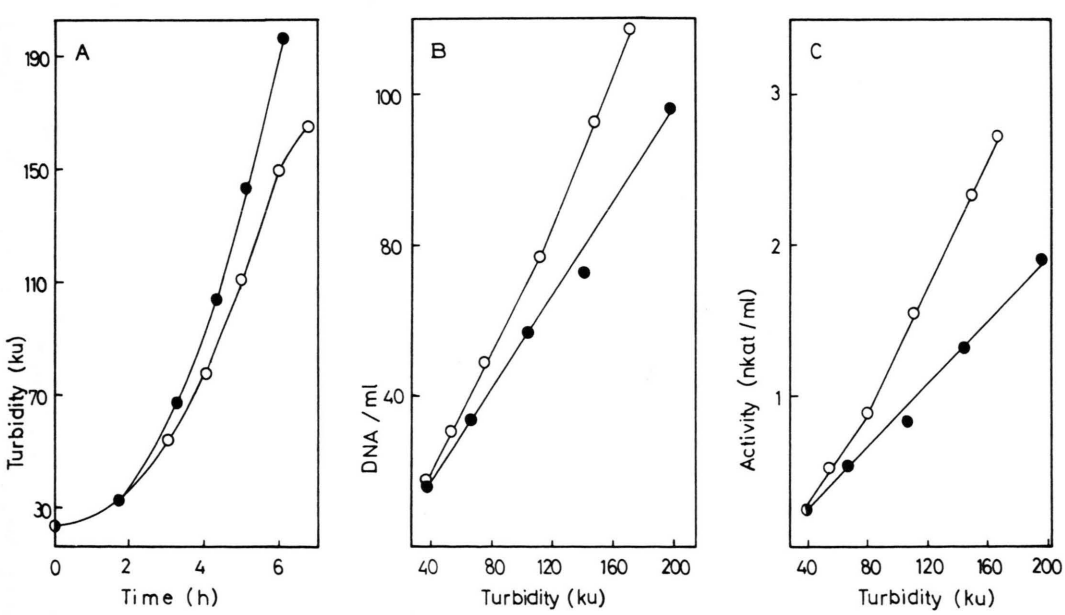

Fig. 1. Effect of penicillin on *E. coli* K 12. A culture grown overnight in the minimal medium was diluted with a fresh portion of the same medium. The resulting cell suspension was immediately divided into 2 portions. One served as a control; to the other penicillin G 30 µg/ml was added at zero time. Closed symbols represent the control culture. A: cell mass, B: differential rate of synthesis of DNA, C: differential rate of synthesis of inorganic pyrophosphatase.

lead to reactions necessary for septum formation and cell division. We are not able to explain how penicillins loosen the relationships between DNA synthesis and cell division and cause the supernormal DNA/cell mass ratio.

We used the turbidity of the culture as a measure of the total cell mass. The ratio between the mass and the turbidity changed somewhat as the cells turned into filaments, but we found this change to be small, always less than 10%. Furthermore, this change could not invalidate our results because the cells of the temperature sensitive cell division mutants at 42 °C were elongated as much as those of wild type strain cultivated in the presence of penicillin, but the supernormal DNA/cell mass ratio appeared only in the latter case.

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