NOTES

Penicillin-Induced Lysis of *Escherichia coli* in Medium That Does Not Support Sustained Growth

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Received for publication 10 December 1975

Exponentially growing cells of an *Escherichia coli* auxotroph that are washed and resuspended in buffer containing a carbon source undergo sufficient growth to render them susceptible to penicillin-induced lysis, even in the absence of the required metabolite. This lysis is probably responsible for the reduction in intracellular enzyme activity previously attributed to a direct effect of penicillin upon the enzyme.

Baich (1) has reported that penicillin inhibits the first step in proline biosynthesis in *Escherichia coli* W. This was based upon the observation that midlog- to late log-phase cells, resuspended in buffer under conditions that do not support growth, produce less Δ^1 -pyrroline-5-carboxylate (PCA), an intermediate in proline biosynthesis, when incubated in the presence of penicillin than when incubated in its absence. Her interpretation was predicated upon the unstated assumption that the cells were not undergoing penicillin-induced lysis.

Our interest in the effects of penicillin and other antibiotics upon proline metabolism (4, 5) led us to attempt to reproduce the findings of Baich. Although we, also, have observed an effect of penicillin on PCA production, we have found that *E. coli* cells lyse in buffer under the conditions that Baich reported lead to a reduction in enzyme activity. Therefore, the reported inhibitory effect of penicillin upon the first step in proline biosynthesis is actually due to penicillin-induced lysis of the cells.

We employed a *proC*⁻ derivative of *E. coli* K-12 W3110 (*thy*⁻), CB0441 (2), in all experiments. *ProC*⁻ mutants are blocked in the conversion of PCA to proline and accumulate PCA, which is excreted into the medium (7). The level of PCA in the medium can be measured by a colorimetric test involving orthoaminobenzaldehyde (7). Media and growth conditions were as described previously (4). The resuspension buffer contained 0.02 M phosphate (pH 7.0) supplemented with glucose (0.5%) and glutamic acid (1 mg/ml) (1).

In our first experiment, cells were grown in L broth to midlog phase, harvested, and resus-

pended at a high titer (approximately 10^{10} cells/ml) in tris(hydroxymethyl)aminomethane buffer, supplemented as previously found to be optimal for PCA excretion (6). When aliquots of this resuspension were incubated without or with penicillin (up to 20,000 U/ml), appreciable PCA production was observed only in the presence of 200 U or less of penicillin per ml (Fig. 1). We also observed a striking decrease in optical density in the presence of 2,000 U or more of penicillin per ml. Since Baich reported that

FIG. 1. Inhibition of PCA production by varying levels of penicillin (1 U corresponds to 0.6 μ g). Mid-log-phase cells were resuspended in supplemented buffer. After 2 h of incubation in the absence of penicillin, there was 0.9 μ mol of PCA produced per 10^9 cells.

FIG. 2. Effect of 2,000 U of penicillin per ml on log-phase cells resuspended in supplemented buffer. (A) Cell density. In the absence of penicillin, an increase in cell density of approximately 10% occurred in 90 min. In the presence of penicillin, a decrease in cell density of approximately 50% occurred during this interval. (B) Cell titer. In this experiment, a decrease in cell density of approximately 60% occurred during this interval.

penicillin above 2,000 U/ml maximally inhibits PCA synthesis, we suspected that this inhibition might be due to penicillin-induced lysis (which Baich did not detect since viable count assays were not performed and since the resuspensions were too dilute to be followed turbidometrically).

As a further test, we repeated the experiment using conditions as close as possible to those used by Baich: growth in glucose minimal medium plus proline (and also thymine), harvest-

ing at specific growth phase, washing twice in distilled water, and resuspension in phosphate buffer supplemented with glutamic acid and glucose at about 10^8 cells/ml. Over a 90-min period, there was an increase in cell density in the absence of penicillin and lysis in the presence of 2,000 U of penicillin per ml (Fig. 2).

It is clear from our observations that Baich assumed incorrectly that proline auxotrophs would not be susceptible to lysis when resuspended in buffer (containing an energy source but lacking proline).

Baich also reported that late log-phase cells were less susceptible to penicillin inhibition of PCA synthesis than were midlog-phase cells. Similarly, we find that there is less increase in cell density (in the absence of penicillin) of late than of midlog-phase cells resuspended in buffer. Baich also reported that high osmolarity served to protect the cells from penicillin inhibition of PCA synthesis. This observation is consistent with the previous demonstration that penicillin does not cause cell lysis in hypertonic medium (3).

It is clear that Baich was measuring the extent of residual growth and concomitant penicillin-induced lysis, rather than an inhibition of enzyme activity. Thus, although we have reproduced the effects reported by Baich, we can account for them on the basis of the well-known lytic effect of penicillin, without resorting to any hypothetical effect of penicillin on proline biosynthesis.

Our observations illustrate the sensitivity of *E. coli* to penicillin-induced lysis, even under conditions where there is no exogenous source of required metabolites. Unless the endogenous pool is also depleted (4), cells can metabolize and undergo limited growth. Hence, log-phase cells, even though washed and resuspended in buffer, can be susceptible to penicillin-induced killing.

This work was supported by Public Health Service grant AI-10862 from the National Institute of Allergy and Infectious Diseases.

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